Fungal Biology

Bhim Pratap Singh ·
Ahmed M. Abdel-Azeem ·
Vibhav Gautam · Garima Singh ·
Santosh Kumar Singh *Editors*

Endophytic Fungi

The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries

Springer

Fungal Biology

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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Editors
Bhim Pratap Singh
Department of Agriculture
and Environmental Sciences
National Institute of Food Technology
Entrepreneurship and Management
(NIFTEM)
Sonipat, Haryana, India

Vibhav Gautam
Centre of Experimental Medicine
and Surgery
Institute of Medical Sciences
Banaras Hindu University
Varanasi, India

Santosh Kumar Singh
Centre of Experimental Medicine
and Surgery
Institute of Medical Sciences
Banaras Hindu University
Varanasi, India

Ahmed M. Abdel-Azeem
Botany and Microbiology Department
Faculty of Science
Suez Canal University
Ismailia, Egypt

Garima Singh
Department of Botany
Pachhunga University College
Mizoram University
Mizoram, India

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Foreword

With immense joy and anticipation, I am delighted to present this extraordinary addition to Springer Nature's esteemed collection of works. This book opens the door to a realm of knowledge and exploration in the field of endophytic fungal research, inviting readers to comprehend the crucial role and significance of these fungi across various industries.

Encompassing contributions from distinguished scholars, this volume delves into a myriad of topics, ranging from the symbiotic relationships of endophytes with their hosts to their potential applications in agriculture and pharmaceuticals. The chapters are meticulously arranged to facilitate a comprehensive understanding of the roles and importance of endophytic fungi.

This book serves as an all-in-one solution for researchers and students engaged in the study of endophytic fungi, while also providing valuable reference material for professionals in the agriculture and pharmaceutical sectors. I extend my heartfelt appreciation to the editors and contributors who have poured their passion and expertise into laying the foundation for this remarkable volume. Their collective contributions enrich the academic discourse that shapes our world.

Lastly, I hope this book will inspire and empower readers to reach new heights of expertise in endophytic fungal research. May it ignite a spark of curiosity that leads to remarkable advancements in this field.

Panjab Singh
President, FAARD Foundation, Varanasi, India
Chancellor, Rani Lakshmi Bai Central Agricultural University, Jhansi, India
Ex-Secretary, Department of Agriculture Research and Education, Varanasi, India
Ex-Director General, Indian Council of Agricultural Research, Varanasi, India
Ex-Vice Chancellor, Banaras Hindu University, Varanasi, India

Preface

Every book has a beginning, and this one is no exception. As we read the compiled chapters on various aspects of endophytic fungi, we are filled with their importance in the agriculture, health, and pharmaceutical industries. This preface captures the intentions and motivations driving us to bring book volume on *Endophytic Fungi: The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries*.

The genesis of this book lies in a confluence of experiences and questions. It is born out of countless conversations, moments of introspection, and a deep-seated desire to share insights that have shaped our understanding of the role of endophytic fungi in our life. In this book volume, you will find contributions from academicians working in the area and understanding the potential of endophytic fungi for sustainable development.

In this book, you will encounter chapters on broad aspects of endophytic fungi that may challenge, resonate, or inspire you and will generate your interest in this group of microorganisms. Others might encourage you to pause, reflect, and weave your interpretations into the narrative.

Ultimately, this book is a gesture of sharing – an offering of thoughts, perspectives, and facts on the latest developments on the role of endophytic fungi in agriculture and pharmaceutical industries. We hope readers will find the content appropriate for teaching and research.

Sonipat, Haryana, India — Bhim Pratap Singh
Ismailia, Egypt — Ahmed M. Abdel-Azeem
Varanasi, India — Vibhav Gautam
Mizoram, India — Garima Singh
Varanasi, India — Santosh Kumar Singh

Acknowledgments

The completion of this book volume marks a significant milestone, and it is with great humility and gratitude that we acknowledge the individuals whose contributions have made this achievement possible.

First and foremost, we extend our heartfelt appreciation to all the contributors who contributed to this book volume in the form of a book review. Your knowledge and invaluable insights have been instrumental in shaping the direction and depth of this book. We also thank the Springer Nature team for their continuous support and cooperation in bringing the volume in final shape.

Finally, we extend our gratitude to the readers who will embark on this journey. We hope the contents covered in this volume will match the reader's expectations.

Bhim Pratap Singh
Ahmed M. Abdel-Azeem
Vibhav Gautam
Garima Singh
Santosh Kumar Singh

Contents

Contributors

Charu Agnihotri Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India

Shekhar Agnihotri Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India

Vivek Ambastha Biology Department, Washington University in St. Louis, St. Louis, MO, USA

Awmpuizeli Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Kaizar J. Bharmal Institute of Chemical Technology Marathwada Campus, Jalna, Maharashtra, India

Pranav Bhaskar Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, VA, USA

Beirachhita Bohia Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Richa Bora Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Babli Borah Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Maini Borah Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Jyoti Lakshmi Hati Boruah Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Vishva Deepak Chaturvedi Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Kangra, Himachal Pradesh, India

Deepjyoti Das Natural Product Chemistry Group, Chemical Sciences and Technology Division, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Mriganka Das Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Mayuresh Dorle Department of Technology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India

Arti Gaur Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India

Vibhav Gautam Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Navoneel Giri Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Parishmita Gogoi Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Priyanka Gogoi Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Upasana Goswami Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Rohit Prasad Gupta Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Divya Jain Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali, Rajasthan, India

Aman Jaiswal Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

M. N. Jha Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

Ojaswinin Kalita Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Swapnil C. Kamble Department of Technology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India

Manveer Kaur Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Rajeev Kaushik Division of Microbiology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Sibashish Kityania Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Amrender Kumar Agricultural Knowledge Management Unit (AKMU), ICAR-Indian Agricultural Research Institute, New Delhi, India

Awnish Kumar Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Deepak Kumar Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Geeta Kumari Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

Ketan Kumar Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India

M. Kumar ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, MauNath Bhanjan, Uttar Pradesh, India

Niraj Kumar Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Rahul Kumar Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Rajiv Kumar Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

C. Lalbiaktluangi Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Nancy Lalhriatpuii Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Rosie Lalmuanpuii Department of Botany, Mizoram University, Aizawl, Mizoram, India

Felicia Lalremruati Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Lalrokimi Department of Biotechnology, Mizoram University, Aizawl, Mizoram, India

A. K. Lavanya ICAR-Central Research Institute for Jute and Allied Fibers, Barrackpur, Kolkata, West Bengal, India

Cecilia Lulu Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Himanshu Mahawar ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh, India

Vishnu Mishra Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA

Cheris Mistry Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Anish Nagar Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Mayur G. Naitam Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

Deepa Nath Department of Botany, Guru Charan College, Silchar, Assam, India

Rajat Nath Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Rupshikha Nath Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Urvi R. Parlikar Institute of Chemical Technology Marathwada Campus, Jalna, Maharashtra, India

Ajit Kumar Passari Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Ciuded de Mexico, México

Swati Patel Department of Plant Biotechnology, ASPEE Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, Gujarat, India

Ratna Prabha Agricultural Knowledge Management Unit (AKMU), ICAR-Indian Agricultural Research Institute, New Delhi, India

Chandrabhan Prajapati Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Vijay Kumar Prajapati Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India

Ramannagari Visweswara Maruthi Prasad Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India

Rajneesh Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Abhishek Rao Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India

Ritika Agricultural Knowledge Management Unit (AKMU), ICAR-Indian Agricultural Research Institute, New Delhi, India

Ratul Saikia Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Lovelesh Singh Saini Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India

Poorvi Saini Department of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad, India

Rashmi Saini Department of Zoology, Gargi College, University of Delhi, Delhi, India

Joyita Sarkar Institute of Chemical Technology Marathwada Campus, Jalna, Maharashtra, India

Udita Sarkar Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Sai Sarvan Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Rashid Khalid Shaikh Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Apurva Singh Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Bhim Pratap Singh Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India

Dhananjaya P. Singh ICAR-Indian Institute of Vegetable Research, Varanasi, India

Prashant Kumar Singh Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Rajan Singh ICAR-Indian Institute of Vegetable Research, Varanasi, India

Santosh Kumar Singh Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Satyendra Singh Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India

Shwetank Singh Department of Plant Pathology, Acharya Narendra Dev University of Agriculture and Technology, Ayodhya, Uttar Pradesh, India

Vishal Kumar Singh Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Kunuthuru Sivakumar Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India

Reetesh Srivastava Faculty of Agricultural Sciences, Institute of Applied Sciences & Humanities, GLA University, Mathura, UP, India

Anupam Das Talukdar Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Pooja Talukdar Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Harshita Tiwari Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Rahul Tiwari Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Viabhav Kumar Upadhyay Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

Ashish Verma Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Ashish Warghane School of Applied Sciences and Technology, Gujarat Technological University, Ahmedabad, Gujarat, India

Priti Warghane Graduate School of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India

Archana Yadav Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Mukesh Kumar Yadav Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

Zirlianngura Department of Chemistry, Government Zirtiri Residential Science College, Aizawl, Mizoram, India

Zothanpuia Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India

Chapter 1
Endophytic Fungi: Symbiotic Bioresource for Production of Plant Secondary Metabolites

Lovelesh Singh Saini, Swati Patel, Arti Gaur, Priti Warghane, Rashmi Saini, and Ashish Warghane

Abstract Endophytes are a diverse group of microbes that asymptomatically colonize the interior organs of higher plants. Fungi and bacteria are also considered endophytes, although the former is more common, adaptable, and pervasive microorganisms that colonize plants growing in practically all geoclimatic situations. Endophytic fungi are a kind of symbiotic fungus that lives inside the tissues of a plant. These fungi have a symbiotic relationship with the plant, providing nutrients and protection while receiving shelter and food from its host. Endophytic fungi can play a significant role in the sustainability of a plant species. Different strains of endophytic fungus are being researched, and the accompanying restrictions are being addressed for maximum use/multidimensional applications as beneficial metabolites with multifaceted environmental effects are progressively being discovered. The current chapter reveals that endophytic fungi are a chemical reservoir of novel compounds and elicit plant secondary metabolites with numerous applications in the pharmaceutical and agrochemical industries. Various bioactive metabolites produced by endophytic fungi have shown socioeconomic value and found uses in agriculture and the environment, as well as biofuels and biocatalysts.

L. S. Saini · A. Gaur
Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India

S. Patel
Department of Plant Biotechnology, ASPEE Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, Gujarat, India

P. Warghane
Graduate School of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India

R. Saini
Department of Zoology, Gargi College, University of Delhi, Delhi, India

A. Warghane (✉)
School of Applied Sciences and Technology, Gujarat Technological University, Ahmedabad, Gujarat, India

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1 Introduction

Higher plants provide complex, multilayered, diversified environments in spatial and temporal habitats that are home to assemblages of microorganisms of various species. Plants inside and outside their tissues contain many microorganisms, including bacteria, fungi, archaea, algae, and protists. Complex interactions between these species have progressively developed over a long-term, resulting in their symbiosis as a group rather than leaving them as separate species (Hassani et al. 2018). The interactions between these microbes and plants positively impact plant sustainability, biodiversity, and ecological stability (Rosier et al. 2016; Bai et al. 2017; Sasse et al. 2017).

Microorganisms, known as endophytes, inhabit plants for at least a portion of their life cycle without producing disease symptoms (Bacon and White 2000). Thus, "endophytism" is a special plant-microbe relationship defined by "location" (not "function") that is momentarily symptomless, inconspicuous, and established within the living host plant tissues (Kusari and Spiteller 2012). Plants that possess poisonous alkaloids and interact with endophytes show high resistance to biotic and abiotic stresses (Carroll 1988; Chagas et al. 2018). Later on, a large body of evidence suggested that endophytic associations were crucial for the development of the plant immune system (Soliman et al. 2015), the control of disease (Terhonen et al. 2016), the uptake of nutrients (Hiruma et al. 2016), and to enhance the ability to withstand abiotic pressures (Khan et al. 2013).

In the accumulations of plants and microbes, microfungi predominate, colonizing the surfaces of leaves and twigs (epiphytes), the tissues inside leaves (foliar endophytes), the young and old bark (bark endophytes), and the wood (xylem endophytes and wood decomposers) (Stone et al. 2004). Endophytic fungus is highly varied and polyphyletic; it includes organisms that can live asymptomatically in the above- and belowground tissues of plants and play a wide range of ecological tasks (Saikkonen et al. 1998). Numerous endophytes can produce a range of bioactive compounds that may be employed directly or indirectly as therapeutic agents against a variety of ailments (Strobel et al. 2004; Staniek et al. 2008; Aly et al. 2010; Kharwar et al. 2011; Kusari and Spiteller 2012; Passari et al. 2015, 2016). Additionally, a large number of endophytic fungi are sources of cytotoxic compounds and secondary metabolites that are biologically active, like paclitaxel, podophyllotoxin, deoxypodophyllotoxin, camptothecin, hypericin, emodin, and azadirachtin (Stierle et al. 1993; Eyberger et al. 2006; Puri et al. 2005, 2006; Kusari et al. 2008, 2009, 2012; Shweta et al. 2010). Various coniferous and deciduous tree hosts for endophytic *Pezicula* species strains produce bioactive secondary

metabolites in culture (Noble et al. 1991; Schulz et al. 1995). Cytochalasins and indole diterpenes with significant biological activity are commonly produced by endophytic species of the *Xylariaceae* (Brunner and Petrini 1992).

Endophyte synthesis of bioactive substances, mainly those unique to their host plants, is significant from a biochemical, pharmacological, and ecological standpoint. Exciting opportunities exist to use endophytic fungus to produce a wide range of recognized and undiscovered physiologically active secondary metabolites.

2 Evolution of Endophytic Fungi in Plants

Endophytic fungi, which dwell inside plant tissues permanently or for a specific time during their life cycles, colonize plants, especially perennials (Stone et al. 2004; Demain 2014), causing no apparent harm or morphological alterations. These microorganisms typically coexist alongside diseases and comprise fungi and bacteria (Zhang et al. 2006; Gouda et al. 2016). In plant tissues, fungal endophytes exist internally, intercellularly or intracellularly, and asymptomatically. Endophytes are distinguishable from mycorrhizae by missing external hyphae or mantels, and they often reside in aboveground plant tissues but can also occasionally be found in roots. Over the past 10 years, the definition of "endophyte" has undergone several changes (Sinclair and Cerkauskas 1996; Bills 1996; Saikkonen et al. 1998).

Parasitic or pathogenic fungi are believed to have originated endophytes on both grasses and woody plants (Carroll 1986, 1991, 1992). Woody plant endophytes are closely related to pathogenic fungi and are thought to have descended from them by lengthening their latency periods and decreasing their pathogenicity (Petrini et al. 1992). It is also believed that the fungal grass diseases of the genus *Epichloe* are the ancestors of the *Neotyphodium* grass endophytes. But there doesn't seem to be a clear coevolutionary route between the host plant and the endophyte. Plants have faced a variety of abiotic and biotic stressors throughout evolution. Since they cannot move, plants have relied on vegetative growth, sophisticated physiology, and seed dissemination to avoid or lessen stress's effects. All plants are known to sense signals, transfer them, and react to stresses, including disease, salt, heat, and drought (Bohnert et al. 1995; Bartels and Sunkar 2005).

Surprisingly complex microscopic specimens have been found in the Canadian Arctic. The earliest documented appearance of fungus may have occurred around 1 billion years ago, more than 500 million years earlier than previously thought, according to tiny fossils discovered in remote Arctic Canada. Endophytes have developed unique biotransformation skills due to the long-term coevolution of fungal endophytes and host plants, which can significantly affect the metabolism and makeup of plants.

Geographical considerations, interactions with other species in the community, phylogenetic and life history restrictions, and abiotic factors all affect the continuum of antagonistic-mutualistic interactions between any two interacting species (Thompson and Pellmyr 1992; Thompson 1994). Similar to this, even during the

life span of the microbe and host plant, complex microbial mutualisms with host plants fluctuate along a continuum from pathogenic to mutualistic (Sinclair and Cerkauskas 1996). Although endophytic fungal-host plant interactions are complex and variable, evolutionary traits like mode of transmission and infection patterns as well as ecological factors like host condition, competition with other microorganisms, population structure, and prevailing abiotic factors allow predictions of where endophyte-plant associations are likely to fall along the continuum.

The byproducts of main metabolic pathways are termed primary metabolites, encompassing lipids, proteins, carbohydrates, and amino acids. They are crucial to the metabolism of building blocks and an organism's growth. Without them, the organism's growth and development are very likely to have flaws. The fact that the by-products of several crucial stages serve as precursors for producing secondary metabolites is an essential function of basic metabolism. These precursors are used by both endophytes and their host plants in their separate secondary metabolites (SMs) biosynthesis processes. According to Kirby and Keasling (2009) and Deepika et al. (2016), SMs in EFs may imitate the host pathways and use those pathways as their biosynthetic route. Using blocking mutant and radiolabeling approaches, researchers have explored the synthesis of certain phytochemicals, including ergot alkaloids, aflatoxin, and lovastatin (Keller et al. 2005; Rekadwad et al. 2022). Although varied, a few shared biosynthetic pathways synthesize SMs, and endophytic fungal communities and their host plants' metabolomic pathways are comparable. It is unclear whether these low-molecular-weight phytochemicals are produced by plants directly or through symbiosis with microbes inside them.

3 Biodiversity of Endophytic Fungi

Nearly every plant on the earth has endophytes, which are the most distinctive microbes. It has mainly been extracted from the soil of large and small trees, coastal grasses, and lichens. Many different microbes, such as bacteria, actinobacteria, fungi, and algae, are found inside the plant tissue (Saini et al. 2015; Zhang et al. 2018; Passari et al. 2020; Sriravali et al. 2022). They all establish symbiotic or asymbiotic biological relationships with the host-plant body. Prokaryotic cells connected with plants through vertical or horizontal transmission through stomata and colonizing the internal plant tissue make up the wide endophytic variety in our ecosystem. Endophytic microorganisms target various parts of the host-plant body, so they can enter and establish a habitat.

Fungal endophytes are a common type of endophyte. Endophytic fungi can sustainably increase crop output and growth by enduring severe biotic and abiotic stress conditions, including drought, high temperatures, and salinity (Rodriguez et al. 2009). Due to their extensive adaption, the fungal endophytes colonize the plant tissue's intra- and intercellular regions, forming a symbiotic or mutualistic

relationship with the host (Aly et al. 2011). The host plant provides the fungal strain with food and protection, and the fungal endophyte confers resistance to pathogens and numerous abiotic stresses. The transfer of a fungal cell to a damaged wound region through surface contact or channels is called external fungal endophytes. Endogenous fungal endophytes, however, travel through inner organelles like mitochondria and chloroplasts (Yadav 2020). There are two ways that fungal endophytes can spread: vertically or systemically from the host plant body to the offspring or seeds of the host plant or horizontally or nonsystematic through sexual reproduction or infection (Malik et al. 2023).

Endophytic fungi belong to diverse phyla, including Ascomycota, Basidiomycota, and Mucoromycota. The majority of endophytic fungi belong to Ascomycota (89%), followed by Basidiomycota (9%), and the remaining to Mucoromycota (2%) (Rana et al. 2019). The diversity of fungal endophyte species in these phyla is summarized in Table 1.1.

The variety of all fungal endophytes can be divided into two major groups. These include *Clavicipitaceae* (CE), which are widely dispersed and occur in asymptomatic tissues of nonvascular plants, conifers, ferns, and angiosperms, which infect specific grasses restricted to chilly climates and nonclavicipitaceous endophytes (NCE). NCE, however, is reportedly only found in the Ascomycota and Basidiomycota groups (Maldonado-González et al. 2015). On every continent, fungi have been found to colonize terrestrial plants. They have been isolated from ferns, gymnosperms, angiosperms, arctic habitats, tropical climes, various xeric environments, and boreal woods (Suryanarayanan et al. 2000; Mohali et al. 2005; Šraj-Kržič et al. 2006; Selim et al. 2017).

Different fungal species with a variety of chemical productions are found in the various fungal areas of plants. A study of the microbial diversity in *Paris polyphylla* var. *yunnanensis* plants (Liu et al. 2017) found that *Trichoderma viride* and *Leptodontidium* sp. coexisted with the dominating species *Fusarium oxysporum* in the rhizospheric endophytes. Along with these three predominant fungi, the presence of *Alternaria* sp., *Pyrenochaeta* sp., *Truncatella* sp., *T. viride*, *Chaetomium* sp., *Penicillium swiecickii*, and *Cylindrocarpon* sp. was also noted.

4 Interaction of Endophytic Fungi with Host Plant

Filamentous fungus and vesicular–arbuscular mycorrhiza (VAM) are the most paramount groups included and investigated as endophytes. Certain fungi that belong to the genus *Trichoderma*, *Colletotrichum*, *Penicillium*, *Aspergillus*, *Purpureocillium*, *Fusarium*, *Claviceps*, *Metarhizium*, *Xylaria*, *Curvularia*, *Cladosporium*, *Dreschlera*, *Alternaria*, etc. colonizes either roots, shoots, or leaves (Uzma et al. 2018; Attia et al. 2020; Baron and Rigobelo 2021). They are populating in the endosphere of plants and are transmitted horizontally or vertically. Endophytes can uphold in

Table 1.1 Biodiversity of different groups of endophytic fungi associated with different hosts

Phyla	Species	References
Ascomycota	*Curvularia* sp., *Setosphaeria* sp., *Guehomyces* sp., *Annulohypoxylon* sp., *Trichoderma* sp., *Xylaria* sp., *Septoria* sp., *Trichosporon* sp., *Filobasidium* sp., *Mucor* sp., *Neurospora* sp., *Ampelomyces* sp., *Microdochium* sp., *Helminthosporium* sp., *Tilletiopsis* sp., *Anthostomella* sp., *Ophiocordyceps* sp., *Plectosphaerella* sp., *Emericella* sp., *Talaromyces* sp., *Glomus* sp., *Stagonospora* sp., *Gibberella* sp., *Alternaria* sp., *Cryptococcus* sp., *Nectria* sp., *Sordariomycetes* sp., *Ilyonectria* sp., *Davidella* sp., *Chaetomium* sp., *Rhodosporidium* sp., *Eurotium* sp., *Stemphylium* sp., *Didymella* sp., *Rigidoporus* sp., *Bipolaris* sp., *Coniothyrium* sp., *Ulocladium* sp., *Cercospora* sp., *Engyodontium* sp., *Porostereum* sp., *Paraconiothyrium* sp., *Phaeosphaeria* sp., *Boeremia* sp., *Cochliobolus* sp., *Exserohilum* sp., *Calonectria* sp., *Paraphoma* sp., *Diaporthe* sp., *Eutypella* sp., *Cladophialophora* sp., *Macrophomina* sp., *Rhizopus* sp., *Phaeosphaeriopsis* sp., *Corynespora* sp., *Nigrospora* sp., *Lasiodiplodia* sp., *Wallemia* sp., *Paecilomyces* sp., *Puccinia* sp., *Williopsis* sp., *Lecanicillium* sp., *Leptospira* sp., *Fusarium* sp., *Nemania* sp., *Neofusicoccum* sp., *Dichotomopilus* sp., *Cylindrocarpon* sp., *Leptosphaeria* sp., *Aspergillus* sp., *Pleosporales* sp., *Peyronellaea* sp., *Marasmius* sp., *Crinipellis* sp., *Mortierella* sp., *Eupenicillium* sp., *Bipolaris* sp., *Clonostachys* sp., *Phomopsis* sp.	Sieber et al. (1988), Fisher and Petrini (1992), Larran et al. (2001), Wakelin et al. (2004), Tian et al. (2004), Nassar et al. (2005), Pan et al. (2008), Saunders and Kohn (2008), Naik et al. (2009), Yuan et al. (2010), Khan et al. (2011, 2012), Gao et al. (2011, 2012), de Souza Leite et al. (2013), Tenguria and Firodiya (2013), Impullitti and Malvick (2013), Amin (2013), Zhao et al. (2012, 2013, 2014), Tian et al. (2014), Fernandes et al. (2015), Colla et al. (2015), Gonzaga et al. (2015), Köhl et al. (2015), Chadha et al. (2015), Pierre et al. (2016), Marcenaro and Valkonen (2016), dos Santos et al. (2016), Parsa et al. (2016), Keyser et al. (2016), Ofek-Lalzar et al. (2016), Wang et al. (2016), Renuka and Ramanujam (2016), Bogner et al. (2016), Rothen et al. (2017), Potshangbam et al. (2017), Hamayun et al. (2017), Singh and Gaur (2017), Spagnoletti et al. (2017), Narayan et al. (2017), Comby et al. (2017), Larran et al. (2002, 2007, 2018), Yang et al. (2014a, 2018), Zhao et al. (2018), Xing et al. (2018), and Rana et al. (2019)
Basidiomycota	*Cryptococcus* sp., *Trichosporon* sp., *Puccinia* sp., *Porostereum* sp., *Rigidoporus* sp., *Filobasidium* sp., *Rhodotorula* sp., *Guehomyces* sp., *Tilletiopsis* sp., *Crinipellis* sp., *Marasmius* sp., *Wallemia* sp., *Rhodosporidium* sp., *Sporobolomyces* sp., *Cryptococcus* sp., *Cystobasidium* sp., *Sporobolomyces* sp., *Rhizoctonia* sp.	
Mucoromycota	*Glomus* sp., *Mucor* sp., *Mortierella* sp.	

environments like high temperatures, temperate forests, mangrove forests, and tropical forests (Arnold 2008), insinuating they can survive under diverse climatic conditions. Endophytic fungi are distinct in their colonization due to the expression of genes required for the molecules necessitated for their colonization. However, there are the least details available for the accountable genes (Behie and Bidochka 2014). It can enter the plant system via wounding of plant tissues that further secrete nutrient metabolites and chemoattractants of endophytes. There is the germination of fungal mycelia in roots and its extensive penetration into the root cortex. Thereby, it commences its colonization. It spreads through the cell wall to the adjacent cells of plants and moves further in the plant system (Yan et al. 2019). Endophytes are observed in almost all plant parts, such as root, shoot, stem, leaves, and reproductive tissues. The existence of endophytes is validated via surface sterilization of plant tissue followed by its growth on a specific media or with metagenome analysis. The internal parts of the plant are the protective, secure zones for the endophytic fungus to get the required nourishment and ameliorate competition. In turn, the fungi also favor plants through direct and indirect courses. They benefit plants directly with nutrient acquisition, secreting molecules that facilitate plant growth. Indirectly with the production of important secondary metabolites and other compounds, endophytic fungus protects plants from biotic and abiotic stress (Fig. 1.1).

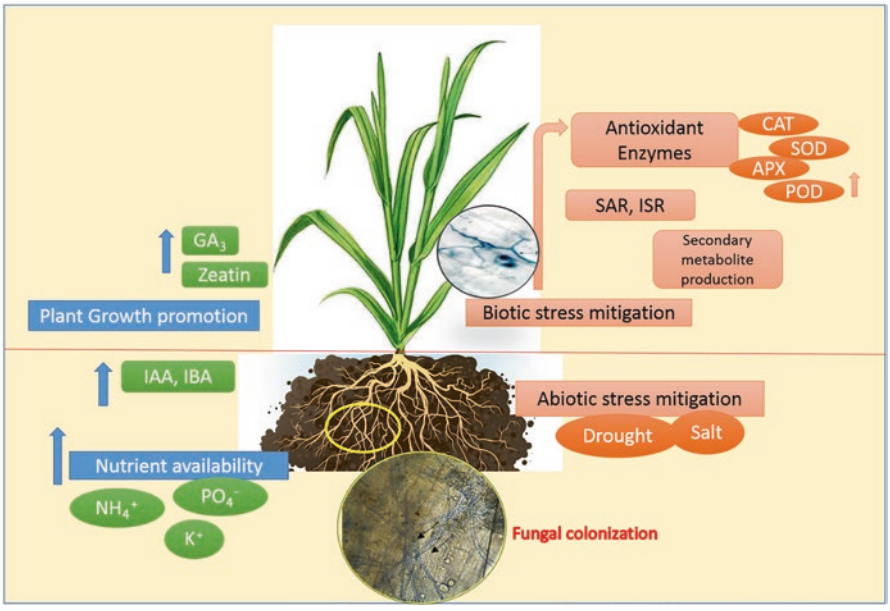

Fig. 1.1 Direct and indirect benefits offered by endophytic fungal colonization. Parameters in blue and green boxes show the direct effects of plant growth promotion. Pink and orange boxes indicate the indirect use of biotic and biotic stress tolerance to the colonized plant

4.1 For Sustainable Agriculture

A global climate change concern is due to deforestation, domestication, urbanization, soil salinization, and soil pollution through the extensive use of chemical fertilizers and pesticides. Plants are also losing important valuable microorganisms due to the abovementioned situations. Therefore, they are not acquiring the direct and indirect advantages imparted by them, which makes them more resistant to stress.

Nutrient Availability

Endophytes do have a role in facilitating macroelements (nitrogen, potassium, phosphorous, calcium, magnesium, sulfur) and microelements (zinc, iron, copper, etc.) for the plants, which make efficient use of fertilizers applied. They can also play a role as biofertilizers. The first report of colonization by the endophytic fungus *Piriformospora indica* revealed that external hyphae of the fungus possess phosphate transporter (*PiPT*) expression that helps to absorb phosphate and make it available to maize plants. Mycorrhizal fungi *Metarhizium* and *Beauveria* have been reported to augment the availability of nitrogen and phosphorous in their symbiosis (Behie and Bidochka 2014). Under conditions of low nitrogen, the genes associated with nitrogen uptake and metabolism, including *OSAMT1;1*, *OSAMT2;2*, OSNR1, and OSGS1, are upregulated in rice due to endophytic colonization by Phomopsis liquidambari. This upregulation is accompanied by elevated levels of total nitrogen, amino acids, proteins, and free $NH4^+$ (Yang et al. 2014b).

Plant Growth Promotion

Phytohormone production is also characteristic of many endophytic fungi. They can produce auxin, cytokinin, and gibberellic acid, mainly with siderophore (Mishra et al. 2016; Tochhawng et al. 2019; Abdalla et al. 2020). The endophytic fungi *Aspergillus fumigatus TS1* and *Fusarium proliferatum* symbionts of *Oxalis corniculata* roots have been screened to have indole acetic acid production and siderophore production with an eclectic derivative of gibberellic acid, such as GA_1, GA_3, and GA_7 (Bilal et al. 2018). Endophytic fungus belongs to the genus *Fusarium*, *Alternaria*, *Xylogone*, and *Didymella* isolated from a medicinally important plant *Sophora flavescens* found to produce a significant concentration of IAA (indole acetic acid), and it has been proven with application and observation of primary root length in *Arabidopsis* plant (Turbat et al. 2020). Endophytic colonization in root cortical cells with *Chaetomium globosum* strain ND35 fostered the growth of cucumber plants with the production of hormones zeatin, gibberellic acid, indole-3 acetic acid (IAA), and indole butyric acid (IBA) (Tian et al. 2022).

4.2 For Stress Management

A mutualistic relationship of plants with endophytic fungus has been observed to produce considerable bioactive compounds and metabolites that impart stress tolerance to the plant. Under abiotic stress conditions like drought and salt stress, these endophytes encountered to regulate the levels of antioxidant enzymes catalase (CAT), peroxidase (POD), ascorbic peroxidase (APX), glutathione (GSH), and superoxide dismutase (SOD) to mitigate stress-induced injury for the cell. The global loss due to plant disease is expected to be 16% (Fontana et al. 2021), and endophytic fungi have been documented to activate induced systemic resistance (ISR) or systemic acquired resistance (SAR) to fight against biotic stress. The banana (*Musa* spp.) crop faces a significant loss due to a fungal pathogen. Endophytic root colonization with *Serendipita indica* increases SOD, POD, CAT, and APX activities, thereby obtaining resistance to *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (Cheng et al. 2020). Under extreme agroecosystems of salt and drought conditions, endophytic colonization with fungi belonging to genus *Periconia macrospinosa*, *Neocamarosporium chichastianum*, and *N. goegapense* obtained from Salt Lake plants alleviates the adverse effect of stress in *Hordeum vulgare* L. that reminisces with the improvement of biomass, shoot length, proline content, and antioxidant enzyme activity (Moghaddam et al. 2022). Endophytic fungi bares the prospect as a biocontrol agent through the secretion of several enzymes (cellulase, amylase, protease, and xylanase), hydrogen cyanide, and certain secondary metabolites; this will reduce the use of synthetic insecticides or pesticides (Yadav et al. 2010). *Penicillium* sp. NAUSF2 can solubilize hard phosphate sources in saline conditions and makes phosphate available to plants with endophytic colonization. It also reduces the disease severity index for bacterial leaf spots caused by *Xanthomonas axonopodis* pv. *V. radiate* in *Vigna radiata* with a significant increase in jasmonic acid and antioxidant enzyme concentration (Patel et al. 2021).

5 Production of Secondary Metabolites by Endophytic Fungi

Plant secondary metabolites are a class of substances that are not essential for basic bodily processes but are crucial for plants to adapt to their environment (Bourgaud et al. 2001). Plants generate low-molecular-weight antimicrobial molecules known as phytoalexins, which comprise a variety of chemicals such as flavonoids, terpenoids, etc. Several studies spotlight the production of phytoalexins by pathogens under numerous nonbiological stress stimuli, such as UV radiation, heavy metal ions, or salt stress (Abraham et al. 1999). Co-culturing with an endophytic elicitor is an additional strategy for enhancing plant secondary metabolites and boosting plant resistance (Li and Tao 2009).

Endophytic fungi are categorized by biological processes that affect plant systems and the proliferation of endophytic fungi. Group I endophytic fungi move a genetic element into plant systems by vertical gene transfer, whereas group II endophytes generally help to combat external stresses. Group III endophytic fungi acquire genes from other fungal species via horizontal gene transfer that produces bioactive chemicals. Depending on the plant's state and age, endophytes present in plant systems create secondary metabolites. Endophytic fungi generate a variety of metabolites from different structural classes, such as terpenoids, steroids, aliphatic chemicals, flavonoids, alkaloids, quinines, phenols, coumarins, peptides, etc. (Calhoun et al. 1992). These metabolites are produced in different pathways like shikimate pathway (alkaloids, flavonoids) (Tohge et al. 2013; Peek and Christendat 2015) and TCA cycle (isoprenoids, polyketide, terpenoids) (Meena et al. 2019). Tejesvi et al. (2007) found that endophytic fungi of medicinal plants create secondary metabolites that can be researched for treating various ailments. All of these studies show that endophytic fungi are a chemical reservoir of novel compounds that have numerous applications in the pharmaceutical and agrochemical industries, including those for antimicrobial, antiviral, antifungal, anticancer, antiparasitic, antitubercular, antioxidant, immunomodulatory, and insecticidal properties (Fig. 1.2) (Calhoun et al. 1992).

In addition to providing novel sources for cytotoxic chemicals, including anticancer and antibacterial compounds (Uzma et al. 2018; Radic and Strukelj 2012), endophytic fungi (EFs) also operate as biostimulants for the production of essential oils (Enshasy et al. 2019). This has led to a great deal of attention in the field. They might function as biological control agents (Poveda and Baptista 2021), encourage plant growth (Mehta et al. 2019), increase nutrient solubilization in the rhizosphere

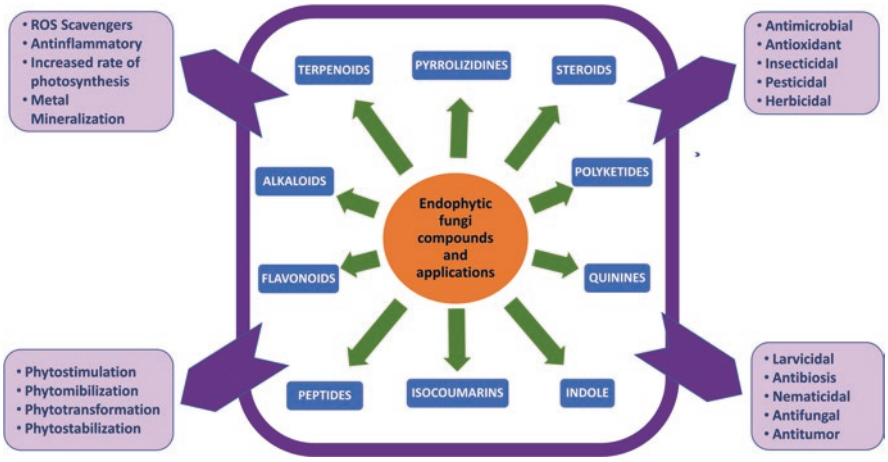

Fig. 1.2 Applications of different bioactive secondary metabolites compounds produced by endophytic fungi

of the plant (Poveda et al. 2021), or activate systemic plant defenses against biotic (Poveda et al. 2020) or abiotic (Cui et al. 2021) stresses.

5.1 Symbiotic Interaction

Endophytes create various connections with their host plants throughout their growth inside the living tissues of the plant, including symbiotic, mutualistic, or parasitic ones. The host plant's cells or intercellular space are home to fungal endophytes, which appear to inflict no harm (Saikkonen et al. 1998). In mutualistic symbiosis, EF partners and host plants benefit from this advantageous symbiotic continuum and eventually succeed in evolution and the environment (Fig. 1.3) (Jia et al. 2016). The host plants' metabolic processes are changed by EFs, which also increase drought and metal tolerance, growth, and nutrient uptake (Poveda et al. 2021; Cui et al. 2021).

However, EFs can also sporulate quickly and interact with host plants in a latent pathogenic or commensalism relationship, with or without appreciable positive impacts on plant physiology (Fig. 1.3) (Hiruma et al. 2016). They can also colonize and flourish asymptomatically inside healthy plant tissues (Saikkonen et al. 1998; Kogel et al. 2006). These endophytes can trigger host plant disease symptoms under stress (Schulz and Boyle 2005), such as those caused by *Cordana*, *Deightoniella*, *Verticillium*, *Curvularia*, *Nigrospora*, *Periconiella*, *Colletotrichum*, *Guignardia*, *Phoma*, *Cladosporium*, and *Fusarium* (Photita et al. 2004; Cui et al. 2021). Equilibrium between these organisms has been achieved during the long-term coevolution of endophytes and plants. Thus, the real endophyte will exist once a balance is reached between fungal activity and the plant response and is sustained throughout time (Gimenez et al. 2007).

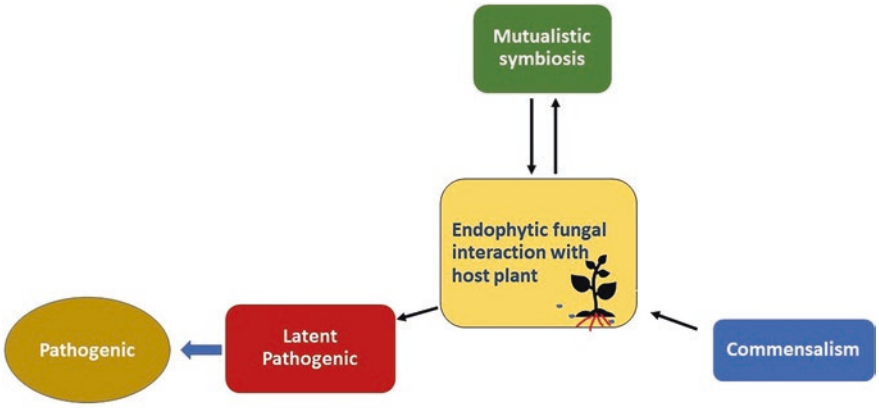

Fig. 1.3 Interaction of endophytic fungi with host plant

5.2 *In Stress Conditions*

Plants create many different pathways, such as jasmonic acid, abscisic acid, and salicylic acid, due to abiotic stress stimuli and function as defense signaling chemicals. As fungal endophytes may develop from plant pathogenic fungi, they may act as pathogens and cause plants to defend themselves. To promote plant development under stressful conditions, fungal endophytes produce siderophores, antibiotics, and phytohormones; mineralize nutrients; and perform other tasks (Yung et al. 2021). Siderophores increase iron intake and phosphate solubilization and plant uptake of these nutrients, promoting plant development and executing defense against many pathogens (Chowdappa et al. 2020). Endophytes also accelerate biomass formation and nitrogen intake while biodegrading the trash (Idbella et al. 2019). Over 500 siderophores from different fungi have been identified (Chowdappa et al. 2020). *Aspergillus fumigatus*, *Aspergillus niger*, *Curvularia*, *Trichoderma*, and other fungal endophytes have all been recently discovered to solubilize and mobilize phosphorus, potassium, and zinc salts, which in turn promote plant growth and high crop production (Mehta et al. 2019; Haro and Benito 2019).

Phytohormone gibberellins are secreted by *Penicillium* sp., which inhabits *Suaeda japonica*, as an example of the reduced amount of plant growth-promoting compounds the fungal endophytes release during stress. The fungi *Penicillium* sp., *Ascomycete* sp., *Aspergillus* sp., *Verticillium* sp., *Cladosporium* sp., and *Fusarium* sp., which live on *Panax ginseng*, also release triterpenoid, ginsenosides, and saponins, which improve stress resistance and root development (Sahoo et al. 2017). To increase the phytoimmobilization and availability of zinc, nitrogen, and phosphorus for the host, siderophores can biodegrade biomass and recycle it in the environment (Yung et al. 2021). They can also lower levels of the hormone ethylene by inhibiting 1-aminocyclopropane-1-carboxylate deaminase (ACC) in plants. Hence by immobilizing osmolytes and regulating membrane ion conductivity during stress, phytoimmobilization by endophytes eventually aids in withstanding abiotic stressors by plants.

Endophytic fungi are also accountable for protecting crops from biotic stress in the wake of the chain of events (Singh et al. 2021a). The three main ways fungi defend themselves from phytopathogens are competition within the biological niche, antibiotics production, and mycoparasitism, which strengthens plant defenses and raises tolerance to virulence factors generated by pathogenic bacteria. The primary endophytes that begin to tolerate biotic stress while simultaneously enhancing the host plant's development and yield components are *Trichoderma* species, *Epicoccum* species, *Aspergillus* species, *Colletotrichum* species, *Gliocladium* species, *Fusarium* species, *Petriella* species, *Piriformospora* species, *Epichloe* species, etc.; mildews, rots, nematodes, blights, and leaf mosaics are just a few of the diseases that *P. indica* can successfully treat (Ali et al. 2019). They ought to be considered as potential biocontrol agents as a result. According to Laihonen et al. (2022), *Epichloe* sp. controls herbivorous insects and offers its host plant biotic resilience. Host plants' roots, twigs, and stems are colonized by the filamentous anamorphic

saprophytic fungus known as *Trichoderma* sp. due to its antibacterial, antifungal, and cytotoxic qualities; it can be utilized as a biocontrol agent.

6 Biotic Potential of Secondary Metabolites Produced by Entophytic Fungi

Many secondary metabolites are produced by endophytic fungi, such as phenols, alkaloids, polyketides, quinones, steroids, enzymes, and peptides, which have a higher therapeutic value than primary metabolites (Xu et al. 2021). They can protect the plants from disease-causing invaders. This protection is made possible by producing secondary metabolites, which act as a defense against the invasion of pathogens (Kaur et al. 2022). These secondary metabolites, such as bioactive compounds, are the primary source of the beneficial characteristics of endophytic fungi. Endophytes can stop the development of resistance mechanisms in plants, which can lead to disease. The production of these bioactive compounds also allows for the release of enzymes, antioxidants, and other beneficial compounds that help protect the plant from external threats (Wen et al. 2022). Additionally, these secondary metabolites can be used for plant growth and development as well as for the improvement of crop yields. Endophytic fungi are crucial for protecting plants from disease and promoting growth, and their ability to produce secondary metabolites is key to their beneficial qualities (Manganyi and Ateba 2020).

Endophytic fungi are an essential source of secondary metabolites, including terpenoids, polyketides, shikimic acid derivatives, and terpenes. They are found in many plants and play an important role in the pharmaceutical and drug industries through the production of alcohol, antibiotics, enzymes, and other medicinal ingredients (Singh et al. 2021b). These secondary metabolites can create new drugs and treatments and provide a valuable treasure for medical research. Endophytic fungi benefit both the environment and humans, since they are natural sources of these compounds, which can decrease the need for chemical synthesis. Additionally, they offer substitutes for chemical-based drugs, which can have a number of health hazards. Endophytic fungi are also valuable in developing treatments for diseases such as cancer and Alzheimer's, since they can produce compounds that can be used to fight these diseases. These compounds have potential applications in drug discovery and can be used to treat various disease conditions. Endophyte-derived natural products can also be used as pesticides, insecticides, and herbicides to control agricultural pests (Zheng et al. 2021; Wen et al. 2022)

Endophytic fungi potentially produce novel bioactive compounds. Suitable media, growth parameters, and nutrient limitations should be explored to gain insight into fungal metabolism and discover novel pharmaceutical products; such compounds can be used to treat many diseases. Furthermore, endophytic fungi provide a sustainable source of novel bioactive compounds which is environmentally friendly (Adeleke and Babalola 2021).

7 Application of Secondary Metabolites Produced from Symbiotic Fungi

Endophytic fungi have recently gained tremendous attention due to their ability to produce novel bioactive compounds with a wide range of biological properties. These compounds have been used in a variety of applications, especially in the fields of medicine, pharmaceuticals, and agriculture. In addition to their bioactive compounds, endophytic fungi have also been found to possess many other beneficial attributes, including the ability to increase a plant's resistance to pathogens, reduce the amount of fertilizer needed, and promote crop yield (Manganyi and Ateba 2020). Moreover, endophytic fungi can act as a natural source of antibiotics, providing potential alternatives to traditional antibiotics. Endophytic fungi can also be used for bioremediation and to clean up contaminated soil and water. Overall, endophytic fungi have a wide range of potential applications, and their ability to produce novel bioactive compounds is an invaluable asset to many industries (Stępniewska and Kuźniar 2013).

The antimicrobial and antifungal properties of endophytic fungi metabolites have been especially noted, as these compounds have the potential to provide novel solutions to existing and emerging drug-resistant microbial and fungal infections (Deshmukh et al. 2022). For instance, endophytic fungi metabolites have been proven to effectively inhibit the growth of several drug-resistant bacterial and fungal pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and *Candida albicans*. In addition, the antifungal activities of endophytic fungi metabolites have been demonstrated against several fungal species, such as *Aspergillus flavus*, *Fusarium solani*, and *Rhizoctonia solani*. Additionally, the antiprotozoal activity of endophytic fungi metabolites has been shown against several protozoan species, such as *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Leishmania*. Moreover, the antiparasitic activity of endophytic fungi metabolites has been demonstrated against several parasitic species, such as *Plasmodium falciparum*, *Schistosoma mansoni*, and *Fasciola hepatica* (Liu et al. 2019; Deshmukh et al. 2022).

Several studies have also reported the antioxidant, immunosuppressant, and anticancer activities of endophytic fungi metabolites. Endophytic fungi metabolites have been demonstrated to possess antioxidant activities, which are beneficial in reducing oxidative stress and protecting against numerous diseases (Almustafa and Yehia 2023). In addition, the immunosuppressant activities of these metabolites have been demonstrated in several studies, as these compounds have been found to reduce inflammation and suppress the immune system. Finally, the anticancer activities of endophytic fungi metabolites have been demonstrated in several studies, as these compounds have been found to inhibit the growth of cancer cells (Table 1.2) (Sharma et al. 2020).

Table 1.2 Examples of biotechnologically relevant fungal secondary metabolites

S. no.	Secondary metabolite	Producing fungus	Application
1	Cephalosporin C	*Acremonium chrysogenum*	Resource for the production of Cephalosporins
2	*B*-carotene	*Blakeslea trispora*	Pigment
3	Astaxanthin	*Phaffia rhodozyma*	Pigment
4	Griseofulvin	*Penicillium griseofulvum*	Antifungal agent
5	Cyclosporine A	*Tolypocladium inflatum*	Immunosuppressant
6	Gibberellic acid	*Gibberella fujikuroi*	Plant growth regulator
7	Penicillin G	*Penicillium rubens*	Antibiotic
8	Taxol	*Taxomyces andreanae*	Anticancer drug
9	Lovastatin	*Aspergillus terreus*	Cholesterol-lowering drug

8 Challenges and Future Perspectives of Endophytic Fungi

To regulate and manipulate the biosynthesis process for increased production, we must elucidate the entire biosynthesis pathway, including all of the enzymes and associated genes. To solve the issues of poor yield and attenuation, the two main obstacles to commercial success, we need to learn more about the functions of host plant-endophyte interactions. For the successful industrial-scale synthesis of pharmaceutically valuable compounds or leads, scientists working in this area and the pharmacological business must collaborate. The pharmaceutical sector must prioritize the endophyte-dependent production of natural plant chemicals. For the pharmaceutical and healthcare sectors, as well as for a "green drug revolution," the concept of endophyte-dependent improved in vivo and in vitro production of plant-derived useful metabolites is crucial.

9 Conclusion

In conclusion, endophytic fungi represent a fascinating group of microorganisms that reside within the tissues of plants without causing any apparent harm. These fungi have coevolved with their host plants, establishing mutualistic relationships that can profoundly affect the fungi and the plants. Over the years, extensive research has revealed various applications for endophytic fungi in various fields. Moreover, endophytic fungi have demonstrated remarkable potential as a source of bioactive compounds with pharmaceutical and industrial importance. Many endophytic fungi produce secondary metabolites with antimicrobial, antiviral, anticancer, and antioxidant properties. These bioactive compounds promise to develop new drugs, nutraceuticals, and natural products for various applications, including medicine, cosmetics, and agriculture.

References

Abdalla MA, Aro AO, Gado D, Passari AK, Mishra VK, Singh BP, McGaw LJ (2020) Isolation of endophytic fungi from South African plants, and screening for their antimicrobial and extracellular enzymatic activities and presence of type I polyketide synthases. S Afr J Bot 134:336–342. https://doi.org/10.1016/j.sajb.2020.03.021

Abraham KJ, Pierce ML, Essenberg M (1999) The phytoalexins desoxyhemigossypol and hemigossypol are elicited by *Xanthomonas* in *Gossypium* cotyledons. Phytochemistry 52:829–836. https://doi.org/10.1016/S0031-9422(99)00331-3

Adeleke BS, Babalola OO (2021) Pharmacological potential of fungal endophytes associated with medicinal plants: a review. J Fungi (Basel) 7(2):147. https://doi.org/10.3390/jof7020147

Ali A, Bilal S, Khan AL, Mabood F, Al-Harrasi A, Lee IJ (2019) Endophytic *Aureobasidium pullulans* BSS6 assisted developments in phytoremediation potentials of *Cucumis sativus* under Cd and Pb stress. J Plant Interact 14:303–313. https://doi.org/10.1080/17429145.2019.1633428

Almustafa HI, Yehia RS (2023) Antioxidant, cytotoxic, and DNA damage protection activities of endophytic fungus *Pestalotiopsis neglecta* isolated from *Ziziphus spina-christi* medicinal plant. Microorganisms 11(1):117. https://doi.org/10.3390/microorganisms11010117

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41:1–16. https://doi.org/10.1007/s13225-010-0034-4

Aly A, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90(6):1829–1845. https://doi.org/10.1007/s00253-011-3270-y

Amin N (2013) Diversity of endophytic fungi from root of Maize var. Pulut (waxy corn local variety of South Sulawesi, Indonesia). Int J Curr Microbiol App Sci 2:148–154

Arnold A (2008) Hidden within our botanical richness, a treasure trove of fungal endophytes. Plant Press 32:13–15

Attia EA, Singh BP, Dashora K, Ahmed M, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17(1):119–132

Bacon CW, White JF (2000) Microbial endophytes. Marcel Deker Inc., New York. https://doi.org/10.1201/9781482277302

Bai Y, Kissoudis C, Yan Z, Visser RGF, van der Linden G (2017) Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. Plant J 93(4):781–793. https://doi.org/10.1111/tpj.13800

Baron NC, Rigobelo EC (2021) Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. Mycology 13(1):39–55. https://doi.org/10.1080/21501203.2021.1945699

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58. https://doi.org/10.1080/07352680590910410

Behie SW, Bidochka MJ (2014) Nutrient transfer in plant–fungal symbioses. Trends Plant Sci 19(11):734–740. https://doi.org/10.1016/j.tplants.2014.06.007

Bilal L, Asaf S, Hamayun M et al (2018) Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis 76:117–127. https://doi.org/10.1007/s13199-018-0545-4

Bills GF (1996) Isolation and analysis of endophytic fungal communities from woody plants. See Ref. 126, pp 31–65

Bogner CW, Kariuki GM, Elashry A, Sichtermann G, Buch A-K, Mishra B, Thines M, Grundler FM, Schouten A (2016) Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. Mycol Prog. https://doi.org/10.1007/s11557-016-1169-9

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell 7(7):1099–1111. https://doi.org/10.2307/3870060

Bourgaud F, Gravot A, Milesi A, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 161-5:839–851. https://doi.org/10.1016/S0168-9452(01)00490-3

Brunner F, Petrini O (1992) Taxonomy of some *Xylaria* species and xylariaceous endophytes by isozyme electrophoresis. Mycol Res 96:723–733. https://doi.org/10.1016/S0953-7562(09)80440-1

Calhoun LA, Findlay JA, Miller JD, Whitney NJ (1992) Metabolites toxic to spruce budworm from balsam fir needle endophytes. Mycol Res 96(4):281–286. https://doi.org/10.1016/S0953-7562(09)80939-8

Carroll GC (1986) The biology of endophytism in plants with particular reference to woody perennials. See Ref. 61a, pp 205–22 26

Carroll G (1988) Fungal endophytes in stems and leaves – from latent pathogen to mutualistic symbiont. Ecology 69(1):2–9. https://doi.org/10.2307/1943154

Carroll GC (1991) Beyond pest deterrence. Alternative strategies and hidden costs of endophytic mutualisms in vascular plants. In: Andrews JH, Monano SS (eds) Microbial ecology of leaves. Springer, New York, pp 358–378. https://doi.org/10.1007/978-1-4612-3168-4_18

Carroll GC (1992) Fungal mutualism. In: Carroll GC, Wicklow DT (eds) The fungal community. Its organization and role in the ecosystem. Dekker, New York, pp 327–354

Chadha N, Prasad R, Varma A (2015) Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with *Piriformosporaindica*. Int J Pharm Bio Sci 6:333–343

Chagas FO, Pessotti RD, Caraballo-Rodriguez AM, Pupo MT (2018) Chemical signaling involved in plant microbe interactions. Chem Soc Rev 47(5):1652–1704. https://doi.org/10.1039/c7cs00343a

Cheng C, Li D, Qi Q et al (2020) The root endophytic fungus *Serendipita indica* improves resistance of Banana to *Fusarium oxysporum* f. sp. *cubense* tropical race 4. Eur J Plant Pathol 156:87–100. https://doi.org/10.1007/s10658-019-01863-3

Chowdappa S, Jagannath S, Konappa N, Udayashankar AC, Jogaiah S (2020) Detection and characterization of antibacterial siderophores secreted by endophytic fungi from *Cymbidium aloifolium*. Biomol Ther 10(10):1412. https://doi.org/10.3390/biom10101412

Colla G, Rouphael Y, Bonini P, Cardarelli M (2015) Coating seeds with endophytic fungi enhances growth, nutrient uptake, yield and grain quality of winter wheat. Int J Plant Prot 9:171–189

Comby M, Gacoin M, Robineau M, Rabenoelina F, Ptas S, Dupont J, Profizi C, Baillieul F (2017) Screening of wheat endophytes as biological control agents against *Fusarium* head blight using two different in vitro tests. Microbiol Res 202:11–20. https://doi.org/10.1016/j.micres.2017.04.014

Cui R, Lu X, Chen X, Malik WA, Wang D, Wang J et al (2021) A novel raffinose biological pathway is observed by symbionts of cotton=Verticillium dahliae to improve salt tolerance genetically on cotton. J Agron Crop Sci 207:956–969. https://doi.org/10.1111/jac.12556

De Souza LT, Cnossen-Fassoni A, Pereira OL, Mizubuti ESG, de Araújo EF, de Queiroz MV (2013) Novel and highly diverse fungal endophytes in soybean revealed by the consortium of two different techniques. J Microbiol 51:56–69. https://doi.org/10.1007/s12275-013-2356-x

Deepika VB, Murali TS, Satyamoorthy K (2016) Modulation of genetic clusters for synthesis of bioactive molecules in fungal endophytes: a review. Microbiol Res 182:125–140. https://doi.org/10.1016/j.micres.2015.10.009

Demain AL (2014) Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 41:185–201. https://doi.org/10.1007/s10295-013-1325-z

Deshmukh SK, Dufossé L, Chhipa H, Saxena S, Mahajan GB, Gupta MK (2022) Fungal endophytes: a potential source of antibacterial compounds. J Fungi (Basel) 8(2):164. https://doi.org/10.3390/jof8020164

Dos Santos TT, de Souza LT, de Queiroz CB, de Araújo EF, Pereira OL, de Queiroz MV (2016) High genetic variability in endophytic fungi from the genus *Diaporthe* isolated from common bean (*Phaseolus vulgaris* L.) in Brazil. J Appl Microbiol 120:388–401. https://doi.org/10.1111/jam.12985

Enshasy HA, Hanapi SZ, Malek RA, Abdelgalil SA, Leng OM (2019) Endophytic fungi: the desired biostimulants for essential oil production. In: Singh B (ed) Advances in endophytic fungal research. Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-030-03589-1_10

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J Nat Prod 69:1121–1124. https://doi.org/10.1021/np060174f

Fernandes EG, Pereira OL, da Silva CC, Bento CBP, de Queiroz MV (2015) Diversity of endophytic fungi in *Glycine max*. Microbiol Res 181:84–92. https://doi.org/10.1016/j.micres.2015.05.010

Fisher P, Petrini O (1992) Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). New Phytol 120:137–143. https://doi.org/10.1111/j.1469-8137.1992.tb01066.x

Fontana DC, de Paula S, Torres AG, de Souza VHM, Pascholati SF, Schmidt D, Dourado Neto D (2021) Endophytic fungi: biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens 10(5). https://doi.org/10.3390/pathogens10050570

Gao Y, Zhao JT, Zu YG, Fu YJ, Wang W, Luo M, Efferth T (2011) Characterization of five fungal endophytes producing Cajaninstilbene acid isolated from pigeon pea [*Cajanus cajan* (L.) Millsp.]. PLoS One 6:e27589. https://doi.org/10.1371/journal.pone.0027589

Gao Y, Zhao J, Zu Y, Fu Y, Liang L, Luo M, Wang W, Efferth T (2012) Antioxidant properties, superoxide dismutase and glutathione reductase activities in HepG2 cells with a fungal endophyte producing apigenin from pigeon pea [*Cajanus cajan* (L.) Millsp.]. Food Res Int 49:147–152. https://doi.org/10.1016/j.foodres.2012.08.001

Gimenez C, Cabrera R, Reina M, Gonzalez-Coloma A (2007) Fungal endophytes and their role in plant protection. Curr Org Chem 11(8):707–720. https://doi.org/10.2174/138527207780598765

Gonzaga L, Costa L, Santos T, Araújo E, Queiroz M (2015) Endophytic fungi from the genus *Colletotrichum* are abundant in the *Phaseolus vulgaris* and have high genetic diversity. J Appl Microbiol 118:485–496. https://doi.org/10.1111/jam.12696

Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538. https://doi.org/10.3389/fmicb.2016.01538

Hamayun M, Hussain A, Khan SA, Kim H-Y, Khan AL, Waqas M, Irshad M, Iqbal A, Rehman G, Jan S (2017) Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00686

Haro R, Benito B (2019) The role of soil fungi in K^+ plant nutrition. Int J Mol Sci 20:3169. https://doi.org/10.3390/ijms20133169

Hassani MA, Duran P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6(1):58. https://doi.org/10.1186/s40168-018-0445-0

Hiruma K, Gerlach N, Sacristan S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramirez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. Cell 165(2):464–474. https://doi.org/10.1016/j.cell.2016.02.028

Idbella M, Zotti M, Cesarano G et al (2019) Fungal endophytes affect plant response to leaf litter with contrasting chemical traits. Community Ecol 20:205–213. https://doi.org/10.1556/168.2019.20.2.10

Impullitti A, Malvick D (2013) Fungal endophyte diversity in soybean. J Appl Microbiol 114:1500–15064

Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 7:906. https://doi.org/10.3389/fmicb.2016.00906

Kaur S, Samota MK, Choudhary M, Choudhary M, Pandey AK, Sharma A, Thakur J (2022) How do plants defend themselves against pathogens-biochemical mechanisms and genetic interventions. Physiol Mol Biol Plants 28(2):485–504. https://doi.org/10.1007/s12298-022-01146-y

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism from biochemistry to genomics. Nat Rev Microbiol 3:937–947. https://doi.org/10.1038/nrmicro1286

Keyser CA, Jensen B, Meyling NV (2016) Dual effects of *Metarhizium* spp. and *Clonostachys rosea* against an insect and a seed-borne pathogen in wheat. Pest Manag Sci 72:517–526. https://doi.org/10.1002/ps.4015

Khan AL, Hamayun M, Kim YH, Kang SM, Lee IJ (2011) Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of *Glycine max* L. Plant Physiol Biochem 49:852–861. https://doi.org/10.1016/j.plaphy.2011.03.005

Khan AL, Hamayun M, Khan SA, Kang S-M, Shinwari ZK, Kamran M, ur Rehman S, Kim JG, Lee IJ (2012) Pure culture of *Metarhizium anisopliae* LHL07 reprograms soybean to higher growth and mitigates salt stress. World J Microbiol Biotechnol 28:1483–1494. https://doi.org/10.1007/s11274-011-0950-9

Khan AL, Hussain J, Al-Harrasi A, Al-Rawahi A, Lee IJ (2013) Endophytic fungi: resource for gibberellins and crop abiotic stress resistance. Crit Rev Biotechnol 35(1):62–74. https://doi.org/10.3109/07388551.2013.800018

Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28:1208–1228. https://doi.org/10.1039/C1NP00008J

Kirby J, Keasling JD (2009) Biosynthesis of plant isoprenoids: perspectives for microbial engineering. Annu Rev Plant Biol 60:335–355. https://doi.org/10.1146/annurev.arplant.043008.091955

Kogel KH, Franken P, Hückelhoven R (2006) Endophyte or parasite--what decides? Curr Opin Plant Biol 9(4):358–363. https://doi.org/10.1016/j.pbi.2006.05.001

Köhl J, Lombaers C, Moretti A, Bandyopadhyay R, Somma S, Kastelein P (2015) Analysis of microbial taxonomical groups present in maize stalks suppressive to colonization by toxigenic *Fusarium* spp.: a strategy for the identification of potential antagonists. Biol Control 83:20–28. https://doi.org/10.1016/j.biocontrol.2014.12.007

Kusari S, Spiteller M (2012) Metabolomics of endophytic fungi producing associated plant secondary metabolites: progress, challenges and opportunities. In: Roessner U (ed) Metabolomics. InTech, Rijeka, pp 241–266. https://doi.org/10.5772/31596

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod 71:159–162. https://doi.org/10.1021/np070669k

Kusari S, Zühlke S, Kosuth J, Cellárová E, Spiteller M (2009) Light independent metabolomics of endophytic *Thielavia subthermophila* provides insight into microbial hypericin biosynthesis. J Nat Prod 72:1825–1835. https://doi.org/10.1021/np9002977

Kusari S, Verma VC, Lamshöft M, Spiteller M (2012) An endophytic fungus from *Azadirachta indica* A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28:1287–1294. https://doi.org/10.1007/s11274-011-0876-2

Laihonen M, Saikkonen K, Helander M, Vázquez de Aldana BR et al (2022) *Epichloë* endophyte-promoted seed pathogen increases host grass resistance against insect herbivory. Front Microbiol 12:786619. https://doi.org/10.3389/fmicb.2021.786619

Larran S, Monaco C, Alippi H (2001) Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. World J Microbiol Biotechnol 17:181–184. https://doi.org/10.1023/A:1016670000288

Larran S, Perello A, Simon M, Moreno V (2002) Isolation and analysis of endophytic microorganisms in wheat (*Triticum aestivum* L.) leaves. World J Microbiol Biotechnol 18:683–686. https://doi.org/10.1023/A:1016857917950

Larran S, Perelló A, Simón MR, Moreno V (2007) The endophytic fungi from wheat (*Triticum aestivum* L.). World J Microbiol Biotechnol 23:565–572. https://doi.org/10.1007/s11274-006-9266-6

Larran S, Siurana MPS, Caselles JR, Simón MR, Perelló A (2018) *Fusarium sudanense*, endophytic fungus causing typical symptoms of seedling blight and seed rot on wheat. J King Saud Univ Sci. https://doi.org/10.1016/j.jksus.2018.07.005

Li YC, Tao WY (2009) Paclitaxel-producing fungal endophyte stimulates the accumulation of taxoids in suspension cultures of *Taxus cuspidate*. Sci Hortic 121:97–102. https://doi.org/10.1016/j.scienta.2009.01.016

Liu T, Greenslade A, Yang S (2017) Levels of rhizome endophytic fungi fluctuate in Paris polyphylla var. Yunnanensis as plants age. Plant Divers 39(1):60–64. https://doi.org/10.1016/j.pld.2016.11.006

Liu P, Zhang D, Shi R, Yang Z, Zhao F, Tian Y (2019) Antimicrobial potential of endophytic fungi from *Astragalus chinensis*. 3 Biotech 9(11):405. https://doi.org/10.1007/s13205-019-1948-5

Maldonado-González MM, Bakker PA, Prieto P, Mercado-Blanco J (2015) *Arabidopsis thaliana* as a tool to identify traits involved in *Verticillium dahliae* biocontrol by the olive root endophyte *Pseudomonas* fluorescens PICF7. Front Microbiol 6:266. https://doi.org/10.3389/fmicb.2015.00266

Malik S, Kishore S, Dutta AK, Dhasmana A (2023) Sustainable agriculture approach through endophytes. In: Endophytic Association: what, why and how. https://doi.org/10.1016/B978-0-323-91245-7.00019-5

Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: a review of novel bioactive compounds with biological applications. Microorganisms 8(12):1934. https://doi.org/10.3390/microorganisms8121934

Marcenaro D, Valkonen JP (2016) Seedborne pathogenic fungi in common bean (Phaseolus *vulgaris* cv. INTA Rojo) in Nicaragua. PLoS One 11:e0168662. https://doi.org/10.1371/journal.pone.0168662

Meena H, Hnamte S, Siddhardha B (2019) Advances in endophytic fungal research. Fungal Biol. https://doi.org/10.1007/978-3-030-03589-1_7

Mehta P, Sharma R, Putatunda C, Walia A (2019) Endophytic fungi: role in phosphate solubilization. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 183–209. https://doi.org/10.1007/978-3-030-03589-1_9

Mishra VK, Singh G, Passari AK, Yadav MK, Gupta VK, Singh BP (2016) Distribution and antimicrobial potential of endophytic fungi associated with ethnomedicinal plant *Melastoma malabathricum* L. J Environ Biol 37(2):229–237

Moghaddam MS, Safaie N, Rahimlou S, Hagh-Doust N (2022) Inducing tolerance to abiotic stress in *Hordeum vulgare* L. by halotolerant endophytic fungi associated with salt lake plants. Front Microbiol 13:906365. https://doi.org/10.3389/fmicb.2022.906365

Mohali S, Burgess T, Wingfield M (2005) Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers. For Pathol 35:385–396. https://doi.org/10.1111/j.1439-0329.2005.00418.x

Naik BS, Shashikala J, Krishnamurthy Y (2009) Study on the diversity of endophytic communities from rice (Oryza sativa L.) and their antagonistic activities in vitro. Microbiol Res 164:290–296. https://doi.org/10.1016/j.micres.2006.12.003

Narayan OP, Verma N, Singh AK, Oelmüller R, Kumar M, Prasad D, Kapoor R, Dua M, Johri AK (2017) Antioxidant enzymes in chickpea colonized by *Piriformospora indica* participate in defense against the pathogen *Botrytis cinerea*. Sci Rep 7(1):13553. https://doi.org/10.1038/s41598-017-12944-w

Nassar AH, El-Tarabily KA, Sivasithamparam K (2005) Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. Biol Fertil Soils 42:97–108. https://doi.org/10.1007/s00374-005-0008-y

Noble HM, Langley D, Sidebottom PJ, Lane SJ, Fisher PJ (1991) An echinocandin from an endophytic *Cryptosporiopsis* sp. and *Pezicula* sp. in *Pinus sylvestris* and *Fagus sylvatica*. Mycol Res 95:1439–1440. https://doi.org/10.1016/S0953-7562(09)80401-2

Ofek-Lalzar M, Gur Y, Ben-Moshe S, Sharon O, Kosman E, Mochli E, Sharon A (2016) Diversity of fungal endophytes in recent and ancient wheat ancestors *Triticum dicoccoides* and *Aegilops sharonensis*. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiw152

Pan JJ, Baumgarten AM, May G (2008) Effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*). New Phytol 178:147–156. https://doi.org/10.1111/j.1469-8137.2007.02350.x

Parsa S, García-Lemos AM, Castillo K, Ortiz V, López-Lavalle LAB, Braun J, Vega FE (2016) Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. Fungal Biol 120:783–790. https://doi.org/10.1016/j.funbio.2016.01.017

Passari AK, Mishra VK, Saikia R, Gupta VK, Singh BP (2015) Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential. Front Microbiol 6(273):1–18. https://doi.org/10.3389/fmicb.2015.00273

Passari AK, Chandra P, Zothanpuia MVK, Leo VV, Gupta VK, Kumar B, Singh BP (2016) Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with Solanum lycopersicum and their plant-growth-promoting effect. Res Microbiol 167(2016):692–705. https://doi.org/10.1016/j.resmic.2016.07.001

Passari AK, Leo VV, Singh G, Samanta L, Ram H, Siddaiah CN, Hashem A, Al-Arjani A-BF, Alqarawi AA, Fathi Abd Allah E, Singh BP (2020) In vivo studies of inoculated plants and in vitro studies utilizing methanolic extracts of endophytic Streptomyces sp. Strain DBT34 obtained from *Mirabilis jalapa* L. exhibit ROS-scavenging and other bioactive properties. Int J Mol Sci 21:7364. https://doi.org/10.3390/ijms21197364

Patel S, Parekh V, Patel K, Jha S (2021) Plant growth-promoting activities of *Penicillium* sp. NAUSF2 ameliorate *Vigna radiata* salinity stress in phosphate-deficient saline soil. Appl Biochem Microbiol 57:500–507. https://doi.org/10.1134/S000368382104013X

Peek J, Christendat D (2015) The shikimate dehydrogenase family: functional diversity within a conserved structural and mechanistic framework. Arch Biochem Biophys 566:85–99. https://doi.org/10.1016/j.abb.2014.12.006

Petrini O, Sieber TH, Toti L, Viret O (1992) Ecology, metabolite production, and substrate utilization in endophytic fungi. Nat Toxins 1:185–196. https://doi.org/10.1002/nt.2620010306

Photita W, Lumyong S, Lumyong P, McKenzie EHC, Hyde KD (2004) Are some fungi isolated as endophytes of *Musa acuminata* latent pathogens? Fungal Divers 16:131–140

Pierre E, Louise NW, Marie TKR, Valere T, Arc-en-ce J, Fekam B (2016) Integrated assessment of phytostimulation and biocontrol potential of endophytic *Trichoderma* spp against common bean (*Phaseolus vulgaris* L.) root rot fungi complex in centre region, Cameroon. Int J Pure Appl Biosci 4:50–68

Potshangbam M, Devi SI, Sahoo D, Strobel GA (2017) Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00325

Poveda J, Baptista P (2021) Filamentous fungi as biocontrol agents in olive (*Olea europaea* L.) diseases: mycorrhizal and endophytic fungi. Crop Prot 146:105672. https://doi.org/10.1016/j.cropro.2021.105672

Poveda J, Zabalgogeazcoa I, Soengas P, Rodríguez VM, Cartea ME, Abilleira R et al (2020) Brassica oleracea var. acephala (kale) improvement by biological activity of root endophytic fungi. Sci Rep 10:20224. https://doi.org/10.1038/s41598-020-77215-7

Poveda J, Eugui D, Abril-Urías P, Velasco P (2021) Endophytic fungi as direct plant growth promoters for sustainable agricultural production. Symbiosis 85:1–19. https://doi.org/10.1007/s13199-021-00789-x

Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. J Nat Prod 68:1717–1719. https://doi.org/10.1021/np0502802

Puri SC, Nazir A, Chawla R, Arora R, Riyaz-Ul-Hasan S, Amna T, Ahmed B, Verma V, Singh S, Sagar R et al (2006) The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol 122:494–510. https://doi.org/10.1016/j.jbiotec.2005.10.015

Radic N, Strukelj B (2012) Endophytic fungi: the treasure chest of antibacterial substances. Phytomedicine 19:1270–1284. https://doi.org/10.1016/j.phymed.2012.09.007

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, Rastegari AA, Singh K, Saxena AK (2019) Endophytic fungi: biodiversity, ecological significance, and potential industrial

applications. In: Yadav AN et al (eds) Recent advancement in white biotechnology through fungi, fungal biology. https://doi.org/10.1007/978-3-030-10480-1_1

Rekadwad B, Gonzalez JM, Shah S, Suryavanshi MV, Khobragade CN, Warghane A (2022) Microbiome perspective: cross- disciplinary exploitations of chitinases. Mycology: current and future developments – sustainable utilization of fungi in agriculture and industry. Bentham Science Publishers Pte. Ltd., Singapore, pp 202–219. https://doi.org/10.2174/9789815040340122020016

Renuka S, Ramanujam B (2016) Fungal endophytes from maize (*Zea mays* L.): isolation, identification and screening against maize stem borer, *Chilo partellus* (Swinhoe). J Pure Appl Microbiol 10:523–529

Rodriguez RJ, White JF Jr, Arnold AE, Redman ARA (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rosier A, Bishnoi U, Lakshmanan V, Sherrier DJ, Bais HP (2016) A perspective on inter-kingdom signaling in plant-beneficial microbe interactions. Plant Mol Biol 90(6):537–548. https://doi.org/10.1007/s11103-016-0433-3

Rothen C, Miranda V, Aranda-Rickert A, Fracchia S, Rodríguez M (2017) Characterization of dark septate endophyte fungi associated with cultivated soybean at two growth stages. Appl Soil Ecol 120:62–69. https://doi.org/10.1016/j.apsoil.2017.07.033

Sahoo S, Sarangi S, Kerry RG (2017) Bioprospecting of endophytes for agricultural and environmental sustainability (Chapter-19). In: Patra JK (ed) Microbial biotechnology, pp 429–458. https://doi.org/10.1007/978-981-10-6847-8_19

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Annu Rev Ecol Syst 29(1):319–343. https://doi.org/10.1146/annurev.ecolsys.29.1.319

Saini R, Dudeja SS, Giri R, Kumar V (2015) Isolation, characterization, and evaluation of bacterial root and nodule endophytes from chickpea cultivated in Northern India. J Basic Microbiol 55(1):74–81. https://doi.org/10.1002/jobm.201300173

Sasse J, Martinoia E, Northen T (2017) Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci 23(1):25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Saunders M, Kohn LM (2008) Host-synthesized secondary compounds influence the in vitro interactions between fungal endophytes of maize. Appl Environ Microbiol 74:136–142. https://doi.org/10.1128/AEM.01538-07

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109(6):661–686. https://doi.org/10.1017/S095375620500273X

Schulz B, Sucker J, Aust HJ, Krohn K, Ludewig K, Jones PG, Doring D (1995) Biologically active secondary metabolites of endophytic Pezicula species. Mycol Res 99:1007–1015. https://doi.org/10.1016/S0953-7562(09)80766-1

Selim KA, Nagia MM, Ghwas DEE (2017) Endophytic fungi are multifunctional biosynthesizers: ecological role and chemical diversity. In: Endophytic fungi: diversity, characterization and biocontrol. Nova Publishers, New York, pp 39–92

Sharma S, Rani V, Saini R, Verma ML (2020) Bioprospecting and biotechnological applications of microbial endophytes. In: Arora P (ed) Microbial technology for health and environment. Microorganisms for sustainability, vol 22. Springer, Singapore. https://doi.org/10.1007/978-981-15-2679-4_7

Shweta S, Zuehlke S, Ramesha BT, Priti V, Mohana Kumar P, Ravikanth G, Spiteller M, Vasudeva R, Uma Shaanker R (2010) Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71:117–122. https://doi.org/10.1016/j.phytochem.2009.09.030

Sieber T, Riesen T, Müller E, Fried P (1988) Endophytic fungi in four winter wheat cultivars (*Triticum aestivum* L.) differing in resistance against *Stagonospora nodorum* (Berk.) Cast. & Germ.= *Septoria nodorum* (Berk.) Berk. J Phytopathol 122:289–306. https://doi.org/10.1111/j.1439-0434.1988.tb01021.x

Sinclair JB, Cerkauskas RF (1996) Latent infection vs. endophytic colonization by fungi. See Ref 126, pp 3–29

Singh SP, Gaur R (2017) Endophytic *Streptomyces* spp. underscore induction of defense regulatory genes and confers resistance against *Sclerotium rolfsii* in chickpea. Biol Control 104:44–56. https://doi.org/10.1016/j.biocontrol.2016.10.011

Singh A, Singh DK, Kharwar RN, White JF, Gond SK (2021a) Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: insights, avenues, and challenges. Microorganisms 9(1):197. https://doi.org/10.3390/microorganisms9010197

Singh N, Singh A, Dahiya P (2021b) Plant growth-promoting endophytic fungi from different habitats and their potential applications in agriculture. In: Yadav AN (ed) Recent trends in mycological research. Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-030-60659-6_3

Soliman SSM, Greenwood JS, Bombarely A, Mueller LA, Tsao R, Mosser DD, Raizada MN (2015) An endophyte constructs fungicide-containing extracellular barriers for its host plant. Curr Biol 25(19):2570–2576. https://doi.org/10.1016/j.cub.2015.08.027

Spagnoletti F, Tobar N, Di Pardo AF, Chiocchio V, Lavado R (2017) Dark septate endophytes present different potential to solubilize calcium, iron and aluminum phosphates. Appl Soil Ecol 111:25–32. https://doi.org/10.1016/j.apsoil.2016.11.010

Šraj-Kržič N, Pongrac P, Klemenc M, Kladnik A, Regvar M, Gaberščik A (2006) Mycorrhizal colonisation in plants from intermittent aquatic habitats. Aquat Bot 85:331–336. https://doi.org/10.1016/j.aquabot.2006.07.001

Sriravali K, Jindal AB, Singh BP, Paul AT (2022) Plant-associated endophytic fungi and its secondary metabolites against drug-resistant pathogenic microbes. In: Kumar V, Shriram V, Paul A, Thakur M (eds) Antimicrobial resistance. Springer, Singapore. https://doi.org/10.1007/978-981-16-3120-7_10

Staniek A, Woerdenbag HJ, Kayser O (2008) Endophytes: exploiting biodiversity for the improvement of natural product-based drug discovery. J Plant Interact 3:75–93. https://doi.org/10.1080/17429140801886293

Stępniewska Z, Kuźniar A (2013) Endophytic microorganisms--promising applications in bioremediation of greenhouse gases. Appl Microbiol Biotechnol 97(22):9589–9596. https://doi.org/10.1007/s00253-013-5235-9

Stierle A, Strobel GA, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214–216. http://www.jstor.org/stable/2881310

Stone JK, Polishook JD, White JF (2004) Endophytic fungi. In: Foster M, Bills G (eds) Biodiversity of fungi. Elsevier Academic Press, Burlington, pp 241–270. https://doi.org/10.1016/B978-012509551-8/50015-5

Strobel GA, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268. https://doi.org/10.1021/np030397v

Suryanarayanan T, Senthilarasu G, Muruganandam V (2000) Endophytic fungi from *Cuscuta reflexa* and its host plants. Fungal Divers 4:117–123

Tejesvi VM, Nalini MS, Basavanna M, Prakash HS (2007) New hopes from endophytic fungal secondary metabolites. Bol Soc Quím Méx 1(1):19–26

Tenguria RK, Firodiya A (2013) Diversity of endophytic fungi in leaves of *Glycine max* (L.) merr. from central region of Madhya Pradesh. World J Pharm Pharm Sci 2:5928–5934

Terhonen E, Sipari N, Asiegbu FO (2016) Inhibition of phytopathogens by fungal root endophytes of Norway spruce. Biol Control 99:53–63. https://doi.org/10.1016/j.biocontrol.2016.04.006

Thompson JN (1994) The coevolutionary process. Univ. Chicago Press, Chicago, 376 pp

Thompson JN, Pellmyr O (1992) Multiple occurrences of mutualism in the yucca moth lineage. Proc Natl Acad Sci 89:2927–2929. https://doi.org/10.1073/pnas.89.7.2927

Tian X, Cao L, Tan H, Zeng Q, Jia Y, Han W, Zhou S (2004) Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. World J Microbiol Biotechnol 20:303–330. https://doi.org/10.1023/B:WIBI.0000023843.83692.3f

Tian X, Yao Y, Chen G, Mao Z, Wang X, Xie B (2014) Suppression of *Meloidogyne incognita* by the endophytic fungus *Acremonium implicatum* from tomato root galls. Int J Pest Manag 60:239–245. https://doi.org/10.1080/09670874.2014.958604

Tian Y, Fu X, Zhang G, Zhang R, Kang Z, Gao K, Mendgen K (2022) Mechanisms in growth-promoting of cucumber by the endophytic fungus *Chaetomium globosum* strain ND35. J Fungi (Basel) 8(2). https://doi.org/10.3390/jof8020180

Tochhawng L, Mishra VK, Passari AK, Singh BP (2019) Endophytic fungi: role in dye decolorization. In: Singh BP (ed) Advances in endophytic fungal research, fungal biology. Published by Springer Nature Switzerland AG 2019. https://doi.org/10.1007/978-3-030-03589-1_1. ISBN 978-3-030-03588-4, ISBN 978-3-030-03589-1 (eBook)

Tohge T, Watanabe M, Hoefgen R, Fernie AR (2013) Shikimate and phenylalanine biosynthesis in the green lineage. Front Plant Sci 4:62. https://doi.org/10.3389/fpls.2013.00062

Turbat A, Rakk D, Vigneshwari A, Kocsubé S, Thu H, Szepesi Á, Bakacsy L, Škrbić BD, Jigjiddorj E-A, Vágvölgyi C et al (2020) Characterization of the plant growth-promoting activities of endophytic fungi isolated from *Sophora flavescens*. Microorganisms 8(5):683. https://doi.org/10.3390/microorganisms8050683

Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV, Singh BP, Mudili V, Gupta VK, Siddaiah CN, Chowdappa S, Alqarawi AA, Abd Allah EF (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:309. https://doi.org/10.3389/fphar.2018.00309

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fertil Soils 40:36–43. https://doi.org/10.1007/s00374-004-0750-6

Wang W, Zhai Y, Cao L, Tan H, Zhang R (2016) Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). Microbiol Res 188:1–8. https://doi.org/10.1016/j.micres.2016.04.009

Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, Hu Y (2022) Endophytic fungi: an effective alternative source of plant-derived bioactive compounds for pharmacological studies. J Fungi (Basel) 8(2):205. https://doi.org/10.3390/jof8020205

Xing HQ, Ma JC, Xu BL, Zhang SW, Wang J, Cao L, Yang XM (2018) Mycobiota of maize seeds revealed by rDNA-ITS sequence analysis of samples with varying storage times. Microbiologyopen 7(6):e00609. https://doi.org/10.1002/mbo3.609

Xu K, Li XQ, Zhao DL, Zhang P (2021) Antifungal secondary metabolites produced by the fungal endophytes: chemical diversity and potential use in the development of biopesticides. Front Microbiol. https://doi.org/10.3389/fmicb.2021.689527

Yadav AN (2020) Agriculturally important fungi for sustainable agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-45971-0

Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Saxena AK, Johri AK (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. J Biol Chem 285(34):26532–26544. https://doi.org/10.1074/jbc.M110.111021

Yan L, Zhu J, Zhao XX, Shi JL, Jiang CM, Shao DY (2019) Beneficial effects of endophytic fungi colonization on plants. Appl Microbiol Biotechnol 103(8):3327–3340. https://doi.org/10.1007/s00253-019-09713-2

Yang Y, Yan M, Hu B (2014a) Endophytic fungal strains of soybean for lipid production. Bioenergy Res 7:353–361. https://doi.org/10.1007/s12155-013-9377-5

Yang B, Wang X-M, Ma H-Y, Jia Y, Li X, Dai C-C (2014b) Effects of the fungal endophyte *Phomopsis liquidambari* on nitrogen uptake and metabolism in rice. Plant Growth Regul 73:165–179. https://doi.org/10.1007/s10725-013-9878-4

Yang H, Ye W, Ma J, Zeng D, Rong Z, Xu M, Wang Y, Zheng X (2018) Endophytic fungal communities associated with field-grown soybean roots and seeds in the Huang-Huai region of China. Peer J 6:e4713. https://doi.org/10.7717/peerj.4713

Yuan ZL, Zhang CL, Lin FC, Kubicek CP (2010) Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. Appl Environ Microbiol 76:1642–1652. https://doi.org/10.1128/AEM.01911-09

Yung L, Sirguey C, Azou-Barré A, Blaudez D (2021) Natural fungal endophytes from *Noccaea caerulescens* mediate neutral to positive effects on plant biomass, mineral nutrition and Zn phytoextraction. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.689367

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771. https://doi.org/10.1039/B609472B

Zhang Y, Kang X, Liu H, Liu Y, Li Y, Yu X, Zhao K, Gu Y, Xu K, Chen C, Chen Q (2018) Endophytes isolated from ginger rhizome exhibit growth promoting potential for Zea mays. Arch Agron Soil Sci 64(9):1302–1314. https://doi.org/10.1080/03650340.2018.1430892

Zhao J, Fu Y, Luo M, Zu Y, Wang W, Zhao C, Gu C (2012) Endophytic fungi from pigeon pea [*Cajanus cajan* (L.) Millsp.] produce antioxidant cajaninstilbene acid. J Agric Food Chem 60:4314–4319. https://doi.org/10.1021/jf205097y

Zhao J, Li C, Wang W, Zhao C, Luo M, Mu F, Fu Y, Zu Y, Yao M (2013) *Hypocrea lixii*, novel endophytic fungi producing anticancer agent cajanol, isolated from pigeon pea (*Cajanus cajan* [L.] Millsp.). J Appl Microbiol 115:102–113. https://doi.org/10.1111/jam.12195

Zhao J, Ma D, Luo M, Wang W, Zhao C, Zu Y, Fu Y, Wink M (2014) In vitro antioxidant activities and antioxidant enzyme activities in HepG2 cells and main active compounds of endophytic fungus from pigeon pea [*Cajanus cajan* (L.) Millsp.]. Food Res Int 56:243–251. https://doi.org/10.1016/j.foodres.2013.12.028

Zhao L, Xu Y, Lai X (2018) Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. Braz J Microbiol 49:269–278. https://doi.org/10.1016/j.bjm.2017.06.007

Zheng R, Li S, Zhang X, Zhao C (2021) Biological activities of some new secondary metabolites isolated from endophytic fungi: a review study. Int J Mol Sci 22(2):959. https://doi.org/10.3390/ijms22020959

Chapter 2
Fungal Endophytes as Potential Anticancer Candidate over Synthetic Drugs: Latest Development and Future Prospects

Chandrabhan Prajapati, Deepak Kumar, Vivek Ambastha, and Santosh Kumar Singh

Abstract Industrialization imparted a significant impact on human health. Since the industrial revolution, there has been an exponential rise in the number of cases of cancer and other diseases. The manufacturing process and changes in lifestyle have introduced several carcinogenic substances that account for an increase in cancer worldwide. On a worldwide scale, cancer ranks as the second leading cause of mortality, with the predicted cancer burden on the world population to be 28.4 million cases in 2040, a 47% rise from 2020. The most advanced cancer treatment involves surgery, chemotherapy, and radiotherapy. Besides surgery and radiotherapy, cancer chemotherapy, which uses cytotoxic agents to kill fast-growing cancer cells, is an also effective treatment that inhibits or delays metastasis. Searching the new natural or synthetic cytotoxic as chemotherapeutics has become a new field of research. Endophytes are a natural source of potentially effective cytotoxic agents. Endophytes are microorganisms that dwell in living plant tissues and are rich in natural biologically active compounds. Fungal endophytes survive inside the plant tissues and do not have any harmful effects on plants. The cytotoxic activity of all these endophytic fungus-derived bioactive compounds accounts for their anticancer properties against different cancer cells. These endophytic microbes have not received much attention in the past, but their application in the pharmaceutical industry remains promising. Their ease of transformation mechanisms makes them an easily accessible source of anticancer compounds. They use special mechanisms to enter the tissues of their host plants or even coexist in a symbiotic association

C. Prajapati · S. K. Singh
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
e-mail: santosh.singh01@bhu.ac.in

D. Kumar
Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

V. Ambastha (✉)
Biology Department, Washington University in St. Louis, St. Louis, MO, USA
e-mail: avivek@wustl.edu

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with them. Using these endophytic fungi, distinctive derivatives of bioactive metabolites can be produced. The ability of a fungal endophyte to establish itself successfully in the host plats depends on a delicate balance being retained between its pathogenicity as well as the plant's defense mechanism, which is accomplished mostly by producing fungal endophyte metabolites. Consequently, this chapter aims to attract attention to a new area of research for developing anticancer metabolites by endophytic fungi. Insights gained from this research would eventually aid in creating safe, highly reliable, and cost-effective anticancer drugs.

Keywords Medicinal plants · Endophytic fungi · Fungal metabolite · Anticancer activity · Apoptosis

1 Introduction

Cancer develops whenever a cell surpasses the normal barriers that limit uncontrolled cell growth and metastasis. Recent advancements in understanding the detrimental changes in cell function have also been extraordinary (Weinberg 1996). In 2012, there were more than 14 million new cancer cases globally. According to the World Health Organization (WHO), 9.6 million people died in the year 2018 from cancer (WHO 2018), and this number raised to 10 million in the year 2020 (Sung et al. 2021). Further, this number is expected to rise to 19.3 million by 2025 (Gulland 2014). Among different types of cancer, carcinoma is the deadliest, which is unregulated cell growth and division of skin or tissues lining the organs, leading to tumors that approach and impair normal tissues (Chen et al. 2016). Carcinogenic cells can propagate toward different body parts and form tumors. This uncontrolled, unregulated growth of malignant tumors eventually causes the patient's death (Kharwar et al. 2011).

Proto-oncogenes, genes needed during chromosomal editing, and tumor suppressor genes play a crucial role in the genesis of tumors (Tysnes and Bjerkvig 2007). DNA damage has been assumed to be the main factor that causes cancer (Wiseman et al. 1995). DNA damage occurs sequentially and is caused by different hereditary and environmental causes, leading to mutation in either a tumor suppressor gene or oncogene. Target cells transform as a consequence of both apoptotic and DNA repair genes that get overexpressed in those cells (Knudson 2001; Croce 2008). Next, these cells spread cancer through metastatic as well as invasions, utilizing several mechanisms such as cellular division rapidly, multiple genetic abnormalities, clonal proliferation, and also the production of subclones with distinct characteristics (Fearon and Vogelstein 1990; Wood et al. 2007). Among the most widely prescribed chemotherapies for therapeutic cancer treatment attempt to eliminate malignant cells in tumors by inhibiting cellular division pathways. Cancer therapy is risky due to drug resistance with nonspecific toxicity generated by

anticancer medications (Nygren and Larsson 2003). The mammoth costs of cancer research and anticancer medicine facilities allow considerable financial pressure on consumers and government healthcare budgets.

On the other hand, plant extracts, including natural products, have become beneficial in managing the malignant tendency of cancers due to their treatment effectiveness and low cytotoxicity (Mbaveng et al. 2011). Drugs made from plants, such as Taxol and Camptothecin, are most effective in the treatment of certain types of cancer (Srivastava et al. 2005). Taxol, a tubulin-binding diterpene, is found in the African fern pine *Podocarpus gracilior* Pilger (Podocarpaceae). Taxol trigger tubulin polymerization by binding to a heterodimer of tubulin β-subunits and inhibiting tumor cell proliferation (Stahlhut et al. 1999). The extract of *Amoora rohituka* leaves showed anticancer properties against breast cancer (Singh et al. 2020). Several new anticancer drugs have been discovered by modifying natural products or isolating specific anticancer agents using medicinal plants (Srivastava et al. 2005). Although overharvesting specific plants for isolating drugs disturbs the ecological balance of their habitat, the conservation of threatened medicinal plant species has already become necessary. Consequently, it is required to explore alternative non-plant sources for anticancer medications (Kala 2000).

With the abovementioned restrictions involved with developing and producing anticancer drugs from plants, scientists have turned to microbes as a potential source of novel bioactive resources. These microbes, particularly fungi, provide an inexhaustible supply and renewable source of new bioactive molecules with possible therapeutic characteristics (Chandra 2012). Using microorganisms as the source for a potentially interesting output has various advantages. The everlasting preservation of most microorganisms of interest under favorable conditions ensures their indefinite availability (Okami 1986). The process of extracting a compound from a fungal endophyte and advancing it to clinical trials requires various stages of research and development, which involve assessing its therapeutic potential by characterizing its properties and structure (Fig. 2.1). Omics tools in combination with biostatistics and mass spectrometry have produced a detailed description of the identification as well as quantification of bioactive molecules derived from endophytic fungi (Gupta et al. 2021). Now endophytic fungi are the most favored natural alternative source of biologically active molecules (Rai et al. 2021; Keshri et al. 2021). The endophytic fungus was first recorded 400 million years ago in the Early Devonian Rhynie chert deposits, suggesting that the origin of endophyte and plant interactions can be linked to the development of plant species (Krings et al. 2007). Cross talk among plants with endophytes is also used to stimulate the production process of bioactive metabolites (Kusari et al. 2012). Routine procedures for growing microorganisms have demonstrated promising outcomes in increasing the production of desired products. The chapter concentrates on that fascinating area called endophytic fungi, which can generate many new bioactive molecules with anticancer activity. This also describes how endophytic fungi interact with their host plant, offering the full account of the most recent research on fungal endophytes' production of anticancer bioactive molecules and the chemical characteristics of numerous significant anticancer compounds. Efficient approaches for producing fungal endophytic-origin

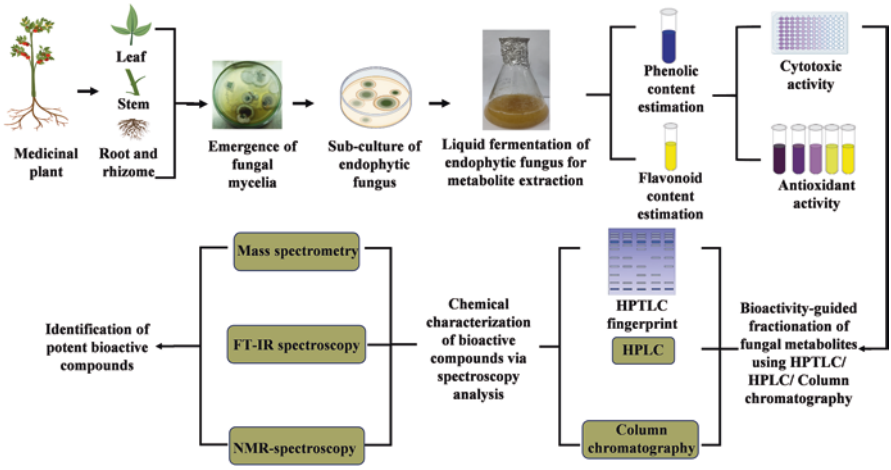

Fig. 2.1 Various plant parts are used to isolate fungal endophytes and identify the bioactive compounds produced from them exhibiting anticancer activity. Healthy tissues like leaves, stems, roots, and rhizomes are collected and cut into small pieces. The tissue pieces are then surface sterilized to remove any contaminants. To prevent bacterial contamination, the sterilized tissues are placed on PDA plates supplemented with antibiotics until fungal mycelial growth occurs. For obtaining pure fungal colonies, each isolate is subcultured onto fresh PDA plates. Molecular studies are conducted to identify the fungal strains at the species level. The pure fungal culture is then subjected to liquid fermentation to produce a large amount of metabolites, and various organic solvents are used for the extraction of fungal metabolites. Several biological activities of the extracted metabolites are used to identify the most potent fungal endophyte. Additionally, the fungal crude extracts are subjected to sophisticated techniques such as high-performance thin-layer chromatography (HPTLC) for biochemical profiling. Further purification is achieved using high-performance liquid chromatography (HPLC) and column chromatography, followed by bioactivity-guided fractionation. Spectroscopic techniques like mass spectrometry, Fourier transform infrared (FT-IR) spectroscopy, and 1D and 2D nuclear magnetic resonance (NMR) are employed to characterize the potent novel bioactive compound. The isolated bioactive compounds are then tested for their anticancer potential using suitable in vivo models

anticancer chemicals on a substantial scale have also been suggested. The main goal of this chapter is to draw attention to a new area of study focused on fungal endophytes that produce novel anticancer compounds.

2 Fungal Endophyte

The microorganisms living in unique and unconventional environments produce metabolites that help them sustain those conditions. Such microorganisms are responsible for the development of new bioactive compounds. The term "endophyte," coined by Anton de Bary about 1866, refers to many microbes (fungi, cyanobacteria, actinomycetes, or even bacteria) that can survive in living plants tissue without causing harm (Bary 1866). Fungal endophytes have received

considerably less attention than infectious fungi, because they commonly persist within host species, and the host remains asymptomatic (Swamy et al. 2016; Uzma et al. 2018). As a result, these microorganisms represent potentially new resources for developing new medications (Kharwar et al. 2011). These endophytes are essential to an emerging micro-ecosystem community (Tan and Zou 2001; Zhang et al. 2006; Rodriguez et al. 2009). Following the innovation of the "golden" anticancer agent Paclitaxel (Taxol), which is derived from the endophytic fungi *Taxomyces andreanae*, researchers' interest in biologically active and new metabolites from endophytic fungi has exploded (Stierle et al. 1993). Several possible secondary metabolites with various therapeutic qualities, including anticancer activity, have been discovered in endophytic fungus over the past several decades (Swamy et al. 2016). At the biological level, endophytic fungi constantly interact with their plant host and aid the host plant's growth by the production of secondary metabolites which promote host defense (Chandra et al. 2010; Swamy et al. 2016). Secondary metabolites derived from fungal endophytes are synthesized by a number of metabolic processes (Tan and Zou 2001). Fungal endophytes synthesizes similar physiologically active molecules as the host plant synthesizes. For example, in the early 1930s, the phytohormones "gibberellins" were isolated from *Fusarium fujikuroi* (Kharwar et al. 2008). Endophytes of bacterial and fungal origin have been found in moss, fern, algae, and various other vascular plants. Endophytic fungi are abundant in tropical environments. When specific stimulating substances from the host plant or the fungal endophyte have been coupled, both species' metabolite production significantly increased (Li et al. 2009; Zhang et al. 2009). The endophytic fungi evolved in their specific microhabitats through genetic diversity, including the acquisition of certain plant genetic material inside their genome during the prolonged association with its host plants, which is evident in the case of *Fusarium oxysporum* mediated synthesis of Podophyllotoxin (Germaine et al. 2004). According to a recent study, the endophytic fungus and the host plant employ similar but distinct metabolic mechanisms in synthesizing bioactive compounds (Jennewein et al. 2001).

Fungal endophytes comprise microorganisms that dwell within living host plants and do not affect them. Certain saprophytic fungi, mycorrhizal fungi, as well as potentially poisonous fungi which reside in living host plants at various phases of their life cycle are also included in the above category (Petrini and Fisher 1990; Akhtar and Siddiqui 2008; Akhtar and Panwar 2011). These fungal endophytes synthesize a wide range of distinct biologically active compounds, comprising alkaloids, chinones, phenolics, benzopyranones, terpenoids, quinones, steroids, flavonoids, tetralones, xanthones, and many others (Tan and Zou 2001; Swamy et al. 2016). Among these potential bioactive metabolites are potent antiparasitic, antitumor, herbicides and pesticides, antimicrobial, immunological psychotropic drugs, and antioxidants (Gunatilaka 2006; Attia et al. 2020). But recently, the endophytic fungus has drawn the attention of researchers as a reservoir of anticancer bioactive compounds that may be utilized to treat many different types of cancers (Schulz et al. 2002; Strobel et al. 2004; Kharwar et al. 2011).

3 Host-Fungal Endophyte Symbiosis

Endophytic fungi have evolved special penetrating mechanisms to survive in their hosts' tissues. Endophytes secrete exoenzymes, which assist in colonization and proliferation within their hosts' apoplastic washing fluid content (Chandra 2012). Endophytes have a mutualistic relationship with their host once they invade the roots of that plant. This mutualistic interaction promotes plant development, while endophytes get nourishment for survival as well as colonization inside the tissues of their host plants (Chandra 2012). Infectious fungal species invasions into a host plant resulted in lower levels of plant defense metabolites than the control group, such as fungal endophytes, which do not affect the host defense metabolites (Schulz et al. 2002). The host defense mechanisms and fungus virulence have reached a condition of stability. When an imbalance happens, the disease develops as virulence rises, and the plant's defense mechanisms are reduced (Chandra 2012). By competing with epiphytes and pathogens, the endophyte synthesizes compounds that aid in colonizing its host species, regulating metabolism in an equilibrium relation (Chandra 2012). Plant variety, fungal identification, and ethnobotany research are required to use potential endophytes and identify the host species that will produce positive outcomes. Secondary metabolite production can emerge when endophytes communicate with hosts in metabolic processes (Priti et al. 2009). The long-term mutualistic relationships of plant species with endophytes result in the production of bioactive molecules as byproducts. Genetic material is transferred due to the host plant's specific interactions with long-term cohabitation with the endophyte (Wang and Dai 2011; Nadeem et al. 2012). Flowering plants generate a number of defensive mechanisms to assist themselves in responding to environmental pressures, including pathogenic illness; such strategies also comprise the production of poisonous compounds. Certain defensive systems are present in a few healthy plants, while in certain other plants, harmful chemicals are synthesized during pathogenesis (Chandra 2012).

Endophytes are recognized to be tolerant of the specific metabolites of their hosts. Numerous endophytes can detoxify different plant defense bioactive chemicals, which affect their efficiency in colonizing host plants (Wang and Dai 2011). The endophytes can alter harmful metabolites synthesized by their plant host species with distinct bioactive compounds with therapeutic properties (Zikmundova et al. 2002; Saunders and Kohn 2009). Endophytes can be processed to generate bioactive molecules, which are much more established and structured than their host plant species' active compounds (Wang and Dai 2011; Swamy et al. 2016). The endophytic fungus uses effective biotransformation enzymes to alter the conformation of various molecules (Chen et al. 2016). Some researchers looked into the possibility of using endophytic fungi to produce chemicals having increased activity. Several investigations have demonstrated that different kinds of endophytic fungi may be used to create a wide range of metabolites, including stereospecific processes (Agusta et al. 2005; Borges et al. 2008; Verza et al. 2009; Swamy et al. 2016).

The endophytic fungus can be used to produce novel molecules that cannot be synthesized chemically through stereospecific as well as region-specific responses.

As a result, byproducts derived from microorganism bioactive molecules have diverse features that make them appealing candidates for commercial production processes (Tejesvi et al. 2007). Under specific and standardized cultural conditions, improvements in endophytic fungal strains lead to a significantly larger production of secondary metabolites (Peñalva et al. 1998). Endophytic fungi also seem unexplored, and additional study on their natural range and biodiversity, including their chemical and microbiological profiles, is required in plant species harboring them.

4 Environmental and Biotic Clues Affecting the Survival of Endophytic Fungi

Humidity, warmth, or even light can indirectly affect the development of endophytic fungi within the host plant's tissues (Wu et al. 2013). *Curvularia protuberata*, a fungal endophyte derived from the plant *Dichanthelium lanuginosum*, can survive in Yellowstone National Park under extreme temperatures (Márquez et al. 2007). *Diplodia mutila*, an endophytic fungus derived from *Iriartea deltoidea*, is sensitive to light intensity and shows increased tissue necrosis and cell death in the presence of bright light (408 ± 17.3) μmol $m^{-2}s^{-1}$ ± SE) by increasing the generation of ROS (reactive oxygen species). In contrast, low illumination (208.2 ± 6.1 μmol $m^{-2}s^{-1}$ ± SE) promotes endosymbiotic growth (Alvarez-Loayza et al. 2011). Thermophilic fungal endophytes isolated from hot desert adapted *Cullen plicata* Delile enhance plants' drought and heat stress tolerance by ecophysiological mechanisms and improve the growth of their host plants (Ali et al. 2019). In tropical forests, canopy and leaf age of plants influence fungal endophyte colonization (Arnold and Herre 2003). Fungal endophyte colonization varies depending on the host plant species and environmental conditions and can significantly impact plant health and ecosystem functioning (Collado et al. 1999; Lau et al. 2013). The host plant's production of defense compounds can significantly impact fungal endophyte colonization (Saunders and Kohn 2009). Climate may also influence symbiotic fungal endophyte colonization (Giauque and Hawkes 2013). The endophyte stimulates the secretion of apoplastic proteins and nucleotides in the plant, facilitating fungal endophyte colonization (Nizam et al. 2019).

5 Fungal-Endophyte-Derived Anticancer Compounds

On a worldwide scale, cancer ranks as the second leading cause of mortality. Because of the significant cancer mortality rate, research seeking effective anticancer drugs seems to be ongoing. So far, medicinal plant-associated fungal endophytes have yielded numerous bioactive compounds that have been isolated and characterized, out of which some of the selected anticancer compounds of various chemical classes are described in subsequent sections (Fig. 2.2). *Aspergillus iizukae*, an

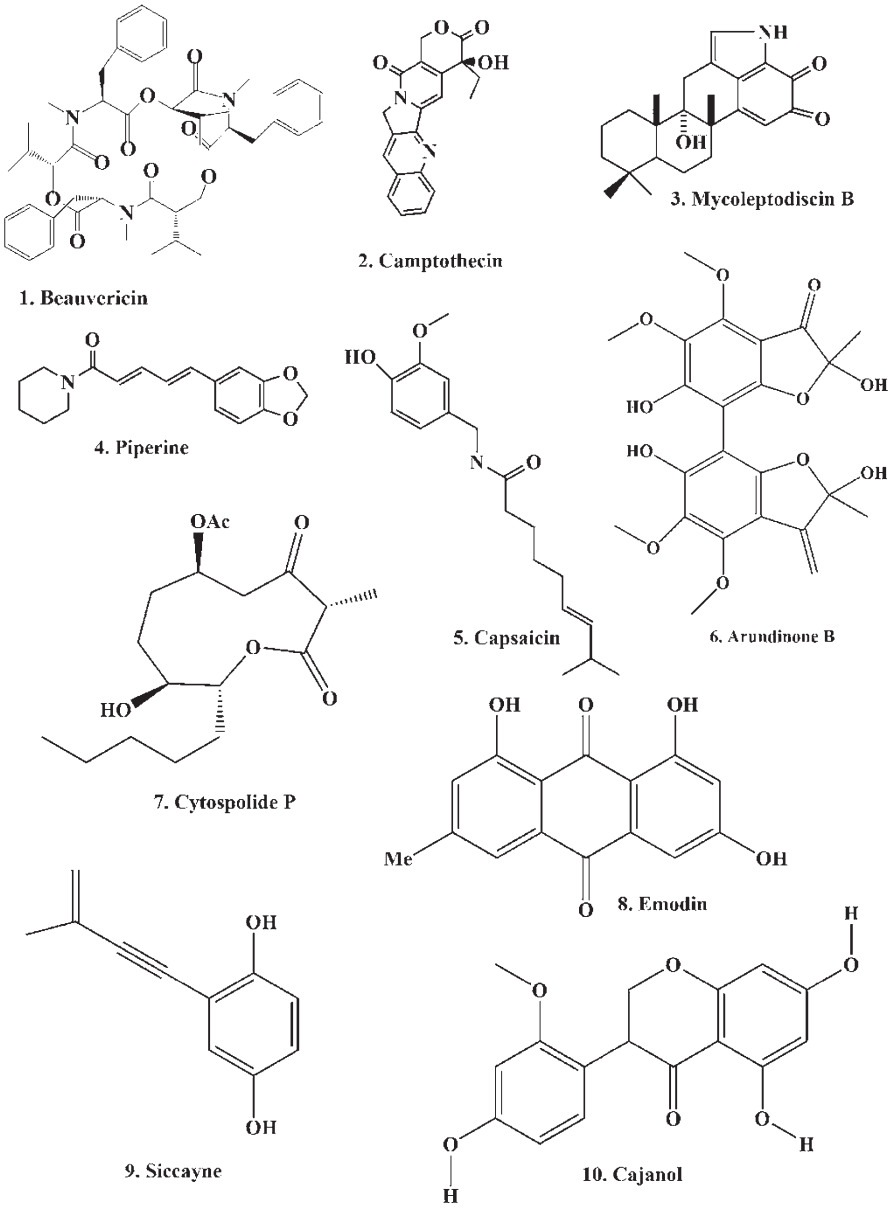

Fig. 2.2 The chemical structure of selected anticancer compounds extracted from plant-associated fungal endophytes

endophytic fungus, was isolated from host plant *Silybum marianum* and had the ability to produce bioactive compounds Silybin A and Silybin B. Silybin A and also Silybin B have anti-inflammatory as well as antitumor effects (El-Elimat et al. 2014; Surai 2015). Endophytic fungi that produced biologically active compounds have

previously been identified as a precursor for various antitumor drugs. Camptothecin is the precursor of several chemicals, including the anticancer drugs Irinotecan and Topotecan. Camptothecin, as well as its analogues, has been shown in clinical trials to be especially effective toward solid tumors of lung, liver, and ovary (Choi et al. 2011). Although Camptothecin is insoluble in water, its water-soluble analogues, camptosar and hycamtin, are utilized for the treatment of colorectal as well as ovarian cancers, respectively (Li et al. 2017). Seven novel azalomycin F analogues isolated from *Streptomyces* sp. 211,726 have a wide range of antibacterial as well as anti-HCT-116 properties, having IC_{50} values that ranged between 1.81 and 5.00 μM (Yuan et al. 2013). Production of secondary metabolites having biosynthetic potential has been explored from endophytic actinobacteria which further shows the potential exists in this group (Passari et al. 2017).

6 Novel Resource for Anticancer Compounds: Fungi Endophytes

6.1 Alkaloids and Nitrogen Containing Heterocyclic Compounds

The alkaloids produced in plants contain a wide range of bioactivities, including toxicity, therapeutic characteristics, and recreational applications. Many alkaloids derived from plants have already been explored for their potential use in anticancer drugs, and many of these molecules have evolved in fungal endophytes (Kharwar et al. 2011). Many alkaloids with antitumor activities have been studied for usage as commercially effective medications, with Camptothecin (CPT) as well as Vincristine, being the two best examples. The endophytic fungus has yielded new alkaloids with anticancer properties as reported recently. Beauvericin, derived by *Fusarium oxysporum*, is cytotoxic to A549, PC-3, and PANC-1 with IC_{50} values of 10.4 ± 1.6 μM, 49.5 ± 3.8 μM, and 47.2 ± 2.9 μM, respectively (Wang et al. 2011a, b). *Eurotium rubrum*, an endophytic fungus isolated from the plant *Hibiscus tiliaceus*, was shown to contain Variecolorin, grand alkaloid E-7, as well as a Dioxopiperazine alkaloid (Li et al. 2008). One quinoline-derived alkaloid that assists through inhibiting enzyme topoisomerase I could be Camptothecin (CPT) (Chandra 2012). *Fusarium solani* MTCC 9667 is a strain of endophytic fungus derived from the plant *Apodytes dimidiata*. Camptothecin and 9-methoxycamptothecin are alkaloids produced by its mycelial extract (Shweta et al. 2010). *Phomopsis* sp., *Fomitopsis* sp., and *Alternaria alternata* were identified as endophytic fungi from *Miquelia dentata* stages of fruit components. These fungal endophytes are responsible for the synthesis of 9-methoxy-camptothecin, Camptothecin, as well as 10-hydroxy-camptothecin. Using these fungal isolates with ethyl acetate or even methanol showed significant cytotoxic activity toward cell lines of colon as well as breast cancer (Shweta et al. 2013). From an

endophytic fungus, *Chaetomium globosum* TY1 was isolated from the plant *Ginkgo biloba*. Numerous alkaloids like azaphilone alkaloids, Chaetomugilides A–C, and Chaetoviridin E were derived from the previously mentioned endophytic fungus. Whenever Chaetomugilide alone was applied to HepG2 cell line, it produced significant cytotoxicity with the following IC_{50} value 1.7 μM. When Chaetoviridin E was introduced for the same HepG2 cell line, it produced a moderate level of cytotoxicity with IC_{50} value ranging 19.8–53.4 μM (Li et al. 2013). The alkaloid Mycoleptodiscin B has been derived from an endophytic fungus *Mycoleptodiscus* sp., which is isolated from the plant *Desmotes incomparabilis*. Mycoleptodiscin B inhibited cancer cell line growth considerably, having an IC_{50} value of 0.60–0.78 μM (Ortega et al. 2013). The Citriquinochroman metabolite was produced by cultivating an endophytic fungus *Penicillium citrinum* upon a bean and rice media. *Penicillium citrinum*, an endophytic fungus, was first identified using the juvenile stem of the plant *Ceratonia siliqua*. Citriquinochroman demonstrated a cytotoxic effect against cell line L5178Y with an IC_{50} value of 6.1 μM (El-Neketi et al. 2013). *Pestalotiopsis* sp. contained the metabolites Pestalactam A and Pestalactam B, which had considerable in vitro efficacy against MCF-7 and NFF mammal cancer cells during experimental testing (Davis et al. 2010). Cytochalasin analogues, Aspochalasin D, and Aspochalasin J inhibited HeLa cells with IC_{50} values of 5.72 μM and 27.4 μM, respectively. Both metabolites are derived from an endophytic fungus *Trichoderma gamsii* isolated from the host plant *Panax notoginseng* (Ding et al. 2012). *Fusarium solani* S-019, an endophytic fungus strain, dwell within the plant host *Camptotheca acuminata* had significant cytotoxic action on cancer cells and was shown to synthesize Camptothecin (CPT). On Vero cells, the fungus CPT was also effective in suppressing cell proliferation and inducing apoptosis (Ran et al. 2017). Piperine, an alkaloid with anticancer activities, is generated from a fungal endophyte *Colletotrichum gloeosporioides* derived from the plant *Piper nigrum* (Chithra et al. 2014). In one of the previous study, a fungal endophyte *Alternaria alternata*, associated with the plant *Capsicum annum* produces alkaloid that have been reported for their cytotoxic effect against different origin of cancer cells as well as induces apoptosis in HL-60 cells (Devari et al. 2014). Camptothecin is an alkaloid with anticancer properties obtained from an endophytic fungus *Entrophospora infrequens*, which is derived from the plant *Nothapodytes foetida* in Jammu as well as Mahabaleshwar region of the Indian country (Amna et al. 2006). The cytotoxic activity of novel bioactive compounds derived from fungal endophytes has been given in Table 2.1.

6.2 Coumarins

The endophytic fungus *Penicillium* sp. 091402 was isolated from the mangrove plant *Bruguiera sexangula* Linn. It generates (3R*,4S*)-6,8-dihydroxy-3,4,7-trimethylisocoumarin, which exhibits modest cytotoxicity against cancer cell line K562 with an IC_{50} value of 18.9 μg/mL (Han et al. 2009). 5-methyl-8-(3-methylbut-2-enyl) furanocoumarin has been named after a unique furanocoumarin molecule

Table 2.1 List of anticancer compounds isolated from the plant-associated fungal endophytes

Plant hosts	Endophytic fungi	Bioactive metabolites	Anticancer activity	References
Cinnamomum kanehirae	*Fusarium oxysporum*	Beauvericin	Cytotoxic to A549 PC-3 and PANC-1 cell lines	Wang et al. (2011a, b)
Hibiscus tiliaceus	*Eurotium rubrum*	Dehydrovariecolorin L and dehydroechinulin	Cytotoxic to Hela, Du145, SMMC7721, MCF-7, SW1990, as well as NCI-H460 cell lines	Li et al. (2008)
Apodytes dimidiata (*Icacinaceae*)	*Fusarium solani* MTCC 9667	Camptothecin (CPT) and 9-methoxycamptothecin	Anticancer activity in lymphocytic leukaemia in the mouse.	Shweta et al. (2010)
Miquelia dentata	*Alternaria alternata*, *Fomitopsis* sp., and *Phomopsis* sp.	9-methoxy CPT (9-MeO-CPT), CPT, as well as 10-hydroxy CPT (10-OH-CPT)	Cytotoxic to HCT-116 and SW-480 (colon cancer cell lines) as well as MCF-7 (breast cancer cell line)	Shweta et al. (2013)
Ginkgo biloba	*Chaetomium globosum* TY1	Chaetomugilides A–C and their derivative	Cytotoxic to HepG2 (liver cancer cell line)	Li et al. (2013)
Desmotes incomparabilis	*Mycoleptodiscus* sp.	Mycoleptodiscin B	Cytotoxic to H460, A2058, H522-T1, as well as PC-3 human cancer cell lines	Ortega et al. (2013)
Ceratonia siliqua	*Penicillium citrinum*	Citriquinochroman	Cytotoxic effect against murine lymphoma L5178Y cells	El-Neketi et al. (2013)
Panax notoginseng	*Trichoderma gamsii*	Aspochalasin D and Aspochalasin J	Cytotoxic effect against HeLa cells of the cervical cancer cell line	Ding et al. (2012)
Camptotheca acuminata	*Fusarium solani* S-019	Camptothecin (CPT)	Cytotoxic effect against Vero cell and PC-3 cell line	Ran et al. (2017)

(continued)

Table 2.1 (continued)

Plant hosts	Endophytic fungi	Bioactive metabolites	Anticancer activity	References
Piper nigrum	*Colletotrichum gloeosporioides*	Piperine	Anticancer activities against K562 derivative of MDR cells	Chithra et al. (2014)
Capsicum annuum	*Alternaria alternata*	Capsaicin	Cytotoxic effect on human promyelocytic leukemia and HL-60 cell line	Devari et al. (2014)
Avicennia sp.	*Penicillium* sp. ZH16	5-methyl-8-(3-methylbut-2-enyl) furanocoumarin	Cytotoxicity against KB and KBV200 cells	Huang et al. (2012)
Ulmus macrocarpa	*Microsphaeropsis arundinis*	Arundinone B	Cytotoxicity to T24, as well as A549 cell lines	Luo et al. (2013)
Sinopodophyllum hexandrum	*Pestalotiopsis adusta*	Pestalustaine B	Cytotoxic to HeLa, HCT116, as well as A549 human cancer cell lines	Xiao et al. (2018)
Ilex canariensis	*Cytospora* sp.	Cytospolide P	Cytotoxic to A549 cancer cell line	Lu et al. (2011)
Pandanus amaryllifolius	*Colletotrichum gloeosporioides*	Tyrosol C	Cytotoxic activities against HT29, HCT116, as well as A549 cancer cell lines	Bungihan et al. (2013)
Sonneratia caseolaris	*Bionectria ochroleuca*	Chloro-derivative of Pullularin E, Pullularin A, Pullularin C, and Verticillin D	Cytotoxic to cell line L5178Y	Ebrahim et al. (2012)
Bruguiera gymnorhiza	*Aspergillus terreus* (GX7-3B)	Beauvericin	Cytotoxicity to cell lines HeLa, A549, MCF-7, as well as KB	Deng et al. (2013) and Kharwar et al. (2011)
Sonneratia apetala	*Talaromyces flavus*	Talaperoxide B, and Talaperoxide D	Cytotoxic to PC-3, HepG2, MCF-7, MDA-MB-435, and also HeLa cell lines	Li et al. (2011)

(continued)

Table 2.1 (continued)

Plant hosts	Endophytic fungi	Bioactive metabolites	Anticancer activity	References
Hopea hainanensis	*Guignardia* sp. IFB-E028	Guignasulphide	Cytotoxic to HepG2 (human liver cancer cell line)	Wang et al. (2010)
Curcuma wenyujin	*Chaetomium globosum*	Chaetoglobosin X	Cytotoxicity against cell lines MFC as well as H22	Wang et al. (2012)
Aquilaria sinensis	*Nodulisporium* sp. A4	(2R*, 4R*)-3,4-dihydro-4-methoxy-2- methyl-2H-1-benzopyran-5-ol	Cytotoxicity against the cell line SF-268	Wu et al. (2010)
Kandelia candel	*Aspergillus tubingensis* (GX1-5E)	TMC 256 A1	Cytotoxicity to MCF-7, Hep3B, SNB19, MDA-MB-435, Huh7, as well as U87 MG cell lines	Liu et al. (2011) and Huang et al. (2011b)
Aegiceras corniculatum	*Alternaria* sp. ZJ9-6B	Alterporriol L and Alterporriol K	Cytotoxicity against MDA-MB-435, as well as MCF-7 cell lines	Huang et al. (2011a)
Hibiscus tiliaceus	*Eurotium rubrum*	12-demethyl-12-oxo-eurotechinulin B, 9-dehydroxyeurotinone, Variecolorin G, alkaloid E-7, and Emodin	Cytotoxic to MCF-7, SW1990, HepG2, NCI-H460, SMMC7721, Hela, as well as Du145 cancer cell lines	Yan et al. (2012)
Mentha pulegium	*Stemphylium globuliferum*	Altersolanol A	Cytotoxic to A549, and K562 cell lines	Teiten et al. (2013)
Laurencia sp. (*red algae*)	*Penicillium chrysogenum* QEN-24S	Penicisteroid A, and Penicisteroid B	Cytotoxicity to HeLa, SW1990, as well as NCI-H460 cancer cell lines	Gao et al. (2011)

(continued)

Table 2.1 (continued)

Plant hosts	Endophytic fungi	Bioactive metabolites	Anticancer activity	References
Avicennia marina	*Aspergillus Niger* MA-132	Nigerapyrones B, D, E, and Asnipyrone A	Cytotoxic to HeLa, HepG2, MCF-7, DU145, NCI-H460, A549, MDA-MB-231, and also SW1990 cancer cell lines	Liu et al. (2011)
Camellia sinensis	*Pestalotiopsis fici*	Siccayne	Cytotoxic to HeLa, and HT29 cell lines	Liu et al. (2013)
Bruguiera gymnorhiza	*Aspergillus terreus* GX7-3B	3β,5α-dihydroxy-(22E,24R)-ergosta-7,22-dien-6-one	Cytotoxicity against cell lines MCF-7, HeLa, A549, as well as KB	Deng et al. (2013)
Solanum nigrum	*Aspergillus flavus* (SNFSt)	Solamargine	Cytotoxicity against cell lines PC-3, HT-29, HCT-15, LNCaP, T47D, and also MDA-MB-231	El-Hawary et al. (2016)
Taxus brevifolia	*Taxomyces andreanae*	Taxol and Taxane	Cytotoxicity to cell line 9 KB	Stierle et al. (1993)
Fagus sp.	*Paraconiothyrium* sp. MY-42	Isopimarane diterpenes	Cytotoxic activity on promyelocytic HL60 cells	Shiono et al. (2011)
Ceratodon purpureus (*moss*)	*Smardaea* sp. AZ0432	Sphaeropsidin A and Sphaeropsidin D and 6-O-acetylsphaeropsidin A	Cytotoxicity to cell line MDA-MB-231	Wang et al. (2011a)
Salacia oblonga	*Alternaria*, *Fusarium*, and *aspergillus Niger*	Taxol and Taxane	Cytotoxicity against 9 KB cell lines	Roopa et al. (2015) and Stierle et al. (1993)
Annona squamosa L.	*Xylarialean* sp. A45	Xylariacin A, Xylariacin B, and Xylariacin C	Cytotoxicity against HepG2 cell line	Lin et al. (2011)

(continued)

Table 2.1 (continued)

Plant hosts	Endophytic fungi	Bioactive metabolites	Anticancer activity	References
Annona muricata	*Periconia* sp.	(+)-(3S,6S,7R,8S)-periconone A, and (−)-(1R,4R,6S,7S)-2-caren-4,8-olide	Cytotoxic to HCT-8, Bel-7402, BGC-823, A549, A2780, as well as MCF-7 cancer cell lines	Ge et al. (2011)
Bidens pilosa	*Botryosphaeria rhodina*	Botryorhodine A and Botryorhodine B	Cytotoxic to HeLa cell lines	Abdou et al. (2010)
Camellia sinensis	*Pestalotiopsis fici*	Pestalofones F, G, and H and Pestalodiol C	Cytotoxic activity against cell lines MCF-7, as well as HeLa	Shu-Chun et al. (2011)
Cajanus cajan	*Hypocrea lixii*	Cajanol	Cytotoxic to A549 cancer cell line	Zhao et al. (2013)
Podophyllum hexandrum	*Fusarium solani*	Podophyllotoxin	Cytotoxicity to P-388, A-549, HT-29, as well as MEL-28 cell lines	Mohammad et al. (2012)
Garcinia hombroniana	*Guignardia bidwellii* (PSU-G11)	Guignarenone A	Cytotoxicity against KB and Vero cell line	Sommart et al. (2012)
Juniperus recurve	*Fusarium oxysporum*	Podophyllotoxin	Cytotoxicity to cell lines P-388, A-549, HT-29, as well as MEL-28	Kour et al. (2008)
Tabebuia rosea	*Aspergillus* TRL1	Pulchranin A	Cytotoxic effects in vitro against MCF-7, Hep-G2, as well as HCT cancer cell lines	Moussa et al. (2020)

originating from the endophytic fungus strain *Penicillium* sp. ZH16. Its bioactive compound has been found to be cytotoxic toward KB cells and KBV200 cells with IC_{50} values of 5.0 μM and 10.0 μM, respectively (Huang et al. 2012). *Microsphaeropsis arundinis*, an endophytic fungus isolated from the *Ulmus macrocarpa*, produces novel bioactive compounds Arundinone B. This compound exhibited cytotoxic effect toward cancerous cell lines T24 and A549 (Luo et al. 2013). *Pestalotiopsis adusta*, a fungal endophyte that survives within its plant host *Sinopodophyllum hexandrum*, produced the novel biologically active molecule Pestalustaine B, as

well as novel sesquiterpene and coumarin derivatives that have been shown to induce apoptosis. Pestalustaines A and B exhibited mild to moderate cytotoxic effects against three cancer cells in vitro, A549, HeLa, and HCT116, having IC_{50} values usually ranging from 21.01 to 55.43 μM (Xiao et al. 2018).

6.3 Lactones

Cytospora sp. is a fungal endophyte that dwells within the plant host *Ilex canariensis* and produces the nonanolide metabolite Cytospolide P. The Cytospolide P has been shown to arrest the cell in the G1 phase by the role of C-2 methylation in treated cell line A549 (Lu et al. 2011). The endophytic fungus *Colletotrichum* sp. has been isolated from the plant *Pandanus amaryllifolius*, which generated a biologically active molecule that is macrolides. These same chemical compounds cytosporone or even Dothiorelone C have been found inside the fungal endophyte *Chaetomium globosum*. The multiple compounds like Dothiorelone C, Cytosporone, as well as Colletotriolide had no significant activities when tested on A549, HT29, and also HCT116 cell lines (Bungihan et al. 2013).

6.4 Peptides

The fungal endophyte *Bionectria ochroleuca* has been isolated from the plant *Sonneratia caseolaris*, was shown to contain the bioactive compounds Pullularin A, Pullularin B, and also Verticillin D (Ebrahim et al. 2012). Verticillin D exhibited strong cytotoxic effects on the cancerous cell line L5178Y. The Pullularin A, Pullularin C, and the chloro-derivative of Pullularin E have also been found to have cytotoxic effect having IC_{50} values varied from 0.1 to 6.7 μM (Ebrahim et al. 2012). The fungal endophytic strain, *Aspergillus terreus* (GX7-3B), was identified from the plant *Bruguiera gymnorhiza* and produces an anticancer compound Beauvericin (Deng et al. 2013). Beauvericin was previously discovered in a variety of different fungus species (Kharwar et al. 2011). Beauvericin was also extracted from an endophytic fungus *Epicoccum nigrum*, which dwells within its host plant *Entada abyssinica*. This compound exhibited cytotoxic activity toward cell lines Vero cell, THP-1, and RAW 264.7 with IC_{50} values of 86.56 ± 3.94 μg/ml, 76.56 ± 5.76 μg/ml, and 64.48 ± 6.17 μg/ml, respectively (Dzoyem et al. 2017).

6.5 Peroxides

XG8D is an endophytic fungal strain isolated from the mangrove plant *Xylocarpus granatum*. Two novel endoperoxide compounds Merulin A and Merulin C were extracted using endophytic fungal strain XG8D. Both compounds had been found

to exhibit cytotoxic effect against cancerous cell lines BT474 and SW620 with IC_{50} values ranging from 1.57 to 4.98 μg/mL (Chokpaiboon et al. 2010). The nor-sesquiterpene peroxides, Talaperoxide B and Talaperoxide D, were obtained using an endophytic fungus *Talaromyces flavus* that lives within its plant host *Sonneratia apetala* (Li et al. 2011). Both compounds have been shown to be cytotoxicity toward following cell lines such as HeLa, PC-3, MDA-MB-435, and HepG2, along with MCF-7 having IC_{50} values that varied between 0.70 and 2.78 μM (Li et al. 2011).

6.6 Polyketides

Pestalpolyol I, a novel polyketide derivative extracted from an endophytic fungus, *Pestalotiopsis clavispora*, found in the mangrove plant *Rhizophora harrisonii*. The pestalpolyol I exhibited cytotoxic activity against cell line L5178Y with an IC_{50} value of 4.10 μM (Hemphill et al. 2016). The Guignasulphide was extracted from the endophytic fungal strain *Guignardia* sp. IFB-E028, which dwells within the juvenile leaf of the *Hopea hainanensis* plant (Wang et al. 2010). Guignasulphide exhibited cytotoxic effect toward the hepatic cancer cell line HepG2 with an IC_{50} value of 5.2 ± 0.4 μM (Wang et al. 2010). Chaetoglobosin is a polyketide with binding specificity for actin filaments in muscle. The bioactive compound Chaetoglobosin X had been obtained from an endophytic fungus *Chaetomium globosum*, which dwells within the plant *Curcuma wenyujin*. The Chaetoglobin X showed a high cytotoxic effect on cancer cell lines MFC as well as H22 cells (Wang et al. 2012).

6.7 Pyrans and Pyrones

The fungal endophyte *Nodulisporium* sp. A4 was isolated from stem of *Aquilaria sinensis* and revealed the existence of a novel benzopyran, (2R*, 4R*)-3,4-dihydro-4-methoxy-2-methyl-2H-1benzopyran-5-ol. Compared to Cisplatin, this metabolite had comparatively modest cytotoxicity against the cell line SF-268 at 100 mg/ml (Wu et al. 2010). Pyrone compounds and the compounds derived from pyrones are produced mainly by fungal endophytes of the *Aspergillus* species (Liu et al. 2011). During in vitro experiments, naphtho-gamma-pyrone, TMC 256 A1, a monomeric chemical derived by the marshes fungal endophyte *Aspergillus tubingensis* (GX1-5E), was shown to be a cytotoxic effect on cancer cell lines U87 MG, SNB19, MDA-MB-435, MCF-7, Hep3B, and Huh7 with IC_{50} values that are ranging from 19.92 to 47.98 μM (Huang et al. 2011b). The α-pyrone derivative compounds, namely, Nigerapyrones B, D, E, and congeners, Asnipyrone A, were isolated from an endophytic fungus *Aspergillus niger* MA-132, which dwells inside plant host *Avicennia marina*. Nigerapyrone E has been shown to be cytotoxicity against cell lines SW1990, A549, and MDA-MB-231 having IC_{50} values of 38, 43, and 48 μM, respectively. The above drug had also been revealed to have a weak to medium effect on the cell lines MCF7, MDA-MB-231, HepG2, NCI-H460, or even

Du145 having IC_{50} values of 105, 48, 86, 43, and also 86 μM, respectively. Nigerapyrone B, Asnipyrone A, and Nigerapyrone D were all compounds found to exhibit cytotoxic effects against various cell lines. Nigerapyrone B exhibited cytotoxic effect against cell line HepG2 with an IC_{50} value of 62 μM. Asnipyrone A showed cytotoxicity against A549 cells with an IC_{50} value of 62 μM. Nigerapyrone D cytotoxicity was demonstrated against many cancer cell lines such as MCF-7, A549, and HepG2 with IC_{50} values of 121 μM, 81 μM, and 81 μM, respectively (Liu et al. 2011).

6.8 Quinones

Aegiceras corniculatum, a wetland host plant, has been found to contain an endophytic fungus strain *Alternaria* sp. ZJ9-6B. The bioactive compounds anthraquinones, Alterporriol L, and Alterporriol K were extracted from endophytic fungal strain *Alternaria* sp. ZJ9-6B (Huang et al. 2011a). During the experimental research, Alterporriol K and Alterporriol L were shown to have a moderate level of cytotoxic effects against cell lines MDA-MB-435 as well as MCF-7 cells with an IC_{50} value ranging from 13.1 to 29.1 μM (Huang et al. 2011a). The *Eurotium rubrum*, an endophytic fungus isolated from the plant host *Hibiscus tiliaceus*, had produced 9-dehydroxyeurotinone, a unique molecule derived from anthraquinone, Variecolorin G, 12-demethyl-12-oxo-eurotechinulin B, along with Emodin and some other anthraquinone molecule. These molecules were shown to be cytotoxic effects on cell lines, such as 12-demethyl-12-oxo-eurotechinulin B, which was cytotoxic to SMMC-7721; 9-dehydroxyeurotinone cytotoxic against SW1990; variecolorin G cytotoxic against HepG2, NCl-H460, and Hela cells; alkaloid E-7 cytotoxic against MCF-7, SW1990, SMMC-7721, and HeLa cells; and Emodin cytotoxic against Du145 (Yan et al. 2012). Altersolanol A, derived from the fungal endophyte *Stemphylium globuliferum*, was also cytotoxic on cancer cell lines A549 and K562. The isolate was produced from the medicinal plant *Mentha pulegium* (Lamiaceae). When antiapoptotic protein levels were reduced, caspase-3 and caspase-9 were cleaved, resulting in cell death via apoptosis caused by Altersolanol A (Teiten et al. 2013).

6.9 Steroids

Laurencia, an alga that hosts an endophytic fungus strain *Penicillium chrysogenum* QEN-24S, has been used to isolate various novel steroidal compounds such as Penicisteroid A and also Penicisteroid B (Gao et al. 2011). Penicisteroid A showed significant antifungal and cytotoxic effects during initial experiments (Gao et al. 2011). The presence of phytoecdysteroids was observed in the fungal endophyte strain *Aspergillus terreus* GX7-3B, which dwell within the host plant *Bruguiera*

gymnorhiza. Cytotoxicity was demonstrated on cell lines MCF-7, KB, A549, and HeLa with IC_{50} values of 4.98 μM, 1.50 μM, 1.95 μM, and 0.68 μM, respectively (Deng et al. 2013). An endophytic fungus strain *Aspergillus flavus* (SNFSt) derived from the plant *Solanum nigrum* has the potential to generate solamargine, a derivative of the steroidal solasodine with anticancer properties in a variety of cancers. Over 11 generations of subcultivation, the endophytic fungal strain SNFSt had produced Solamargine and yielded (7 mg, ~300 μg l^{-1}) considerable levels of Solamargine, suggesting that it may have been utilized as an alternate source for the synthesis of Solamargine as the anticancer compound (El-Hawary et al. 2016).

6.10 Diterpenes

A fungal endophyte *Taxomyces andreanae*, derived from the plant *Taxus brevifolia*, yields Taxol and Taxane (Stierle et al. 1993). A fungal endophyte strain *Paraconiothyrium* sp. MY-42 had been used to extract Sphaeropsidin A and Sphaeropsidin D diterpenes that displayed moderate cytotoxicity on human HL60 cells (Shiono et al. 2011). Both diterpenes Sphaeropsidin A and Sphaeropsidin D are produced by a moss tissue with active photosynthesis *Ceratodon purpureus*, which is the host of an endophytic fungus strain *Smardaea* sp. AZ0432. Many cancer cell lines with human fibroblast were used to test these two metabolites, and also 6-O-acetylsphaeropsidin A, for effective anticancer activities. The three diterpenes were shown to have a significant amount of cytotoxicity. Remarkably, Sphaeropsidin A was shown to be cytotoxicity against breast cancer cell line MDA-MB-231 at sub-cytotoxic doses (Wang et al. 2011a, b). *Salacia oblonga*, a medicinal plant, was used to isolate the endophytic fungus. Using morphological and ITS sequencing data, species characterization, and identification indicated that the main species in the samples were *Alternaria*, *Fusarium*, and *Aspergillus niger*. One sample showed bands for the BAPT gene, indicating that the endophyte was able to produce Taxol or a similar chemical. Some of the samples that demonstrate amplification with the DBAT primers may create a Taxol precursor, such as Baccatin III or a similar compound, which may be converted into a Taxane. Another option is that this precursor would be utilized in the semisynthetic synthesis of a Taxane/Taxol (Roopa et al. 2015).

6.11 Triterpenes

The fermented triterpene by-products Xylariacin A, Xylariacin B, and Xylariacin C were extracted using the fungal endophyte *Xylarialean* sp. A45 (Lin et al. 2011). In laboratory studies, cytotoxicity testing was performed on HepG2 (human tumor cells), which showed medium cytotoxic effects (Lin et al. 2011). *Periconia* sp., an endophytic fungus derived from the medicinal plant *Annona muricata*, contains

novel distinct terpenoids, (+)-(3S,6S,7R,8S)-periconone A, as well as (−)-(1R,4R,6S,7S)-2-caren-4,8-olide. Such compounds have been found to be slightly cytotoxic to human cancer cell lines on HCT-8, MCF-7, Bel-7402, A2780, A549, and BGC-823 (Ge et al. 2011).

6.12 Miscellaneous

The bioactive compound, Piperine is derived from a fungal endophyte *Periconia* sp., which is isolated from the plant *Piper longum* L. (Verma et al. 2011a, b). An endophytic fungus *Colletotrichum gloeosporioides* has been isolated from the leaf tissue of the plant *Oroxylum indicum* (L.) Kurz. The ethyl acetate extract of *Colletotrichum gloeosporioides* demonstrated the cytotoxic effect against cancer cell lines HCT116, HeLa, as well as HepG2, having IC_{50} values of 76.59, 176.20, and 1750.70 μg/mL, respectively (Rai et al. 2022a, b). Further study conducted by the group showed the cytotoxic and apoptosis-inducing potential of ethyl acetate extract of *C. gloeosporioides* against human breast cancer cells MDA-MB-231 and MCF-7. The findings suggest that the bioactive compounds produced from endophyte *C. gloeosporioides* induces apoptosis in breast cancer cells by elevating the excessive production of reactive oxygen species, depolarizing the mitochondrial membrane potential and altering the genes associated with extrinsic as well as intrinsic pathways (Rai et al. 2023a). *Penicillium oxalicum*, an endophytic fungus, has been derived from the leaves of the plant *Amoora rohituka*. The ethyl acetate extract of *Penicillium oxalicum* showed cytotoxic effect against different origin of cancer cells such as HuT-78, MDA-MB-231, and MCF-7 with IC_{50} values of 56.81 ± 0.617 μg/mL, 37.24 ± 1.26 μg/mL, and 260.627 ± 5.415 μg/mL, respectively (Verma et al. 2022). Several endophytic fungi isolated from the plant *Moringa oleifera* and *Withania somnifera* showed significant antibacterial activity (Atri et al. 2020). The endophytic fungi are a natural alternative source for novel or potent anticancer properties (Rai et al. 2022a, b). Streptomyces, an endophytic actinomycete isolated from the roots of the *Azadirachta indica* A. Juss, was found to be a promising biocontrol and a plant growth promoter (Verma et al. 2011a, b). Similarly, endophytic actinobacterium, *Streptomyces thermocarboxydus* strain BPSAC147, showed disease resistance potential and growth promotion in tomato (Passari et al. 2019). Mycosterol is derived from endophytic fungus of the plant *Gymnema sylvestre* (Ranjan et al. 2019). *Argemone mexicana* L. seed extracts were tested for their effectiveness against certain bacterial pathogens. Seeds extracted in chloroform showed varying degrees of antibacterial properties, with minimum inhibitory concentrations of 2.0–5.0 mg/ml toward both gram-positive and gram-negative bacteria (Singh et al. 2009). From an endophytic fungi *Pestalotiopsis fici*, alkyne molecules Siccayne had been produced (Liu et al. 2013). Siccayne had been demonstrated to be toxic to cells in the cancer cell lines such as HeLa and HT29, which showed IC_{50} values of 48.2 μM and 33.9 μM, respectively (Liu et al. 2013). *Botryosphaeria rhodina*, which dwells inside the medicinal herb *Bidens pilosa*, was shown to have the

compounds Botryorhodine A and Botryorhodine B. Both bioactive molecules had been shown to be cytotoxicity against cancer cell line HeLa, with IC_{50} values of 96.97 μM as well as 36.41 μM, respectively (Abdou et al. 2010). An endophytic fungus, *Pestalotiopsis fici* produces Pestalofones F, G, or H, and Pestalodiols C, are derivatives of epoxy isoprene. These four compounds exhibited cytotoxic activity against MCF-7 as well as HeLa cell lines (Shu-Chun et al. 2011). An isoflavone called Cajanol has been identified within an endophytic fungus fungal *Hypocrea lixii* derived from the plant *Cajanus cajan*. On the cancer cell line A549, it was found that Cajanol had a highly potent cytotoxic effect that depended on the time period as well as concentration (Zhao et al. 2013). Teniposide, Etopophos phosphate, and Etoposide are all anticancer drugs that comprise precursors of Podophyllotoxin. *Fusarium solani*, an endophytic fungus isolated from the root cells of *Podophyllum hexandrum*, has been shown to produce Podophyllotoxin (Nadeem et al. 2012). The appearance of Guignarenone A, a distinct tricycloalternarene molecule derived first from an endophytic fungus strain *Guignardia bidwellii* PSU-G11, produced a substantial cytotoxic effect in African green monkey and responsible for oral cancer as well as renal fibroblast in Vero cells (Sommart et al. 2012). Podophyllotoxin is produced by *Fusarium oxysporum*, a fungal endophyte dwelling within the plant host *Juniperus recurva* (Kour et al. 2008). A recent study showed the first-time isolation and identification of a lipid mediator bioactive compound *N*-(2-Hydroxyethyl)hexadecanamide (commonly known as palmitoylethanolamide) produced from *Oroxylum indicum*-associated endophyte *C. gloeosporioides* potentiates apoptosis in human breast cancer cells MDA-MB-231 and MCF-7 (Rai et al. 2023b). Furthermore, a strain of the fungal endophyte *Aspergillus* TRL1, which was derived from the plant *Tabebuia rosea*, can produce the novel bioactive compound pulchranin A, which has been discovered to inhibit cyclin-dependent kinases like CDK1, CDK2, as well as CDK4 in the cell cycle. Pulchranin A demonstrated potential cytotoxic effects in vitro on cancer cell lines MCF-7, HCT, and Hep-G2, with IC_{50} values of 63, 91, as well as 80 μg/mL, respectively (Moussa et al. 2020). An endophytic fungus *Aspergillus nidulan*s derived from the host plant, *Nyctanthes arbor-tristis* Linn, was used to produce Sterigmatocystin. In vitro cytotoxicity tests demonstrated that Sterigmatocystin was only cytotoxic activity on cell line MCF-7, with an IC_{50} value of 50 ± 2.5 μM/mL (Sana et al. 2019). The biogenic synthesis of silver nanoparticles mediated by *Penicillium oxalicum* (POAgNPs), an endophytic fungus isolated from the leaf of the host plant *Amoora rohituka*, exhibited cytotoxicity toward breast cancer cell lines MDA-MB-231 and MCF-7, having IC_{50} values of 20.080 ± 0.761 and 40.038 ± 1.022 μg/mL, respectively (Gupta et al. 2022). *Pestalotiopsis microspora* VJ1/VS1, a fungal endophyte strain, was isolated from the leaf tissues of the plant host *Gymnema sylvestre*. The synthesized silver nanoparticles have a high negative zeta potential value (−35.7 mV) utilizing aqueous culture filtrate of *Pestalotiopsis microspora*. The biosynthesized AgNPs were shown to havve cytotoxic effect on cancer cell lines B16F10, SKOV3, A549, and PC3, with IC_{50} values of 26.43 ± 3.41 μg/mL, 16.24 ± 2.48 μg/mL, 39.83 ± 3.74 μg/mL, and 27.71 ± 2.89 μg/mL, respectively (Netala et al. 2016). Additionally, fungal endophyte-derived

natural bioactives are used in the various neurodegenerative disorders, suggesting their neuroprotective activity (Verma et al. 2023). Furthermore, for the effective Alzheimer's disease treatment, lipid-coated mesoporous silica nanoparticles (MSNs) comprising natural compound berberine (BBR) have been synthesized (Singh et al. 2021). A strain of the fungal endophyte *Fusarium solani* ATLOY-8 had been isolated by the plant *Chonemorpha fragrans*. Green gold nanoparticles showed dose-dependent cytotoxicity against cancer cell lines such as HeLa and MCF-7 with IC_{50} values of 0.8 ± 0.5 μg/mL as well as 1.3 ± 0.5 μg/mL, respectively (Clarance et al. 2020). The strain of a fungal endophyte *Guignardia mangiferae* (Bios PTK 4) was isolated from *Citrus* sp. leaves and successfully utilized in the myogenic production of silver nanoparticles. After 24 hours of incubation, silver nanoparticles exhibited cytotoxic effects on cell lines Vero, HeLa, as well as MCF-7, having IC_{50} values of 63.37 μg/mL, 27.54 μg/mL, and 23.84 μg/mL, respectively (Balakumaran et al. 2015). Copper oxide nanoparticles (CuONPs) were created using a fungal endophyte strain *Aspergillus terreus* FCBY1 derived from the medicinal plant *Aegle marmelos*. Copper oxide nanoparticles were found to be cytotoxic on cell line HT-29, including an IC_{50} value of 22 μg/mL (Mani et al. 2021).

7 Conclusion and Future Prospects

Endophytic fungi exist in the inner tissues of healthy plants and interact with them in a number of different ways. Endophytic fungi acquire numerous essential and novel traits during their prolonged association with their hosts. Endophytic fungi secrete various enzymes that help in colonization and growth to sustain a stable relationship. Endophytic fungi have a high potential for the production of novel bioactive compounds due to their distinct environments. The formation of such identical biological active secondary metabolites by endophytic fungi gives support to the idea that for most of the coevolution of endophytic fungi and their host plants, endophytes adapted to their distinct microenvironments through genetic variation, including the incorporation of some host DNA into their own genomes (Germaine et al. 2004). Such gene transfer may have resulted in the potential among some endophytic fungi to biosynthesize compounds made by the plant host (Stierle et al. 1993). Tan and Zou (2001) also said that several fungal endophytic species may be isolated from a single plant, with at least one of them showing host specificity. To scale up production, the scientific community will concentrate their efforts on molecular analysis of endophytic fungi and optimization of the fermentation process (Tan and Zou 2001). Large-scale microbe culture in the tank fermenter can provide unlimited usable products (Okami 1986). It has been demonstrated with the principal anticancer medicines derived from plants, significant manufacturing costs contribute to high therapy expenditures. For example, the monthly cost of eribulin for breast cancer treatment or liposarcoma has now reached USD 10,000, whereas brentuximab for the treatment of lymphoma surpasses USD 30,000 (Howard et al. 2015; Gordon et al. 2018). Endophytic fungi, as a potential alternative source of

anticancer compounds for the synthesis of anticancer medicines, play an important role in limiting cancer death rates and lowering cancer therapy costs. The fungus may generally release enormous quantities of secondary metabolites that can convert metal ions to metallic nanoparticles. A fungal endophyte was isolated from the medicinal plant *Catharanthus roseus* (Linn.) and was used to create silver nanoparticles (AgNPs). Under in vitro situations, the produced AgNPs were efficacious in neutralizing free radicals and inducing apoptotic indicators such as nuclear and DNA fragmentation in lung (A549) cancer cell lines. AgNPs were shown to be cytotoxic to A549 cells, with an IC_{50} of 40 μg/mL. This is the first study to show that AgNPs from *Botryosphaeria rhodina* may cause apoptosis in many kinds of cancer cells as a potential cancer therapy technique (Akther et al. 2019). Bioinformatics plays a significant role in recent pharmaceutical research by providing high-throughput data of DNA, RNA, and proteins and also give data on gene expression and gene sequencing (Barik et al. 2020). Recent advancements in pharmacological interventions and high-throughput approaches have made significant contributions to endophyte research to solve the predicted challenges. Endophyte genetic modification, which includes gene overexpression and gene modifications, strain mutations, and cocultivation of compatible strains, is focused on improving specific endophytic fungi strains and increasing the supply of specified metabolites. Because of the scarcity of natural resources and the shortage of natural remedies, the bioprospection of endophytic fungi and their genetic improvement is an essential field of research in drug development programs.

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References

Abdou R, Scherlach K, Dahse HM, Sattler I, Hertweck C (2010) Botryorhodines A–D, antifungal and cytotoxic depsidones from Botryosphaeria rhodina, an endophyte of the medicinal plant Bidens pilosa. Phytochemistry 71(1):110–116

Agusta A, Maehara S, Ohashi K, Simanjuntak P, Shibuya H (2005) Stereoselective oxidation at C-4 of flavans by the endophytic fungus Diaporthe sp. isolated from a tea plant. Chem Pharm Bull 53(12):1565–1569

Akhtar M, Panwar J (2011) Arbuscular mycorrhizal fungi and opportunistic fungi: efficient root symbionts for the management of plant parasitic nematodes. Adv Sci Eng Med 3(3):165–175

Akhtar MS, Siddiqui ZA (2008) Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht, pp 61–97

Akther T, Mathipi V, Kumar NS, Davoodbasha M, Srinivasan H (2019) Fungal-mediated synthesis of pharmaceutically active silver nanoparticles and anticancer property against A549 cells through apoptosis. Environ Sci Pollut Res 26:13649–13657

Ali AH, Radwan U, El-Zayat S, El-Sayed MA (2019) The role of the endophytic fungus, Thermomyces lanuginosus, on mitigation of heat stress to its host desert plant Cullen plicata. Biologia Futura 70(1):1–7

Alvarez-Loayza P, White JF Jr, Torres MS, Balslev H, Kristiansen T, Svenning JC, Gil N (2011) Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, Iriartea deltoidea. PloS One 6(1):e16386

Amna T, Puri SC, Verma V, Sharma JP, Khajuria RK, Musarrat J, Spiteller M, Qazi GN (2006) Bioreactor studies on the endophytic fungus Entrophospora infrequens for the production of an anticancer alkaloid camptothecin. Can J Microbiol 52(3):189–196

Arnold AE, Herre EA (2003) Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in Theobroma cacao (Malvaceae). Mycologia 95(3):388–398

Atri N, Rai N, Singh AK, Verma M, Barik S, Gautam V, Singh SK (2020) Screening for endophytic fungi with antibacterial efficiency from Moringa oleifera and Withania somnifera. J Sci Res 64(1):127–133

Attia EA, Singh BP, Dashora K, Ahmed M, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17(1):119–132

Balakumaran MD, Ramachandran R, Kalaichelvan PT (2015) Exploitation of endophytic fungus, Guignardia mangiferae for extracellular synthesis of silver nanoparticles and their in vitro biological activities. Microbiol Res 178:9–17

Barik S, Rai N, Mishra P, Singh SK, Gautam V (2020) Bioinformatics: how it helps to boost modern biological research. Curr Sci 118:698–699

Bary A (1866) Morphologie und physiologie der pilze, flechten und myxomyceten, vol 1. W. Engelmann

Borges KB, Borges WDS, Pupo MT, Bonato PS (2008) Stereoselective analysis of thioridazine-2-sulfoxide and thioridazine-5-sulfoxide: an investigation of rac-thioridazine biotransformation by some endophytic fungi. J Pharm Biomed Anal 46(5):945–952

Bungihan ME, Tan MA, Takayama H, dela Cruz, T.E.E. and Nonato, M.G. (2013) A new macrolide isolated from the endophytic fungus Colletotrichum sp. Philipp Sci Lett 6(1):57–73

Chandra S (2012) Endophytic fungi: novel sources of anticancer lead molecules. Appl Microbiol Biotechnol 95:47–59

Chandra S, Bandopadhyay R, Kumar V, Chandra R (2010) Acclimatization of tissue cultured plantlets: from laboratory to land. Biotechnol Lett 32:1199–1205

Chen L, Zhang QY, Jia M, Ming QL, Yue W, Rahman K, Qin LP, Han T (2016) Endophytic fungi with antitumor activities: their occurrence and anticancer compounds. Crit Rev Microbiol 42(3):454–473

Chithra S, Jasim B, Sachidanandan P, Jyothis M, Radhakrishnan EK (2014) Piperine production by endophytic fungus Colletotrichum gloeosporioides isolated from Piper nigrum. Phytomedicine 21(4):534–540

Choi CH, Lee YY, Song TJ, Park HS, Kim MK, Kim TJ, Lee JW, Lee JH, Bae DS, Kim BG (2011) Phase II study of belotecan, a camptothecin analogue, in combination with carboplatin for the treatment of recurrent ovarian cancer. Cancer 117(10):2104–2111

Chokpaiboon S, Sommit D, Teerawatananond T, Muangsin N, Bunyapaiboonsri T, Pudhom, K (2010) Cytotoxic nor-chamigrane and chamigrane endoperoxides from a basidiomycetous fungus. J Nat Prod 73(5):1005–1007

Clarance P, Luvankar B, Sales J, Khusro A, Agastian P, Tack JC, Al Khulaifi MM, Al-Shwaiman HA, Elgorban AM, Syed A, Kim HJ (2020) Green synthesis and characterization of gold nanoparticles using endophytic fungi Fusarium solani and its in-vitro anticancer and biomedical applications. Saudi J Biol Sci 27(2):706–712

Collado J, Platas G, González I, Peláez F (1999) Geographical and seasonal influences on the distribution of fungal endophytes in Quercus ilex. New Phytol 144(3):525–532
Croce CM (2008) Oncogenes and cancer. N Engl J Med 358(5):502–511
Davis RA, Carroll AR, Andrews KT, Boyle GM, Tran TL, Healy PC, Kalaitzis JA, Shivas RG (2010) Pestalactams A–C: novel caprolactams from the endophytic fungus Pestalotiopsis sp. Org Biomol Chem 8(8):1785–1790
Deng CM, Liu SX, Huang CH, Pang JY, Lin YC (2013) Secondary metabolites of a mangrove endophytic fungus Aspergillus terreus (No. GX7-3B) from the South China Sea. Mar Drugs 11(7):2616–2624
Devari S, Jaglan S, Kumar M, Deshidi R, Guru S, Bhushan S, Kushwaha M, Gupta AP, Gandhi SG, Sharma JP, Taneja SC (2014) Capsaicin production by Alternaria alternata, an endophytic fungus from Capsicum annum; LC–ESI–MS/MS analysis. Phytochemistry 98:183–189
Ding G, Wang H, Li L, Chen AJ, Chen L, Chen H, Zhang H, Liu X, Zou Z (2012) Trichoderones A and B: two pentacyclic cytochalasans from the plant endophytic fungus Trichoderma gamsii. Eur J Org Chem 2012(13):2516–2519
Dzoyem JP, Melong R, Tsamo AT, Maffo T, Kapche DG, Ngadjui BT, ... Eloff JN (2017) Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus Epicoccum nigrum associated with Entada abyssinica. Revista Brasileira de Farmacognosia 27:251–253
Ebrahim W, Kjer J, El Amrani M, Wray V, Lin W, Ebel R, Lai D, Proksch P (2012) Pullularins E and F, two new peptides from the endophytic fungus Bionectria ochroleuca isolated from the mangrove plant Sonneratia caseolaris. Mar Drugs 10(5):1081–1091
El-Elimat T, Raja HA, Graf TN, Faeth SH, Cech NB, Oberlies NH (2014) Flavonolignans from Aspergillus iizukae, a fungal endophyte of milk thistle (Silybum marianum). J Nat Prod 77(2):193–199
El-Hawary SS, Mohammed R, AbouZid SF, Bakeer W, Ebel R, Sayed AM, Rateb ME (2016) Solamargine production by a fungal endophyte of Solanum nigrum. J Appl Microbiol 120(4):900–911
El-Neketi M, Ebrahim W, Lin W, Gedara S, Badria F, Saad HEA, Lai D, Proksch P (2013) Alkaloids and polyketides from Penicillium citrinum, an endophyte isolated from the Moroccan plant Ceratonia siliqua. J Nat Prod 76(6):1099–1104
Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV, Singh BP, Mudili V, Gupta VK, Siddaiah CN, Chowdappa S, Alqarawi AA, Abd Allah EF (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:328484. https://doi.org/10.3389/fphar.2018.00309
Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61(5):759–767
Gao SS, Li XM, Li CS, Proksch P, Wang BG (2011) Penicisteroids A and B, antifungal and cytotoxic polyoxygenated steroids from the marine alga-derived endophytic fungus Penicillium chrysogenum QEN-24S. Bioorg Med Chem Lett 21(10):2894–2897
Ge HL, Zhang DW, Li L, Xie D, Zou JH, Si YK, Dai J (2011) Two new terpenoids from endophytic fungus Periconia sp. F-31. Chem Pharm Bull 59(12):1541–1544
Germaine K, Keogh E, Garcia-Cabellos G, Borremans B, van der Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ER, Campbell CD (2004) Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol Ecol 48(1):109–118
Giauque H, Hawkes CV (2013) Climate affects symbiotic fungal endophyte diversity and performance. Am J Bot 100(7):1435–1444
Gordon N, Stemmer SM, Greenberg D, Goldstein DA (2018) Trajectories of injectable cancer drug costs after launch in the United States. J Clin Oncol 36(4):319–325
Gulland A (2014) Global cancer prevalence is growing at "alarming pace," says WHO, vol 348
Gunatilaka AL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):509–526
Gupta P, Rai N, Verma A, Saikia D, Singh SP, Kumar R, Singh SK, Kumar D, Gautam V (2022) Green-based approach to synthesize silver nanoparticles using the fungal endophyte Penicillium

oxalicum and their antimicrobial, antioxidant, and in vitro anticancer potential. ACS omega, vol 7, p 46653
Gupta P, Verma A, Rai N, Singh AK, Singh SK, Kumar B, Kumar R, Gautam V (2021) Mass spectrometry-based technology and workflows for studying the chemistry of fungal endophyte derived bioactive compounds. ACS Chem Biol 16(11):2068–2086
Han Z, Mei W, Zhao Y, Deng Y, Dai H (2009) A new cytotoxic isocoumarin from endophytic fungus Penicillium sp. 091402 of the mangrove plant Bruguiera sexangula. Chem Nat Compd 45:805–807
Hemphill CFP, Daletos G, Liu Z, Lin W, Proksch P (2016) Polyketides from the mangrove-derived fungal endophyte Pestalotiopsis clavispora. Tetrahedron Letters 57(19):2078–2083
Howard DH, Bach PB, Berndt ER, Conti RM (2015) Pricing in the market for anticancer drugs. J Econ Perspect 29(1):139–162
Huang CH, Pan JH, Chen B, Yu M, Huang HB, Zhu X, Lu YJ, She ZG, Lin YC (2011a) Three bianthraquinone derivatives from the mangrove endophytic fungus Alternaria sp. ZJ9-6B from the South China Sea. Mar Drugs 9(5):832–843
Huang HB, Xiao ZE, Feng XJ, Huang CH, Zhu X, Ju JH, Li MF, Lin YC, Liu L, She ZG (2011b) Cytotoxic naphtho-γ-pyrones from the mangrove endophytic fungus Aspergillus tubingensis (GX1-5E). Helv Chim Acta 94(9):1732–1740
Huang Z, Yang J, Cai X, She Z, Lin Y (2012) A new furanocoumarin from the mangrove endophytic fungus Penicillium sp.(ZH16). Nat Prod Res 26(14):1291–1295
Jennewein S, Rithner CD, Williams RM, Croteau RB (2001) Taxol biosynthesis: taxane 13α-hydroxylase is a cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci 98(24):13595–13600
Kala CP (2000) Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. Biol Conserv 93(3):371–379
Keshri PK, Rai N, Verma A, Kamble SC, Barik S, Mishra P, Singh SK, Salvi P, Gautam V (2021) Biological potential of bioactive metabolites derived from fungal endophytes associated with medicinal plants. Mycol Prog 20(5):577–594
Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28(7):1208–1228
Kharwar RN, Verma VC, Strobel G, Ezra D (2008) The endophytic fungal complex of Catharanthus roseus (L.) G. Don. Curr Sci:228–233
Knudson AG (2001) Two genetic hits (more or less) to cancer. Nat Rev Cancer 1(2):157–162
Kour A, Shawl AS, Rehman S, Sultan P, Qazi PH, Suden P, Khajuria RK, Verma V (2008) Isolation and identification of an endophytic strain of Fusarium oxysporum producing podophyllotoxin from Juniperus recurva. World J Microbiol Biotechnol 24:1115–1121
Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007) Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol 174(3):648–657
Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798
Lau MK, Arnold AE, Johnson NC (2013) Factors influencing communities of foliar fungal endophytes in riparian woody plants. Fungal Ecol 6(5):365–378
Li DL, Li XM, Li TG, Dang HY, Wang BG (2008) Dioxopiperazine alkaloids produced by the marine mangrove derived endophytic fungus Eurotium rubrum. Helv Chim Acta 91(10):1888–1893
Li F, Jiang T, Li Q, Ling X (2017) Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer? Am J Cancer Res 7(12):2350
Li H, Huang H, Shao C, Huang H, Jiang J, Zhu X, Liu Y, Liu L, Lu Y, Li M, Lin Y (2011) Cytotoxic norsesquiterpene peroxides from the endophytic fungus Talaromyces flavus isolated from the mangrove plant Sonneratia apetala. J Nat Prod 74(5):1230–1235
Li X, Tian Y, Yang SX, Zhang YM, Qin JC (2013) Cytotoxic azaphilone alkaloids from Chaetomium globosum TY1. Bioorg Med Chem Lett 23(10):2945–2947

Li YC, Tao WY, Cheng L (2009) Paclitaxel production using co-culture of Taxus suspension cells and paclitaxel-producing endophytic fungi in a co-bioreactor. Appl Microbiol Biotechnol 83:233–239

Lin T, Lin X, Lu CH, Shen YM (2011) Three new triterpenes from Xylarialean sp. A45, an endophytic fungus from Annona squamosa L. Helv Chim Acta 94(2):301–305

Liu D, Li XM, Meng L, Li CS, Gao SS, Shang Z, Proksch P, Huang CG, Wang BG (2011) Nigerapyrones A–H, α-pyrone derivatives from the marine mangrove-derived endophytic fungus Aspergillus niger MA-132. J Nat Prod 74(8):1787–1791

Liu S, Guo L, Che Y, Liu L (2013) Pestaloficiols Q–S from the plant endophytic fungus Pestalotiopsis fici. Fitoterapia 85:114–118

Lu S, Sun P, Li T, Kurtán T, Mándi A, Antus S, Krohn K, Draeger S, Schulz B, Yi Y, Li L (2011) Bioactive nonanolide derivatives isolated from the endophytic fungus Cytospora sp. J Org Chem 76(23):9699–9710

Luo J, Liu X, Li E, Guo L, Che Y (2013) Arundinols A–C and arundinones A and B from the plant endophytic fungus Microsphaeropsis arundinis. J Nat Prod 76(1):107–112

Mani VM, Kalaivani S, Sabarathinam S, Vasuki M, Soundari AJPG, Das MA, Elfasakhany A, Pugazhendhi A (2021) Copper oxide nanoparticles synthesized from an endophytic fungus Aspergillus terreus: bioactivity and anticancer evaluations. Environ Res 201:111502

Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ (2007) A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science 315(5811):513–515

Mbaveng AT, Kuete V, Mapunya BM, Beng VP, Nkengfack AE, Meyer JJM, Lall N (2011) Evaluation of four Cameroonian medicinal plants for anticancer, antigonorrheal and antireverse transcriptase activities. Environ Toxicol Pharmacol 32(2):162–167

Moussa AY, Mostafa NM, Singab ANB (2020) Pulchranin A: first report of isolation from an endophytic fungus and its inhibitory activity on cyclin dependent kinases. Nat Prod Res 34(19):2715–2722

Nadeem M, Ram M, Alam P, Ahmad MM, Mohammad A, Al-Qurainy F, Khan S, Abdin MZ (2012) Fusarium solani, P1, a new endophytic podophyllotoxin-producing fungus from roots of Podophyllum hexandrum. Afr J Microbiol Res 6(10):2493–2499

Netala VR, Bethu MS, Pushpalatha B, Baki VB, Aishwarya S, Rao JV, Tartte V (2016) Biogenesis of silver nanoparticles using endophytic fungus Pestalotiopsis microspora and evaluation of their antioxidant and anticancer activities. Int J Nanomedicine 11:5683

Nizam S, Qiang X, Wawra S, Nostadt R, Getzke F, Schwanke F ... Zuccaro A (2019) Serendipita indica E5′ NT modulates extracellular nucleotide levels in the plant apoplast and affects fungal colonization. EMBO Reports 20(2):e47430

Nygren P, Larsson R (2003) Overview of the clinical efficacy of investigational anticancer drugs. J Intern Med 253(1):46–75

Okami Y (1986) Marine microorganisms as a source of bioactive agents. Microb Ecol 12:65–78

Ortega HE, Graupner PR, Asai Y, TenDyke K, Qiu D, Shen YY, Rios N, Arnold AE, Coley PD, Kursar TA, Gerwick WH (2013) Mycoleptodiscins A and B, cytotoxic alkaloids from the endophytic fungus Mycoleptodiscus sp. F0194. J Nat Prod 76(4):741–744

Passari AK, Mishra VK, Singh G, Singh P, Kumar B, Gupta VK, Sharma RK, Saikia R, Donovan AO, Singh BP (2017) Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. Sci Rep 7:11809. https://doi.org/10.1038/s41598-017-12235-4

Passari AK, Upadhyaya K, Singh G, Abdel-Azeem AM, Thankappan S, Uthandi S et al (2019) Enhancement of disease resistance, growth potential, and photosynthesis in tomato (*Solanum lycopersicum*) by inoculation with an endophytic actinobacterium, *Streptomyces thermocarboxydus* strain BPSAC147. PLoS One 14(7):e0219014. https://doi.org/10.1371/journal.pone.0219014

Peñalva MA, Rowlands RT, Turner G (1998) The optimization of penicillin biosynthesis in fungi. Trends Biotechnol 16(11):483–489

Petrini O, Fisher PJ (1990) Occurrence of fungal endophytes in twigs of Salix fragilis and Quercus robur. Mycol Res 94(8):1077–1080

Priti V, Ramesha BT, Singh S, Ravikanth G, Ganeshaiah KN, Suryanarayanan TS, Uma Shaanker R (2009) How promising are endophytic fungi as alternative sources of plant secondary metabolites? Curr Sci 97(4):477–478

Rai N, Gupta P, Keshri PK, Verma A, Mishra P, Kumar D, Kumar A, Singh SK, Gautam V (2022a) Fungal endophytes: an accessible source of bioactive compounds with potential anticancer activity. Appl Biochem Biotechnol 194(7):3296–3319

Rai N, Gupta P, Verma A, Singh SK, Gautam V (2023a) Isolation and characterization of N-(2-hydroxyethyl) hexadecanamide from Colletotrichum gloeosporioides with apoptosis-inducing potential in breast cancer cells. Biofactors 49:663

Rai N, Gupta P, Verma A, Tiwari RK, Madhukar P, Kamble SC, Kumar A, Kumar R, Singh SK, Gautam V (2023b) Ethyl acetate extract of Colletotrichum gloeosporioides promotes cytotoxicity and apoptosis in human breast cancer cells, vol 8. ACS Omega, p 3768

Rai N, Keshri PK, Gupta P, Verma A, Kamble SC, Singh SK, Gautam V (2022b) Bioprospecting of fungal endophytes from Oroxylum indicum (L.) Kurz with antioxidant and cytotoxic activity. PLoS One 17(3):e0264673

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S, Kumar Singh S, Gautam V (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology 12(3):139–159

Ran X, Zhang G, Li S, Wang J (2017) Characterization and antitumor activity of camptothecin from endophytic fungus Fusarium solani isolated from Camptotheca acuminate. Afr Health Sci 17(2):566–574

Ranjan A, Singh RK, Khare S, Tripathi R, Pandey RK, Singh AK, Gautam V, Tripathi JS, Singh SK (2019) Characterization and evaluation of mycosterol secreted from endophytic strain of Gymnema sylvestre for inhibition of α-glucosidase activity. Sci Rep 9(1):17302

Rodriguez RJ, White JF Jr, Arnold AE, Redman ARA (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330

Roopa G, Madhusudhan MC, Sunil KCR, Lisa N, Calvin R, Poornima R, Zeinab N, Kini KR, Prakash HS, Geetha N (2015) Identification of Taxol-producing endophytic fungi isolated from Salacia oblonga through genomic mining approach. J Genet Eng Biotechnol 13(2):119–127

Sana T, Siddiqui BS, Shahzad S, Farooq AD, Siddiqui F, Sattar S, Begum S (2019) Antiproliferative activity and characterization of metabolites of Aspergillus nidulans: an endophytic fungus from Nyctanthes arbor-tristis Linn. against three human cancer cell lines. Med Chem 15(4):352–359

Saunders M, Kohn LM (2009) Evidence for alteration of fungal endophyte community assembly by host defense compounds. New Phytol 182(1):229–238

Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004

Shiono Y, Kikuchi M, Koseki T, Murayama T, Kwon E, Aburai N, Kimura KI (2011) Isopimarane diterpene glycosides, isolated from endophytic fungus Paraconiothyrium sp. MY-42. Phytochemistry 72(11–12):1400–1405

Shu-Chun LIU, Xin YE, Liang-Dong GUO, Ling LIU (2011) Cytotoxic isoprenylated epoxycyclohexanediols from the plant endophyte Pestalotiopsis fici. Chin J Nat Med 9(5):374–379

Shweta S, Gurumurthy BR, Ravikanth G, Ramanan US, Shivanna MB (2013) Endophytic fungi from Miquelia dentata Bedd., produce the anticancer alkaloid, camptothecine. Phytomedicine 20(3–4):337–342

Shweta S, Zuehlke S, Ramesha BT, Priti V, Kumar PM, Ravikanth G, Spiteller M, Vasudeva R, Shaanker RU (2010) Endophytic fungal strains of Fusarium solani, from Apodytes dimidiata E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71(1):117–122

Singh AK, Singh SS, Rathore AS, Singh SP, Mishra G, Awasthi R, Mishra SK, Gautam V, Singh SK (2021) Lipid-coated MCM-41 mesoporous silica nanoparticles loaded with berberine improved inhibition of acetylcholine esterase and amyloid formation. ACS Biomater Sci Eng 7(8):3737–3753

Singh RK, Ranjan A, Srivastava AK, Singh M, Shukla AK, Atri N, Mishra A, Singh AK, Singh SK (2020) Cytotoxic and apoptotic inducing activity of Amoora rohituka leaf extracts in human breast cancer cells. J Ayurveda Integr Med 11(4):383–390

Singh SK, Pandey VD, Singh A, Singh C (2009) Antibacterial activity of seed extracts of Argemone mexicana L. on some pathogenic bacterial strains. Afr J Biotechnol 8(24)

Sommart U, Rukachaisirikul V, Trisuwan K, Tadpetch K, Phongpaichit S, Preedanon S, Sakayaroj J (2012) Tricycloalternarene derivatives from the endophytic fungus Guignardia bidwellii PSU-G11. Phytochem Lett 5(1):139–143

Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SP (2005) Plant-based anticancer molecules: a chemical and biological profile of some important leads. Bioorg Med Chem 13(21):5892–5908

Stahlhut R, Park G, Petersen R, Ma W, Hylands P (1999) The occurrence of the anticancer diterpene taxol in Podocarpus gracilior Pilger (Podocarpaceae). Biochem Syst Ecol 27(6):613–622

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67(2):257–268

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249

Surai PF (2015) Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants 4(1):204–247

Swamy MK, Akhtar MS, Sinniah UR (2016) Response of PGPR and AM fungi toward growth and secondary metabolite production in medicinal and aromatic plants. In: Plant, soil and microbes: volume 2: mechanisms and molecular interactions. Springer, Cham, pp 145–168

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18(4):448–459

Teiten MH, Mack F, Debbab A, Aly AH, Dicato M, Proksch P, Diederich M (2013) Anticancer effect of altersolanol A, a metabolite produced by the endophytic fungus Stemphylium globuliferum, mediated by its pro-apoptotic and anti-invasive potential via the inhibition of NF-κB activity. Bioorg Med Chem 21(13):3850–3858

Tejesvi MV, Nalini MS, Mahesh B, Prakash HS, Kini KR, Shetty HS, Subbiah V (2007) New hopes from endophytic fungal secondary metabolites. Bol Soc Quím Méx 1(1):19–26

Tysnes BB, Bjerkvig R (2007) Cancer initiation and progression: involvement of stem cells and the microenvironment. Biochim Biophys Acta 1775(2):283–297

Verma A, Gupta P, Rai N, Tiwari RK, Kumar A, Salvi P, Kamble SC, Singh SK, Gautam V (2022) Assessment of biological activities of fungal endophytes derived bioactive compounds isolated from Amoora rohituka. J Fungi 8(3):285

Verma A, Rai N, Kamble SC, Mishra P, Barik S, Kumar R, Singh SK, Salvi P, Gautam V (2023) Beyond the synthetic drugs: fungal endophytes derived bioactive compounds in the management of neurodegenerative disorders. In: Traditional medicine for neuronal health. Bentham Science Publishers Pte. Ltd., Singapore, p 82

Verma VC, Lobkovsky E, Gange AC, Singh SK, Prakash S (2011a) Piperine production by endophytic fungus Periconia sp. isolated from Piper longum L. J Antibiot 64(6):427–431

Verma VC, Singh SK, Prakash S (2011b) Bio-control and plant growth promotion potential of siderophore producing endophytic Streptomyces from Azadirachta indica A. Juss. J Basic Microbiol 51(5):550–556

Verza M, Arakawa NS, Lopes NP, Kato MJ, Pupo MT, Said S, Carvalho I (2009) Biotransformation of a tetrahydrofuran lignan by the endophytic fungus Phomopsis Sp. J Braz Chem Soc 20:195–200

Wang FW, Ye YH, Ding H, Chen YX, Tan RX, Song YC (2010) Benzophenones from Guignardia sp. IFB-E028, an endophyte on Hopea hainanensis. Chem Biodivers 7(1):216–220

Wang QX, Li SF, Zhao F, Dai HQ, Bao L, Ding R, Gao H, Zhang LX, Wen HA, Liu HW (2011b) Chemical constituents from endophytic fungus Fusarium oxysporum. Fitoterapia 82(5):777–781

Wang XN, Bashyal BP, Wijeratne EK, U'Ren JM, Liu MX, Gunatilaka MK, Arnold AE, Gunatilaka AL (2011a) Smardaesidins A–G, isopimarane and 20-nor-isopimarane diterpe-

noids from Smardaea sp., a fungal endophyte of the moss Ceratodon purpureus. J Nat Prod 74(10):2052–2061
Wang Y, Dai CC (2011) Endophytes: a potential resource for biosynthesis, biotransformation, and biodegradation. Ann Microbiol 61(2):207–215
Wang Y, Xu L, Ren W, Zhao D, Zhu Y, Wu X (2012) Bioactive metabolites from Chaetomium globosum L18, an endophytic fungus in the medicinal plant Curcuma wenyujin. Phytomedicine 19(3–4):364–368
Weinberg RA (1996) How cancer arises. Sci Am 275(3):62–70
Wiseman H, Kaur H, Halliwell B (1995) DNA damage and cancer: measurement and mechanism. Cancer Lett 93(1):113–120
Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N (2007) The genomic landscapes of human breast and colorectal cancers. Science 318(5853):1108–1113
World Health Organization (2018) Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. International agency for research on cancer. World Health Organization, Geneva, pp 1–4
Wu L, Han T, Li W, Jia M, Xue L, Rahman K, Qin L (2013) Geographic and tissue influences on endophytic fungal communities of Taxus chinensis var. mairei in China. Curr Microbiol 66:40–48
Wu ZC, Li DL, Chen YC, Zhang WM (2010) A new isofuranonaphthalenone and benzopyrans from the endophytic fungus *Nodulisporium* sp. A4 from Aquilaria sinensis. Helv Chim Acta 93(5):920–924
Xiao J, Lin LB, Hu JY, Duan DZ, Shi W, Zhang Q, Han WB, Wang L, Wang XL (2018) Pestalustaines A and B, unprecedented sesquiterpene and coumarin derivatives from endophytic fungus Pestalotiopsis adusta. Tetrahedron Lett 59(18):1772–1775
Yan HJ, Li XM, Li CS, Wang BG (2012) Alkaloid and anthraquinone derivatives produced by the marine-derived endophytic fungus Eurotium rubrum. Helv Chim Acta 95(1):163–168
Yuan G, Hong K, Lin H, She Z, Li J (2013) New azalomycin F analogs from mangrove Streptomyces sp. 211726 with activity against microbes and cancer cells. Mar Drugs 11(3):817–829
Zhang HC, Liu JM, Lu HY, Gao SL (2009) Enhanced flavonoid production in hairy root cultures of *Glycyrrhiza uralensis* Fisch by combining the over-expression of chalcone isomerase gene with the elicitation treatment. Plant Cell Rep 28:1205–1213
Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771
Zhao J, Li C, Wang W, Zhao C, Luo M, Mu F, Fu Y, Zu Y, Yao M (2013) *Hypocrea lixii*, novel endophytic fungi producing anticancer agent cajanol, isolated from pigeon pea (Cajanus cajan [L.] Millsp.). J Appl Microbiol 115(1):102–113
Zikmundova M, Drandarov K, Bigler L, Hesse M, Werner C (2002) Biotransformation of 2-benzoxazolinone and 2-hydroxy-1, 4-benzoxazin-3-one by endophytic fungi isolated from *Aphelandra tetragona*. Appl Environ Microbiol 68(10):4863–4870

Chapter 3
Fungal Endophytes as an Alternative Natural Resource for the Discovery of Bioactive Compounds of Pharmacological Importance

Jyoti Lakshmi Hati Boruah, Deepjyoti Das, Parishmita Gogoi, Niraj Kumar, Babli Borah, Maini Borah, Pooja Talukdar, Cecilia Lulu, Priyanka Gogoi, Archana Yadav, Upasana Goswami, Bhim P. Singh, and Ratul Saikia

Abstract Fungal endophytes are microorganisms that live in symbiotic relationships with the host plant for at least a part of their life cycle. One or more fungal endophytes are present in every plant across the globe. Fungal endophytes have emerged as a potential natural reservoir for bioactive secondary metabolites to meet the never-ending need for effective treatments. Secondary metabolites are divided into a number of classes, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones, xanthones, etc. Endophytic fungi such as *Alternaria* sp., *Aspergillus* sp., *Bipolaris* sp., *Cephalosporium* sp., *Chaetomium* sp., *Colletotrichum* sp., *Emericella* sp., *Fusarium* sp., *Guignardia* sp., *Hormonema* sp., *Metarhizium* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp., *Phomopsis* sp., *Talaromyces* sp., *Taxomyces* sp., *Tolypocladium* sp., *Xylaria* sp., etc. have been reported to possess potential bioactive compounds. Thus, they are potential sources of several natural drugs available

J. L. H. Boruah · B. Borah · M. Borah · P. Talukdar · A. Yadav · U. Goswami
Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

D. Das
Natural Product Chemistry Group, Chemical Sciences and Technology Division, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

P. Gogoi · N. Kumar · C. Lulu · P. Gogoi · R. Saikia (✉)
Biological Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India
e-mail: ratulsaikia@neist.res.in

B. P. Singh
Department of Agriculture & Environmental Sciences, National Institute of Food Technology, Entrepreneurship & Management (NIFTEM), Sonipat, Haryana, India

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in the market for antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, and antidiabetic agents. This chapter summarizes about various fungal endophytes, their association with plants, and the pharmaceutical application of their secondary metabolites.

Keywords Fungal endophytes · Bioactive compounds · Metabolites · Natural products · Therapeutic agents

1 Introduction

Plants have long been used as a source of therapeutic bioactive substances for treating a wide range of diseases. Ironically, rather than actual plants, microbes connected to plants have been shown to give materials and products with tremendous medicinal potential. Native microorganisms, referred to as endophytes, can survive inside the tissue of plants without causing any obvious exterior symptoms and are in charge of processing nutrients, inducing defense mechanisms, and synthesizing secondary metabolites. Endophytes are a kind of endosymbiotic microbes that colonize the intercellular and/or intracellular spaces of plants and are widely known for facilitating host growth and nutrient absorption. The endophytic fungi provide their host plant significant resistance to numerous pathogenic, parasitic, and pest threats by employing secondary metabolites produced by them under normal and stress conditions. The focus of recent biotechnological advancements is on discovering and using new bioactive substances that can be derived from endophytic fungi. Despite this, only a small percentage of endophytic fungi have been isolated and studied for their biological activity (Gouda et al. 2016; Pandey et al. 2014; Uzma et al. 2018; Manganyi and Ateba 2020; Abdalla et al. 2020). This chapter emphasizes the numerous sources of endophytes, their secondary metabolites, and their potential as antibacterial, antifungal, antiviral, antioxidant, anticancer, and antidiabetic medications. The current quest of many researchers to identify distinct endophytes from intriguing and unexpected hosts and settings is well captured in these illustrations.

2 Plant-Fungal Endophytes Associations

Most interactions between endophytic fungus and plants can be divided into neutral, beneficial, and destructive. While different kinds of associations exist in ecological positions (Schulz and Boyle 2005), certain fungal endophytes cooperate, but most endophytes are commensals (Brader et al. 2014). Based on the length of the endophytic existence period, fungal endophytes are divided into systemic and nonsystemic endophytes (Mostert et al. 2000; Wani et al. 2015). Long-term or temporary relationships with the plants are possible for fungus endophytes. The population

size and species richness of nonsystemic endophytes can change over time depending on host variables such as plant growth and development, environmental enrichment, and biotic factors, such as pathogen attack. The nonsystemic endophytes, however, may change from mutualism to parasitism and become specifically damaging to the host.

Conversely, systemic endophytes are mutualists and have no adverse effects on plants. As a result, endophytes can be passed from one host to another, albeit some colonize their hosts and become an integral part of the plant endobiome (Mostert et al. 2000). The taxonomy, transmission route, endophytic life, host defense response, ecological role, and diversity are the bases for this classification of endophytic fungus (Wani et al. 2015).

3 Bioactive Metabolites from Endophytic Fungi

Endophytes have historically received less attention, but more recently, their potential has come to light due to their positive impact on the production of pharmacologically significant bioactive metabolites that contain antimicrobial, antiviral, natural antioxidant, antitumor, immunosuppressant, antidiabetic, and insecticidal agents (Gouda et al. 2016; Yadav et al. 2014; Fadiji and Babalola 2020). The discovery of Taxol in 1993 from an endophytic fungus in *Taxomyces andreanae* rekindled interest in medicinal substances derived from endophytic sources (Stierle et al. 1993). Researchers have discovered a variety of endophytic fungi that may contain a wide range of bioactive compounds that are beneficial to human health. Additionally, studies have demonstrated that endophytes can create, induce, and alter the host's molecular structures. Numerous bioactive secondary metabolites with distinctive structural characteristics, such as flavonoids, terpenoids, phenolic compounds, chinones, steroids, alkaloids, benzopyrenes, quinones, tetralones, and others, are now playing a significant role in scientific advancements in the pharmaceutical industry (Lugtenberg et al. 2016). *Tolypocladium inflatum*, an endophytic fungus, was found to produce the highly beneficial immunosuppressant cyclosporine (Borel et al. 1991). Endophytic fungi from *Muscodor albus*, a species of tropical tree, produces several volatile organic compounds, including the antibiotic-rich aciphyllene, 2-butanone, and 2-methyl furan (Atmosukarto et al. 2005). A list of the bioactive substances produced by endophytes is mentioned in Table 3.1.

4 Biological Properties of Endophytic Fungi

Endophytic fungus has been reported to demonstrate a variety of biological activities, including antibacterial, antifungal, antiviral, antioxidant, anticancer, and antidiabetic properties due to their abundance of bioactive chemicals. In this section, we look at endophytic fungus as a potential source of a wide range of bioactive substances that may benefit human health.

Table 3.1 List of some bioactive compounds obtained from fungal endophytes possessing biological activities

Endophytic fungus	Host	Bioactive compounds	Biological activities	References
Phomopsis sp.	*Plumeria acutifolia*	Terpenoid	Antibacterial activity	Nithya and Muthumary (2010)
Penicillium janthinellum	*Melia azedarach*	Polyketide citrinin	Antibacterial activity	Kumar et al. (2017)
Xylaria sp.	*Garcinia dulcis*	Sordaricin	Antifungal activity against *Candida albicans*	Digra and Nonzom (2023)
Tolypocladium inflatum	–	Cyclosporine	Immunosuppressant	Yang et al. (2018)
Aspergillus clavatonanicus	*Torreya maire*	Clavatol	Antimicrobial activity	Zhang et al. (2008)
Strain QJ18	*Gentiana macrophylla*	Secoiridoids mainly gentiopicrin	Choleretic, anti-hepatotoxic, anti-inflammatory, antifungal, and antihistamine activities	Yin et al. (2009)
Fusarium oxysporum	*Ginkgo biloba*	Gingkolides	Anti-inflammatory	Cui et al. (2012)
Metarhizium anisopliae, *Taxomyces andreanae*	*Taxus baccata*	Taxol (paclitaxel)	Anti-cancerous	Das et al. (2022)
Neurospora sp.	*Nothapodytes foetida*	Alkaloid (camptothecin)	Anticancer, anti-HIV, antimalarial, antibacterial, antioxidant, anti-inflammatory, antifungal, and anemia	Durga et al. (2020) and Rehman et al. (2008)
Muscodor albus	*Cinnamomum zeylanicum*	Volatile organic compounds, such as aciphyllene, 2-butanone, and 2-methyl furan	Antibacterial, antifungal compounds	Atmosukarto et al. (2005)
Alternaria tenuissima QUE1Se	*Quercus emoryi*	Altertoxins	Anti-HIV activity	Bashyal et al. (2014)
2L-5	*Ocimum basilicum*	Ergosterol, cerevesterol	Antimicrobial	Haque et al. (2005)
Emericella sp.	*Aegiceras corniculatum*	Aegiceras corniculatum	Antiviral	Zhang et al. (2011)
Serratia marcescens	*Rhyncholacis penicillata*	Oocydin A	Antifungal	Strobel et al. (2003)

4.1 Antibacterial Activity

The quest for new nonresistant antibiotics has persisted for scientists, professionals, and clinical specialists. This has become a topic of concern during the recent SARS-CoV2 pandemic. In the history of medicine, the secondary metabolites from endophytic fungi have played an essential role, such as the discovery of penicillin, an antimicrobial metabolite from *Penicillium chrysogenum* that was used against microbial infections and saved millions of lives (Manganyi and Ateba 2020). According to earlier reviews, investigating antimicrobial bioactive compounds from less explored microbial systems paves a new pathway to treat multidrug-resistant contagious pathogens (Deshmukh et al. 2015; Jakubczyk and Dussart 2020). Endophytic fungi from various sources have been shown to exhibit potent inhibition against pathogenic bacterial strains, drug-resistant pathogens, in combating antibiotic inefficiency against bacterial organisms. Compared to conventional antibacterials, metabolites of endophytic fungi are more efficient, less expensive, and have a lower chance of bacterial resistance. Using bioactive compounds of fungal endophytes as the source for industrial production of antibacterials will not only be economically viable for pharmaceutical industries but would also help in the reduction of bacterial diseases in public health systems at a much lower cost. The bioactive metabolites produced by endophytic fungi responsible for the activity profiles include flavonoids, alkaloids, peptides, terpenoids, phenols, xanthones, quinines, etc. Table 3.2 provides a list of antibacterial chemicals from diverse classes of endophytic fungi.

4.2 Antifungal Activity

Fungi infections are frequently overlooked, despite the fact that they might cause subsequent illnesses. Due to their opportunistic nature and potential impact on immunocompromised people, pathogenic fungi significantly burden the world's existing healthcare systems (Giacomazzi et al. 2016). Endophytic fungi are a potential source for producing various bioactive compounds with significant antifungal activities. Triterpene glycoside has been reported to show promising antifungal inhibitory activity against *Candida* and *Aspergillus* sp. in mice model (Peláez et al. 2000). Additionally, *Streptomyces* spp. TQR12–4 isolated from elite *Citrus nobilis* fruit was found to have broad-spectrum antimicrobial activity against fungal organisms, including *Colletotrichum truncatum*, *Geotrichum candidum*, *F. oxysporum*, and *F. udum* (Singh et al. 2017). Numerous antifungal properties are found in the bioactive substances made by *Xylaria* sp. These compounds include multiplolides A and B and sordaricin. Sordaricin (Pongcharoen et al. 2008) and multiplolides A and B (Boonphong et al. 2001) have antifungal efficacy against *Candida albicans*. The griseofulvin produced by the *Xylaria* sp. F0010, a spirobenzofuran antibiotic, is isolated from *Abies holophylla* (Macías-Rubalcava and Sánchez-Fernández 2017).

Table 3.2 List of some antibacterial compounds obtained from fungal endophytes

Class	Endophytic fungus	Source	Compounds isolated	Biological target	Reference
Ascomycetes	*Diaporthe* sp. GDG-118	*Sophora tonkinensis*	21-Acetoxycytochalasin J3	*B. anthracis*, *E. coli*	Huang et al. (2021)
	Xylaria sp. (GDG-102)	Leaves of *S. tonkinensis*	6-Heptanoyl-4-methoxy-2Hpyran-2-one	*E. coli*, *S. aureus*	Zheng et al. (2018)
	Chaetomium sp. HQ-1	*Astragalus chinensis*	Differanisole A	*L. monocytogenes S. aureus*, MRSA	Liu et al. (2019a)
	Talaromyces purpureogenus XL-25	*Panax notoginseng*	Talaroconvolutin A	*B. subtilis*, *Micrococcus lysodeikticus*, *Vibrio parahaemolyticus*	Feng et al. (2020)
Anamorphic ascomycetes	*Aspergillus flavus*	*Cephalotaxus fortunei*	5-hydroxymethylfuran-3-carboxylic acid, 5-acetoxymethylfuran-3- carboxylic acid	*S. aureus*	Ma et al. (2016)
	Penicillium ochrochloronthe	*Roots of Taxus media*	3,4,6-trisubstituted α-pyrone derivatives, namely, 6-(20R-hydroxy3 0E,50E-diene-10 -heptyl)-4-hydroxy-3-methyl-2H-pyran-2-one, 6-(20 Shydroxy-50E-ene-10-heptyl)-4- hydroxy-3-methyl-2H-pyran2-one, 6-(20S-hydroxy −10 -heptyl)-4 -hydroxy-3-methyl-2H-pyran-2-one, trichodermic acid	*E. coli*, *Enterobacter aerogenes*	Zhao et al. (2019)
	Fusarium sp. TP-G1	*Dendrobium officinale*	Trichosetin, beauvericin, beauvericin A, Enniatin H, enniatin I, enniatin MK1688	*S. aureus*, MRSA	Shi et al. (2018)

	Alternaria alternata ZHJG5	*Cercis chinensis*	Isotalaroflavone, 4-hydroxyalternariol-9-methyl ether, verrulactone A	*Xanthomonas oryzae pv. oryzae*, *Xanthomonas oryzae pv. oryzicola Ralstonia solanacearum* (Rs)	Zhao et al. (2021)
	Cladosporium sp.	*Rauwolfia serpentina*	Methyl ether of fusarubin	*S. aureus*, *E. coli*, *P. aeruginosa*, *B. megaterium*	Khan et al. (2016)
	Pestalotiopsis sp.	*Melaleuca quinquenervia*	(1S,3R)-austrocortirubin, (1S,3S)-austrocortirubin, 1-deoxyaustrocortirubin	Gram-positive bacteria	Beattie et al. (2016)
	Phoma sp. JS752	*Phragmites communis*	Barceloneic acid C	*Listeria monocytogenes*, *Staphylococcus pseudintermedius*	Xia et al. (2015)
	Colletotrichum gloeosporioides B12	*Illigera rhodantha*	Colletolides A and B, and 3-methylene isoindolinon	*Xanthomonas oryzae pv. oryzae*	Li et al. (2019)
	Bipolaris eleusines	Potato	(S)-5-Hydroxy-2-(1-hydroxyethyl)-7- methylchromone, 5,7-dihydroxyl-2,6,8- trimethylchromone	*Staphylococcus aureus* subsp. *aureus*	He et al. (2019)
Basidiomycete	*Psathyrella candolleana*	*Ginkgo biloba*	Quercetin, carboxybenzene, and nicotinamide	*S. aureus*	Pan et al. (2020)
Zygomycetes	*Mucor irregularis*	–	Chlorflavonin	–	Rehberg et al. (2018)

It has been used to treat both human- and animal-related mycotic illnesses (Reshma et al. 2019). The endophytic fungi *Chaetomium globosum* and *Phomopsis* species that live on *Ginkgo biloba* have been found to have compounds cytosporone B and C and chaetomugilin A and D that have antifungal effects on *Candida albicans* and *Fusarium oxysporum* (Kumar et al. 2017). It has also been shown that the endophytic fungus *Armillaria mellea* produces antimicrobial chemicals with strong bioactivity against Gram-positive bacterial and fungal infections (Singh et al. 2017). The antifungal activities of some specific endophytic fungi have been highlighted and presented in Table 3.3.

4.3 Antiviral Activity

Viral infections are considered a global health hazard due to their devastating effects (Lacerda et al. 2022). The current challenge is the development of new antiviral drugs that target the virus's mutation rate. The search, screening, and development of bioactive compounds with antiviral effects benefit drug research. The potential of the viruses to produce mutant-resistant strains against the antiviral is inevitable. An antiviral drug should inhibit the virus infection without cytotoxicity and cause minimal harm to the host cells. If the drug partially inhibits the infection, it leads to the development of drug-resistant mutant strains. Recently, broad-spectrum antivirals are being developed that target multiple viruses. The broad antiviral spectrum with similar structure may lead to the synthesis of potent drug derivatives against different viruses (Toghueo 2020). To address such problems, cost-effective and potent antiviral drugs or vaccines are being developed. Various reports have shown antiviral properties of endophyte-produced bioactive compounds such as pestalotheols A-D, a crude organic extract of *Pestalotiopsis theae* (LN560) which showed inhibitory effect on HIV-1 LAI replication in C8166 cells named (Li et al. 2008). Asperterphenyllins A, isolated from *Aspergillus candidus* LDJ-5, displayed antiviral efficacy against the H1N1 strain of the influenza A virus (Zhou et al. 2021). Another endophytic fungus *Curvularia papendorfii* isolated from *Vernonia amygdalina* is reported to be effective against the human coronavirus HCoV 229E and the feline coronavirus FCV F9. Table 3.4 provides a list of endophyte-produced bioactive compounds with antiviral activities.

4.4 Antioxidant Activity

Antioxidants have a plethora of benefits, from anti-inflammatory to disease elimination, antiaging to food enrichment. Antioxidants prevent oxidation, BY inhibiting reactive oxygen species (ROS), and free radicals from damaging cells. Naturally obtained antioxidants are gaining more recognition in pharmacology, food, and cosmetics. Antioxidants such as subglutinols A and B, which provide immunosuppressive

Table 3.3 List of some fungal endophytes possessing antifungal properties

Endophytic fungi	Host plant	Bioactive compound	Effects	Reference
Hormonema sp.	*Juniperus communis*	Triterpene glycoside	Active against *Candida* sp., *Aspergillus* sp., moderate efficacy against candidiasis	Peláez et al. (2000)
Xylaria sp.	–	Sordaricin, multiplolides A and B, 1, 8-dihydroxynaphthol 1-O-a-glucopyranoside, and mellisol	Effective against *Candida albicans*	Molina et al. (2012)
Xylaria sp. F0010	*Abies holophylla*	Griseofulvin	Active against both human and animal mycotic illnesses	Macías-Rubalcava and Sánchez-Fernández (2017) and Reshma et al. (2019)
Chaetomium globosum, *Phomopsis* sp.	*Ginkgo biloba*	Cytosporone B and C, chaetomugilin A and D	Effective against *Candida albicans* and *Fusarium oxysporum*	Kumar et al. (2017)
Pestalotiopsis adusta	–	Pestalachlorides A, pestalachlorides B	Active against *Verticillium albo-atrum*, *Gibberella zeae*, and *Fusarium culmorum*	Puri et al. (2018)
Penicillium citrinum, *Penicillium digitatum*	*Citrus limon*	Deoxytryptoquialanone, tryptoquialanine A, tryptoquialanine C, 15-dimethyl-2-epi-fumiquinazoline A, citrinadin A, ceoxycitrinadin A, and chrysogenamide A	Antifungal activity	Costa et al. (2019) and Huang et al. (2007)
Pestalotiopsis mangiferae	*Mangifera indica*	4-(2,4,7-trioxa-bicyclo [4.1.0] heptan-3-yl) phenol	Active against C. albicans (MIC, 0.039 g/mL), Nystatin (MIC 10.0 g/mL)	Subban et al. (2013)

Table 3.4 List of some antiviral compounds obtained from fungal endophytes

Endophytic fungus	Host	Bioactive compounds from endophytes	Antiviral properties	Reference
Aspergillus terreus	Soybean root	Aspergillide B1, 3α-Hydroxy-3, 5-dihydromonacolin L	SARS-COV2	El-Hawary et al. (2021)
Talaromyces purpureogenus	*Taxus baccata* Linn.	Polycyclic meroterpenoids, talaromyolides E- K	Anti-pseudorabies virus	Cao et al. (2020)
Pleospora tarda	*Ephedra aphylla*	Alternariol, alternariol-(a)-methyl ether	Against herpes simplex virus type-2 (HSV-2), vesicular stomatitis virus (VSV)	Selim et al. (2018)
Phoma sp.	*Aconitum vilmorinianum*	Phomanolide	H1N1 influenza virus	Liu et al. (2019a, b)
Alternaria alternate PGL-3	*Punica granatum*	Alternariol, Alternariol 9-methyl ether	Hepatitis C virus (HCV)	Abou El-kassem et al. (2019)
Guignardia mangiferae	*Carapa guianensis*	Crude extract	Yellow fever virus	Ferreira et al. (2015)
Alternaria alternata	*Sclerocarya birrea* and Hypoxis plants	Crude extract	Anti-HIV	Nzimande et al. (2022)
Aspergillus candidus	Mangrove	Asperterphenyllins A	Anti-influenza virus A (H1N1)	Zhou et al. (2021)
Daldinia eschscholtzii	*Musa paradisiaca*	Aureonitol	Anti-HIV	Chigozie et al. (2020)
Pestalotiopsis theae	–	Pestalotheol A-D	Anti-HIV	Li et al. (2008)
Paecilomyces sp. and *aspergillus clavatus*	*Taxus mairei* and *Torreya grandis*	Brefeldin-A	Anti-poliovirus	Cuconati et al. (1998) and Wang et al. (2002)
Alternaria sp.	*Sacrophyton* sp. soft coral	Tetrahydroaltersolanol C, alterporriol Q	Anti-porcine reproductive and respiratory syndrome virus	Zheng et al. (2012)

activity, are obtained from *Fusarium subglutinans* (Sadrati et al. 2013). The fungal endophytes, namely, *Chaetomium globosum*, *Penicillium* sp., *Nigrospora sphaerica*, *Aspergillus flavus*, *Nigrospora sphaerica*, *Acremonium* sp., *Aspergillus* sp., *Ulocladium chartarum*, *Pleospora tarda*, *Scopulariopsis* sp., *Chaetomium spirale*, *Mucor fuscus*, *Absidia corymbifera*, *Cochliobolus lunatus*, *Phoma leveillei*, *Fusarium* sp., and *Chaetomium globosum*, have been reported to be a promising strain for anti-oxidant along with *Pleospora tarda* to be affective for antiviral activity (Selim et al. 2018). Bioactive compounds secreted by endophytic fungi such as borneol,

corynesidones A and B, coumarin, 2,14-dihydroxy-7-drimen-12,11-olide, 5-(hydroxymethyl)-2-furanocarboxylic acid, isopestacin, lapachol, p-tyrosol, pestacin, phloroglucinol, tetrahydroxy-1-methylxanthone, tyrosol, and rutin have been reported to show antioxidant activity against inflammation and tumors (Toghueo 2020). This depicts that it is essential to continuously search for more novel bioactive compounds from endophytic fungi with effective antioxidant properties. Some bioactive compounds and host of endophytic fungi with antioxidant properties are listed in Table 3.5.

Table 3.5 List of bioactive compounds antioxidants from fungal endophytes

Bioactive compounds as antioxidants	Host	Fungi	Reference
Flavonoid, tannins	*Swietenia mahagoni* Jacq	*Colletotrichum* sp., *Rhizoctonia* sp.	Hastuti et al. (2022)
Phenolic compound	*Adiantum capillus-veneris*	*Chaetomium globosum*, *Penicillium* sp.	Selim et al. (2018)
Ascorbic acid	*Ocimum basilicum*, *Eugenia jambolana Tabebuia argentea*	*Aspergillus fumigatus*, Y. *Chaetomium* sp., *Aspergillus* sp., *Alternaria alternate*, *Aspergillus niger*	Sharaf et al. (2022), Yadav et al. (2014), and Sadananda et al. (2011)
Xyloketals B	*Avicennia marina*	*Xylaria* sp.	Zhou et al. (2018)
Anthraquinones	*Tabebuia argentea*,	*Alternaria alternata*, *Aspergillus niger*	Sadananda et al. (2011)
Pestacin (1,3-dihydroisobenzofuran), isopestacin (isobenzofuranone)	*Terminalia morobensis*	*Pestalotiopsis microspore*	Pandey et al. (2014) and Toghueo and Boyomet (2019)
β–carotene– linoleate	*Avicennia officinalis*, *Triticum durum*	*Aspergillus flavus*, Penicillium sp., Aspergillus sp.	Ravindran et al. (2012) and Sadrati et al. (2013)
Pyrogallol, α-tocospiro A	*Albizia zygia*	ZA161	Ibrahim et al. 2021
Graphislactone	*Trachelospermum jasminoides*	*Cephalosporium* sp.	Joseph and Priya (2011)

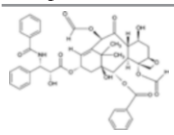
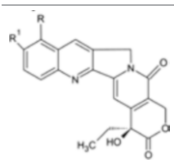
4.5 Anticancer Activity

Over the years, several endophytic fungi have been identified as a potential source of bioactive molecules with various pharmacological activities, including anticancerous properties. Taxol (paclitaxel) is a potent anticancer drug that was originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia*); however, an endophytic fungus known as *Taxomyces andreanae* that resides within the bark of *Taxus* species can also produce it (Wheeler et al. 1992). The discovery of Taxol from endophytic fungi has opened new possibilities for the sustainable production of this important anticancer drug. Camptothecin is another natural compound with potent anticancer properties that was first isolated from the endophytic fungus *Fusarium solani*, which lives within the bark of the Chinese Happy Tree (*Camptotheca acuminata*). While camptothecin has demonstrated significant anticancer activity in preclinical studies, its use as an anticancer drug has been limited due to its poor solubility, stability, and toxicity. Derivatives of camptothecin, such as topotecan and irinotecan, have been developed to overcome these limitations and have demonstrated improved efficacy and safety in clinical trials (Li et al. 2017). Cordycepin, a nucleoside analogue found in several different species of fungi, including endophytic fungi, is reported to exhibit various pharmacological activities, including antiviral, anti-inflammatory, and anticancer properties. Cordycepin was first isolated from the fungus *Cordyceps militaris*, a parasitic fungus that grows on the larvae of insects (Olatunji et al. 2018). Cordycepin has been the subject of numerous preclinical studies, and it has shown promise as a therapeutic agent in various cancer models (Panda et al. 2022). While griseofulvin, an isolate of the endophytic fungus *Penicillium griseofulvum*, is not typically used as an anticancer drug, it has been found to exhibit some anticancer activity in various preclinical studies (Saxena et al. 2019). Griseofulvin is a member of the class of compounds known as polyketides, known to have diverse pharmacological activities, including antibacterial, antifungal, antiviral, and anticancer properties (Nisa et al. 2015).

The discovery of new compounds from endophytic fungi, including those with anticancer properties, remains an active area of research. Podophyllotoxin is a naturally occurring lignan compound that has been found to exhibit anticancer activity in preclinical studies. It was first isolated from the roots of the American mandrake plant (*Podophyllum peltatum*), but it has also been found in endophytic fungi associated with the plant. Endophytic fungi such as *Alternaria alternata*, *Phoma* sp., and *Fusarium* sp. have been found to produce podophyllotoxin (Rana et al. 2020). The podophyllotoxin obtained from these fungi has been shown to have similar anticancer properties as the compound isolated from the *Podophyllum* plant. Podophyllotoxin is currently used clinically as a precursor to the development of several other anticancer drugs, including etoposide and teniposide (Cragg and Newman 2005). Fumonisin B1 which is a mycotoxin produced by various species of endophytic

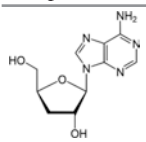
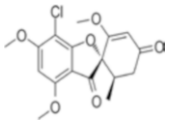
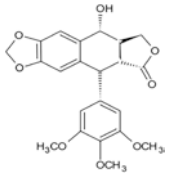
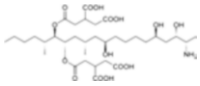
fungi, including *Fusarium moniliforme* and *Fusarium proliferatum* (Bacon and Nelson 1994), has been found to exhibit some potential anticancer activity in preclinical studies. However, it is important to note that fumonisin B1 is a toxic compound and can cause serious health problems if consumed in high amounts. Lovastatin first isolated from the fungus *Aspergillus terreus* is an endophytic fungus commonly found in soil (Goswami et al. 2013). Lovastatin is a statin drug widely used to lower cholesterol levels and reduce the risk of heart disease. However, it has also exhibited anticancer properties in various preclinical studies. In addition to its cholesterol-lowering effects, lovastatin has been found to inhibit the growth and proliferation of different cancer cell lines, including breast, prostate, and colon cancer cells (Tobert 2003). While lovastatin is not yet used clinically as an anticancer drug, its potential as a novel anticancer agent is an active area of research. The discovery of new compounds from endophytic fungi, including those with anticancer properties, holds great promise for developing new and effective cancer treatments. Anticancer compounds from different sources of endophytic fungi and their mode of action are presented in Table 3.6.

Table 3.6 Products of fungal endophytes with anticancer activities

Anticancer bioactive compound	Role	Endophytic fungus source	Host plant	Reference
 Taxol (paclitaxel)	Binds to microtubule assembly and prevents the separation of chromosomes during cell division (Schiff and Horwitz 1980). Efficacy against cancers, including ovarian, breast, and lung cancers. Taxane-based anticancer drugs have been developed, including docetaxel and cabazitaxel (Ojima et al. 2016).	*Fusarium redolens*	*Taxus wallichiana*	Garyali et al. (2013)
		Metarhizium anisopliae	*Taxus chinensis*	Liu et al. (2009)
		Taxomyces andreanae	*Taxus brevifolia*	Stierle et al. (1993)
		Bartalinia robillardoides Tassi	*Aegle marmelos*	Gangadevi and Muthumary (2008)
		Colletotrichum capsici	*Capsicum annuum*	Kumaran et al. (2011)
 Camptothecin	Inhibits DNA topoisomerase I enzyme leading to the accumulation of DNA damage and apoptosis (Kharwar et al. 2011).	*Nothapodytes foetida*	*Nothapodytes foetida*	Puri et al. (2005)
		Phomopsis spp.	*Miquelia dentata*	Shweta et al. (2013)
		Diaporthe sp.	*Nothapodytes nimmoniana*	Degambada et al. (2021)
		Fusarium solani ATLOY-8	*Chonemorpha fragrans*	Clarance et al. (2019)

(continued)

Table 3.6 (continued)

Anticancer bioactive compound	Role	Endophytic fungus source	Host plant	Reference
 Cordycepin	Inhibits cell proliferation, induce apoptosis, and inhibit angiogenesis (Panda et al. 2022).	*Cordyceps militaris*	*Tetheella fluctuosa*, *Ochropacha duplaris*, *Tethea ocularis*	Kryukov et al. (2011)
		Irpex lacteus	*Cordyceps hawkesii* Gray	Liu et al. (2022)
		Cordyceps ninchukispora	*Beilschmiedia erythrophloia* Hayata	Chang et al. (2017)
 Griseofulvin	Antioxidant induces cell cycle arrest and apoptosis (Nisa et al. 2015).	*Nigrospora oryzae*	*Emblica officinalis*	Rathod et al. (2014)
		Penicillium griseofulvum	*Mentha pulegium* L.	Zerroug et al. (2018)
		Xylaria sp.	*Abies holophylla*	Park et al. (2005)
 Podophyllotoxin	Inhibits the activity of an enzyme called topoisomerase II inhibiting cell division, or mitosis (Cragg and Newman 2005).	*Fusarium solani*	*Podophyllum hexandrum*	Nadeem et al. (2012)
		Phialocephala fortinii	*Podophyllum peltatum*	Eyberger et al. (2006)
		Trametes hirsuta	*Podophyllum hexandrum*	Puri et al. (2006)
 Fumonisin (Fumonisin B1)	Induces apoptosis and inhibit angiogenesis (Pandey et al. 2011).	*Fusarium moniliforme*	*Zea mays* L.	Jurrado et al. (2010)
		Fusarium verticillioides	*Zea mays* L.	Bezuidenhout et al. (1988)

4.6 Antidiabetic Properties

The prevalence of diabetes has increased recently all over the world. Diabetes occurs when the body develops insulin resistance or produces insufficient amounts of insulin, which disrupts glucose homeostasis and leads to serious complications like neuropathy, retinopathy, and nephropathy. According to the World Health Organization, the number of people with diabetes mellitus has increased dramatically over the past several decades. It is predicted to reach 629 million by 2045 (Agrawal et al. 2022). Various classes of drugs are available to treat type II diabetes. As these drugs are associated with certain side effects, conventional antidiabetic agents have limitations (Hussain et al. 2021), necessitating a continuous quest for novel antidiabetic agents from natural sources such as medicinal plants and fungal

endophytes. Several investigations have shown that endophytic fungal extracts exhibit antidiabetic activities and anti-lipidemic properties. Isolated from the African rainforest, *Pseudomassaria* species is reported to synthesize demethylasterriquinone B-1 (L-783,281) which have similar properties to insulin (Gupta et al. 2020). Another report on endophyte *Aspergillus awamori*, isolated from *Acacia nilotica*, has experimentally proven to synthesize biomolecules active against people with diabetes (Singh and Kaur 2016). Table 3.7 provides a list of endophyte-produced bioactive compounds with antidiabetic properties.

Table 3.7 List of fungal endophytes possessing antidiabetic properties

Endophytic fungus	Host	Bioactive compounds	Antidiabetic activity	Reference
Nigrospora oryzae	*Combretum dolichopetalum*	Fungal extract, (S)-(+)-2-cis-4-trans-abscisic acid, 7′-hydroxy-abscisic acid, and 4-des-hydroxyl altersolanol A	The extract and the compounds (1–3) significantly ($p < 0.001$) reduced the fasting blood sugar of the alloxan-induced diabetic mice.	Uzor et al. (2017)
Cladosporium sp.	*Loranthus micranthus*	Carbohydrates, tannin, phenolic glycosides, terpenoids, alkaloids, phenol, anthraquinones	The CsAgNPs reduced the activity of α-amylase, α-glucosidase and dipeptidyl peptidase IV in vitro. It showed significant glucose uptake in 3T3L1 cell line with inhibitory activity against AChE and BChE.	Popli et al. (2018)
Cladosporium uredinicola		Flavonoids, tannins, alkaloids, glycosides, phenols, terpenoids, coumarin, coumaric acid, hymecromone, alloisoimperatorin	The extract significantly reduced β-glucosidase and acetyl cholinesterase activity.	Govindappa et al. (2019)
Penicillium brevicaule alba ThomCC200	*Celosia cristata*	Saponins, Terpenoids, cardiac Glycosides	Fractions rich in triterpene saponins in an extract of *Penicillium brevicaule alba*. ThomCC200 showed the highest (76.5%) inhibition activity against α-amylase.	Nasmetova et al. (2020)

(continued)

Table 3.7 (continued)

Endophytic fungus	Host	Bioactive compounds	Antidiabetic activity	Reference
Aspergillus egypticus-HT166S	*Helianthus tuberosus*	Polymethoxylated flavones (PMF)	Purified K-10 PMF sample showed high inhibitory activity against pancreatic α-amylase (IC50 = 4.82 mg/ml) compared to total methanol extract (IC50 = 5.53 mg/ml), which was comparable to the activity of acarbose (IC50 = 4.74 mg/ml).	Ruzieva et al. (2021)
Curvularia lunata	*Ficus religiosa*	Flavonoids, alkaloids, phenols, terpenes, terpenoids, saponins, steroids	Ethyl acetate extract of *C. lunata* showed inhibition against α-amylase (IC50 = 0.35 ± 0.46 mg/ml) which was comparable to that of acarbose (0.31 ± 0.24 mg/ml).	Jayant and Vijayakumar (2021)
Trichoderma longibrachiatum isolate TL10 Aspergillus versicolor isolate BAB-6580	*Tinospora cordifolia*	Alkaloids, flavonoids, carbohydrates, tannins	Methanolic fractions of both the fungus, T1EA, T1nB, T2EA, T2nB potentially inhibited α-amylase, α-glucosidase, and aldose reductase enzyme(s.	Habbu et al. (2021)

5 Conclusion and Future Prospect

Medicinal plants and phytochemicals have an established history of their uses in traditional medicine. The ecological distributions, variability of the majority of these medicinal plants, and deterioration of plant material with time have been described as significant difficulties. Also, the uncontrolled use of medicinal and endangered plants to search for new bioactive molecules extensively adds to the environmental burden. These challenges have prompted researchers to look for new and alternative sources of bioactive molecules from environmentally friendly endophytic fungi. With widespread distribution and easy accessibility, fungal endophytes have emerged as a cost-effective and renewable source of bioactive molecules. Endophytic fungi produce a variety of secondary metabolites, such as alkaloids, terpenoids, steroids, quinones, phenols, and coumarins, among others, with a wide range of biological properties that could be investigated as prodrugs to lessen the significant financial load on public healthcare systems. Several studies have reported novel, beneficial bioactive compounds from fungal endophytes exhibiting biological properties, such as antibacterial, antidiabetic, antifungal, anti-inflammatory,

antiviral, anticancer activities, etc. Thus, fungal endophytes will be a potential and affordable natural source for drug development in the drug discovery process. Many endemic, endangered and medicinally valuable plants are on the verge of extinction, and so are the endophytes that harbor in them. To restore and sustain the pursuit of discovering fungal endophytes for pharmaceutical application, there is need to follow up this research with advanced biotechnological interventions, genome mining, metabolic engineering, and metagenomics which might help to induce and optimize secondary metabolites production from endophytic fungi under laboratory conditions in sustained manner. As endophytes reside in versatile interactions with host plants and other endophytes, future research on systematic studies on biochemical and genetic framework behind endophyte-endophyte and endophyte-host species cross talk is also essential in identifying new bioactive compounds from fungal endophytes. Thus, further research on production of bioactive metabolites from fungal endophytes through biotechnological tools and bioprospecting them as pharmaceutical entities may advance our knowledge but would also address sustained supply of bioactive prodrug against present and emerging diseases to ensure sustainable health.

References

Abou El-Kassem L, Hawas UW, El-Souda S, Ahmed EF, El-Khateeb W, Fayad W (2019) Anti-HCV protease potential of endophytic fungi and cytotoxic activity. Biocatal Agric Biotechnol 19:101170

Agrawal S, Samanta S, Deshmukh SK (2022) The antidiabetic potential of endophytic fungi: future prospects as therapeutic agents. Biotechnol Appl Biochem 69(3):1159–1165

Atmosukarto I, Castillo U, Hess WM, Sears J, Strobel G (2005) Isolation and characterization of *Muscodor albus* I-41.3 s, a volatile antibiotic producing fungus. Plant Sci 169(5):854–861

Abdalla MA, Aro AO, Gado D, Passari AK, Mishra VK, Singh BP, McGaw LJ (2020) Isolation of endophytic fungi from South African plants, and screening for their antimicrobial and extracellular enzymatic activities and presence of type I polyketide synthases. S Afr J Bot 134:336–342. https://doi.org/10.1016/j.sajb.2020.03.021

Bacon CW, Nelson PE (1994) Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum*. J Food Prot 57(6):514–521

Bashyal BP, Wellensiek BP, Ramakrishnan R, Faeth SH, Ahmad N, Gunatilaka AL (2014) Altertoxins with potent anti-HIV activity from *Alternaria tenuissima* QUE1Se, a fungal endophyte of *Quercus emoryi*. Bioorg Med Chem 22(21):6112–6116

Beattie KD, Ellwood N, Kumar R, Yang X, Healy PC, Choomuenwai V, Quinn RJ, Elliott AG, Huang JX, Chitty JL, Fraser JA (2016) Antibacterial and antifungal screening of natural products sourced from Australian fungi and characterisation of pestalactams D-F. Phytochemistry 124:79–85

Boongphong S, Isaka M, Pittayakhajonwut D, Tanticharoen M, Thebtaranonth Y (2001) Multiplolides A and B, new antifungal 10- membered lactones from *Xylaria* multiplex. J Nat Prod 64(7):965–967

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37

Cao X, Shi Y, Wu S, Wu X, Wang K, Sun H, He S, Dickschat JS, Wu B (2020) Polycyclic meroterpenoids, talaromyolides E− K for antiviral activity against pseudorabies virus from the endophytic fungus *Talaromyces purpureogenus*. Tetrahedron 76(30):131349

Chigozie V, Okezie M, Ajaegbu E, Okoye F, Esimone C (2020) Isolation, identification, and evaluation of biological activities of *Daldinia eschscholtzii*, an endophytic fungus isolated from the leaves of *Musa paradisiaca*. GSC Biological and Pharmaceutical Sciences 12(1):216–228

Costa JH, Wassano CI, Angolini CFF, Scherlach K, Hertweck C, Pacheco Fill T (2019) Antifungal potential of secondary metabolites involved in the interaction between citrus pathogens. Sci Rep 9(1):1–11

Cragg GM, Newman DJ (2005) Plants as a source of anticancer agents. J Ethnopharmacol 100(1–2):72–79

Cuconati A, Molla A, Wimmer E (1998) Brefeldin A inhibits cell-free, de novo synthesis of poliovirus. J Virol 72(8):6456–6464

Cui Y, Yi D, Bai X, Sun B, Zhao Y, Zhang Y (2012) Ginkgolide B produced endophytic fungus (*Fusarium oxysporum*) isolated from Ginkgo biloba. Fitoterapia 83(5):913–920

Das T, Dey A, Pandey DK, Panwar JS, Nandy S (2022) Fungal endophytes as biostimulants of secondary metabolism in plants: a sustainable agricultural practice for medicinal crops. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 283–314

Deshmukh SK, Verekar SA, Bhave SV (2015) Endophytic fungi: a reservoir of antibacterials. Front Microbiol 5:715

Digra S, Nonzom S (2023) An insight into endophytic antimicrobial compounds: an updated analysis. Plant Biotechnology Reports 14:1–31

Durga B, Julius A, Raghavendra JS (2020) Review of pharmacological aspects of *Nothapodytes nimmoniana* species. Eur J Mol Clinic Med 7(03):1727–1732

El-Hawary SS, Mohammed R, Bahr HS, Attia EZ, El-Katatny MMH, Abelyan N, Al-Sanea MM, Moawad AS, Abdelmohsen UR (2021) Soybean-associated endophytic fungi as potential source for anti-COVID-19 metabolites supported by docking analysis. J Appl Microbiol 131(3):1193–1211

Feng LX, Zhang BY, Zhu HJ, Pan L, Cao F (2020) Bioactive metabolites from *Talaromyces purpureogenus*, an endophytic fungus from *Panax notoginseng*. Chem Nat Compd 56:974–976

Ferreira MC, Vieira MDLA, Zani CL, de Almeida Alves TM, Junior PAS, Murta SM, Romanha AJ, Gil LHVG, de Oliveira Carvalho AG, Zilli JE, Vital MJS (2015) Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). Biochem Syst Ecol 59:36–44

Giacomazzi J, Baethgen L, Carneiro LC, Millington MA, Denning DW, Colombo AL, In Pasqualotto AC, Association with the LIFE Program (2016) The burden of serious human fungal infections in Brazil. Mycoses 59(3):145–150

Goswami S, Vidyarthi AS, Bhunia B, Mandal T (2013) A review on lovastatin and its production. J Biochem Technol 4(1):581–587

Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538

Govindappa M, Thanuja V, Tejashree S, Soukhya CA, Barge S, Manojkumar A, Ravishankar Rai V (2019) In vitro and in silico antioxidant, antidiabetic, anti-hiv and antialzheimer activity of endophytic fungi, *Cladosporium uredinicola* phytochemicals. Int J Pharmacol Phytochem Ethnomed 13:13–34

Gupta S, Chaturvedi P, Kulkarni MG, Van Staden J (2020) A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotechnol Adv 39:107462

Habbu PV, Madagundi SD, Kulkarni VH (2021) Antioxidant and antidiabetic potential of endophytic fungal fractions recovered from *Tinospora cordifolia* (Willd.) leaves. *RGUHS*. J Pharm Sci 11(2)

Haque MA, Hossain MS, Rahman MZ, Rahman MR, Hossain MS, Mosihuzzaman M, Nahar N, Khan SI (2005) Isolation of bioactive secondary metabolites from the endophytic fungus of *Ocimum basilicum*. J Pharm Sci 4(2):127–130

Hastuti US, Sulisetijono S, Thoyibah C, Pratiwi SH, Khotimah K (2022, May) Histological observation, identification, and secondary metabolites content in Endophytic fungi of mahogany

tree (*Swietenia mahagoni* Jacq). In: 7th international conference on biological science (ICBS 2021). Atlantis Press, pp 156–164
He J, Li ZH, Ai HL, Feng T, Liu JK (2019) Antibacterial chromones from cultures of the endophytic fungus *Bipolaris eleusines*. Nat Prod Res 33(24):3515–3520
Huang WY, Cai YZ, Xing J, Corke H, Sun M (2007) A potential antioxidant resource: endophytic fungi from medicinal plants. Econ Bot 61(1):14–30
Huang X, Zhou D, Liang Y, Liu X, Cao F, Qin Y, Mo T, Xu Z, Li J, Yang R (2021) Cytochalasins from endophytic *Diaporthe* sp. GDG-118. Nat Prod Res 35(20):3396–3403
Hussain H, Nazir M, Saleem M, Al-Harrasi A, Elizbit and Green, I.R. (2021) Fruitful decade of fungal metabolites as antidiabetic agents from 2010 to 2019: emphasis on α-glucosidase inhibitors. Phytochem Rev 20:145–179
Ibrahim M, Oyebanji E, Fowora M, Aiyeolemi A, Orabuchi C, Akinnawo B, Adekunle AA (2021) Extracts of endophytic fungi from leaves of selected Nigerian ethnomedicinal plants exhibited antioxidant activity. BMC Complement Med Ther 21(1):1–13
Jakubczyk D, Dussart F (2020) Selected fungal natural products with antimicrobial properties. Molecules 25(4):911
Jayant KK, Vijayakumar BS (2021) In-vitro antioxidant and antidiabetic potential of endophytic fungi associated with *Ficus religiosa*. Ital J Mycol 50:10–20
Joseph B, Priya RM (2011) Bioactive compounds from endophytes and their potential in. Am J Biochem Mol Biol 1(3):291–309
Khan MIH, Sohrab MH, Rony SR, Tareq FS, Hasan CM, Mazid MA (2016) Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. Toxicol Rep 3:861–865
Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28(7):1208–1228
Kumar G, Chandra P, Choudhary M (2017) Endophytic fungi: a potential source of bioactive compounds. Chem Sci Rev Lett 6:2373–2381
Lacerda ÍCDS, Polonio JC, Golias HC (2022) Endophytic fungi as a source of antiviral compounds–a review. Chem Biodivers 19(6):e202100971
Li E, Tian R, Liu S, Chen X, Guo L, Che Y (2008) Pestalotheols A− D, bioactive metabolites from the plant endophytic fungus *Pestalotiopsis theae*. J Nat Prod 71(4):664–668
Li F, Jiang T, Li Q, Ling X (2017) Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer? Am J Cancer Res 7(12):2350
Li Y, Wei W, Wang RL, Liu F, Wang YK, Li R et al (2019) Colletolides A and B, two new γ-butyrolactone derivatives from the endophytic fungus *Colletotrichum gloeosporioides*. Phytochem Lett 33:90–93
Liu P, Zhang D, Shi R, Yang Z, Zhao F, Tian Y (2019a) Antimicrobial potential of endophytic fungi from *Astragalus chinensis*. 3 Biotech 9:1–9
Liu SS, Jiang JX, Huang R, Wang YT, Jiang BG, Zheng KX, Wu SH (2019b) A new antiviral 14-nordrimane sesquiterpenoid from an endophytic fungus *Phoma* sp. Phytochem Lett 29:75–78
Lugtenberg BJ, Caradus JR, Johnson LJ (2016) Fungal endophytes for sustainable crop production. FEMS Microbiol Ecol 92(12)
Liu P, Zhang D, Shi R, Yang Z, Zhao F, Tian Y. Antimicrobial potential of endophytic fungi from Astragalus chinensis. 3 Biotech. 2019 Nov;9:1-9.
Ma YM, Ma CC, Li T, Wang J (2016) A new furan derivative from an endophytic *Aspergillus flavus of Cephalotaxus fortunei*. Nat Prod Res 30(1):79–84
Macías-Rubalcava ML, Sánchez-Fernández RE (2017) Secondary metabolites of endophytic *Xylaria* species with potential applications in medicine and agriculture. World J Microbiol Biotechnol 33:1–22
Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: a review of novel bioactive compounds with biological applications. Microorganisms 8(12):1934

Molina G, Pimentel MR, Bertucci TC, Pastore GM (2012) Application of fungal endophytes in biotechnological processes. Chem Eng Trans 2012;27

Mostert L, Crous PW, Petrini O (2000) Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia 52(1):46–58

Nasmetova S, Gulyamova T, Ruzieva D, Mukhammedov I, Abdulmyanova L (2020) Isolation of α-amylase inhibitors from methanol fraction of the endophytic fungus penicillium brevicaule alba thom. Eur J Mol Clin Med 7(2):2174–2181

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microb Pathog 82:50–59

Nithya K, Muthumary J (2010) Secondary metabolite from *Phomopsis* sp. isolated from *Plumeria acutifolia* Poiret. Recent Res Sci Technol 2(4)

Nzimande B, Kumalo HM, Ndlovu SI, Mkhwanazi NP (2022) Secondary metabolites produced by endophytic fungi, *Alternaria alternata*, as potential inhibitors of the human immunodeficiency virus. Front Genet 13

Ojima I, Lichtenthal B, Lee S, Wang C, Wang X (2016) Taxane anticancer agents: a patent perspective. Expert Opin Ther Pat 26(1):1–20

Olatunji OJ, Tang J, Tola A, Auberon F, Oluwaniyi O, Ouyang Z (2018) The genus *Cordyceps*: An extensive review of its traditional uses, phytochemistry and pharmacology. Fitoterapia 129:293–316

Pan Y, Zheng W, Yang S (2020) Chemical and activity investigation on metabolites produced by an endophytic fungi *Psathyrella candolleana* from the seed of *Ginkgo biloba*. Nat Prod Res 34(21):3130–3133

Panda SK, Sahoo G, Swain SS, Luyten W (2022) Anticancer activities of mushrooms: a neglected source for drug discovery. Pharmaceuticals 15(2):176

Pandey PK, Singh S, Yadav RNS, Singh AK, Singh MCK (2014) Fungal endophytes: promising tools for pharmaceutical science. Int J Pharm Sci Rev Res 25(2):128–138

Pandey PR, Okuda H, Watabe M, Pai SK, Liu W, Kobayashi A, Xing F, Fukuda K, Hirota S, Sugai T, Wakabayashi G (2011) Resveratrol suppresses growth of cancer stem-like cells by inhibiting fatty acid synthase. Breast Cancer Res Treat 130:387–398

Peláez F, Cabello A, Platas G, Díez MT, del Val AG, Basilio A, Martán I, Vicente F, Bills GF, Giacobbe RA, Schwartz RE (2000) The discovery of enfumafungin, a novel antifungal compound produced by an endophytic *Hormonema* species biological activity and taxonomy of the producing organisms. Syst Appl Microbiol 23(3):333–343

Pongcharoen W, Rukachaisirikul S, Phongpaichit S, Kuhn T, Pelzing M, Sakayaroj J, Taylor WC (2008) Metabolites from the endophytic fungus *Xylaria* sp. PSU-D14. Phytochemistry 69(9):1900–1902

Popli D, Anil V, Subramanyam AB, MN N, VR R, Rao SN, Rai RV, Govindappa M (2018) Endophyte fungi, Cladosporium species-mediated synthesis of silver nanoparticles possessing in vitro antioxidant, anti-diabetic and anti-Alzheimer activity. Artificial cells, nanomedicine, and biotechnology, 46(sup1): 676–683

Puri SK, Habbu PV, Kulkarni PV, Kulkarni VH (2018) Nitrogen containing secondary metabolites from endophytes of medicinal plants and their biological/pharmacological activities-a review. Syst Rev Pharm 9(1):22–30

Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN, Yadav N, Singh K, Saxena AK (2020) Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Microbial endophytes. Woodhead, pp 273–305

Ravindran C, Naveenan T, Varatharajan GR, Rajasabapathy R, Meena RM (2012) Antioxidants in mangrove plants and endophytic fungal associations. Bot Mar 55(3):269–279

Rehberg N, Akone HS, Ioerger TR, Erlenkamp G, Daletos G, Gohlke H, Proksch P, Kalscheuer R (2018) Chlorflavonin targets acetohydroxyacid synthase catalytic subunit IlvB1 for synergistic killing of *Mycobacterium tuberculosis*. ACS Infect Dis 4(2):123–134

Rehman S, Shawl AS, Kour A, Andrabi R, Sudan P, Sultan P, Verma V, Qazi GN (2008) An endophytic *Neurospora* sp. from *Nothapodytes foetida* producing camptothecin. Appl Biochem Microbiol 44:203–209

Reshma J, Vinaya C, Linu M (2019) Agricultural applications of endophytic microflora. Biol Biotechnol, Seed Endophytes, pp 385–403

Ruzieva D, Gulyamova T, Nasmetova S, Mukhammedov I, Rasulova G, Ruziyeva DM (2021) Identification of bioactive compounds of the endophytic fungus *Aspergillus egypticus-HT166S* inhibiting the activity of pancreatic α-amylase Pankreatik a-amilaz aktivitesini inhibe eden endofitik mantar *Aspergillus egypticus-HT166S*′nin biyoaktif bileşiklerinin

Sadananda TS, Nirupama R, Chaithra K, Govindappa M, Chandrappa CP, Vinay Raghavendra B (2011) Antimicrobial and antioxidant activities of endophytes from *Tabebuia argentea* and identification of anticancer agent (lapachol). J Med Plants Res 5(16):3643–3652

Sadrati N, Daoud H, Zerroug A, Dahamna S, Bouharati S (2013) Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Triticum durum*). J Plant Protect Res 53(2)

Saxena S, Chhibber M, Singh IP (2019) Fungal bioactive compounds in pharmaceutical research and development. Curr Bioact Compd 15(2):211–231

Schiff PB, Horwitz SB (1980) Taxol stabilizes microtubules in mouse fibroblast cells. Proc Natl Acad Sci 77(3):1561–1565

Schulza B, Boyle C (2005) The endophyte continuum. Mycol Res 109(6):661–686

Selim KA, Elkhateeb WA, Tawila AM, El-Beih AA, Abdel-Rahman TM, El-Diwany AI, Ahmed EF (2018) Antiviral and antioxidant potential of fungal endophytes of Egyptian medicinal plants. Fermentation 4(3):49

Sharaf MH, Abdelaziz AM, Kalaba MH, Radwan AA, Hashem AH (2022) Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from *Ocimum basilicum*. Appl Biochem Biotechnol:1–19

Shi S, Li Y, Ming Y, Li C, Li Z, Chen J, Luo M (2018) Biological activity and chemical composition of the endophytic fungus *Fusarium* sp. TP-G1 obtained from the root of *Dendrobium officinale* Kimura Migo. Rec Nat Prod 12:549–556

Singh B, Kaur A (2016) Antidiabetic potential of a peptide isolated from an endophytic Aspergillus awamori. J Appl Microbiol 120(2):301–311

Singh M, Kumar A, Singh R, Pandey KD (2017) Endophytic bacteria: a new source of bioactive compounds. 3 Biotech **7**:1–14

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Strobel GA, Morrison SI, Cassella M (2003). Methods for protection of plants from Oomyocyte pathogens by use of *Serratia marcescens* and isolates. US Patent Appl US2003/0049230 A1

Subban K, Subramani R, Johnpaul M (2013) A novel antibacterial and antifungal phenolic compound from the endophytic fungus *Pestalotiopsis mangiferae*. Nat Prod Res 27(16):1445–1449

Tobert JA (2003) Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nat Rev Drug Discov 2(7):517–526

Toghueo RMK (2020) Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. Mycology 11(1):1–21

Toghueo RMK, Boyom FF (2019) Endophytes from ethno-pharmacological plants: sources of novel antioxidants-A systematic review. Biocatal Agric Biotechnol 22:101430

Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Praveen V, Kamath PV, Singh BP, Mudili V, Gupta VK, Siddaiah CH, Chowdappa S, Alqarawi AA, Abd Allah E.F. (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:328484. https://doi.org/10.3389/fphar.2018.00309

Uzor PF, Osadebe PO, Nwodo NJ (2017) Antidiabetic activity of extract and compounds from an endophytic fungus *Nigrospora oryzae*. Drug Res 67(05):308–311

Wang J, Huang Y, Fang M, Zhang Y, Zheng Z, Zhao Y, Su W (2002) Brefeldin A, a cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunol Med Microbiol 34(1):51–57

Wani ZA, Ashraf N, Mohiuddin T, Riyaz-Ul-Hassan S (2015) Plant-endophyte symbiosis, an ecological perspective. Appl Microbiol Biotechnol 99:2955–2965

Wheeler NC, Jech K, Masters S, Brobst SW, Alvarado AB, Hoover AJ, Snader KM (1992) Effects of genetic, epigenetic, and environmental factors on taxol content in *Taxus brevifolia* and related species. J Nat Prod 55(4):432–440

Xia X, Kim S, Bang S, Lee HJ, Liu C, Park CI, Shim SH (2015) Barceloneic acid C, a new polyketide from an endophytic fungus *Phoma* sp. JS752 and its antibacterial activities. J Antibiot 68(2):139–141

Yadav M, Yadav A, Yadav JP (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. Asian Pac J Trop Med 7:S256–S261

Yang X, Feng P, Yin Y, Bushley K, Spatafora JW, Wang C (2018) Cyclosporine biosynthesis in *Tolypocladium inflatum* benefits fungal adaptation to the environment. MBio 9(5):e01211–e01218

Yin H, Zhao Q, Sun FM, An T (2009) Gentiopicrin-producing endophytic fungus isolated from *Gentiana macrophylla*. Phytomedicine 16(8):793–797

Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, Gu Q (2011) Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegiceras corniculatum*. Phytochemistry 72(11–12):1436–1442

Zhang CL, Zheng BQ, Lao JP, Mao LJ, Chen SY, Kubicek CP, Lin FC (2008) Clavatol and patulin formation as the antagonistic principle of Aspergillus clavatonanicus, an endophytic fungus of Taxus mairei. Appl Microbiol Biotechnol 78:833–840

Zheng CJ, Shao CL, Guo ZY, Chen JF, Deng DS, Yang KL, Chen YY, Fu XM, She ZG, Lin YC, Wang CY (2012) Bioactive hydroanthraquinones and anthraquinone dimers from a soft coral-derived *Alternaria* sp. fungus. J Nat Prod 75(2):189–197

Zhao S, Wang B, Tian K, Ji W, Zhang T, Ping C, Yan W, Ye Y (2021) Novel metabolites from the *Cercis chinensis* derived endophytic fungus *Alternaria alternata* ZHJG5 and their antibacterial activities. Pest Manag Sci 77(5):2264–2271

Zhao T, Xu LL, Zhang Y, Lin ZH, Xia T, Yang DF, Chen YM, Yang XL (2019) Three new α-pyrone derivatives from the plant endophytic fungus *Penicillium ochrochloronthe* and their antibacterial, antifungal, and cytotoxic activities. J Asian Nat Prod Res 21(9):851–858

Zheng N, Yao F, Liang X, Liu Q, Xu W, Liang Y, Liu X, Li J, Yang R (2018) A new phthalide from the endophytic fungus *Xylaria* sp. GDG-102. Nat Prod Res 32(7):755–760

Zhou G, Zhang X, Shah M, Che Q, Zhang G, Gu Q, Zhu T, Li D (2021) Polyhydroxy p-terphenyls from a mangrove endophytic fungus *Aspergillus candidus* LDJ-5. Mar Drugs 19(2):82

Zhou J, Diao X, Wang T, Chen G, Lin Q, Yang X, Xu J (2018) Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. PLoS One 13(6):e0197359

Chapter 4
Pharmaceutically Important Fungal Endophytes Associated with Mushrooms: Current Findings and Prospects

Swapnil C. Kamble and Mayuresh Dorle

Abstract Traditional medication knowledge base has propelled the pharma sector to actively look into natural bioactive. Apart from various plants, certain mushrooms have also been documented. Many edible mushroom varieties that are used in traditional medication grow in the wild and have proved to be difficult for cultivation. Endophytes associated with such mushrooms could potentiate the availability of mushroom-specific bioactive via their fermentation route. This concept is based on the similarity of endophytic fungi of plants that sometimes possess host-similar pathways to produce pharmaceutically important bioactive. Isolation, characterization, and growing of mushroom-isolated endophytes in laboratory conditions may allow bypassing the need for mushroom cultivation. The traditional approach of isolating, cultivating and characterizing of endophyte has led to limited research on their endophytic fungi. Recent methods of high-throughput sequencing have allowed discovery of previously unknown microbial strains associated with mushrooms. Subsequent analyses for bioactive discovery are being eagerly looked forward. Here, we attempt to understand the available literature and present the scope of research in this domain. Specifically, we examine the pharmaceutically important bioactive of endophytes present in *Tricholoma matsutake*, *Cordyceps sinensis*, *Cordyceps hawkesii* Gray, *Cordyceps militaris*, *Lycoperdon umbrinum* and *Morchella crassipes*. Cultivating such endophytes may require environmental equivalence as that of wild mushroom host or the presence of metabolites from the host mushroom. Analysing these interactors will provide for measures to cultivate the endophytes that will allow harvesting the beneficial bioactive. We conclude with possible research domains that may be taken up by avid researchers.

Keywords Macrofungi · Fruiting bodies · Bioactive · Cultivable endophytes · Endophyte extract

S. C. Kamble (✉) · M. Dorle
Department of Technology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India
e-mail: sckamble@unipune.ac.in

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1 Introduction

The discovery and development of a new drug is a resource-consuming process. Traditional medicinal systems like traditional Indian medicines, traditional Korean medicine, traditional African medicine, ancient Iranian medicine and traditional Chinese medicines have been looked up by the pharma industry for isolation and identification of bioactive for management of various maladies. Apart from plants, the significance of mushrooms in traditional medicinal systems is well documented. Many of the mushrooms are not cultivable and are to be foraged. Exhaustive mushroom foraging has led to depletion of their natural number in the wild, resulting in their enlisting in the International Union for Conservation of Nature Red List. Considering the fact that wild mushrooms have the tendency to accumulate trace elements, it is apparent that these mushrooms could be toxic upon consumption (Fu et al. 2020). Though this depends on the region from where the mushrooms are collected, it is difficult for a global end consumer to know as many of the mushrooms are geographically restricted. In the presence of pharmaceutically important bioactive in such mushrooms, it is possible that their values are less explored. Nonetheless, the popularity of mushrooms has not decreased due to their known richness of bioactive.

In high similarity to the endophyte-plant association, certain fungi have been reported in mushrooms. Wildly grown mushrooms have endophytic association within their environmental niche. Endophytes are traditionally the fungi or bacteria that coexist inside the asymptomatic plant tissues (Wilson 1995). To define the mushroom isolated fungi as 'endophytes' may be in direct contrast to the definition proposed by Wilson, where the relationship is between plant and fungi. Not discounting the symbiotic relationship, a new definition may be required to classify such fungi. Interestingly, some of the fungi isolated from mushroom have been reported in plants as well. It is yet to be evaluated as to what relationship may have preceded the other over time.

The fungal and bacterial endophytes are diverse and have been difficult to isolate, identify and characterize within the experimental conditions (Riva et al. 2019). It is estimated that all plants have fungal endophytes associated with one or the other organ. They inhabit the healthy tissues of the plant without presenting any disease condition. The relationship between the host and endophyte is a balanced symbiosis and may improve general well-being of the plant (Rho et al. 2018). For example, water scarcity (environmental stress condition) increases the magnitude of effect of the endophytes in the host plants, resulting in the increase of plant biomass, height, yield, water content, enzymes like superoxide dismutase, etc. (Dastogeer 2018). Certain endophytic fungi produce alkaloids that aid in survival of the host plants against various herbivores and insects. For example, Clavicipitaceae (Ascomycota) predominates the fungal endophytes in temperate grasses (Johnson et al. 2013). The underlying large reservoir of bioactive in the fungal endophytes forms the basis of a symbiotic relationship and mutual survival in strenuous environmental conditions. These bioactive compounds obtained from fungal endophytes

are classified into different functional groups like alkaloids, steroids, flavonoids, phenolic acids, benzopyranones, quinines, tannins, xanthones, terpenoids, and many others. Some endophytic fungi are useful to produce laccases which are extracellular enzymes required in dye decolorization. Some of the dyes are non-degradable and toxic, and decolorization of these dyes is possible with the help of fungal endophytes by using various processes like biomagnification, biosorption, bioaccumulation and enzymatic degradation (Ngieng et al. 2013).

The presence of pharmaceutically significant bioactive has attracted the attention of biosimilar/drug discovery scientists alike. Many bioactive have failed to be artificially synthesized. One plausible reason is the lack of knowledge of which all bioactive are associated with health benefits. This can be evaluated by generation of suitable solvent-based extracts and subsequent assays. The bioactive can be purified and characterized through high-performance liquid chromatography, thin-layer chromatography, nuclear magnetic resonance, infrared spectroscopy and matrix-assisted laser desorption/ionization – time of flight, electron spray ionization, fast atom bombardment, etc. (Rai et al. 2021a, b). Another limitation for the artificial bioactive synthesis could be the absence of a well-elucidated pathway responsible for production of bioactive in mushrooms. Current advances in 'omics' may usher the knowledge that is being currently sought.

The expertise available in scaling up by fermentation process requires a bridging for the presence of similar pathways in microbes. Fermentation-based production of such bioactive for pharmaceutical applications is an open-space research. Isolation of endophytes from respective mushrooms could pave way for their cultivation and possible fermentation-based scaling. Different bioactive present and findings from respective mushroom-endophytes are provided in the subsequent sections.

2 Endophytic Fungi in *Tricholoma matsutake* (Pine Mushroom or Matsutake Mushroom)

Tricholoma matsutake (Agaricales, Tricholomataceae) is a popular medicinal mushroom that is consumed worldwide. It tends to form a symbiotic relationship with coniferous trees and may dominate over certain fungal species of the area (Zhou et al. 2021). It is known to produce bioactive that prevent cancer progression (Ren et al. 2014; Wang et al. 2016). Considering the decreasing availability from the forests and failure in its lab-based cultivation, matsutake mushroom is a highly priced mushroom (Park et al. 2020). Though the composition of different geolocation sourced matsutake has been attempted by Li (2016), not all of them have been evaluated for their beneficiary effects (Qiang Li et al. 2016b). However, this does not reduce the significance of the bioactive in it, and hence, endophytes associated with it may direct toward alternative sources. The fruiting bodies of *T. matsutake* collected from Sichuan, China, had 13 fungi, 15 yeast and 14 bacterial strains (Li

Table 4.1 Strains of fungi isolated from *Tricholoma matsutake*

S. no.	Strain (GenBank Accession Number)	Species
1.	13 (KJ936996)	*Fusarium solani*
2.	05 (KJ936995)	
3.	01 (KJ936994)	
4.	18 (KJ936997)	
5.	*B1 (KJ936998)*	*Phomopsis* sp.
6.	J1 (KJ936999)	*Sordariomycetes* sp.
7.	M3 (KJ937001)	*Daldinia loculata*
8.	ML3 (KJ937002)	
9.	J28 (KJ937000)	Uncultured fungus
10.	X28 (KJ937003)	*Aspergillus versicolor*
11.	15 (KJ936991)	*Nectria haematococca*
12.	J3 (KJ936992)	*Mortierella* sp.
13.	XN (KJ936993)	*Exophiala* sp.

et al. 2016a) (Table 4.1). The related strains of the reported fungi have been associated with production of bioactive compounds.

3 Endophytic Fungi in *Cordyceps sinensis* (Caterpillar Fungus)

Cordyceps sinensis is a routine ingredient in traditional Chinese preparations for management of chronic kidney disease. It has potential in clearing serum creatinine, thereby reducing proteinuria (Zhang et al. 2014a). Extracts from *C. sinensis* and cordycepsin stimulate Leydig cell steroidogenesis as well (Chen et al. 2017). The lab of Fang Deng and Yun Deng, Chengdu University of Traditional Chinese Medicine, China identified the presence of 28 endophytic fungal strains from *C. sinensis* (Guo et al. 2019). Among the identified strains, the authors found *Penicillium herquei* as the only fungus to be sensitive to 5-aza-2-deoxycytidin that gave more metabolites than others. Further, the authors identified three α-pyrone derivatives from it in the presence of 5-aza-2-deoxycytidin. These are the following:

1. (S)-6-(sec-butyl)-5-(hydroxymethyl)-4-methoxy-2H-pyran-2-one (yellow gum)
2. (5S,7R)-7-ethyl-4,5-dimethoxy-7-methyl-5,7-dihydro-2H-furo[3,4-b]pyran-2-one (yellow gum)
3. (5R,7R)-7-ethyl-4,5-dimethoxy-7-methyl-5,7-dihydro-2H-furo[3,4-b]pyran-2-one (yellow gum)

The structures of these new compounds were elucidated by extensive spectroscopic analyses and quantum chemical ECD calculations, and cytotoxic analysis showed compounds 1–3 only exhibited weak cytotoxic activity against MV4-11 cell.

The group reported another fungus, *Aspergillus fumigatus* (GenBank accession No. MG519287), from the fruiting body of *C. sinensis* from which new compounds were discovered. The fermentation broth of *A. fumigatus* (Li et al. 2019a) had the following four isochromanes (Fig. 4.1). Compounds 1a and 2 are reported to have moderate growth inhibition against MV4–11 cell line.

1a. (S)-3,6-dihydroxy-8-methoxy-3-methylisochroman-4-one (yellow gum)
1b. (R)-3,6-dihydroxy-8-methoxy-3-methylisochroman-4-one (yellow gum)
2. 6-methoxy-3-methylisochromane-3,8-diol (yellow gum)
3. 3,6-dimethoxy-3-methylisochroman-8-ol (yellow gum)
4. 3,6,7,8-tetramethoxy-3-methylisochromane (yellow gum)

In addition, five novel polyketides were identified (Guo et al. 2018) (Fig. 4.2). These are the following:

1. 3R,4S-3,8-Dimethoxy-3-methylisochromane-4,6-diol (1a)
2. 3R,4R-3,8-Dimethoxy-3-methylisochromane-4,6-diol (1b)
3. 3S,4R-3,8-Dimethoxy-3-methylisochromane-4,6-diol (2a)
4. 3R,4S-3,8-Dimethoxy-3-methylisochromane-4,6-diol (2b)
5. 3,6,8-Trimethoxy-3-methylisochromane (3)

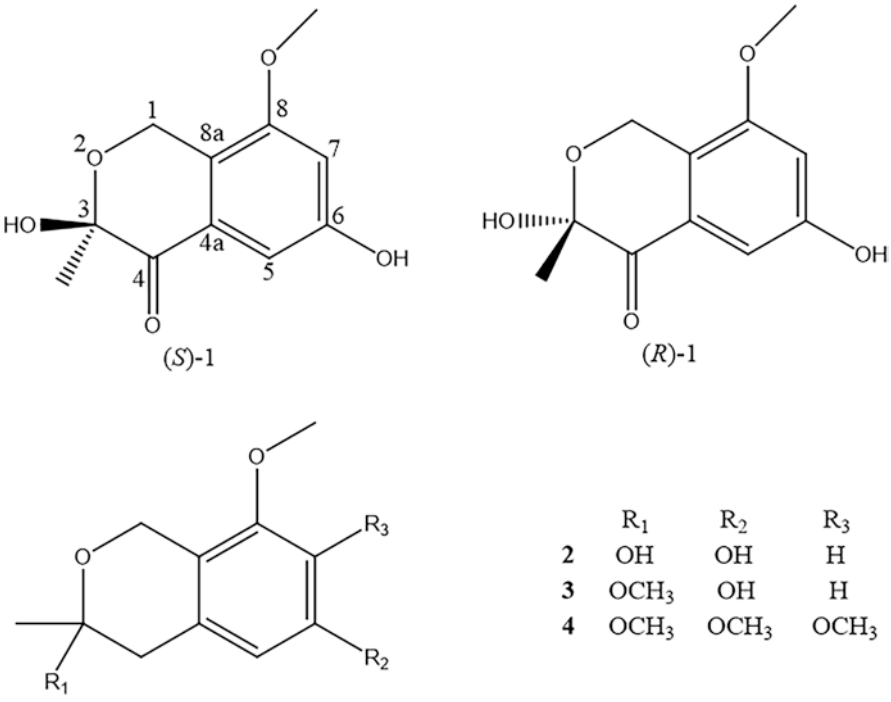


Fig. 4.1 Structure of compounds obtained from *Aspergillus fumigatus*. (Adapted from Li et al. 2019a)

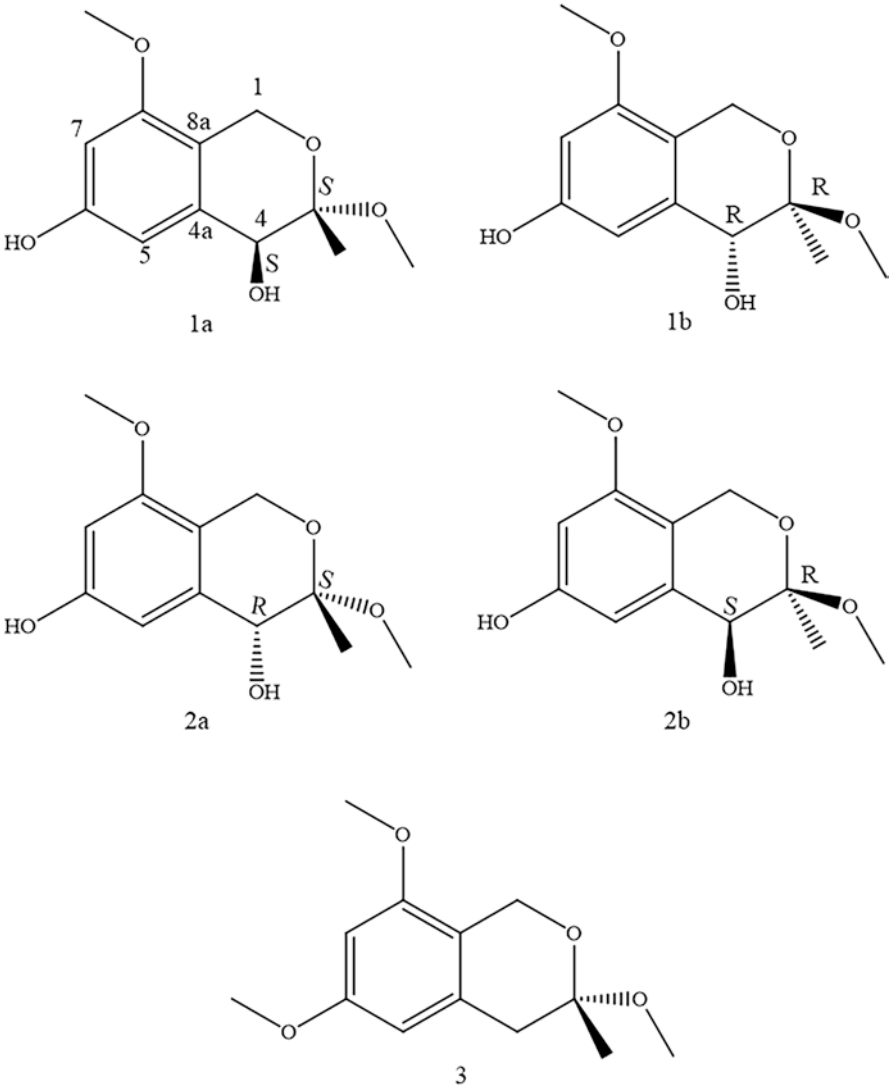


Fig. 4.2 Structure of compounds obtained from *Aspergillus fumigatus*. (Adapted from Guo et al. 2018)

4 Endophytic Fungi in *Cordyceps hawkesii* Gray (Fawn Vegetable Caterpillar)

Another *Cordyceps* genus member was assessed for endophytes capable of producing cordycepin. Recent research documents the presence of 22 endophytes from *C. hawkesii* Gray collected from the hill of Mutian Village in China in

subtropical monsoon climate (Liu et al. 2022). Aiming at the cordycepin-producing endophytes, four such endophytic fungi, *Irpex lacteus* CHG05, *Arthrinium arundinis*, *Arthrinium* sp., and *Bjerkandera* sp., were identified. The best yield of cordycepin was obtained from *Irpex lacteus* CHG05 at 162.05 mg/L when pH, culture temperature and culture times were 5.6, 23 °C and 13 d, respectively. It is possible to perform strain improvement to achieve a higher yield of cordycepin.

5 Fungi in *Cordyceps militaris*

C. militaris is widely consumed cultivable cordyceps. It imparts anti-cancer activity through inhibition of Hedgehog signalling pathway (Jo et al. 2020; Wu et al. 2022). The carotenoids present in it protect retinal endothelial cells from oxidative stress induced by hydrogen peroxide (Lan et al. 2022). The extract of *C. militaris* has been used for various diseases including allergies and colitis (Phull et al. 2022). Structure of various polysaccharides present in *C. militaris* and their associated functions has been described in detail by Miao and team (Miao et al. 2022). The details of pathways associated with cordycepin and polysaccharides derived from *C. militaris* have been tabulated (Table 4.2).

High-throughput sequencing of 16S rRNA and ITS genes showed the presence of bacterial and fungal communities from sample constituting C. militaris and soil on which it grew (region of Songming County, China) (Zhang et al. 2021). 94 fungal genera were detected in different samples, and it was dominated by *Cordyceps*, *Trichopaea*, *Isaria*, *Saitozyma* and *Amanita*. 89 other genera accounted for <0.25% of the total fungal community. A detailed follow-up study is looked forward to.

Cordycepin derived from *C. sinensis* and *C. militaris* is most evaluated for potential anti-cancer activities. Here we have attempted to collate the pathways that are identified through which anti-cancer activity is achieved (Table 4.3).

Table 4.2 Anti-diabetic bioactive present in *C. militaris*

Bioactive	Pathway	Diabetes effect
Cordycepin	11β-HSD1 and PPARγ ↓ co-stimulatory molecules ICAM-1 and B7–1/−2 ↓ with cordycepin increment	T2D inhibition of diabetic regulating genes in activated macrophages (Shin et al. 2009)
Polysaccharides	Increasing pyruvate kinase (PK) activity↑ rate-limiting enzyme for glycolysis	Promotion of glucose absorption (Zhang et al. 2006)

Table 4.3 Pathways responsible for anti-cancer role of cordycepin

S. no.	Mushroom	Pathway and action	Type of cancer
1.	*C. sinensis*	Downregulated the mRNA levels of *FGF9*, *FGF18*, *FGFR2* and *FGFR3* genes in MA-10 cells. Inhibited MA-10 cell proliferation by suppressing FGFs/FGFRs pathway. Decreased FGF9-induced MA-10 cell proliferation by inhibiting the expressions of p-ERK1/2, p-Rb and E2F1 and subsequently reducing the expressions of cyclins and CDKs. Decreased FGF9-induced FGFR1-4 protein expressions in vitro and in vivo.	Testicular cancers (Chang et al. 2020)
2.	*C. militaris*	CD4+ T, CD8+ T, M1-type macrophages, NK cells were upregulated. Inhibited phagocytosis immune checkpoint CD47 protein expression by reducing BNIP3 expression. Inhibited the expression of TSP1 in tumour cells and Jurkat cells, which may reduce the binding of TSP1 to CD47, thereby reducing T cell apoptosis and allowing more T cells to infiltrate into tumours. Enhanced the phagocytosis of CT26 cells by macrophages. Inhibited colon cancer cell proliferation by downregulating MYC mRNA/protein expression and upregulating miR-26a in both HCT116 and Caco-2 cells. Increased JNK and p21WAF-1, miR-26a expression. Cordycepin increased sub-G1 and G2/M phase arrest. Downregulated MYC mRNA/protein expression.	Colon Cancer (Deng et al. 2022; Lee et al. 2010, 2013; Zhang et al. 2022)
3.	*C. militaris*	G2/M arrested and apoptosis of MCF-7 and MDA-MB-231 cells resulting in restraining the proliferation of the cells. Downregulated the expression levels of nuclear factor erythroid 2-related factor (Nrf2) and a series of downstream genes, such as heme oxygenase-1 (HO-1), to enhance ROS in breast cancer cells exposed to irradiation. Cordycepin treatment sensitized breast carcinoma cells toward irradiation via Nrf2/HO-1/ROS axis. Inactivated the EMT signalling pathway by inhibiting TWIST1 and SLUG expression. Regulated the growth and metastasis formation. Inhibited the Hedgehog pathway. Induced DNA double-strand breaks. Increased caspase-3, caspase-9, mitochondrial translocation of BAX and cytochrome c.	Breast Cancer (Choi et al. 2011; Kim et al. 2011; Lee et al. 2012; Wei et al. 2022; Wu et al. 2022)

(continued)

Table 4.3 (continued)

S. no.	Mushroom	Pathway and action	Type of cancer
4.	*C. militaris*	Suppressed cell proliferation. Induced Bax, caspase-3, caspase-9, and caspase-12 upregulation at the mRNA and protein levels while simultaneously downregulating anti-apoptotic Bcl-2 expression.	Tongue cancer (Zheng et al. 2020b)
5.	*C. militaris*	Apoptosis induction by activating caspase-3, caspase-9 and cytochrome c. MAPK pathway was blocked by cordycepin. Inhibited the expression of Ras. S-phase was arrested. Activated Chk2 (checkpoint kinase 2) pathway. Downregulated cyclin A2 and CDK2 phosphorylation. Inhibited pancreatic cancer cells growth. Blocked Ras/ErK pathway.	Pancreatic cancer (Li et al. 2020)
6.	*C. militaris*	Inhibited migration and invasion and decreased the expression of CXCR4. Downregulated the activation of phosphorylated (p-) NF-κB inhibitor α (IκBα) and p-P65 and also the principal components of the NF-κB signalling pathway. Suppressed the nuclear translocation of P65. Downregulated CXCR4 expression. Impaired migration and invasion abilities of liver cancer cells and attenuated reactivity to SDF1. Inhibited cell proliferation and migration/invasion. Increased expression of epithelial marker, E-cadherin. Expression of focal adhesion kinase (FAK) slightly reduced, phosphorylated FAK are significantly reduced. Suppressed the expression of integrin α3, integrin α6 and integrin β1. Apoptosis was induced by increased caspase-3, caspase-8, caspase-9, FADD, and Bid; and decreased Bcl-2. Increased PARP cleavage.	liver cancer (Guo et al. 2020; Shao et al. 2016; Yao et al. 2014)
7.	*C. militaris*	Inhibited cell growth and induced apoptosis. P53, BAX, Caspase-3, and Caspase-9 were upregulated. BCL-2 was suppressed. Induced the generation of reactive oxygen species. Suppressed antioxidant genes GPX, SOD, and catalase. Activation of autophagy.	Brain Cancer (Zhao et al. 2017; Chaicharoenaudomrung et al. 2018)

(continued)

Table 4.3 (continued)

S. no.	Mushroom	Pathway and action	Type of cancer
8.	*C. sinensis*	Induced apoptosis. Increased the total protein levels of p53 and phosphorylated p53. Increased levels of cleaved caspase-7 and poly (ADP-ribose) polymerase (PARP).	Glioma (Chen et al. 2014)
9.	*C. militaris*	Inhibited the MPNST cell growth. Arrested cell cycle at G2/M and S phases. The levels of ERK, survivin, pAKT, Sp1, tubulin proteins were decreased.	Malignant peripheral nerve sheath tumour (Lee et al. 2021)
10.	*C. militaris*	Increased p38 MAPK, caspase-3,8, PARP cleavage. Decreased Akt and Bcl-2 levels.	Glioblastoma (Baik et al. 2016)
11.	*C. militaris*	Apoptosis induced. Cell cycle arrest by Caspase-sub-G1 phase cell-cycle arrest. Increased caspase-3 and PARP cleavage.	Neuroblastoma (Li et al. 2015)
12.	*C. sinensis*	Inhibited cell viability, proliferation and colony formation ability and induced cell cycle arrest and early apoptosis. Upregulation of Bax, cleaved caspase-3, cleaved caspase-9 and cleaved PARP. Downregulation of Bcl-2.	Human pancreatic cancer MIAPaCa-2 and Capan-1 (Zhang et al. 2018)
13.	*C. militaris*	Inhibition of NSCLC cell cycle progression and inducing cancer cell apoptosis without apparent adverse effect on normal lung cells. Activation of AMP-activated protein kinase. Downregulation of c-FLIPL which suppresses the activity of caspase-8. Suppression of mTOR signalling pathway. Activation of caspase −3, −8 and −9 activities. Accumulation of Sub G1. Apoptosis induction by ERK/JNK or ERK/Slug. Increased CAV1, JNK/Foxo3a, BAX, caspase-3 cleavage and GSK-3β. Decreased ERK, Bcl-2, H-Ras. Increased MMP-9 and caspase-3.	lung cancer (Cho and Kang 2018; Hwang et al. 2017b; Joo et al. 2017; Tao et al. 2016; Wei et al. 2019; Yu et al. 2017)
14.	*C. sinensis*	Suppressed cell proliferation. Induced chromatin condensation in oesophageal cancer cells and significantly increased the number of apoptotic cells. Activation of caspase cascades. Altered cyclin-dependent kinase1 and cyclin B1 expression, which resulted in a G2/M phase blockade.	Oesophageal cancer (Xu et al. 2019)
15.	*C. militaris*	Inhibited the phosphorylations of PI3K, Akt, mTOR. Reduced the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver and serum. Reduced the levels of interleukin-6 (IL-6), IL-1β, tumour necrosis factor-α (TNF-α), methane dicarboxylic aldehyde (MDA).	Nitrosodiethylamine (NDEA)-induced hepatocellular carcinomas (Zeng et al. 2017)

(continued)

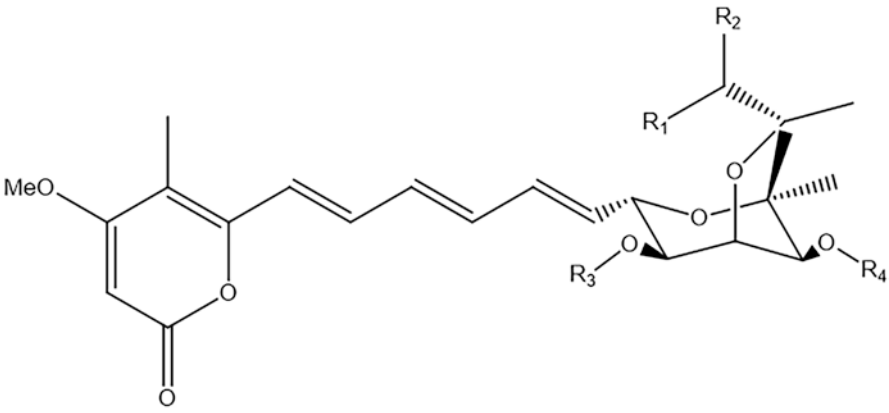
Table 4.3 (continued)

S. no.	Mushroom	Pathway and action	Type of cancer
16.	*C. sinensis*	Increased apoptotic rate, inhibited cell proliferation and increased cleaved poly (ADP-ribose) polymerase (PARP) level. Suppressed the activation of ERKs and mTOR.	Hepatocellular carcinoma (Zhou et al. 2017)
17.	*C. sinensis*	Induced apoptosis. Percentage of G1 phase cell declined. Percentages of G2M and subG1 phase cell increased.	Human oral squamous cancer (Wu et al. 2007)
18.	*C. militaris*	Inhibited cell proliferation and induced cell death, induced autophagy. Activated DNA-PK and ERK inducing centrosome amplification and aberrant mitosis. Amplified centrosomes, disrupted microtubule arrays and actin networks leading to defective cell adhesion.	Human gestational choriocarcinoma (Wang et al. 2020)
19.	*C. militaris*	Reduced CD34 + CD38-cells. Induced Dkk1 expression via autocrine and paracrine regulation. Downregulated N-cadherin. Apoptosis induction by increased caspase-3, caspase-8 and PARP cleavage and decreased Bcl-2, Akt and β-catenin.	Leukaemia (Jeong et al. 2011; Ko et al. 2013; Liang et al. 2017; Tian et al. 2014)
20.	*C.* spp.	miR-33b attenuated melanoma migration and invasiveness upon cordycepin exposure. Suppression of tumour metastasis and miR-33b/HMGA2/Twist1/ZEB1 axis. miR-33b was upregulated. miR-33b directly binds to HMGA2, Twist1 and ZEB1 3'-UTR to suppress their expression.	Malignant melanoma (Zhang et al. 2015)
21.	*C. militaris*	Promotes apoptosis. Inhibits TNF-α-mediated NF-κB/GADD45B signalling. Activates the MKK7-JNK signalling pathway through inhibition of c-FLIPL expression. Increased JNK and caspase-3. Decreased ERK.	Renal carcinoma (Hwang et al. 2016, 2017a; Yamamoto et al. 2015)
22.	*C. militaris*	Inhibited gastric cancer cell proliferation and migration. Induced apoptosis and arresting the cell cycle at the G2 phase. Upregulated CLEC2.	Gastric cancer (Wang et al. 2019)
23.	*C. militaris*	Induction of apoptosis by activating caspase-8 and -9, increased caspase-3 activity and increased the Bax/Bcl-2 ratio and truncation of Bid and destroyed the integrity of mitochondria, which contributed to the cytosolic release of cytochrome c. Inactivated the phosphoinositide 3-kinase (PI3K)/Akt signalling pathway. G2/M cell-cycle arrest. Increased cJNK.	Human bladder cancer (Kim et al. 2019; Lee et al. 2009)

6 Endophytic Fungus (*Calcarisporium arbuscula*) from the Fruit Bodies of *Russulaceae*

Mushroom-forming basidiomycetes family Russulaceae is consumed in many parts of the world. They form a significant proportion of ectomycorrhizal symbionts in temperate and tropical forests (Looney et al. 2018). Many members of this family are recent discoveries suggesting our scope of expanding our knowledge base (Chen et al. 2022; Li et al. 2021; Wisitrassameewong et al. 2022).

The fruiting bodies of *Russulaceae* have endophyte *Calcarisporium arbuscula* NRRL 3705 (Cheng et al. 2020). It produces a variety of secondary metabolites with anti-cancer, anti-nematode and antibiotic activities. The aurovertin-type mycotoxins are potent against F0F1-ATPase and are applicable in management of breast carcinoma (Huang et al. 2008). Production of such mycotoxins through fermentation-based approaches has been limited. If achieved, it could aid the pharmaceutical industry in obtaining a natural drug against F0F1-ATPase (Fig. 4.3).



Aurovertin A: R_1=H, R_2=H, R_3=Acetyl, R_4=Acetyl
Aurovertin B: R_1=H, R_2=H, R_3=H, R_4=Acetyl
Aurovertin D: R_1=OH, R_2=H, R_3=H, R_4=Acetyl
Aurovertin E: R_1=H, R_2=H, R_3=H, R_4=H
Aurovertin J: R_1=Acetyl, R_2=H, R_3=H, R_4=H
Aurovertin M: R_1=H, R_2=H, R_3=Acetyl, R_4=Propionyl

Fig. 4.3 Chemical structure of arovertins. (Adapted from Cheng et al. 2020)

7 Endophytes Screening in *Morchella crassipes*

Morels are high-value macrofungi that have limited scientific data reports. *Morchella semilibera* (previously *Morchella crassipes*) (May 2017) is a wild edible ascomycetous mushroom. It is a typical yellow morel species possessing medicinal and nutritional values, leading to a high commercial implication. Geographically, it is primarily found in the low-altitude plains of Eurasia. The composition has largely remained uncharted and with limited research on it. The exopolysaccharide of *M. crassipes* cultivated in the lab possesses antioxidant capacity (He et al. 2012). The ascocarps of *M. crassipes* (collected from three different regions of China) were evaluated for the presence of endophytes (Pei-xin et al. 2014). 16 fungal strains of endophytic fungi belonging to 14 taxa were isolated and identified. 15 out of the 16 isolates were ascomycetes or their anamorphs, while only one isolate (*Mortierella alpina*) belonged to Zygomycota. *Chaetomium* fungi were isolated from samples of all three origins and *Lecanicillium fungicola* var. aleophilum was isolated from sporocarps occurring in Mianyang, Sichuan province and Zhengzhou, Henan province.

Mortierella alpina is an oleaginous fungus, and it produces a variety of glycerolipids, glycerophospholipids and sphingolipids (Wang et al. 2011). It is commercially used for production of arachidonic acid, an important dietary lipid. Cyclooxygenases can utilize arachidonic acid for the synthesis of prostaglandin H_2 (PGH_2), which itself is a precursor molecule for bioactive prostaglandins such as like prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Currently, $PGF_{2\alpha}$ is industrially produced by chemical synthesis processes. The presence of *M. alpina* indicates that the presence of high arachidonic acid may be expected in *M. crassipes*. Further research on the presence of bioactive from these endophytes is required. *Chaetomium* fungi is a common endophytic genera (Yadav et al. 2022). *Lecanicillium fungicola* causes dry bubble disease in white-button mushrooms, and its presence in the ascocarp requires attention to prevent disease development (Berendsen et al. 2010).

8 Endophyte *Epicoccum sorghinum* Cultured in Host Mushroom *Thelephora ganbajun* (Ganba Fungus)

Thelephora ganbajun, also called ganba fungus, is a rare wild edible fungus present in Yunnan Province of China. This coral fungus frequently grows on *Pinus yunnanensis* and *Pinus kesiya* var. *langbianensis* (Zang 1987). The extracts prepared by ultrasonic-assisted extraction process in aqueous and ethanol solvents (57.38% ethanol) had the presence of rutin, 2-hydrocinnamic acid and epicatechin. These may have contributed to the high levels of antioxidants and anti-proliferatives (Xu et al. 2016). The anti-cancer potential was evaluated on cancer cell lines of A549 (human lung adenocarcinoma), MCF-7 (malignant breast adenocarcinoma), HepG2 (hepatic carcinoma) and HT-29 (human colon adenocarcinoma). In the absence of non-cancer cell line control from healthy cells like HEK293, it is imperative to

perform a follow-up study to determine if the antiproliferation activity at the given concentrations is specific to cancer cells. Rutin is a citrus flavonoid that targets cancer cells by suppressing pro-inflammatory secretions. It modulates the signalling pathways involving MAPK, PI3K/Akt, epidermal growth factor, Wnt/catenin (Satari et al. 2021), Notch-1 and Hes-1 (Khan et al. 2021) and notch (Singh et al. 2022). Unlike rutin, the role of 2-hydrocinnamic acid is not well elucidated. It was identified to be present in extracts of *Gordonia axillaris* that had anti-proliferative activity in cancer cell lines (Li et al. 2019b). Epicatechin is a pharmaceutically significant molecule and is a component of green tea. It has antioxidant activity (Zhao et al. 2006) and stimulatory effects on splenocytes proliferation (Zhao et al. 2007). In addition, *T. ganbajun* tends to accumulate selenium and zinc, making it a suitable edible agent for overcoming these deficiencies (Zhang et al. 2014b; Zheng et al. 2020a).

Identification of pathways leading to synthesis of bioactive by endophytes has been attempted. Wang et al. cultured *Epicoccum sorghinum* (previously *Phoma sorghina*) in host mushroom *T. ganbajun* containing medium (Wang et al. 2022). In comparison to the media without the mushroom, episorin A and epicosorin A were produced from *E. sorghinum*. Episorin A inhibited production of nitric oxide in lipopolysaccharide-activated macrophages. It was cytotoxic against cancer cell lines of acute myeloid leukaemia HL-60, lung adenocarcinoma A549, breast carcinoma MCF-7, colon carcinoma SW480 and hepatocarcinoma SMMC-7721. Thus, by using some amount of *T. ganbajun* in the media, it is possible to obtain episorin A.

9 Conclusion and Prospects

Bioactive search for treatment of numerous diseases is an urgent need. Endophytes have proven to be a good source for finding such bioactive. Many mushrooms are known for their health benefits. It is possible for them to have some symbiont association in the natural environment. Identification of the presence of various fungal endophytes with omics-based approaches has been limited. Utility of such tools is an urgent requirement for the discovery process. The endophytic fungi present in them could possess the pathways required for bioactive as present in the host mushroom. Successful isolation and cultivation of endophytic fungi is expected to aid in the development of fermentation-based extraction of bioactive. This will not only reduce the dependency on the wild capture of mushrooms but allow the percolation of the precious bioactive in them to a larger population.

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References

Baik JS, Mun SW, Kim KS, Park SJ, Yoon HK, Kim DH, Park MK, Kim CH, Lee YC (2016) Apoptotic effects of cordycepin through the extrinsic pathway and p38 MAPK activation in human glioblastoma U87MG cells. J Microbiol Biotechnol 26:309–314. https://doi.org/10.4014/JMB.1507.07090

Berendsen RL, Baars JJP, Kalkhove SIC, Lugones LG, WöSten HAB, Bakker PAHM (2010) Lecanicillium fungicola: causal agent of dry bubble disease in white-button mushroom. Mol Plant Pathol 11:585–595. https://doi.org/10.1111/J.1364-3703.2010.00627.X

Chaicharoenaudomrung N, Jaroonwitchawan T, Noisa P (2018) Cordycepin induces apoptotic cell death of human brain cancer through the modulation of autophagy. Toxicol In Vitro 46:113–121. https://doi.org/10.1016/J.TIV.2017.10.002

Chang MM, Hong SY, Yang SH, Wu CC, Wang CY, Huang BM (2020) Anti-cancer effect of cordycepin on FGF9-induced testicular tumorigenesis. Int J Mol Sci 21:8336. https://doi.org/10.3390/IJMS21218336

Chen Y, Yang SH, Hueng DY, Syu JP, Liao CC, Wu YC (2014) Cordycepin induces apoptosis of C6 glioma cells through the adenosine 2A receptor-p53-caspase-7-PARP pathway. Chem Biol Interact 216:17–25. https://doi.org/10.1016/J.CBI.2014.03.010

Chen YC, Chen YH, Pan BS, Chang MM, Huang BM (2017) Functional study of Cordyceps sinensis and cordycepin in male reproduction: a review. J Food Drug Anal 25:197. https://doi.org/10.1016/J.JFDA.2016.10.020

Chen B, Liang J, Jiang X, Song J (2022) Morphological characters and molecular phylogeny reveal three new species of subgenus Russula from China. Life (Basel, Switzerland) 12:480. https://doi.org/10.3390/LIFE12040480

Cheng JT, Cheng JT, Cao F, Cao F, Chen XA, Chen XA, Li YQ, Li YQ, Mao XM, Mao XM (2020) Genomic and transcriptomic survey of an endophytic fungus Calcarisporium arbuscula NRRL 3705 and potential overview of its secondary metabolites. BMC Genomics 21:1–13. https://doi.org/10.1186/s12864-020-06813-6

Cho SH, Kang IC (2018) The inhibitory effect of Cordycepin on the proliferation of cisplatin-resistant A549 lung cancer cells. Biochem Biophys Res Commun 498:431–436. https://doi.org/10.1016/J.BBRC.2018.02.188

Choi S, Lim MH, Kim KM, Jeon BH, Song WO, Kim TW (2011) Cordycepin-induced apoptosis and autophagy in breast cancer cells are independent of the estrogen receptor. Toxicol Appl Pharmacol 257:165–173. https://doi.org/10.1016/J.TAAP.2011.08.030

Dastogeer KMG (2018) Influence of fungal endophytes on plant physiology is more pronounced under stress than well-watered conditions: a meta-analysis. Planta 248:1403–1416. https://doi.org/10.1007/s00425-018-2982-y

Deng Q, Li X, Fang C, Li X, Zhang J, Xi Q, Li Y, Zhang R (2022) Cordycepin enhances anti-tumor immunity in colon cancer by inhibiting phagocytosis immune checkpoint CD47 expression. Int Immunopharmacol 107:108695. https://doi.org/10.1016/J.INTIMP.2022.108695

Fu Z, Liu G, Wang L (2020) Assessment of potential human health risk of trace element in wild edible mushroom species collected from Yunnan Province, China. Environ Sci Pollut Res Int 27:29218–29227. https://doi.org/10.1007/S11356-020-09242-W

Guo Z, Chen W, Dai G, Huang Y (2020) Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. Int J Mol Med 45:141–150. https://doi.org/10.3892/IJMM.2019.4391

He P, Geng L, Mao D, Xu C (2012) Production, characterization and antioxidant activity of exopolysaccharides from submerged culture of Morchella crassipes. Bioprocess Biosyst Eng 35:1325–1332. https://doi.org/10.1007/S00449-012-0720-6/FIGURES/5

Huang TC, Chang HY, Hsu CH, Kuo WH, Chang KJ, Juan HF (2008) Targeting therapy for breast carcinoma by ATP synthase inhibitor aurovertin b. J Proteome Res 7:1433–1444. https://doi.org/10.1021/pr700742h

Hwang J-H, Joo JC, Kim DJ, Jo E, Yoo H-S, Lee K-B, Park SJ, Jang I-S (2016) Cordycepin promotes apoptosis by modulating the ERK-JNK signaling pathway via DUSP5 in renal cancer cells. Am J Cancer Res 6:1758

Hwang IH, Oh SY, Jang HJ, Jo E, Joo JC, Lee KB, Yoo HS, Lee MY, Park SJ, Jang IS (2017a) Cordycepin promotes apoptosis in renal carcinoma cells by activating the MKK7-JNK signaling pathway through inhibition of c-FLIPL expression. PLoS One 12:e0186489. https://doi.org/10.1371/JOURNAL.PONE.0186489

Hwang JH, Park SJ, Ko WG, Kang SM, Lee DB, Bang J, Park BJ, Wee CB, Kim DJ, Jang IS, Ko JH (2017b) Cordycepin induces human lung cancer cell apoptosis by inhibiting nitric oxide mediated ERK/Slug signaling pathway. Am J Cancer Res 7:417

Jeong JW, Jin CY, Park C, Hong SH, Kim GY, Jeong YK, Lee JD, Yoo YH, Choi YH (2011) Induction of apoptosis by cordycepin via reactive oxygen species generation in human leukemia cells. Toxicol In Vitro 25:817–824. https://doi.org/10.1016/J.TIV.2011.02.001

Jo E, Jang HJ, Shen L, Yang KE, Jang MS, Huh YH, Yoo HS, Park J, Jang IS, Park SJ (2020) Cordyceps militaris exerts anticancer effect on non-small cell lung cancer by inhibiting hedgehog signaling via suppression of TCTN3. Integr Cancer Ther 19:153473542092375. https://doi.org/10.1177/1534735420923756

Johnson LJ, De Bonth ACM, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ, Tapper BA, Simpson WR, Voisey CR, Card SD (2013) The exploitation of epichloae endophytes for agricultural benefit. Fungal Divers 60:171–188. https://doi.org/10.1007/s13225-013-0239-4

Joo JC, Hwang JH, Jo E, Kim YR, Kim DJ, Lee KB, Park SJ, Jang IS (2017) Cordycepin induces apoptosis by caveolin-1-mediated JNK regulation of Foxo3a in human lung adenocarcinoma. Oncotarget 8:12211–12224. https://doi.org/10.18632/ONCOTARGET.14661

Khan F, Pandey P, Jha NK, Khalid M, Ojha S (2021) Rutin mediated apoptotic cell death in Caski cervical cancer cells via notch-1 and hes-1 downregulation. Life 11:761. https://doi.org/10.3390/life11080761

Kim H, Naura AS, Errami Y, Ju J, Boulares AH (2011) Cordycepin blocks lung injury-associated inflammation and promotes BRCA1-deficient breast cancer cell killing by effectively inhibiting PARP. Mol Med 17:893–900. https://doi.org/10.2119/MOLMED.2011.00032

Kim SO, Cha HJ, Park C, Lee H, Hong SH, Jeong SJ, Park SH, Kim GY, Leem SH, Jin CY, Hwang EJ, Choi YH (2019) Cordycepin induces apoptosis in human bladder cancer T24 cells through ROS-dependent inhibition of the PI3K/Akt signaling pathway. Biosci Trends 13:324–333. https://doi.org/10.5582/BST.2019.01214

Ko BS, Lu YJ, Yao WL, Liu TA, Tzean SS, Shen TL, Liou JY (2013) Cordycepin regulates GSK-3β/β-catenin signaling in human leukemia cells. PLoS One 8:e76320. https://doi.org/10.1371/JOURNAL.PONE.0076320

Lan L, Wang S, Duan S, Zhou X, Li Y (2022) Cordyceps militaris carotenoids protect human retinal endothelial cells against the oxidative injury and apoptosis resulting from H2O2. Evid Based Complement Alternat Med 2022:1–12. https://doi.org/10.1155/2022/1259093

Le Guo D, Li XH, Feng D, Jin MY, Cao YM, Cao ZX, Gu YC, Geng Z, Deng F, Deng Y (2018) Novel polyketides produced by the endophytic fungus Aspergillus Fumigatus from Cordyceps Sinensis. Molecules 23:1709. https://doi.org/10.3390/molecules23071709

Le Guo D, Qiu L, Feng D, He X, Li XH, Cao ZX, Gu YC, Mei L, Deng F, Deng Y (2019) Three new α-pyrone derivatives induced by chemical epigenetic manipulation of Penicillium herquei, an endophytic fungus isolated from Cordyceps sinensis. Nat Prod Res 34:958–964. https://doi.org/10.1080/14786419.2018.1544974

Lee SJ, Kim SK, Choi WS, Kim WJ, Moon SK (2009) Cordycepin causes p21WAF1-mediated G2/M cell-cycle arrest by regulating c-Jun N-terminal kinase activation in human bladder cancer cells. Arch Biochem Biophys 490:103–109. https://doi.org/10.1016/J.ABB.2009.09.001

Lee SJ, Moon GS, Jung KH, Kim WJ, Moon SK (2010) c-Jun N-terminal kinase 1 is required for cordycepin-mediated induction of G2/M cell-cycle arrest via p21WAF1 expression in human colon cancer cells. Food Chem Toxicol 48:277–283. https://doi.org/10.1016/J.FCT.2009.09.042

Lee HJ, Burger P, Vogel M, Friese K, Brüning A (2012) The nucleoside antagonist cordycepin causes DNA double strand breaks in breast cancer cells. Investig New Drugs 30:1917–1925. https://doi.org/10.1007/S10637-012-9859-X

Lee SY, Debnath T, Kim SK, Lim BO (2013) Anti-cancer effect and apoptosis induction of cordycepin through DR3 pathway in the human colonic cancer cell HT-29. Food Chem Toxicol 60:439–447. https://doi.org/10.1016/J.FCT.2013.07.068

Lee M-J, Lee J-C, Hsieh J-H, Lin M-Y, Shih I-A, You H-L, Wang K (2021) Cordycepin inhibits the proliferation of malignant peripheral nerve sheath tumor cells through the p53/Sp1/tubulin pathway. Am J Cancer Res 11:1247

Li Y, Li R, Zhu S, Zhou R, Wang L, Du J, Wang Y, Zhou B, Mai L (2015) Cordycepin induces apoptosis and autophagy in human neuroblastoma SK-N-SH and BE(2)-M17 cells. Oncol Lett 9:2541–2547. https://doi.org/10.3892/OL.2015.3066

Li Q, Chen C, Penttinen P, Xiong C, Zheng L, Huang W (2016a) Microbial diversity associated with Tricholoma matsutake fruiting bodies. Microbiol (Russian Fed) 85:531–539. https://doi.org/10.1134/S0026261716050106

Li Q, Zhang L, Li W, Li X, Huang W, Yang H, Zheng L (2016b) Chemical compositions and volatile compounds of Tricholoma matsutake from different geographical areas at different stages of maturity. Food Sci Biotechnol 25:71–77. https://doi.org/10.1007/s10068-016-0010-1

Li XH, Han XH, Qin LL, He JL, Cao ZX, Gu YC, Le Guo D, Deng Y (2019a) Isochromanes from Aspergillus fumigatus, an endophytic fungus from Cordyceps sinensis. Nat Prod Res 33:1870–1875. https://doi.org/10.1080/14786419.2018.1478824

Li Y, Cao SY, Lin SJ, Zhang JR, Gan RY, Li HB (2019b) Polyphenolic profile and antioxidant capacity of extracts from Gordonia axillaris fruits. Antioxidants 8:1–14. https://doi.org/10.3390/antiox8060150

Li XY, Tao H, Jin C, Du ZY, Liao WF, Tang QJ, Ding K (2020) Cordycepin inhibits pancreatic cancer cell growth in vitro and in vivo via targeting FGFR2 and blocking ERK signaling. Chin J Nat Med 18:345–355. https://doi.org/10.1016/S1875-5364(20)30041-8

Li GJ, Li SM, Buyck B, Zhao SY, Xie XJ, Shi LY, Deng CY, Meng QF, Sun QB, Yan JQ, Wang J, Li M (2021) Three new Russula species in sect. Ingratae (Russulales, Basidiomycota) from southern China. MycoKeys 84:103–139. https://doi.org/10.3897/MYCOKEYS.84.68750

Liang SM, Lu YJ, Ko BS, Jan YJ, Shyue SK, Yet SF, Liou JY (2017) Cordycepin disrupts leukemia association with mesenchymal stromal cells and eliminates leukemia stem cell activity. Sci Rep 7:43930. https://doi.org/10.1038/SREP43930

Liu Z, Leng G, Wen J, Deng G, Jiang J (2022) Cordycepin production by a novel endophytic fungus Irpex lacteus CHG05 isolated from Cordyceps hawkesii Gray. Folia Microbiol (Praha) 10:851. https://doi.org/10.1007/s12223-022-00981-6

Looney BP, Meidl P, Piatek MJ, Miettinen O, Martin FM, Matheny PB, Labbé JL (2018) Russulaceae: a new genomic dataset to study ecosystem function and evolutionary diversification of ectomycorrhizal fungi with their tree associates. New Phytol 218:54–65. https://doi.org/10.1111/NPH.15001

May TW (2017) Report of the nomenclature Committee for Fungi – 20. IMA Fungus 8:189. https://doi.org/10.5598/IMAFUNGUS.2017.08.01.12

Miao M, Yu WQ, Li Y, Sun YL, Guo SD (2022) Structural elucidation and activities of Cordyceps militaris-derived polysaccharides: a review. Front Nutr 9:898674. https://doi.org/10.3389/FNUT.2022.898674

Ngieng NS, Zulkharnain A, Roslan HA, Husaini A (2013) Decolourisation of synthetic dyes by endophytic fungal flora isolated from Senduduk Plant (Melastoma malabathricum). ISRN Biotechnol 2013:1–7. https://doi.org/10.5402/2013/260730

Park KH, Oh SY, Yoo S, Park MS, Fong JJ, Lim YW (2020) Successional change of the fungal microbiome pine seedling roots inoculated with Tricholoma matsutake. Front Microbiol 11:574146. https://doi.org/10.3389/fmicb.2020.574146

Pei-xin H, Wei L, Xin-sheng H, Lu-jing G (2014) Diversity of endophytic fungi in ascocarps of Morchella crassipes. J Zhengzhou Univ Light Ind (Natural Sci Ed) 29:1–6

Phull AR, Ahmed M, Park HJ (2022) Cordyceps militaris as a bio functional food source: pharmacological potential, anti-inflammatory actions and related molecular mechanisms. Microorganisms 10:405. https://doi.org/10.3390/MICROORGANISMS10020405

Rai N, Keshri PK, Gupta P, Verma A, Kamble SC, Singh SK, Gautam V (2021a) Isolation and characterization of bioactive compounds derived from fungal endophytes associated with Oroxylum indicum (L.) Kurz. Front Pharmacol

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S, Kumar Singh S, Gautam V (2021b) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycol An Int J Fungal Biol 12:139–159. https://doi.org/10.1080/21501203.2020.1870579

Ren M, Ye L, Hao X, Ren Z, Ren S, Xu K, Li J (2014) Polysaccharides from Tricholoma matsutake and Lentinus edodes enhance 5-fluorouracil-mediated H22 cell growth inhibition. J Tradit Chinese Med 34:309–316. https://doi.org/10.1016/s0254-6272(14)60095-9

Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim SH (2018) Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. Microb Ecol 75:407–418. https://doi.org/10.1007/s00248-017-1054-3

Riva V, Mapelli F, Bagnasco A, Mengoni A, Borin S (2019) A meta-analysis approach to defining the culturable core of plant endophytic bacterial communities. Appl Environ Microbiol 88:1–10

Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z (2021) Rutin: a flavonoid as an effective sensitizer for anticancer therapy; insights into multifaceted mechanisms and applicability for combination therapy. Evid Based Complement Alternat Med 2021:1. https://doi.org/10.1155/2021/9913179

Shao LW, Huang LH, Yan S, Di Jin J, Ren SY (2016) Cordycepin induces apoptosis in human liver cancer HepG2 cells through extrinsic and intrinsic signaling pathways. Oncol Lett 12:995–1000. https://doi.org/10.3892/OL.2016.4706

Shin S, Lee S, Kwon J, Moon S, Lee S, Lee C-K, Cho K, Ha N-J, Kim K (2009) Cordycepin suppresses expression of diabetes regulating genes by inhibition of lipopolysaccharide-induced inflammation in macrophages. Immune Netw 9:98–105. https://doi.org/10.4110/IN.2009.9.3.98

Singh AK, Shuaib M, Prajapati KS, Kumar S (2022) Rutin potentially binds the gamma secretase catalytic site, down regulates the notch signaling pathway and reduces sphere formation in colonospheres. Meta 12:926. https://doi.org/10.3390/metabo12100926

Tao X, Ning Y, Zhao X, Pan T (2016) The effects of cordycepin on the cell proliferation, migration and apoptosis in human lung cancer cell lines A549 and NCI-H460. J Pharm Pharmacol 68:901–911. https://doi.org/10.1111/JPHP.12544

Tian T, Song L, Zheng Q, Hu X, Yu R (2014) Induction of apoptosis by Cordyceps militaris fraction in human chronic myeloid leukemia K562 cells involved with mitochondrial dysfunction. Pharmacogn Mag 10:325–331. https://doi.org/10.4103/0973-1296.137374

Wang L, Chen W, Feng Y, Ren Y, Gu Z, Chen H, Wang H, Thomas MJ, Zhang B, Berquin IM, Li Y, Wu J, Zhang H, Song Y, Liu X, Norris JS, Wang S, Du P, Shen J, Wang N, Yang Y, Wang W, Feng L, Ratledge C, Zhang H, Chen YQ (2011) Genome characterization of the oleaginous fungus Mortierella alpina. PLoS One 6:e28319. https://doi.org/10.1371/JOURNAL.PONE.0028319

Wang Y, Chen Y, Zhang X, Cai G, An S, Wang X, Teng L, Wang D (2016) Tricholoma matsutake aqueous extract induces hepatocellular carcinoma cell apoptosis via caspase-dependent mitochondrial pathway. Biomed Res Int 2016:11. https://doi.org/10.1155/2016/9014364

Wang Y, Lv Y, Liu TS, Di Yan W, Chen LY, Li ZH, Piao YS, An RB, Lin ZH, Ren XS (2019) Cordycepin suppresses cell proliferation and migration by targeting CLEC2 in human gastric cancer cells via Akt signaling pathway. Life Sci 223:110–119. https://doi.org/10.1016/J.LFS.2019.03.025

Wang CY, Tsai SW, Chien HH, Chen TY, Sheu SY, So EC, Huang BM (2020) Cordycepin inhibits human gestational choriocarcinoma cell growth by disrupting centrosome homeostasis. Drug Des Devel Ther 14:2987–3000. https://doi.org/10.2147/DDDT.S252401

Wang CF, Yang XQ, Shi WZ, Long X, Su S, Cen RH, Yang YB, Ding ZT (2022) The production of broad-spectrum antibiotics from phytopathogen Epicoccum sorghinum by culturing in

host edible mushroom Thelephora ganbajun extract. Phytochemistry 200:113221. https://doi.org/10.1016/J.PHYTOCHEM.2022.113221

Wei C, Yao X, Jiang Z, Wang Y, Zhang D, Chen X, Fan X, Xie C, Cheng J, Fu J, Leung ELH (2019) Cordycepin inhibits drug-resistance non-small cell lung cancer progression by activating AMPK signaling pathway. Pharmacol Res 144:79–89. https://doi.org/10.1016/J.PHRS.2019.03.011

Wei C, Khan MA, Du J, Cheng J, Tania M, Leung ELH, Fu J (2022) Cordycepin inhibits triple-negative breast cancer cell migration and invasion by regulating EMT-TFs SLUG, TWIST1, SNAIL1, and ZEB1. Front Oncol 12:898583. https://doi.org/10.3389/FONC.2022.898583

Wilson D (1995) Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73:274. https://doi.org/10.2307/3545919

Wisitrassameewong K, Manz C, Hampe F, Looney BP, Boonpratuang T, Verbeken A, Thummarukcharoen T, Apichitnaranon T, Pobkwamsuk M, Caboň M, Adamčík S (2022) Two new Russula species (fungi) from dry dipterocarp forest in Thailand suggest niche specialization to this habitat type. Sci Rep 12:2826. https://doi.org/10.1038/S41598-022-06836-X

Wu WC, Hsiao JR, Lian YY, Lin CY, Huang BM (2007) The apoptotic effect of cordycepin on human OEC-M1 oral cancer cell line. Cancer Chemother Pharmacol 60:103–111. https://doi.org/10.1007/S00280-006-0354-Y

Wu W, Li X, Qi M, Hu X, Cao F, Wu X, Fu J (2022) Cordycepin inhibits growth and metastasis formation of MDA-MB-231 xenografts in nude mice by modulating the hedgehog pathway. Int J Mol Sci 23:10362. https://doi.org/10.3390/IJMS231810362

Xu DP, Zheng J, Zhou Y, Li Y, Li S, Li HB (2016) Extraction of natural antioxidants from the Thelephora ganbajun mushroom by an ultrasound-assisted extraction technique and evaluation of antiproliferative activity of the extract against human cancer cells. Int J Mol Sci 17:1664. https://doi.org/10.3390/IJMS17101664

Xu JC, Zhou XP, Wang XA, Xu MD, Chen T, Chen TY, Zhou PH, Zhang YQ (2019) Cordycepin induces apoptosis and G2/M phase arrest through the ERK pathways in esophageal cancer cells. J Cancer 10:2415–2424. https://doi.org/10.7150/JCA.32071

Yadav AN, Kour D, Kaur T, Devi R, Yadav A (2022) Endophytic fungal communities and their biotechnological implications for agro-environmental sustainability. Folia Microbiol (Praha) 67:203–232. https://doi.org/10.1007/S12223-021-00939-0

Yamamoto K, Shichiri H, Uda A, Yamashita K, Nishioka T, Kume M, Makimoto H, Nakagawa T, Hirano T, Hirai M (2015) Apoptotic effects of the extracts of Cordyceps militaris via Erk phosphorylation in a renal cell carcinoma cell line. Phytother Res 29:707–713. https://doi.org/10.1002/PTR.5305

Yao W-L, Ko B-S, Liu T-A, Liang S-M, Liu C-C, Lu Y-J, Tzean S-S, Shen T-L, Liou J-Y (2014) Cordycepin suppresses integrin/FAK signaling and epithelial-mesenchymal transition in hepatocellular carcinoma. Anti Cancer Agents Med Chem 14:29–34. https://doi.org/10.2174/18715206113139990305

Yu X, Ling J, Liu X, Guo S, Lin Y, Liu X, Su L (2017) Cordycepin induces autophagy-mediated c-FLIPL degradation and leads to apoptosis in human non-small cell lung cancer cells. Oncotarget 8:6691–6699. https://doi.org/10.18632/ONCOTARGET.14262

Zang M (1987) Some new and noteworthy higher fungi from eastern Himalayans. Acta Bot Yunnanica 9:8

Zeng Y, Lian S, Li D, Lin X, Chen B, Wei H, Yang T (2017) Anti-hepatocarcinoma effect of cordycepin against NDEA-induced hepatocellular carcinomas via the PI3K/Akt/mTOR and Nrf2/HO-1/NF-κB pathway in mice. Biomed Pharmacother 95:1868–1875. https://doi.org/10.1016/J.BIOPHA.2017.09.069

Zhang G, Huang Y, Bian Y, Wong JH, Ng TB, Wang H (2006) Hypoglycemic activity of the fungi Cordyceps militaris, Cordyceps sinensis, Tricholoma mongolicum, and Omphalia lapidescens in streptozotocin-induced diabetic rats. Appl Microbiol Biotechnol 72:1152–1156. https://doi.org/10.1007/S00253-006-0411-9/FIGURES/3

Zhang HW, Lin ZX, Tung YS, Kwan TH, Mok CK, Leung C, Chan LS (2014a) Cordyceps sinensis (a traditional Chinese medicine) for treating chronic kidney disease. Cochrane Database Syst Rev 2014:CD008353. https://doi.org/10.1002/14651858.CD008353.PUB2

Zhang P, Luo XY, Wu Y, Chen YM, Xu WJ, Yi XX (2014b) Optimization of submerged fermentation of Thelephora ganbajun zang. J Basic Microbiol 54:866–872. https://doi.org/10.1002/JOBM.201200757

Zhang P, Huang C, Fu C, Tian Y, Hu Y, Wang B, Strasner A, Song Y, Song E (2015) Cordycepin (3′-deoxyadenosine) suppressed HMGA2, Twist1 and ZEB1-dependent melanoma invasion and metastasis by targeting miR-33b. Oncotarget 6:9834–9853. https://doi.org/10.18632/ONCOTARGET.3383

Zhang Y, Zhang XX, Yuan RY, Ren T, Shao ZY, Wang HF, Cai WL, Chen LT, Wang XA, Wang P (2018) Cordycepin induces apoptosis in human pancreatic cancer cells via the mitochondrial-mediated intrinsic pathway and suppresses tumor growth in vivo. Onco Targets Ther 11:4479–4490. https://doi.org/10.2147/OTT.S164670

Zhang XM, Tang DX, Li QQ, Wang YB, Xu ZH, Li WJ, Yu H (2021) Complex microbial communities inhabiting natural Cordyceps militaris and the habitat soil and their predicted functions. Antonie van Leeuwenhoek. Int J Gen Mol Microbiol 114:465–477. https://doi.org/10.1007/s10482-021-01534-6

Zhang Z, Li K, Zheng Z, Liu Y (2022) Cordycepin inhibits colon cancer proliferation by suppressing MYC expression. BMC Pharmacol Toxicol 23:12. https://doi.org/10.1186/S40360-022-00551-Z

Zhao M, Yang B, Wang J, Li B, Jiang Y (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chem 98:539–544. https://doi.org/10.1016/j.foodchem.2005.06.028

Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y (2007) Immunomodulatory and anticancer activities of flavonoids extracted from litchi (Litchi chinensis Sonn.) pericarp. Int Immunopharmacol 7:162–166. https://doi.org/10.1016/j.intimp.2006.09.003

Zhao M, Chen L, Chen W, Meng Z, Hu K, Du S, Zhang L, Yin L, Wu B, Guan YQ (2017) Packaging cordycepin phycocyanin micelles for the inhibition of brain cancer. J Mater Chem B 5:6016–6026. https://doi.org/10.1039/C7TB00994A

Zheng L, Ma Y, Zhang Y, Meng Q, Yang J, Gong W, Liu Q, Cai L, Hao L, Wang B, Yang Y (2020a) Distribution of zinc in mycelial cells and antioxidant and anti-inflammatory activities of mycelia zinc polysaccharides from Thelephora ganbajun TG-01. Oxidative Med Cell Longev 2020:1. https://doi.org/10.1155/2020/2308017

Zheng Q, Sun J, Li W, Li S, Zhang K (2020b) Cordycepin induces apoptosis in human tongue cancer cells in vitro and has antitumor effects in vivo. Arch Oral Biol 118:104846. https://doi.org/10.1016/J.ARCHORALBIO.2020.104846

Zhou Y, Guo Z, Meng Q, Lu J, Wang N, Liu H, Liang Q, Quan Y, Wang D, Xie J (2017) Cordycepin affects multiple apoptotic pathways to mediate hepatocellular carcinoma cell death. Anti Cancer Agents Med Chem 17:143–149. https://doi.org/10.2174/1871520616666160526114555

Zhou J, Gui H, Yang S, Yang X, Shi L (2021) Fungal interactions matter: Tricholoma matsutake domination affect fungal diversity and function in mountain forest soils. Biology (Basel) 10:1–13. https://doi.org/10.3390/biology10101051

Chapter 5
Biological Synthesis of Nanoparticles from Fungal Endophytes and Their Application in Pharmaceutical Industries

Kaizar J. Bharmal, Urvi R. Parlikar, and Joyita Sarkar

Abstract Metal-based nanoparticles have extensive applications in pharmaceutical sector like antimicrobial, cancer theranostics, wound healing, etc. However, there is always ever-growing need for development of sustainable, clean, eco-friendly, and nontoxic synthetic approaches. Owing to the bioaccumulation, tolerance, and ability to reduce metal ions, an endophytic fungus has emerged as potential candidate for mycosynthesis of nanoparticles. Endophytes isolated from different parts have been extensively used for synthesis of plethora of metal nanoparticles like silver, zinc, gold, copper, iron, cobalt, etc. Both intracellular (using fungal mycelia) and extracellular (using enzymes in cell-free filtrate) have been employed for this purpose. The nanoparticles thus fabricated exhibited excellent pharmaceutical properties like broad-spectrum antibacterial and antifungal (even for multidrug-resistant strains), antioxidant, anticancer, and wound healing properties, both in vitro and in vivo. In this chapter, we have attempted to summarize the recent advances in endophytic synthesis of metal nanoparticles with focus on their properties obtained and potential application in pharmaceutics. We have also tried to identify the limitations and scope for further improvement in the aforementioned area.

Keywords Endophytes · Metal nanoparticles · Mycosynthesis · Pharmaceutical application

K. J. Bharmal · U. R. Parlikar · J. Sarkar (✉)
Institute of Chemical Technology Marathwada Campus, Jalna, Maharashtra, India
e-mail: j.sarkar@marj.ictmumbai.edu.in

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1 Introduction

Particles less than 100 nm size are referred as nanoparticles which could be of various shapes like spherical (with or without core shell), rods, or cages. They have attained immense applications in several fields like pharmaceutics, biosensors, drug delivery, catalysts, semiconductors, etc. Based on the synthesis material, nanoparticles could be predominantly classified as carbon, metal, ceramic, and polymer and lipid-based. The fabrication techniques of these particles are either top-down or bottom-up. In the case of top-down approaches, larger substances (like naturally occurring iron oxide) are fragmented and disintegrated to generate the nanoparticles, whereas in bottom-up approaches, compounds like salts, etc. are used to synthesize the particle (Mogilevsky et al. 2014; Iravani 2011). The conventional manufacture of nanoparticles is bound with known connotations as higher operating prices, non-environment-friendly, higher energy consumption, use of toxic chemicals, and lower yield. Hence, there is always an ever-growing need for development of sustainable, clean, eco-friendly, and nontoxic synthetic approaches (Khan et al. 2017).

Biosynthesis or green synthesis is one such approach, and currently, endophyte-based biosynthesis of nanoparticles is an emerging field (Nayantara and Kaur 2018). Research on endophytes is gaining popularity, as they are a rich source of novel, natural products that find numerous applications in the pharmaceutical and agricultural sectors (Manganyi and Ateba 2020). Endophytes are living organisms, generally fungi or bacteria, colonizing inside plant cells (Packiam and Dhakshinamoorthy 2021; Suresh and Sona 2021; Swamy and Sandhu 2021). They often nurture a symbiotic relation with the flora, without causing any mutilation or disease to the plant. The term endophyte (Gr. endon, within; python, plant) was originally coined by de Bary in 1866 (Staniek et al. 2008). They are being highly recognized as important sources of new effective secondary metabolites with antimicrobial, antioxidant, anticancer, and various biological activities (Bose and Sezhian 2017). Endophyte has the potential to uptake, tolerate, and accumulate metals that impart the property to synthesize nanoparticles by reduction of metallic ions (Mathur et al. 2021). Table 5.1 provides an overview of different nanoparticles synthesized using endophytes. Biosynthesis mediated by endophytic fungi is earning high popularity in the scientific arena due to its simplicity, rapid rate of synthesis, and eco-friendliness. In this chapter, we have presented the recent developments on the endophytic synthesis of various nanoparticles and their uses in pharmaceutical sector. We have also discussed the limitations and challenges of current approaches as well as scope for further improvement and concerns that need more attention.

2 Fabrication of Nanoparticles Using Endophytes

Bottom-up and top-down approaches for synthesis of nanoparticles further encompass physical, chemical, and biological methods. The physical methods include ball milling, electrochemical synthesis, microwave-assisted synthesis, and ultrasonication, while the chemical synthesis methods include sol-gel synthesis, chemical

Table 5.1 List of nanoparticles synthesized using endophytes and their applications

Sr. no	Nanoparticles and its precursor	Endophytes used for synthesis	Applications	Reference
1.	Cobalt tetroxide (Co_3O_4) *Precursor:* Cobalt sulfate ($COSO_4.7H_2O$)	*Aspergillus terreus*	Antioxidant and antimicrobial activities	Rai et al. (2022)
2.	Nickel oxide (NiO) *Precursor: Nickel sulfate ($NiSO_4.6H_20$)*			
3.	Copper oxide (CuO) *Precursor*: Copper sulfate ($CuSO_4.5H_2O$)			
4.	Ferrosoferric oxide (Fe_2O_4) *Precursor*: *ferric nitrate ($Fe(NO_3)_3$)*			
5.	Zinc oxide (ZnO) *Precursor:* zinc acetate ($ZnC_4H_6O_4.7H_2O$)			
6.	Cobalt oxide (CoO) *Precursor:* cobalt acetylacetonate ($C_{10}H_{14}CoO_4$)	*Aspergillus nidulans*	Antimicrobial, anticancer, antidiabetic, anticholinergic, antifungal activities	Vijayanandan and Balakrishnan (2018), Waris et al. (2021)
7.	Zinc oxide (ZnO) *Precursor*: zinc acetate ($ZnC_4H_6O_4$)	*Cochliobolus geniculata*		Kadam et al. (2019)
8.	Gold (Au) *Precursor:* auric chloride ($AuCl_3$)	*Penicillium citrinum*	Drug development	Manjunath et al. (2017)
9.	Iron (Fe) *Precursor*: ferrous sulfate ($FeSO_4$)	*Penicillium oxalicum*	Dye decolorization	Mathur et al. (2021)
10.	Silver (Ag) *Precursor*: silver nitrate ($AgNO_3$)	*Alternaria* sp.	Antimicrobial, anticancer, antibacterial activities	Singh et al. (2017)
		Nemania sp.		Mohammad and Farokhi (2018)
		Lasiodiplodia theobromae		Ranjani et al. (2021)
		Trichoderma atroviride		Ahmed et al. (2020)
		Pestalotia sp.		Verma et al. (2016)
		Talaromyces purpureogenus		Hu et al. (2019)
		Exserohilum rostratum		Bagur et al. (2020)
11.	ZnS:Gd *Precursor*: zinc sulfate ($ZnSO_4.7H_2O$), gadolinium nitrate $Gd(NO_3)_3$	*Aspergillus flavus*	Metal-based detection (Sensor)	Uddandarao et al. (2019)

(continued)

Table 5.1 (continued)

Sr. no	Nanoparticles and its precursor	Endophytes used for synthesis	Applications	Reference
12.	TiO_2 *Precursor*: Titanium trichloride ($TiCl_3$)	*Bacillus cereus*	Antimicrobial activity	Peiris et al. (2018)
13.	Sulfur and aluminum oxide *Precursor*: sodium thiosulfate ($Na_2S_2O_3$), aluminum chloride ($AlCl_3$)	*Colletotrichum* sp.	Activity against food borne pathogens	Suryavanshi et al. (2017)
14.	Cadmium sulfide (CdS) *Precursor*: cadmium sulfate ($CdSO_4$)	*Fusarium oxysporum*	Optoelectronic applications (biological indicators, photo catalysts)	Ahmad et al. (2002)

reduction, condensation, coprecipitation, and spray pyrolysis. The physical methods applied in nanoparticle synthesis are expensive, while chemical synthesis is expensive as well as requires the use of toxic and hazardous chemicals that cause detrimental effects to the environment and living beings. Furthermore, these methods tend to produce low yields of nanoparticles. To overcome these drawbacks, the use of biological resources to synthesize nanoparticles has been explored. The biological method is majorly employed for synthesis of metal or metal oxide nanoparticles using metal salts as precursors. It is basically a bottom-up technique that utilizes the reducing power of bacteria, algae, fungi, actinomycetes, and other flora for the formation of nanoparticles (Ealias and Saravanakumar 2017). This revolutionized and encouraged research in "green synthesis" of nanomaterials using biological components. This process has proven to be extremely economical, eco-friendly, and rapid (Ahmed et al. 2016; Bhargava et al. 2013). Biosynthesis of nanoparticles involves various biological entities, right from basic prokaryotic bacteria to complex eukaryotic fungi and plants, especially microbes like yeast, fungi, bacteria, and algae are known to accumulate and absorb metals which can be utilized for metal recovery and reduction of pollution (Baker and Satish 2012).

Using fungi instead of other biological options for biosynthesis makes sense as fungi are eukaryotic and easier to culture and maintain/handle in a laboratory. They generate substantial quantities of extracellular enzymes that help reduce metal ions aiding in rapid synthesis of nanoparticles. They also synthesize proteins that help in nanoparticle coating/capping making them more stable and prevent agglomeration (Mariana and de Lima 2019; Netala et al. 2016). For the biogenesis of nanoparticles using fungi, the fungal mycelia (biomass) and cell-free filtrates have been a source for biological molecules that help reduce metal ions. Depending on the source, two mechanisms of synthesis have been elucidated, viz., the intracellular route and the extracellular route. The extracellular route of synthesis uses biological components that the fungus secretes, whereas intracellular synthesis route uses wet mycelia and cytoplasmic/cell membrane biomolecules/enzymes involving the internalization/endocytosis of the metal ions leading to their reduction and subsequent nanoparticle

formation (Messaoudi and Bendahou 2020; Moghaddam et al. 2015). In general, the biosynthesis of nanoparticles using fungi is relatively simple. The fungi are grown on a suitable media under appropriate temperature and incubation period; the obtained fungal biomass is washed with sterilized distilled water to get rid of growth media contaminants. The actively growing biomass is resuspended in deionized water. Metal salts are added to the resuspended wet mycelia for nanoparticle synthesis via intracellular route. For extracellular route, the mycelia are filtered, and the resulting cell-free filtrate is subjected to metal salts for reduction (Du et al. 2011; Kumar et al. 2020).

The exact mechanism for intracellular and extracellular synthesis is not completely understood yet; however, there are findings that try to elucidate the possible mechanisms. It was believed that one of the extracellular mechanisms for silver nanoparticle synthesis by fungi was based on NADH-dependent nitrate reductase enzymes (Zomorodian et al. 2016); however, a new study suggests that NADPH alone is capable of producing silver nanoparticles without the use of specific enzymes (Hietzschold et al. 2019). Another study specific for gold nanoparticles demonstrated the possibility of intracellular synthesis of nanoparticles by using disrupted fungal cellular extracts; this helped in reducing gold ions by molecules such as amino acids, glucose, and other cofactors (all less than 3 kDa). The nanoparticles were seen to be capped by larger proteins (more than 3 kDa); this study even proposed the possibility of growth media remnants having an effect on reducing the metal ion. Processed mycelia thoroughly washed failed to produce nanoparticles by the extracellular route (Molnár et al. 2018). A report explained the manufacture of nickel oxide nanoparticles using dead fungal biomass of *Hypocrea lixii* by both extracellular and intracellular mechanisms. TEM and HRTEM characterization of nickel oxide nanoparticles showed the presence of both extracellular and intracellular nanoparticles in the dead fungal mycelia. The nanoparticles were capped by proteins and peptides (Salvadori et al. 2015). Zhang et al. (2011) presented a report on intracellular synthesis of gold nanoparticles by three fungi, *A. pullulans*, *Fusarium* sp., and *F. oxysporum*. TEM images, Bradford method, and DNSA assay suggested that *A. pullulans* produced nanoparticles with the aid of reducing sugars, whereas *Fusarium* sp. and *F. oxysporum* through protein. LC-MS/MS data showed three proteins, viz., plasma membrane ATPase (100 kDa), β-1, 3-glucan-binding protein (25 kDa), and glyceraldehyde-3-phosphate dehydrogenase (19 kDa), were responsible for nanoparticle synthesis by *Fusarium* sp.

Fabrication of metal nanoparticles using endophytes involves a general strategy, viz., sample collection (collection of part of the plant like shoot, bark, leaves, etc. which contains the required endophyte); endophyte isolation from the sample collected; biomass preparation, where freshly prepared culture of the isolate is inoculated and prepared at the required conditions. For synthesis via intracellular route, the metal salt is directly added to the harvested mycelia, whereas for extracellular route, first an extract/filtrate is prepared from the biomass, and the metal salt is added to the filtrate (Mohanta and Behera 2014). Many factors, such as pH, temperature, precursor concentration, and composition of the culture media, affect the synthesis time, yield, size, and stability of the metal nanoparticles. However, the

effects vary according to the fungal species used and are not conclusive to draw a pattern (Mariana and de Lima 2019).

2.1 Fabrication of Silver Nanoparticles

Silver nanoparticles (AgNPs) are being widely used in various fields like biomedicine, pharmacy, biotechnology, pathology, and many others. AgNPs are known for their anticancer, antifungal, anti-inflammatory, and antibacterial characteristics. Several studies have been conducted regarding synthesis of silver nanoparticles using different species of endophytes. The transmission electron microscope (TEM) image of AgNPs is shown in Fig. 5.1a (Elbahnasawy et al. 2021). AgNPs were mycosynthesized using *Penicillium citrinum* CGJ-C1 isolated from *T. involucrata* plant. Endophytic fungus mycelial biomass was separated by filtration after 72 h of growth in potato dextrose broth (PDB), any indications of media components were removed by extensive washing with distilled water. The silver nanoparticles were fabricated by adding 10 mL of fungal filtrate to 90 mL of 1 mM AgNO3 followed by 24 h incubation at 32–34 °C. Resulting nanostructures were well dispersed, 2 to 5 nm spheres. The nanoparticles exhibited potential larvicidal and dose-dependent

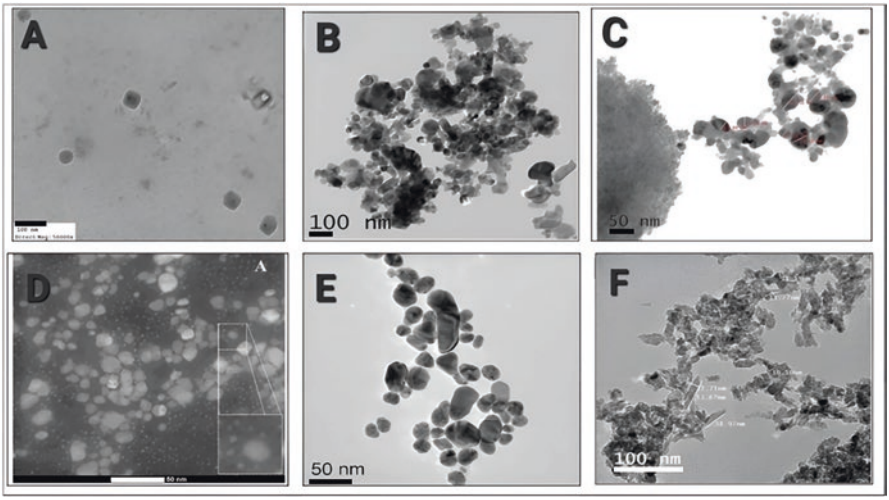

Fig. 5.1 Transmission electron microscopic images of mycosynthesized nanoparticles (**a**) Silver nanoparticles; scale bar: 100 nm (Elbahnasawy et al. 2021). Reproduced with permission. (**b**) Zinc nanoparticles; scale bar: 100 nm (Ganesan et al. 2020). Reproduced with permission. (**c**) Cobalt nanoparticles; scale bar: 50 nm (Vijayanandan and Balakrishnan 2018). Reproduced with permission. (**d**) Iron nanoparticles; scale bar: 50 nm (Arias et al. 2018). Reproduced with permission. (**e**) Gold nanoparticles; scale bar: 50 nm (Priyadarshini et al. 2014). Reproduced with permission. (**f**) Copper nanoparticles; scale bar: 100 nm (Shehabeldine et al. 2022). Reproduced with permission

anticancer activities (Danagoudar et al. 2020). In another study, AgNPs using *Nemania* sp. isolated from *Taxus baccata L.* The plant was collected and disinfected with 70% ethanol for a minute and a disinfectant (0.5% sodium hypochlorite) for 4 min. Following that, it was rinsed three times with distilled water and dried in sterile circumstances. The inner bark was placed on a petri plate with potato dextrose agar (PDA) containing 500 mg/L chloramphenicol. Fungi were cultivated from the bark fragments at 28 °C. At this point, fungi were cleansed using the hyphal tip technique; hyphal tips were picked from various fungal colonies, placed on fresh PDA medium, and cultured at 25 °C for at least 10 days. This method was carried out three times to achieve complete purity. The pure fungal culture was incubated further in malt extract-glucose-yeast extract-peptone broth at 28 °C for 72 h. To the biomass filtrate, 1 mM of silver nitrate ($AgNO_3$) solution was mixed. The mixture's color change suggested the creation of silver nanoparticles. Spherical particles with average size of approximately 35 nm were obtained exhibiting antibacterial activity (Farsi and Farokhi 2018). Devi and Joshi (2015) isolated the three endophytic fungi from *Potentilla fulgens L*, an ethnomedical plant and then grew the biomass aerobically in PDB with an infusion of 250 g potato and 20 g dextrose per liter of distilled water. The inoculation flasks were incubated in an orbital shaker for 96 h at 25 ± 2 °C with 120 rpm agitation. Further following incubation, the collected biomass was filtered through filter paper. The three endophytes isolated were *Aspergillus tamarii* PFL2, *Aspergillus niger* PFR6, and *Penicillium ochrochloron* PFR8. The fungal filtrates were treated with a 1 mM silver nitrate solution and then incubated at room temperature in the dark in an Erlenmeyer flask resulting in spherical particles of different sizes. Similarly, *Talaromyces purpureogenus* isolated from leaves of *Pinus densiflora S. et Z.* was used to fabricate spherical and triangular nanostructures of 25 nm average size with broad-spectrum antibacterial, anticancer, and potential wound healing activity. Silver nanoparticle synthesis using extracellular route was carried out by using *F. oxysporum* biomass. Conical flask with fungal biomass was filled with $AgNO_3$ to provide an overall Ag ion concentration of 10^3 M in the aqueous solution, and the reaction was carried out in the dark. AgNPs films were seen on silicon substrates after reaction with the fungal biomass was complete. This was done by drop-coating the *F. oxysporum* biomass with the nanoparticle solution both before and after the reaction with Ag ions for 72 h. Before reacting with the silver ions, the biomass is found to have a light-yellow color; when the reaction is complete, the color changed to a brownish hue. Spherical particles with a diameter of around 5 to 15 nm were obtained (Ahmad et al. 2003).

2.2 Fabrication of Zinc Nanoparticles

Zinc-based nanoparticles have varied applications in agricultural and pharmaceutical sectors. Different types of zinc nanoparticles exist like zinc oxide (ZnO), zinc sulfide (ZnS), zinc telluride (ZnTe), zinc selenide (ZnSe), etc. These particles are

inexpensive, stable at higher temperatures, exhibit antimicrobial property, and are employed for targeted delivery and diagnostic purposes in animals as well as for alleviating stress conditions and zinc deficiency in plants (Rani et al. 2022; Sturikova et al. 2018; Moezzi et al. 2012). Mycosynthesis of ZnO nanoparticles (ZnONPs) has been carried out using different endophytes isolated from a variety of plant species. One such study reports a sol-gel method using *Balanites aegyptiaca* plant leaves from which *Periconium* sp. were isolated on PDA laced with streptomycin. To obtain the biomass, the fungus was grown for 21 days in PDA followed by drying at 60 °C and grinding into powder. To obtain, the filtrate powder was heated for 2 h at 70 °C in deionized water. Zn $(NO_3)_2$ solution was mixed with fungal extract, and the resultant mixture was then further evaporated to create the solution with pH 5. The final mixture was placed in a hot air oven at 45 °C to allow water molecules to slowly evaporate, creating a gel with a homogenous distribution of Zn^{2+} ions. The gel became yellowish, brittle, and porous after being dried at 125 °C to eliminate any remaining water. Additionally, the dried gel was calcined at 700 °C resulting in the manufacture of ultrafine ZnONPs. The resulting particles were of 16 to 78 nm size (Fig. 5.1b) and exhibited antioxidant properties and broad-spectrum antimicrobial property against gram-positive and gram-negative bacteria as well as fungi (Ganesan et al. 2020). In another instance, the endophyte *Cochliobolus geniculata* was isolated from leaves of a medicinal plant *Nothapodytes foetida* and cultured in the dark on potato dextrose agar along with chloramphenicol at 28 °C. The fungi were inoculated in malt extract-glucose-yeast extract-peptone (MGYP) broth and grown for 72 h to obtain the biomass. The cell-free extract was added to zinc acetate (1 mM) to generate ZnONPs capped with proteins (Kadam et al. 2019).

2.3 Fabrication of Cobalt Nanoparticles

Cobalt and tricobalt tetroxide nanoparticles have promising applications in pharmaceutical industries owing to their antibacterial, antifungal, larvicidal, antileishmanial, antioxidant, and cytotoxic activities (Waris et al. 2021). Intracellular synthesis of spinel cobalt oxide nanoparticles (Co_3O_4NPs) with approximate size of 20 nm was carried out using endophytic fungus *Aspergillus nidulans* isolated from plant *Nothapodytes foetida* (Fig. 5.1c). The plant was cut into small pieces and spread on sterile potato dextrose agar with chloramphenicol in petri dishes and incubated at room temperature. The fungal inoculum from agar plate was grown in potato dextrose broth, and the mycelium obtained from the biomass was treated with 2 mM cobalt acetylacetone solution with stirring (Vijayanandan and Balakrishnan 2018). Superparamagnetic spherical cobalt ferrite nanoparticles of 6.5 nm average size were synthesized using *Monascus purpureus*. Cobalt nitrate and ferric nitrate were dissolved in deionized water in a 1:2 M ratio and introduced dropwise (in an equal volume basis) to the prepared cell-free fungal culture and vigorously stirred at room temperature for several hours (4–6 h) until it turned a dark hue, suggesting

the completion of the reduction process. After that, the mixture was heated for 5 min at 60 °C to start the synthesis of cobalt-ferrite nanoparticles. The obtained mixture was then cooled and left at room temperature overnight, when a black precipitate began to form, suggesting the commencement of nanoparticle creation. Ultracentrifugation at 20000 rpm for 20 min at 4 °C separated the nanoparticles. The acquired nanoparticles were dispersed numerous times in deionized water and ethanol to eliminate any remaining biological compounds. The purified nanoparticles were subsequently dried using a hot air oven at 70 °C, yielding a fine powder. Further, considerable increase in yield of the cobalt ferrite nanoparticles was observed when the fungal culture was irradiated with gamma rays (El-Sayed et al. 2020). Similarly, endophytic fungi *Aspergillus terreus* was employed to fabricate Co_3O_4, CuO, Fe_3O4, NiO, and ZnO nanoparticles (Mousa et al. 2021). In another instance, nitrogen-doped Co_3O_4NPs were synthesized using *Fusarium oxysporum* extract. The particles were 25 nm in size and exhibited photocatalytic activity (Islam et al. 2021).

2.4 Fabrication of Iron Nanoparticles

Owing to the superparamagnetic properties of iron, their nanoparticles have huge applications in pharmaceutical and biomedical fields, such as drug delivery, diagnostic imaging, magnetic resonance imaging, cell separation, tissue repair, levitation-based techniques, etc. Fig. 5.1d shows the TEM image of iron nanoparticles (FeNPs) (Arias et al. 2018). In one study employing mycosynthesis for fabricating FeNPs, endophyte *Penicillium oxalicum* isolated from the plant *Tecomella undulata* was inoculated in 150 ml of PDB and grown at 30 °C. The mycelia obtained was transferred in 150 ml of sterilized distilled water after which it was incubated in a shaker incubator (150 rpm) for 12 h at 30 °C, and the filtrate obtained was utilized for synthesizing of iron nanoparticles. Fungal extract and $FeSO_4$ were mixed and kept overnight on a magnetic stirrer. Post stirring the solution was then centrifuged for 15 min at 12,000 rpm. The precipitate was then dried in an incubator at 50 °C, and a fine powder was acquired. The synthesized nanoparticles were spherical in shape with an average size of 140 nm and effectively caused decolorization of methylene blue (Mathur et al. 2021). In a similar study, endophytic fungi *Fusarium proliferatum* was isolated from *Cymbopogon citratus*, a plant of medicinal value. The fungal extract was added to $FeCl_3$ solution to initiate nanoparticle synthesis. The particles had average size of 20–50 nm and caused effective decolorization of a number of dyes (Schuster and Ting 2022). In another study, $FeCl_3$ was added to the biomass of *Trichoderma* sp. to synthesize FeNPs, and effects of synthesis parameters were analyzed. It was revealed that a pH of 4.5 and temperature of 25 °C agitation are required for optimum synthesis of the FeNPs (Kareem et al. 2020). Another study reported the synthesis of spherical FeNPs of size 10–25 nm using the fungi *Aspergillus oryzae* TFR9 (Tarafdar and Raliya 2013).

2.5 Fabrication of Gold Nanoparticles

Gold nanoparticles (AuNPs) are biocompatible and find applications in therapeutic, imaging, and diagnostic fields. They are used in photothermal and photodynamic therapy; targeted drug delivery; X-ray, fluorescence, and photoacoustic imaging; and as biosensors (Bansal et al. 2020). In a novel approach, mycosynthesis of AuNPs was carried out using endophyte *Penicillium citrinum* isolated from marine brown alga *Sargassum wightii.* Samples of 1 cm length were cut and cultured on PDA containing chloramphenicol at 28 °C for fungal growth and then on potato dextrose agar without antibiotic for 30 days at the same temperature. The fungal mycelia were used to prepare the extract which was subjected to $AuCl_4$ for fabrication of the nanoparticles of 60–80 nm size (Manjunath et al. 2017). In another study, chloroauric acid was added to *Aspergillus terreus* IF0 fungal extract to biosynthesize AuNPs. Under comparable conditions, a control experiment was conducted without the addition of $HAuCl_4$. The initial pH of fungal filtrate was altered to investigate the effect of pH on the size and form distribution of AuNPs. According to the results obtained, it was concluded that acidic pH leads to formation of lesser nucleation points as compared to alkaline pH due to which large polydispersed particles were formed in acidic pH. At PH 10, monodispersed particles of 10–19 nm size were fabricated that exhibited antimicrobial activity against *Escherichia coli* (Fig. 5.1e) (Priyadarshini et al. 2014). In another approach, *Fusarium oxysporum* was isolated from leaves of *Azadirachta indica*, and AuNPs were synthesized using fungal mycelia. Protein-coated nanoparticles of 22 nm size were obtained showing potential antimicrobial activity against *Pseudomonas* sp. (Thakker et al. 2013). Similarly, endophytic fungus *Phoma* sp. isolated from vascular tissues of *Prunus persica* was used for synthesis of AuNPs. The resultant nanoparticles exhibited antifungal and antibacterial activity against *Rhizoctonia solani* AG1-IA and *Xanthomonas oryzae pv.*, respectively (Nejad et al. 2022).

2.6 Fabrication of Copper Oxide Nanoparticles

Copper oxide nanoparticles (CuONPs) have been widely used as potential anticancer and broad-spectrum antimicrobial agent as well as sensors for glucose, hydrogen peroxide, dopamine, etc. (Verma and Kumar 2019). In one approach for the endophytic synthesis of CuONPs, *Aspergillus terreus* was isolated from leaves, bark, and branches of *Aegle marmelos*. The mycelial filtrate was added to copper sulfate (0.1 mM) and incubated for 36–48 h, the pH was maintained at 7.4. Post incubation, a brown precipitate was observed indicting the synthesis of copper oxide nanoparticles. The precipitate was centrifuged at 10,000 rpm for 20 min, and the pellet was vacuum dried for 2 h at 80 °C. The nanoparticles exhibited antibacterial, antioxidant, anticancer, and antiangiogenic properties (Mani et al. 2021). In another study, CuONPs were synthesized using *Aspergillus niger* strain STA9 using

copper sulfate as precursor salt. The resulting 5 to 100 nm particles exhibited antidiabetic, antibacterial, and anticancer activities (Noor et al. 2020). In another approach, *Penicillium chrysogenum* mycelial extract was used to fabricate CuONPs using copper acetate salt as precursor. Both spherical and rod-shaped particles were formed with respective sizes of 4 to 15 nm and 11 to 54 nm (Fig. 5.1f). The particles exhibited excellent broad-spectrum antibacterial as well as anti-biofilm activity (Shehabeldine et al. 2022). In a novel approach, biosynthesis of CuONPs was achieved by using a native white-rot fungus of Chile, *Stereum hirsutum*. The effect of different precursor salts (copper chloride, copper nitrate, and copper sulfate) and different pHs (5, 7, and 9) on the yield of nanoparticles was studied. Copper chloride as precursor at neutral or basic pH was found to be best suited as the synthesis parameters (Cuevas et al. 2015).

2.7 Fabrication of Other Miscellaneous Nanoparticles

In one approach, Thomas et al. (2017) used the fungi *Aspergillus tubingensis* TFR-29 isolated from an agricultural farm to biofabricate molybdenum nanoparticles. The mycelia-free extract of the fungal biomass was subjected to different concentrations (0.1, 0.5, and 1 mM) of ammonium molybdate. It was exhibited that by using 0.5 mM of precursor salt, 2.3 nm monodisperse particles were produced with hexagonal shape and thin layer of protein coating. The particles have the potential to perform as nano-fertilizer as evident by enhancement in grain and biomass yield, enzyme and microbial activity, root length, area, diameter, and number of tips, upon application to chick pea. In another study, the fungal extract of *Colletotrichum* sp. was employed to synthesize aluminum and sulfur nanoparticles using sodium thiosulfate and aluminum chloride as precursors, respectively. The resulting nanoparticles were spherical in shape with average size of 30 and 50 nm for aluminum and sulfur, respectively. The particles were further used to formulate nanofunctionalized essential oil that exhibited significantly higher broad-spectrum antimicrobial activity as compared to bare nanoparticles (Suryavanshi et al. 2017).

3 Pharmaceutical Applications of Biosynthesized Nanoparticles Using Fungal Endophytes

Metal nanoparticles have a plethora of pharmaceutical and biomedical applications. The most common ones are as antibacterial, antifungal, antiplasmodial, antidiabetic, antioxidant, and anticancer agents as well as for clinical diagnosis and wound healing (Singh et al. 2021). In this section, the major pharmaceutical applications of mycosynthesized metal nanoparticles by endophytes are discussed.

3.1 Antimicrobial Activity

Metal-based nanoparticles have no specific mechanism to cause toxicity in microbes; therefore, they have the potential to be used against broad-spectrum species and also against multidrug-resistant pathogens. Of the many mechanisms to cause toxicity in microbial cells, one of the crucial is enhanced production of reactive oxygen species (ROS) owing to the high surface-to-volume ratio of the nanoparticles. The ROS like hydroxyl radical, superoxide ion, etc. cause damage to the cell membrane of the microbes (Mei and Wu 2017). Figure 5.2a shows a schematic of mechanism of toxicity of metal nanoparticles. AgNPs synthesized using *Penicillium* sp. extracted from turmeric were analyzed for their antibacterial activity against multidrug-resistant bacteria *Staphylococcus aureus* and *E. coli*. Agar well diffusion method was used, and results were analyzed based on measuring the zone of inhibition (ZOI). The study showed that the AgNPs exhibited inhibitory activity against both *E. coli* and *S. aureus* thereby indicating broad-spectrum activity (Singh et al. 2014).

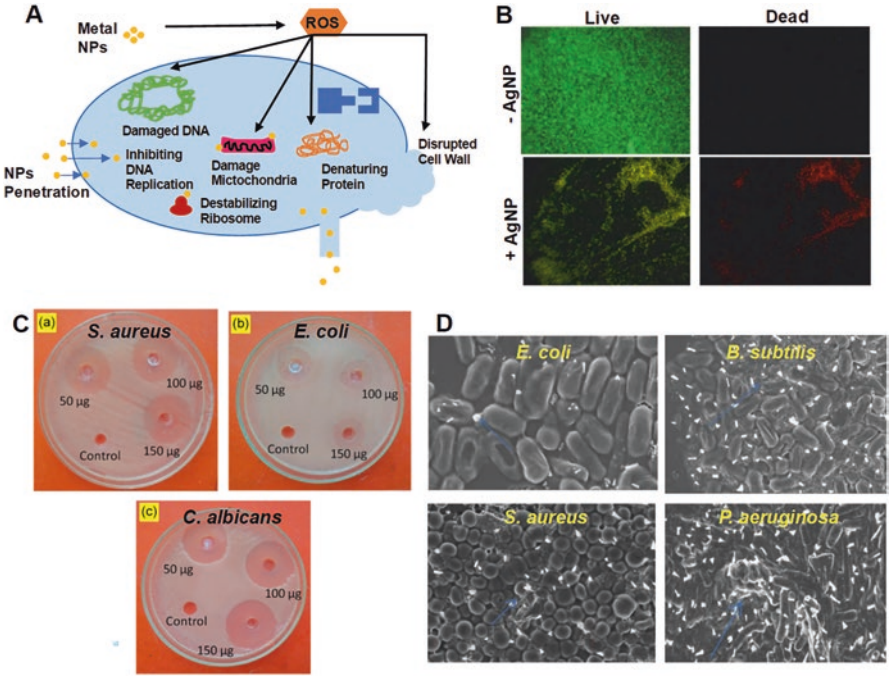

Fig. 5.2 Antimicrobial mycosynthesized nanoparticles. (**a**) Schematic showing mechanism of antimicrobial activity of nanoparticles. (**b**) Fluorescent image showing damage produced on preformed uropathogenic *Escherichia coli* biofilms by *Fusarium scirpi* synthesized AgNPs stained with Live/Dead BacLight Viability kit; magnification: 40 X (Rodríguez-Serrano et al. 2020). Reproduced with permission. (**c**) Zone of inhibition by *Periconium* sp. synthesized ZnO nanoparticles (Ganesan et al. 2020). Reproduced with permission. (**d**) SEM image showing cell wall damage by *Cladosporium cladosporioides* synthesized AuNPs (Joshi et al. 2017). Reproduced with permission

Another study was conducted using mycosynthesized AgNPs by *Fusarium scirpi* to elucidate the antibacterial activity on planktonic and established biofilms of uropathogenic *E. coli* biofilms. For planktonic *E. coli*, a minimal inhibitory concentration (MIC) of 25 mg/mL was adequate to decrease any kind of bacterial activity after 3 h treatment. Further, a nanoparticle concentration of 7.5 mg/mL led to significant damage to the biofilm; 80% of the bacterial cells detached from the surface (Fig. 5.2b) (Rodríguez-Serrano et al. 2020). In another approach, the antibacterial and antifungal activity of mycosynthesized AgNPs by *Aspergillus flavus* was evaluated. Five pathogenic bacteria were procured (*E. coli*, *Bacillus subtilis*, *S. aureus*, *Bacillus cereus*, *Enterobacter aerogenes*) and four concentrations of AgNPs were studied (20, 50, 100, and 150 μM). It was observed that the inhibitory activity of silver nanoparticles increased with increase in concentration, and the activity was most efficient toward *B. subtilis* followed by *E. coli*. Further, a combination of tetracycline and AgNPs was used which enhanced the antibacterial activity. The antifungal activity was tested on *Aspergillus niger* and *Trichoderma* spp. A similar result was observed; increase in concentration of AgNPs led to increase in its inhibitory activity (Fatima et al. 2016). Similarly, antimicrobial activity of AgNPs synthesized by *Aspergillus fumigatus* was tested against gram-negative bacteria *E. coli*, gram-positive bacteria *Bacillus mycoides*, and fungus *Candida albicans*. It was found that *B. mycoides* had a larger ZOI compared to *E. coli* (15–16 mm); this can be accredited to the marked variations in the composition of cell walls. For *C. albicans*, the diameter for ZOI was in a similar range as *B. mycoides* (Othman et al. 2019). The antibacterial and antifungal activity of AgNPs synthesized by Balakumaran et al. (2015) using *Guignardia mangiferae* was also evaluated. MIC of AgNPs was calculated for the bacteria; *B. subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Enterococcus faecalis* exhibited 6.25 μg/ml, 3.12 μg/ml, 6.25 μg/ml, 3.12 μg/ml, and 12.5 μg/ml, respectively. The antifungal activity was tested by measuring the ZOI; satisfactory activity at 1 mg/ml of AgNPs was observed for *Colletotrichum* sp. followed by *Rhizoctonia solani* and *Cochliobolus lunatus*; *Fusarium* sp. showed the least activity.

ZnONPs synthesized using cellular extracts of endophytic fungi; *Alternaria tenuissima* exhibited broad-spectrum antimicrobial activity and significantly better activity as compared to amoxicillin/clavulanic acid and nystatin. MIC for the bacteria *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* was 200 μg/ml, and for *E. coli*, it was 100 μg/ml. In case of fungus, *C. albicans* showed MIC of 50 μg/ml; *Alternaria solani* and *Fusarium oxysporum* exhibited 100 μg/ml, whereas *A. niger s*howed MIC of 200 μg/ml of ZnONP (Abdelhakim et al. 2020). Similarly, ZnONPs biosynthesized utilizing *Periconium* sp. fungal extracts showed large ZOI for *S. aureus* (16 ± 0.01 mm), *E. coli* (14 ± 0.03 mm), and *C. albicans* (24 ± 0.01 mm). A dose-dependent response was observed, and results indicated that nanoparticles had better inhibitory activity against gram-positive bacteria (Fig. 5.2c) (Ganesan et al. 2020). Another study reported the synthesis of two different-shaped zinc oxide nanoparticles, hexagonal by using *Fusarium keratoplasticum* and nano-rod shaped using *A. niger*. Nanoparticles synthesized by separate strains were checked for antibacterial activity toward *B. subtilis*, *S. aureus* (gram positive) and *P. aeruginosa*,

and *E. coli* (gram negative). Results based on the ZOI study indicated that nano-rod ZnO particles demonstrated a more effective inhibition of 23.6 ± 0.4 mm and 19.5 ± 0.2 mm for *B. subtilis* and *S. aureus*, respectively, compared to hexagonal that revealed 22.0 ± 0.6 mm and 18.2 ± 0.7 mm for respective gram-positive bacteria. For gram-negative bacteria, similar results were observed; hexagonal ZnONPs showed 15.7 ± 0.4 mm ZOI for both gram-negative bacteria, while nano-rods had larger zones 17.5 ± 0.3 mm and 16.1 ± 0.4 mm for *E. coli* and *P. aeruginosa*, respectively. The study concluded that the inhibition toward these bacteria was dependent on the shape and size of the nanoparticles (Mohamed et al. 2019). In another study, extracellular extracts of *A. niger* were utilized to synthesize ZnONPs whose antimicrobial effect was tested on six bacterial and four fungal strains. The MIC of ZnONPs using resazurin tincture method was established for both bacterial and fungal strains. *Acinetobacter baumannii*, *E. coli*, *Staphylococcus haemolyticus*, and *S. aureus* had a MIC of 12.5 μg/mL, whereas *K. pneumoniae* and *P. aeruginosa* had a MIC of 50 μg/ml. MIC for fungi *Candida parapsilosis*, *Penicillium marneffei*, and *Candida glabrata* was 12.5 μg/mL, while for *A. niger*, it was 25 μg/mL (Mekky et al. 2021). In another approach, ZnONPs mycosynthesized by *Xylaria acuta* extracts indicated that *S. aureus* and *B. cereus* were more susceptible with MIC of 15.6 μg/mL, followed by *E. coli* and *P. aeruginosa* (31.3 μg/mL). The inhibitory activity of the ZnONPs increased with increase in concentration of the nanoparticles, depicting a dose-dependent response activity. When tested with pathogenic fungi, *F. oxysporum* was found to be less sensitive to the nanoparticles compared to *Aspergillus flavus* and *Phomopsis* sp. (Sumanth et al. 2020). Similarly, biosynthesized ZnONPs utilizing *A. niger* exhibited that both *K. pneumoniae* carbapenemase (KPC)-producing bacteria (test) and *K. pneumoniae* (control) were extremely sensitive to ZnONPs concentration of 7.5 mg/ml (20.8 ± 2.7 mm). KPC is an emerging gram-negative bacteria that is highly resistant to multiple antibiotics. Mean values observed for MIC and minimal bactericidal concentration were 0.7 and 1.8 mg/ml. Scanning electron microscopy (SEM) images revealed that KPC bacteria treated with ZnONPs had several notable morphological changes like change of shape of bacteria from rod to coccus, dents on cell surface, decreased size, and severe damage. Control-untreated cells and imipenem (a carbapenem antibiotic)-treated cells showed no such change in morphology (Rasha et al. 2021).

AuNPs fabricated using endophytic fungi *Cladosporium cladosporioides* exhibited antimicrobial activity at a concentration of 50 mg/ml against bacteria *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, and fungi *A. niger*. The well diffusion method exhibited ZOI of approximately 12, 1.5, 9.5, 10.5, and 10 mm for *S. aureus*, *S. epidermidis*, *B. subtilis*, *E. coli*, and *A. niger*, respectively. Further SEM imaging showed an accumulation of nanoparticles on the membrane of the bacterial cell wall and the subsequent damage by rupturing the cell wall were observed too (Fig. 5.2d). These results provide significant insight on the massive broad-spectrum antimicrobial potential these nanoparticles have as they are effective against both gram-positive and gram-negative bacteria and also fungi (Joshi et al. 2017). A more recent study used *Aspergillus terreus* to synthesize AuNPs; their antimicrobial potential was checked against bacteria *Salmonella typhimurium*, *S. aureus*,

methicillin-resistant *Staphylococcus aureus*, *Vibrio cholerae*, fungi *F. oxysporum*, and *Rhizoctonia solani*. Zone of inhibition results showed that at 400 μg/ml, the largest zones of inhibition were observed for *S. aureus* (8.58 ± 0.28 mm) and *V. cholerae* (9.31 ± 0.14 mm), whereas no visible zones of inhibition were observed for methicillin-resistant *S. aureus* or *S. typhimurium*. For the antifungal activity, AuNPs concentrations of 25, 50, 200, and 200 μg/ml were screened that showed maximum inhibition of 52.5% and 65.46% for *R. solani* and *F. oxysporum*, respectively, at AuNPs concentration of 200 μg/ml (Mishra et al. 2022). FeNPs synthesized using endophytic fungi *Aspergillus flavus* exhibited antibacterial activity against *S. aureus* at a concentration of 100 mg/ml with a ZOI of 10 mm but was ineffective on *E. coli*. However, they showed antifungal activity toward both *C. albicans* and *Aspergillus fumigatus* with ZOI 11 mm and 10 mm, respectively (Ramadan Gouda et al. 2020). In a similar study, ZOI analysis revealed that the FeNPs fabricated using *Alternaria alternata* were extremely effective against both gram-negative and gram-positive bacteria. *B. subtilis* showed the maximum inhibition (16.4 mm), whereas *E. coli*, *P. aeruginosa*, and *S. aureus* exhibited zones of inhibition of 13.2, 10.5, and 12.3 mm, respectively. They further performed TEM for *B. subtilis* after being treated with the iron nanoparticles; the results showed significant morphological changes in the bacterial cells (Mohamed et al. 2015).

CuONPs were synthesized by endophytic fungi *A. terreus* isolated from a medicinal tree *Aegle marmelos*, and its antimicrobial potential was tested using ZOI method against *Proteus mirabilis* (1.2 cm), *E. coli* (2.2 cm), *K. pneumoniae* (1.9 cm), *P. aeruginosa* (2.3 cm), *S. aureus* (0.9 cm), *V. cholerae* (1.9 cm), *S. epidermidis* (1.5 cm), *S. typhi* (1.1 cm), *A. niger* (1.1 cm), and *C. albicans* (1.2 cm). These were then compared to ZOI of the cultural filtrate, biomass crude extracts, and methanol; the CuONPs showed a significantly higher effect compared to the other three. Only *A. niger* showed no significant difference among the four values (Mani et al. 2021). In another study, CuONPs and CoONPs were synthesized using *A. terreus*, and their antimicrobial activity was tested against bacteria (*P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *S. aureus*) and fungi (*C. albicans*, *Aspergillus brasiliensis*, *Alternaria alternata*, and *F. oxysporum*). It was observed that all the bacteria and fungi were sensitive toward CuONPs; however, *E. coli* and *C. albicans* were the most sensitive both having an MIC of 100 μg/ml. The most resistant was *P. aeruginosa* (Mousa et al. 2021).

3.2 Antioxidant Activity

An important activity that has abundant pharmaceutical applications is antioxidant activity. Mycosynthesized nanoparticles are proven antioxidants, the activity most probably is due to coating of biomolecules that constitute the fungal isolates like secondary metabolites (polyphenols, flavonoids, etc.) that have the ability to donate hydrogen (Ramamurthy et al. 2013). Figure 5.3a represents a schematic of mechanism of antioxidant activity of metal nanoparticles. The antioxidant activity of

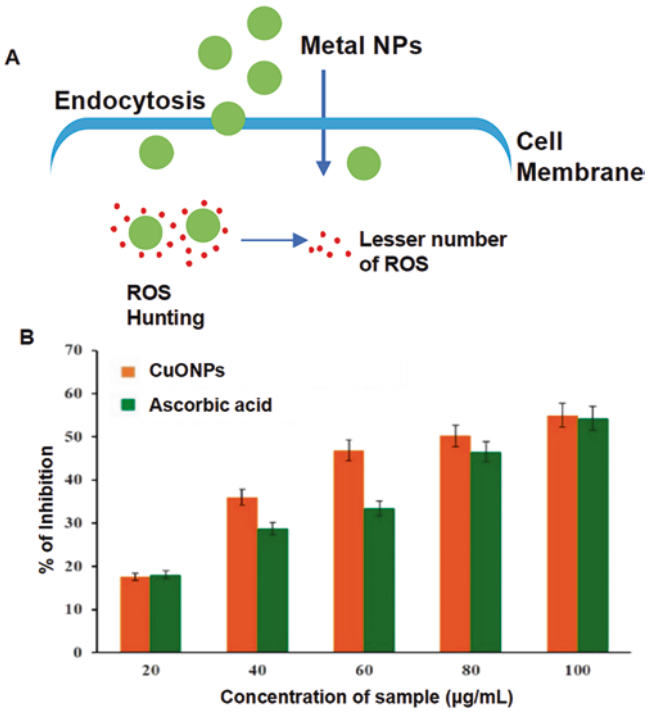

Fig. 5.3 Antioxidant activity mycosynthesized nanoparticles. (**a**) Schematic showing mechanism of antioxidant activity of nanoparticles. (**b**) DPPH radical scavenging activity of *Aspergillus terreus* synthesized CuONPs (Mani et al. 2021). Reproduced with permission

AgNPs synthesized using fungi *Penicillium italicum* was evaluated by three different methods: bleaching/scavenging of radicals of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, and resazurin. For the three methods used, 30 µg/ml was the most potent concentration showing a significant 60%, 53%, and 50% inhibition compared with ascorbic acid for DPPH, hydroxyl, and resazurin radicals, respectively (Taha et al. 2019). *Cladosporium cladosporioides* synthesized AgNPs were employed to study its antioxidant activity by DPPH radical scavenging method. An increasing concentration of AgNPs were used, and the results obtained a direct relation with scavenging activity and increase in nanoparticle concentration. The antioxidant activity was accredited to the phenolic content present in the fungal extract (Manjunath Hulikere and Joshi 2019). Similarly, antioxidant activity of AgNPs synthesized using extract of *Aspergillus versicolor* isolated from plant *Centella asiatica* was compared with that of only fungal extract as well as the precursor salt, silver nitrate. The DPPH radical scavenging activity at 100 µg/ml of AgNPs was found to be 60.4%, compared to the fungal extract (41.19%) indicating that the AgNPs significantly increased the radical scavenging activity. Free silver nitrate does not show any potential antioxidant activity. The nanoparticles mainly have this activity due to the presence of proteins, secondary metabolites such as flavonoids, polyphenols, terpenoids, and other biomolecules as coating (Netala et al. 2016).

For biosynthesized ZnONPs employing *Alternaria tenuissima*, a dose-dependent increase in DPPH-free radical scavenging activity was observed as concentration of nanoparticles increased. The IC_{50} value for nanoparticles was observed to be 102.13 μg/ml and for ascorbic acid 72.95 μg/ml; the least inhibitory concentration was found to be 25 μg/ml for both nanoparticles and ascorbic acid (Abdelhakim et al. 2020). ZnONPs synthesized using *Periconium* sp. exhibited the maximum DPPH radical scavenging activity of 85.52% at 100 μg/ml concentration (Ganesan et al. 2020). Similarly, mycosynthesized ZnONPs from extracts of *A. niger* were analyzed for 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and DPPH radical scavenging activity. DPPH assay results revealed that high concentrations of ZnONPs (100 μg/ml) had a higher antioxidant activity (57.74%) compared to lower concentration (5 μg/ml) having low activity (13.95%). Similar antioxidant activity was observed for ABTS assay, high concentrations having 73.58% activity and lower ZnONP concentration having 11.23% inhibitory activity. Based on these results and FTIR functional group analysis, it was concluded that antioxidant inhibitory potential increased and was dependent on the dose of ZnONPs; this antioxidant activity was attributed to the biological components of *A. niger* that were used to synthesize the nanoparticles (Gao et al. 2019).

Antioxidant activity of AuNPs fabricated using endophytic fungi *Alternaria* sp. was studied using the DPPH radical scavenging and the reducing power assay. At 500 μg/ml nanoparticle concentration, total antioxidant activity was reported to be 83.47 and 69.15 for DPPH and reducing power, respectively, which are higher than that of ascorbic acid (Hemashekhar et al. 2019). In two more studies in which AuNPs were synthesized using *Penicillium citrinum* and *C. cladosporioides*, the antioxidant activity was tested using the DPPH assay. AuNPs concentration (0–110 μg) was screened with ascorbic acid as reference. A dose-dependent response was observed for the radical scavenging activity for both the synthesized nanoparticles. The AuNPs synthesized by *C. cladosporioides* were subjected to the reducing power assay which showed moderate antioxidant activity of 1.15 ± 0.03 mg of ascorbic acid equivalent per gram of sample (Joshi et al. 2017; Manjunath et al. 2017). A recent study utilized *A. terreus* to produce AuNPs and evaluated its antioxidant activity using ABTS assay. Significant antioxidant activity was revealed as reflected by IC_{50} value of 38.61 μg/ml. To further illustrate the activity, DNA nick assay was performed via the generation of highly reactive hydroxyl radical species. These cut circular plasmid DNA to linear DNA; the AuNPs provided a protective effect preventing the supercoiled circular DNA from being cut to linear DNA. Nanoparticle concentrations 25–200 μg/ml were taken, and a dose-dependent protection from DNA nicking was observed, keeping the DNA in its supercoiled form, thus showing the antioxidant activity of the nanoparticles by protecting the DNA from oxidative stress (Mishra et al. 2022).

A. terreus was employed to synthesize CuONPs, and their antioxidant activity was checked using the DPPH, nitric oxide radical scavenging assay, and the reducing power assay. A dose-dependent scavenging activity was established, and IC_{50} values 0.080 mg/ml and 0.096 mg/ml were observed for DPPH and nitric oxide radicals, respectively. For the reducing power assay, a dose-dependent response was

seen with an increase in optical density units (Fig. 5.2b) (Mani et al. 2021). Another study also used *A. terreus* to synthesize CuONPs that showed a dose-dependent increase in DPPH radical scavenging activity with IC_{50} value of 96.74 μg/ml. Similarly, Co_3O_4NPs fabricated by *A. terreus* exhibited an IC_{50} of 85.44 μg/ml (Mousa et al. 2021).

3.3 Anticancer/Tumor Activity

Another activity that has been studied extensively is the cytotoxicity of metal nanoparticles toward a number of cancer cell types. Although the mechanism related for this activity is not completely understood, a study proposed a possibility that metal nanoparticles disrupt and interfere with oxidative phosphorylation via production of ROS, which further creates oxidative stress and damages nucleic acids (Vasanth et al. 2014) (Fig. 5.4a). Potential anticancer activity of AgNPs is demonstrated by many studies. Antitumor activity of different concentrations (0.4–54 μg/ml) of AgNPs synthesized using *Aspergillus fumigatus* was tested on cell lines HCT116 (human colon carcinoma), A549 (human lung carcinoma), PC3 (human prostate cell line), and MCF7 (human breast cancer) cell lines. The 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for cancer cell lines treated with AgNPs indicated a dose-response association with test cells and test concentrations. IC_{50} values at 48 h were found to be 31.1 μg/ml (HCT116), 45.4 μg/ml (A549), 40.9 μg/ml (MCF7), and 33.5 μg/ml (PC3). Restriction in cell growth at lower concentrations was also observed (Othman et al. 2019). AgNPs synthesized using *Guignardia mangiferae* were tested for their antitumor activity, using increasing concentrations of nanoparticles the cell viability of Vero (Monkey kidney), MCF-7, and HeLa (human cervical cancer) cell lines was experimented on. IC_{50} values for the cells were 63.37 μg/ml, 27.54 μg/ml, 23.84 μg/ml for Vero, HeLa, and MCF-7, respectively. MCF-7 cells exhibited maximum inhibition at lower concentrations of AgNPs (Balakumaran et al. 2015). Five concentrations of AgNPs synthesized using *Penicillium italicum* (5, 10, 20, 40, 80 μg/ml) were used by Taha et al. (2019) to demonstrate the anticancer activity. Performing the MTT assay on MCF-7 cells treated with AgNPs, it was found that at 80 μg/ml, the highest cytotoxicity of 87% and the lowest 16% at 5 μg/ml were seen. A 24-h long-term treatment was done to the cells with 10 μg/ml and 20 μg/ml of AgNPs, and a clonogenic survival assay was performed; similar results for cell viability were observed; however, the effect was found to be more potent (26%). The results showed a concentration-dependent increase in cytotoxicity. Anticancer activity and cytotoxicity of AgNPs synthesized using fungal isolates of *Aspergillus* sp. were described by Mohmed et al. (2017). Normal Vero cells and human colorectal adenocarcinoma cells (Caco-2) were treated with increasing concentration of nanoparticles 0.48–1000 μg/ml and MTT assays were performed. Vero cells exhibited 100% viability at 15.62 μg/ml; as AgNPs concentration increased, the viability decreased. For Caco-2 cells, the lowest concentration proved to have 100% viability and 7.65% viability at highest

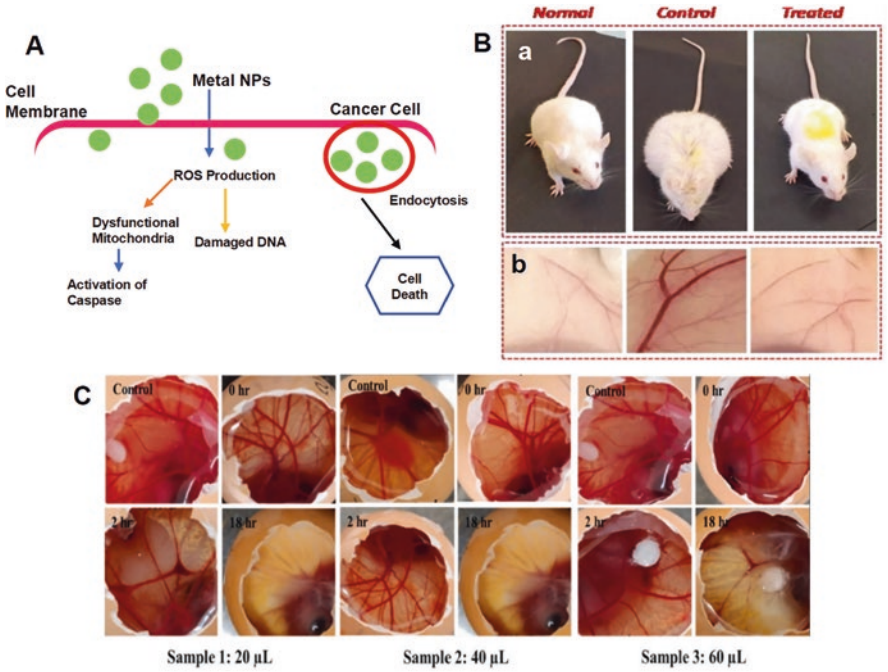

Fig. 5.4 Anticancer activity mycosynthesized nanoparticles. (**a**) Schematic showing mechanism of anticancer activity of nanoparticles. (**b**) In vivo anticancer effect of *Cladosporium* sp. synthesized AuNPs. (a) Changes in morphology of EAC tumor mouse after treatment with nanoparticles. (b) Reduced neovascularization after treatment with nanoparticles (Munawer et al. 2020). Reproduced with permission. (**c**) Antiangiogenic activity of *Aspergillus terreus* synthesized CuONPs (Mani et al. 2021). Reproduced with permission

concentration of AgNPs. The toxicity results for Caco-2 were as expected, highest concentration of nanoparticles having 92.34% toxicity. The IC_{50} value for Caco-2 was 3.75 µg/ml, and for Vero, it was 280 µg/ml.

Anticancer/cytotoxicity activity of ZnONPs synthesized using *Alternaria tenuissima* was demonstrated by Abdelhakim et al. (2020). Three cell lines, one normal Hbf-4 (human melanocyte) and two cancerous MCF-7 and HepG-2 (human liver cancer) cells were treated with nanoparticles, after which an MTT assay was performed. Values for test were compared to the control Taxol activity. Data suggested a concentration-dependent increase in inhibitory activity of the nanoparticles. IC_{50} values of ZnONPs for Hfb-4 (55.76 µg/ml), Hep-G2 (16.87 µg/ml), and MCF-7 (18.02 µg/ml) when compared to Taxol 12.5 µg/ml, 10.35 µg/ml, and 10.42 µg/ml, respectively, for the cell lines showed satisfactory anticancer activity. In another approach, rod- and hexagonal-shaped ZnONPs synthesized using *A. niger* and *Fusarium keratoplasticum*, respectively, exhibited potential anticancer activity against Caco-2, Clone 9 (rat liver), and Vero cell lines. Hexagonal ZnONPs showed significantly higher toxicity toward Caco-2 cancer cell line at low concentrations

compared to nano-rods. The study even recommends safe concentrations to work with for possible applications in animal cells, for hexagonal <20.1 ppm, and for nano-rods <57.6 ppm; it concluded that the cytotoxicity of the nanoparticles is shape dependent (Mohamed et al. 2019). The anticancer activity of ZnONPs synthesized using *Xylaria acuta* isolated from plant *Millingtonia hortensis* L.f. was tested on human MDA-MB 134 (mammary gland carcinoma) cells. The cellular uptake of the nanoparticles by the carcinoma cells was studied under a confocal laser microscope that revealed significant internalization both by endocytosis and cell-surface-binding mechanisms owing to their small size and large surface area, respectively. A positive assay for Caspase 8, 9, and 3 indicated onset of programmed cell death. When exposed for long duration of 12 h, a high concentration of ZnONPs exhibited improved apoptosis as revealed by translocation exposure of phosphatidylserine assay (Sumanth et al. 2020). Similarly, ZnONPs synthesized using endophytic fungi *A. niger* were evaluated for their cytotoxicity against HepG2 and HEK-293 (human kidney) cells. MTT results for HEK-293 cells showed no cytotoxicity, while IC_{50} values for HepG2 cells were found to be 26.75 ± 1.04 μg/mL (24 h), 22.29 ± 0.35 μg/mL (48 h), and 19.16 ± 0.32 μg/mL (72 h). The control anticancer drug, doxorubicin, showed IC_{50} of 5.35 ± 1.01 μg/mL after 72 h of treatment. Treating HepG2 cells with IC_{50} concentrations and staining them by acridine orange/ethidium bromide, an orange-red disrupted nuclei was observed under the microscope, whereas untreated cells had an intact green nucleus. The 4′,6-diamidino-2-phenylindole (DAPI) staining furthermore confirmed the apoptotic potential of ZnONPs; IC_{50} concentration-treated cells showed a clearly fragmented nucleus and formation of apoptotic bodies for 72 h, 24 h, and 48 h a less prominent fragmentation of the nucleus was seen. Flow cytometer data suggested that the cells treated with IC_{50} concentration of ZnONPs had been arrested at the G_0/G_1 phase of the cell cycle (Gao et al. 2019).

A detailed study involving in vitro and in vivo experiments demonstrated the excellent anticancer/tumor activity of AuNPs biofabricated by fungi *Cladosporium* sp. MCF-7 cancer cells were treated with AuNPs (0–320 μg/ml), and the IC_{50} values were determined as 38.23 μg/ml by the MTT assay. Results of dual staining assay of acridine orange/ethidium bromide were taken to comment on the apoptotic state of the treated cells. Lower doses of AuNPs showed less apoptotic bodies; for higher concentrations, clear sign of late apoptotic and necrotic cells was observed, with orange yellow fluorescence, membrane blebbing, condensed nuclei, and apoptotic bodies. Annexin V FITC/PI staining further analyzed cellular apoptosis in the cells by flow cytometry; AuNPs-treated MCF-7 cells for 24 h showed 9.88% late apoptotic and 4.59% early apoptotic cells at IC_{50} x 1 μg/ml and 12.68% late and 8.81% early apoptotic cells at IC_{50} x 2 μg/ml concentration. Comet assay to assess DNA damage of the cells was performed for both IC_{50} concentrations, both showed substantial DNA damage observed by olive moments of 40.44 ± 4.77 for IC_{50} x 1 μg/ml and 78.02 ± 6.74 for IC_{50} x 2 μg/ml, control had a value of 3.10 ± 1.12. DNA fragmentation study results reported that IC_{50} x 1 μg/ml had less DNA fragmentation in comparison to IC_{50} x 2 μg/ml, and control had no DNA fragmentation. These results indicate that AuNPs mitigate cellular apoptosis via DNA fragmentation, and the effect of this activity is dose dependent. The in vivo activity of the AuNPs was

tested on Ehrlich ascites carcinoma (EAC) tumor-bearing mice. Toxicology dose of 2000 mg/kg body weight showed no toxicity or mortality in the mice suggesting the nanoparticles are safe at maximal dose. Nanoparticle-treated mice showed a decrease in weight compared to untreated mice; gain in weight is a hallmark of EAC tumor growth (Fig. 5.4b). The decrease in weight was accredited to a decrease in peritoneal fluid secretion in test mice, thus indicating antitumor potential of the AuNPs. A substantial decrease in ascites tumor cell volume was also observed 5.33 ± 0.2 ml the nanoparticle-treated mice as compared to the 11 ± 0.5 ml of the control. Another study involved checking the prolonged life of the nanoparticle-treated mice compared to the control EAC tumor-bearing mice. The control group had a median survival time of 19 days, as for the mice that were orally given AuNPs for 14 days had a median survival time of 35 days, an 85% increase. Furthermore, nanoparticle-treated mice showed a reduction of neovascularization of the peritoneum compared to the untreated control (Fig. 5.4b). On studying the hematological parameters, mice treated with AuNPs showed a significant decrease in white blood cell and neutrophil counts, accompanied by increases in red blood cells, lymphocytes, monocytes, and hemoglobin, all to normal level. In contrast, the control group reported increase in white blood cells and neutrophils and a decrease in red blood cells, lymphocytes, monocytes, and hemoglobin along with anemia, all of which are potent signs of cancer induction. These results also suggest a protective effect of the AuNPs toward the hematopoietic system. Histopathological analysis of the organs (liver and spleen) of control and test mice revealed an increase in weight of the EAC tumor-bearing mice (control). For liver, the control showed a poorly developed central vein with hepatocytes being infiltrated with mononuclear cells and treated showed less infiltration. Treated mice had a normal spleen with red and white pulp; however, the control showed a decrease in cells. EAC cells from the treated and untreated mice were subject to multiple apoptotic assays. DNA fragmentation assay showed that AuNPs induced apoptosis. Giemsa staining helped visualize apoptotic bodies and cell shrinkage in treated cells. All results suggested that AuNPs have the ability to induce apoptosis in the EAC cells (Munawer et al. 2020). In another study, AuNPs were fabricated by endophytic fungi *Fusarium solani*, and its anticancer activity was determined by various experiments. The cytotoxicity was evaluated against MCF-7, HeLa cells, and HEK293 (normal human kidney) cells using the MTT assay. Resultant IC_{50} values obtained were 1.3 ± 1.0 μg/ml for HeLa cells and 0.8 ± 0.5 μg/ml for MCF-7 cell line, and no significant toxicity was observed in HEK cells. Following this, an acridine orange/EtBr staining of MCF-7 cells treated with nanoparticles (0.5, 1, 2 μg/ml) showed dense red-colored cells undergoing apoptosis. To check nuclear irregularities and fragmenting, DAPI staining was performed on the same cell line treated with similar concentrations of gold nanoparticles; untreated cells showed no significant changes, whereas treated cells showed bright blue patches indicative of nuclear fragmenting and chromatin condensation. Further cell cycle arrest analysis was carried out using flow cytometry; MCF-7 cells were treated with gold nanoparticles 0.5, 1, and 2 μg/ml, and it was observed that 55.13%, 52.11%, and 51.10%, respectively, showed cells in the G0-G1 phase which were reduced compared to the control. This was accredited to reduction in cyclin

expression which is a significant protein involved in cell cycle progression from the G1 to S phase. They also observed reduction in the G2 and M phase simultaneously which is again indicative of cells at an arrest in G1/S phase (Clarance et al. 2020). A similar study synthesized gold nanoparticles from endophytic fungi *Alternaria* spp. isolated from the roots of *Rauvolfia tetraphylla* and checked its antimitotic activity on the root tips of *Allium cepa*. For 10 mg/ml of the nanoparticles, a mitotic index of 26.2% was reported and for 5 mg/ml an index of 21.9%. The results were compared to standard mitotic index of quercetin (17.4% at 1 mg/ml) (Hemashekhar et al. 2019). A report that focused on the synthesis of AuNPs using endophytic fungus *Chaetomium globosum* studied the anticancer activity by treating HeLa cells at the concentration range of 10–100 μg/ml. MTT assay data showed an IC_{50} value of 59.19 μg/mL (Ningaraju et al. 2021).

CuONPs were synthesized using *A. terreus*, and the anticancer activity was elucidated. Human colon cancer cell line HT-29 was treated with the nanoparticles, and the IC_{50} value was determined using the MTT assay; 22 μg/ml was the resultant concentration at which 50% cells experienced a growth inhibition. To further demonstrate the anticancer potential, Hen's egg-chorioallantoic membrane test was performed to show angiogenesis inhibition (Fig. 5.4c). Three concentrations of CuONPs were tested (20, 40, 60 μL) at intervals of 2 h and 18 h. After 2 h, 16.66, 20, and 36.36% and after 18 h, 50, 70, and 81.82% angiogenesis inhibitions were observed for the respective concentrations 20, 40, and 60 μL. These studies demonstrate the incredible anticancer potential of these nanoparticles (Mani et al. 2021).

3.4 Wound-Healing Activity

Many metal nanoparticles also exhibit wound-healing property by wound contraction initiated by fibroblast and causing keratinocyte-mediated reepithelialization. Some nanoparticles are capable of enhancing recruitment and proliferation of keratinocytes as well as differentiation of fibroblasts to myoblasts and thus augmenting wound closure by contraction (Sau et al. 2017) (Fig. 5.5a). The endophytic fungus *Talaromyces purpureogenus* was used to synthesize AgNPs, and the cellular wound-healing potential was demonstrated. NIH3T3 (mouse fibroblast) cells were cultured and allowed to reach a desired confluence; the cells were then scratched with pipette tips. Test groups were treated with different AgNPs concentrations and the controls with plain media. After 48 h of treatment, the wound area decreased for test groups 1, 5, and 10 μg/ml and 1.976, 1.824, and 1.4364 cm^2, respectively. Compared to control group wound area (2.356 cm^2), the test group wound areas were smaller. AgNPs did not show any toxicity toward the cell (Hu et al. 2019). ZnONPs were synthesized using *A. niger* by Rasha et al. (2021), and their wound healing was evaluated based on degree of wound closure on rats infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria. Four groups were made G1 (infected/untreated), G2 (uninfected/untreated), G3 (infected/imipenem ointment treated), and G4 (infected/ZnONPs treated). Wound healing and closure were

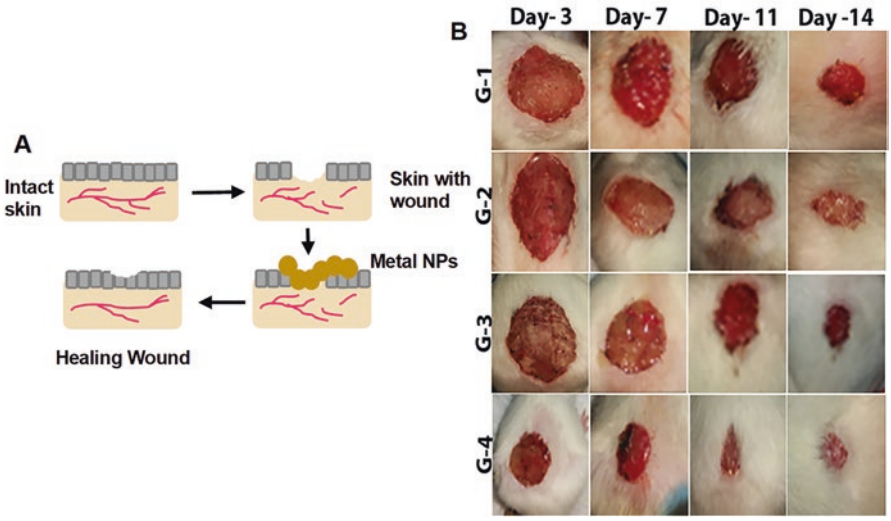

Fig. 5.5 Wound-healing activity mycosynthesized nanoparticles. (**a**) Schematic showing mechanism of wound healing activity of nanoparticles. (**b**) Wound recovery in mice after treatment with *Aspergillus niger* synthesized ZnONPs; G-1, *K. pneumoniae* infected and untreated control; G-2, uninfected and untreated control; G-3, *K. pneumoniae* infected and treated with imipenem; and G-4 (*K. pneumoniae* infected and treated with ZnONPs (Rasha et al. 2021). Reproduced with permission

observed for 3, 7, 11, and14 days. On day 14, wound healing percentage was determined by statistical analysis, G1 (63%), G2 (64%), G3 (70%), and G4 (96%). A histopathological observation of the different groups, on day 14, G4 showed a significant improvement in wound healing with improved dermis and regenerated epidermis (391 μm) (Fig. 5.5b).

4 Conclusion

In this chapter, we have discussed the mycosynthesis of different metal-based nanoparticles using endophytes isolated from plants and their major applications in pharmaceutical sector. The most highlightable feature of this fabrication method is "green synthesis" as the technique is environmentally friendly, nontoxic, and cost-effective. Though many studies have been conducted regarding the endophytic synthesis of nanoparticles, still there is no clear idea about the synthesis parameters as well as the properties of the nanoparticles. It is difficult to explore a vast range of synthesis parameters, such as pH, temperature, precursor concentration, etc., as the technique involves live organisms or enzymes that have maximum activity at optimal condition. Therefore, the yield of the nanoparticles cannot be increased by using extreme parameters. This also limits the properties of the nanoparticles thus

obtained like charge, shape, surface-to-volume ratio, etc. The protein and other biomolecule coating during mycosynthesis though render many beneficial characteristics, but it renders complications on the properties of the fabricated nanoparticles. The large-scale production of nanoparticles is also very challenging especially for intracellular route of synthesis as scale-up of fungal culture is itself cumbersome.

The metal-based nanoparticles fabricated by endophytes exhibited similar pharmaceutical properties as those synthesized by other methods. They presented excellent broad-spectrum antimicrobial, antioxidant, and anticancer effects. However, the same nanoparticle that exhibits antimicrobial and anticancer properties majorly via ROS production also shows potential antioxidant properties. This may prove beneficial and detrimental at the same time. More research is required to establish a therapeutic window of these particles with proper therapeutic index, so that mycosynthesized nanoparticles could be rendered safe and effective. Further, most of the research carried on the pharmaceutical applications of nanoparticles synthesized by endophytes has been conducted in vitro. More detailed in vivo studies are required for complete understanding of pharmacokinetics and pharmacodynamics of these particles. Nonetheless, mycosynthesis of nanoparticles using endophytic fungi has many advantages over the conventional physical and chemical methods. Large amount of research has already been conducted in this area. Proper implementation, execution, and curation of this method could revolutionize the utilization of nanoparticles in pharmaceutical sector.

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References

Abdelhakim HK, El-Sayed ER, Rashidi FB (2020) Biosynthesis of zinc oxide nanoparticles with antimicrobial, anticancer, antioxidant and photocatalytic activities by the endophytic Alternaria tenuissima. J Appl Microbiol 128:1634–1646. https://doi.org/10.1111/JAM.14581

Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry M (2002) Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, Fusarium oxysporum. J Am Chem Soc 124:12108–12109. https://doi.org/10.1021/ja027296o

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M (2003) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf B Biointerfaces 28:313–318. https://doi.org/10.1016/S0927-7765(02)00174-1

Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res 7:17–28. https://doi.org/10.1016/J.JARE.2015.02.007

Ahmed A-A, Nada AA, O'Donovan A, Thakur VK, Elkelish A (2020) Mycogenic silver nanoparticles from endophytic Trichoderma atroviride with antimicrobial activity. J Renew Mater 8:171–185. https://doi.org/10.32604/JRM.2020.08960

Arias LS, Pessan JP, Vieira APM, De Lima TMT, Delbem ACB, Monteiro DR (2018) Iron oxide nanoparticles for biomedical applications: a perspective on synthesis, drugs, antimicrobial activity, and toxicity. Antibiotics (Basel) 7:46. https://doi.org/10.3390/ANTIBIOTICS7020046

Bagur H, Poojari CC, Melappa G, Rangappa R, Chandrasekhar N, Somu P (2020) Biogenically synthesized silver nanoparticles using endophyte fungal extract of Ocimum tenuiflorum and evaluation of biomedical properties. J Clust Sci 31:1241–1255. https://doi.org/10.1007/S10876-019-01731-4

Baker S, Satish S (2012) Endophytes: toward a vision in synthesis of nanoparticle for future therapeutic agents. Int J Bio-Inorg Hybd Nanomat 1:67–77

Balakumaran MD, Ramachandran R, Kalaichelvan PT (2015) Exploitation of endophytic fungus, Guignardia mangiferae for extracellular synthesis of silver nanoparticles and their in vitro biological activities. Microbiol Res 178:9–17. https://doi.org/10.1016/J.MICRES.2015.05.009

Bansal SA, Kumar V, Karimi J, Singh AP, Kumar S (2020) Role of gold nanoparticles in advanced biomedical applications. Nanoscale Adv 2:3764–3787. https://doi.org/10.1039/D0NA00472C

Bhargava A, Jain N, Barathi ML, Akhtar MS, Yun YS, Panwar J (2013) Synthesis, characterization and mechanistic insights of mycogenic iron oxide nanoparticles. J Nanopart Res 15:2031. https://doi.org/10.1007/S11051-013-2031-5

Bose P, Sezhian UG (2017) Mycosynthesis, optimisation and characterization of silver nanoparticles by endophytic fungus isolated from the root of *casuarina Junghuhniana Miq*. Int J Pharm Sci Rev Res 43:107–115

Clarance P, Luvankar B, Sales J, Khusro A, Agastian P, Tack JC, Al Khulaifi MM et al (2020) Green synthesis and characterization of gold nanoparticles using endophytic fungi Fusarium Solani and its in-vitro anticancer and biomedical applications. Saudi J Biol Sci 27:706–712. https://doi.org/10.1016/J.SJBS.2019.12.026

Cuevas R, Durán N, Diez MC, Tortella GR, Rubilar O (2015) Extracellular biosynthesis of copper and copper oxide nanoparticles by Stereum hirsutum, a native white-rot fungus from Chilean forests. J Nanomater 2015:789089. https://doi.org/10.1155/2015/789089

Danagoudar A, Pratap GK, Shantaram M, Ghosh K, Kanade SR, Joshi CG (2020) Characterization, cytotoxic and antioxidant potential of silver nanoparticles biosynthesised using endophytic fungus (Penicillium citrinum CGJ-C1). Mater Today Commun 25:101385. https://doi.org/10.1016/J.MTCOMM.2020.101385

Devi LS, Joshi SR (2015) Ultrastructures of silver nanoparticles biosynthesized using endophytic fungi. J Microsc Ultrastruct 3:29–37. https://doi.org/10.1016/J.JMAU.2014.10.004

Du L, Xian L, Feng JX (2011) Rapid extra-/intracellular biosynthesis of gold nanoparticles by the fungus Penicillium Sp. J Nanopart Res 13:921–930. https://doi.org/10.1007/S11051-010-0165-2/FIGURES/7

Ealias SAM, Saravanakumar MP (2017) A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf Ser: Mater Sci Eng 263:032019. https://doi.org/10.1088/1757-899X/263/3/032019

Elbahnasawy MA, Shehabeldine AM, Khattab AM, Amin BH, Hashem AH (2021) Green biosynthesis of silver nanoparticles using novel endophytic Rothia endophytica: characterization and anticandidal activity. J Drug Deliv Sci Techno 62:102401. https://doi.org/10.1016/j.jddst.2021.102401

El-Sayed ESR, Abdelhakim HK, Zakaria Z (2020) Extracellular biosynthesis of cobalt ferrite nanoparticles by Monascus purpureus and their antioxidant, anticancer and antimicrobial activities: yield enhancement by gamma irradiation. Mater Sci Eng C Mater Biol Appl 107:110318. https://doi.org/10.1016/J.MSEC.2019.110318

Farsi M, Farokhi S (2018) Biosynthesis of antibacterial silver nanoparticles by endophytic Fungus Nemania sp. isolated from Taxus baccata L. (Iranian Yew). Zahedan J Res Med Sci 20. https://doi.org/10.5812/ZJRMS.57916

Fatima F, Verma SR, Pathak N, Bajpai P (2016) Extracellular mycosynthesis of silver nanoparticles and their microbicidal activity. J Glob Antimicrob Resist 7:88–92. https://doi.org/10.1016/J.JGAR.2016.07.013

Ganesan V, Hariram M, Vivekanandhan S, Muthuramkumar S (2020) Periconium Sp. (endophytic fungi) extract mediated sol-gel synthesis of ZnO nanoparticles for antimicrobial and antioxidant applications. Mater Sci Semicond Process 105:104739. https://doi.org/10.1016/J.MSSP.2019.104739

Gao Y, Anand MAV, Ramachandran V, Karthikkumar V, Shalini V, Vijayalakshmi S, Ernest D (2019) Biofabrication of zinc oxide nanoparticles from Aspergillus Niger, their antioxidant, antimicrobial and anticancer activity. J Clust Sci 30:937–946. https://doi.org/10.1007/S10876-019-01551-6

Hemashekhar B, Chandrappa CP, Govindappa M, Chandrashekar N (2019) Endophytic fungus Alternaria Spp isolated from Rauvolfia tetraphylla root arbitrate synthesis of gold nanoparticles and evaluation of their antibacterial, antioxidant and antimitotic activities. Adv Nat Sci Nanosci Nanotechnol 10:035010. https://doi.org/10.1088/2043-6254/AB38B0

Hietzschold S, Walter A, Davis C, Taylor AA, Sepunaru L (2019) Does nitrate reductase play a role in silver nanoparticle synthesis? Evidence for NADPH as the sole reducing agent. ACS Sustain Chem Eng 7:8070–8076. https://doi.org/10.1021/ACSSUSCHEMENG.9B00506/SUPPL_FILE/SC9B00506_SI_001.PDF

Hu X, Saravanakumar K, Jin T, Wang MH (2019) Mycosynthesis, characterization, anticancer and antibacterial activity of silver nanoparticles from endophytic fungus *Talaromyces purpureogenus*. Int J Nanomedicine 14:3427–3438. https://doi.org/10.2147/IJN.S200817

Iravani S (2011) Green synthesis of metal nanoparticles using plants. Green Chem 13:2638–2650. https://doi.org/10.1039/C1GC15386B

Islam SKN, Naqvi SMA, Parveen S, Ahmad A (2021) Endophytic fungus-assisted biosynthesis, characterization and solar photocatalytic activity evaluation of nitrogen-doped Co_3O_4 nanoparticles. Appl Nanosci 11:1651–1659. https://doi.org/10.1007/S13204-021-01824-5

Joshi CG, Danagoudar A, Poyya J, Kudva AK, Dhananjaya BL (2017) Biogenic synthesis of gold nanoparticles by marine endophytic fungus-Cladosporium cladosporioides isolated from seaweed and evaluation of their antioxidant and antimicrobial properties. Process Biochem 63:137–144. https://doi.org/10.1016/J.PROCBIO.2017.09.008

Kadam VV, Ettiyappan JP, Balakrishnan RM (2019) Mechanistic insight into the endophytic fungus mediated synthesis of protein capped ZnO nanoparticles. Mater Sci Eng B 243:214–221. https://doi.org/10.1016/J.MSEB.2019.04.017

Kareem SO, Adeleye TM, Ojo RO (2020) Effects of PH, temperature and agitation on the biosynthesis of iron nanoparticles produced by Trichoderma species. IOP Conf Ser: Mater Sci Eng 805:012036. https://doi.org/10.1088/1757-899X/805/1/012036

Khan NT, Jameel J, Jameel N, Rheman SUA (2017) An overview: biosynthesized nanoparticles with their potential applications. Glob J Nanomed 2:5–8. https://doi.org/10.19080/GJN.2017.02.555577

Kumar S, Aharwal RP, Sandhu SS (2020) Nanoparticles from fungi: a novel approach toward eco-friendly drug designing. Mod Appro Drug Des 3:1. https://doi.org/10.31031/MADD.2020.03.000558

Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: a review of novel bioactive compounds with biological applications. Microorganisms 8:1–25. https://doi.org/10.3390/microorganisms8121934

Mani VM, Kalaivani S, Sabarathinam S, Vasuki M, Soundari AJPG, Das MPA, Elfasakhany A, Pugazhendhi A (2021) Copper oxide nanoparticles synthesized from an endophytic fungus Aspergillus terreus: bioactivity and anti-cancer evaluations. Environ Res 201:111502. https://doi.org/10.1016/J.ENVRES.2021.111502

Manjunath HM, Joshi CG (2019) Characterization, antioxidant and antimicrobial activity of silver nanoparticles synthesized using marine endophytic fungus- Cladosporium cladosporioides. Process Biochem 82:199–204. https://doi.org/10.1016/J.PROCBIO.2019.04.011

Manjunath HM, Joshi CG, Raju NG (2017) Biofabrication of gold nanoparticles using marine endophytic fungus - *Penicillium citrinum*. IET Nanobiotechnol 11:40–44. https://doi.org/10.1049/IET-NBT.2016.0065

Mariana G-C, de Lima R (2019) Synthesis of silver nanoparticles mediated by fungi: a review. Front Bioeng Biotechnol 7:287. https://doi.org/10.3389/FBIOE.2019.00287/BIBTEX

Mathur P, Saini S, Paul E, Sharma C, Mehtani P (2021) Endophytic fungi mediated synthesis of iron nanoparticles: characterization and application in methylene blue decolorization. Curr Res Green Sustain Chem 4:100053. https://doi.org/10.1016/j.crgsc.2020.100053

Mei W, Wu Q (2017) Applications of metal nanoparticles in medicine/metal nanoparticles as anticancer agents. In: Thota S, Crans DC (eds) Metal nanoparticles: synthesis and applications in pharmaceutical sciences. John Wiley & Sons, Inc, Hoboken, pp 169–190. https://doi.org/10.1002/9783527807093.CH7

Mekky AE, Farrag AA, Hmed AA, Sofy AR (2021) Preparation of zinc oxide nanoparticles using *Aspergillus Niger* as antimicrobial and anticancer agents. J Pure Appl Microbiol 15:1547–1566. https://doi.org/10.22207/JPAM.15.3.49

Messaoudi O, Bendahou M (2020) Biological synthesis of nanoparticles using endophytic microorganisms: current development. In: Sen M (ed) Nanotechnology and the environment. InTech Open, London. https://doi.org/10.5772/intechopen.93734

Mishra RC, Kalra R, Dilawari R, Goel M, Barrow CJ (2022) Bio-synthesis of *Aspergillus terreus* mediated gold nanoparticle: antimicrobial, antioxidant, antifungal and in vitro cytotoxicity studies. Materials (Basel) 15:3877. https://doi.org/10.3390/ma15113877

Moezzi A, McDonagh AM, Cortie MB (2012) Zinc oxide particles: synthesis, properties and applications. Chem Eng J 185–186:1–22. https://doi.org/10.1016/J.CEJ.2012.01.076

Moghaddam AB, Namvar F, Moniri M, Md Tahir P, Azizi S, Mohamad R (2015) Nanoparticles biosynthesized by fungi and yeast: a review of their preparation, properties, and medical applications. Molecules 20:16540–16565. https://doi.org/10.3390/MOLECULES200916540

Mogilevsky G, Olga H, Emmons ED, Balboa A, Decoste JB, Schindler BJ, Iordanov I, Karwacki CJ (2014) Bottom-up synthesis of anatase nanoparticles with graphene domains. ACS Appl Mater Interfaces 6:10638–10648. https://doi.org/10.1021/AM502322Y/SUPPL_FILE/AM502322Y_SI_001.PDF

Mohamed YM, Azzam AM, Amin BH, Safwat NA (2015) Mycosynthesis of iron nanoparticles by Alternaria alternata and its antibacterial activity. Afr J Biotechnol 14:1234–1241. https://doi.org/10.5897/AJB2014.14286

Mohamed AA, Fouda A, Abdel-Rahman MA, Hassan SED, El-Gamal MS, Salem SS, Shaheen TI (2019) Fungal strain impacts the shape, bioactivity and multifunctional properties of green synthesized zinc oxide nanoparticles. Biocatal Agric Biotechnol 19:101103. https://doi.org/10.1016/J.BCAB.2019.101103

Mohammad F, Farokhi S (2018) Biosynthesis of antibacterial silver nanoparticles by endophytic fungus Nemania Sp. isolated from Taxus baccata L. (Iranian Yew). Zahedan. J Res Med Sci 20:e57916. https://doi.org/10.5812/ZJRMS.57916

Mohanta YK, Behera SK (2014) Biosynthesis, characterization and antimicrobial activity of silver nanoparticles by Streptomyces sp. SS2. Bioprocess Biosyst 37:2263–2269. https://doi.org/10.1007/s00449-014-1205-6

Mohmed AA, Hassan SE, Fouda A, Elgamal S (2017) Extracellular biosynthesis of silver nanoparticles using Aspergillus Sp. and evaluation of their antibacterial and cytotoxicity. J Appl Life Sci Int 11:1–12. https://doi.org/10.9734/JALSI/2017/33491

Molnár Z, Bódai V, Szakacs G, Erdélyi B, Fogarassy Z, Sáfrán G, Varga T et al (2018) Green synthesis of gold nanoparticles by thermophilic filamentous fungi. Sci Rep 8:3943. https://doi.org/10.1038/s41598-018-22112-3

Mousa SA, El-Sayed ESR, Mohamed SS, El-Seoud MAA, Elmehlawy AA, Abdou DAM (2021) Novel mycosynthesis of Co_3O_4, CuO, Fe_3O_4, NiO, and ZnO nanoparticles by the endophytic Aspergillus terreus and evaluation of their antioxidant and antimicrobial activities. Appl Microbiol Biotechnol 105:741–753. https://doi.org/10.1007/S00253-020-11046-4/TABLES/5

Munawer U, Raghavendra VB, Ningaraju S, Krishna KL, Ghosh AR, Melappa G, Pugazhendhi A (2020) Biofabrication of gold nanoparticles mediated by the endophytic Cladosporium species: photodegradation, in vitro anticancer activity and in vivo antitumor studies. Int J Pharm 588:119729. https://doi.org/10.1016/J.IJPHARM.2020.119729

Nayantara KP, Kaur P (2018) Biosynthesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: a review. Biotechnol Res Innov 2:63–73. https://doi.org/10.1016/J.BIORI.2018.09.003

Nejad MS, Najafabadi NS, Aghighi S, Pakina E, Zargar M (2022) Evaluation of *Phoma* Sp. biomass as an endophytic fungus for synthesis of extracellular gold nanoparticles with antibacterial and antifungal properties. Molecules 27:1181. https://doi.org/10.3390/molecules27041181

Netala VR, Kotakadi VS, Bobbu P, Gaddam SA, Tartte V (2016) Endophytic fungal isolate mediated biosynthesis of silver nanoparticles and their free radical scavenging activity and anti microbial. Studies 3 Biotech 6:132. https://doi.org/10.1007/S13205-016-0433-7

Ningaraju S, Munawer U, Raghavendra VB, Balaji KS, Melappa G, Brindhadevi K, Pugazhendhi A (2021) Chaetomium globosum extract mediated gold nanoparticle synthesis and potent anti-inflammatory activity. Anal Biochem 612:113970. https://doi.org/10.1016/J.AB.2020.113970

Noor S, Shah Z, Javed A, Ali A, Hussain SB, Zafar S, Ali H, Muhammad SA (2020) A fungal based synthesis method for copper nanoparticles with the determination of anticancer, antidiabetic and antibacterial activities. J Microbiol Methods 174:105966. https://doi.org/10.1016/J.MIMET.2020.105966

Othman AM, Elsayed MA, Al-Balakocy NG, Hassan MM, Elshafei AM (2019) Biosynthesis and characterization of silver nanoparticles induced by fungal proteins and its application in different biological activities. J Genet Eng Biotechnol. https://doi.org/10.1186/S43141-019-0008-1

Packiam KK, Dhakshinamoorthy M (2021) Endophytic fungi: a review of survival strategies that influence the biodiversity studies associated with biopotential products, *fungi bio-prospects in sustainable agriculture, environment and nano-technology*. In: Sharma VK, Shah MP, Parmar S, Kumar A (eds) Fungi bio-prospects in sustainable agriculture, environment and nano-technology. Elsevier, Academic Press, Cambridge, MA, pp 1–15. https://doi.org/10.1016/B978-0-12-821394-0.00001-9

Peiris M, Gunasekara T, Jayaweera PM, Fernando S (2018) TiO_2 nanoparticles from Baker's yeast: a potent antimicrobial. J Microbiol Biotechnol 28:1664–1670. https://doi.org/10.4014/JMB.1807.07005

Priyadarshini E, Pradhan N, Sukla LB, Panda PK (2014) Controlled synthesis of gold nanoparticles using Aspergillus terreus If0 and its antibacterial potential against gram negative pathogenic bacteria. J Nanotechnol 2014:1. https://doi.org/10.1155/2014/653198

Rai M, Gupta I, Bonde S, Ingle P, Shende S, Gaikwad S, Razzaghi-Abyaneh M, Gade A (2022) Industrial applications of nanomaterials produced from *Aspergillus* species. In: Razzaghi-Abyaneh M, Rai M (eds) The genus Aspergillus - pathogenicity, mycotoxin production and industrial applications. InTech Open, London. https://doi.org/10.5772/INTECHOPEN.98780

Ramadan Gouda A, Sidkey NM, Shawky HA, Abdel-hady YA (2020) Biosynthesis, characterization and antimicrobial activity of iron oxide nanoparticles synthesized by fungi. Al-Azhar J Pharm Sci 62:164–179. https://doi.org/10.21608/AJPS.2020.118382

Ramamurthy CH, Padma M, Daisy Mariya Samadanam I, Mareeswaran R, Suyavaran A, Suresh Kumar M, Premkumar K, Thirunavukkarasu C (2013) The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. Colloids Surf B Biointerfaces 102:808–815. https://doi.org/10.1016/J.COLSURFB.2012.09.025

Rani S, Kumar P, Dahiya P, Dang AS, Suneja P (2022) Biogenic synthesis of zinc nanoparticles, their applications, and toxicity prospects. Front Microbiol 13:824427. https://doi.org/10.3389/FMICB.2022.824427/BIBTEX

Ranjani S, Shariq Ahmed M, Adnan M, Senthil Kumar N, Ruckmani K, Hemalatha S (2021) Synthesis, characterization and applications of endophytic fungal nanoparticles. Inorg Nano-Met Chem 51:280–287. https://www.ingentaconnect.com/content/tandf/lsrt21/2021/00000051/00000002/art00017

Rasha E, Alkhulaifi MM, AlOthman M, Khalid I, Doaa E, Alaa K, Awad MA, Abdalla M (2021) Effects of zinc oxide nanoparticles synthesized using Aspergillus Niger on Carbapenem-resistant *Klebsiella pneumonia* in vitro and in vivo. Front Cell Infect Microbiol 11:748739. https://doi.org/10.3389/FCIMB.2021.748739/BIBTEX

Rodríguez-Serrano C, Guzmán-Moreno J, Ángeles-Chávez C, Rodríguez-González V, Ortega-Sigala JJ, Ramírez-Santoyo RM, Vidales-Rodríguez LE (2020) Biosynthesis of silver nanoparticles by Fusarium scirpi and its potential as antimicrobial agent against uropathogenic Escherichia coli biofilms. PLoS One 15:e0230275. https://doi.org/10.1371/JOURNAL.PONE.0230275

Salvadori MR, Ando RA, Nascimento CAO, Corrêa B (2015) Extra and intracellular synthesis of nickel oxide nanoparticles mediated by dead fungal biomass. PLoS One 10:e0129799. https://doi.org/10.1371/JOURNAL.PONE.0129799

Sau TK, Biswas A, Ray P (2017) Metal nanoparticles in nanomedicine: advantages and scope. In: Thota S, Crans DC (eds) Metal nanoparticles: synthesis and applications in pharmaceutical sciences. John Wiley & Sons, Inc, Hoboken, pp 121–168. https://doi.org/10.1002/9783527807093.CH6

Schuster S, Ting ASY (2022) Decolourisation of triphenylmethane dyes by biogenically synthesised iron nanoparticles from fungal extract. Mycology 13:56–67. https://doi.org/10.1080/21501203.2021.1948928

Shehabeldine AM, Amin BH, Hagras FA, Ramadan AA, Kamel MR, Ahmed MA, Atia KH, Salem SS (2022) Potential antimicrobial and antibiofilm properties of copper oxide nanoparticles: time-kill kinetic essay and ultrastructure of pathogenic bacterial cells. Appl Biochem Biotechnol 195:467. https://doi.org/10.1007/S12010-022-04120-2/FIGURES/8

Singh D, Rathod V, Ninganagouda S, Hiremath J, Singh AK, Mathew J (2014) Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* Sp. isolated from *Curcuma longa* (Turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorg Chem Appl 2014:408021. https://doi.org/10.1155/2014/408021

Singh T, Kumari J, Patnaik A, Singh A, Chauhan R, Chandel SS (2017) Biosynthesis, characterization and antibacterial activity of silver nanoparticles using an endophytic fungal supernatant of Raphanus sativus. J Genet Eng Biotechnol 15:31–39. https://doi.org/10.1016/J.JGEB.2017.04.005

Singh A, Madhavi BLR, Nithin Sagar MN (2021) An overview of green synthesis mediated metal nanoparticles preparation and its scale up opportunities. J Drug Deliv Ther 11:304–314. https://doi.org/10.22270/JDDT.V11I6.5082

Staniek A, Woerdenbag HJ, Kayser O (2008) Endophytes: exploiting biodiversity for the improvement of natural product-based drug discovery. J Plant Interact 3:75–93. https://doi.org/10.1080/17429140801886293

Sturikova H, Krystofova O, Huska D, Adam V (2018) Zinc, zinc nanoparticles and plants. J Hazard Mater 349:101–110. https://doi.org/10.1016/J.JHAZMAT.2018.01.040

Sumanth B, Lakshmeesha TR, Ansari MA, Alzohairy MA, Udayashankar AC, Shobha B, Niranjana SR, Srinivas C, Almatroudi A (2020) Mycogenic synthesis of extracellular zinc oxide nanoparticles from *Xylaria acuta* and its nanoantibiotic potential. Int J Nanomedicine 15:8519–8536. https://doi.org/10.2147/IJN.S271743

Suresh JI, Sona NM (2021) Fungal endophytes, biodiversity and biopotential applications. In: Sharma VK, Shah MP, Parmar S, Kumar A (eds) Fungi bio-prospects in sustainable agriculture, environment and nano-technology. Elsevier, Academic Press, Cambridge, MA, pp 107–115. https://doi.org/10.1016/B978-0-12-821394-0.00005-6

Suryavanshi P, Pandit R, Gade A, Derita M, Zachino S, Rai M (2017) *Colletotrichum Sp.*- mediated synthesis of sulphur and aluminium oxide nanoparticles and its in vitro activity against selected food-borne pathogens. LWT - Food Sci Technol 81:188–194. https://doi.org/10.1016/J.LWT.2017.03.038

Swamy N, Sandhu SS (2021) Fungal endophytes: entry, establishment, diversity, future prospects in agriculture. In: Sharma VK, Shah MP, Parmar S, Kumar A (eds) Fungi bio-prospects in sustainable agriculture, environment and nano-technology. Elsevier, Academic Press, Cambridge, MA, pp 97–105. https://doi.org/10.1016/B978-0-12-821394-0.00004-4

Taha ZK, Hawar SN, Sulaiman GM (2019) Extracellular biosynthesis of silver nanoparticles from Penicillium italicum and its antioxidant. Antimicrob Cytotoxicity Activities Biotechnol Lett 41:899–914. https://doi.org/10.1007/S10529-019-02699-X

Tarafdar JC, Raliya R (2013) Rapid, low-cost, and ecofriendly approach for iron nanoparticle synthesis using Aspergillus oryzae TFR9. J Nanoparticle 2013:141274. https://doi.org/10.1155/2013/141274

Thakker JN, Dalwadi P, Dhandhukia PC (2013) Biosynthesis of gold nanoparticles using *Fusarium oxysporum* f. Sp. Cubense JT1, a plant pathogenic fungus. Int Sch Res Notices 2013:515091. https://doi.org/10.5402/2013/515091

Thomas E, Rathore I, Tarafdar JC (2017) Bioinspired production of molybdenum nanoparticles and its effect on chickpea (*Cicer arietinum* L). J Bionanosci 11:153–159. https://doi.org/10.1166/jbns.2017.1425

Uddandarao P, Balakrishnan RM, Ashok A, Swarup S, Sinha P (2019) Bioinspired ZnS:Gd nanoparticles synthesized from an endophytic fungi Aspergillus flavus for fluorescence-based metal detection. Biomimetics 4:11. https://doi.org/10.3390/BIOMIMETICS4010011

Vasanth K, Ilango K, MohanKumar R, Agrawal A, Dubey GP (2014) Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. Colloids Surf B Biointerfaces 117:354–359. https://doi.org/10.1016/J.COLSURFB.2014.02.052

Verma N, Kumar N (2019) Synthesis and biomedical applications of copper oxide nanoparticles: an expanding horizon. ACS Biomater Sci Eng 5:1170–1188. https://doi.org/10.1021/ACSBIOMATERIALS.8B01092/ASSET/IMAGES/MEDIUM/AB-2018-010925_0012.GIF

Verma SK, Gond SK, Mishra A, Sharma VK, Kumar J, Singh DK, Kharwar RN (2016) Biofabrication of antibacterial and antioxidant silver nanoparticles (Agnps) by an endophytic fungus *Pestalotia Sp.* Isolated from *Madhuca Longifolia*. J Nanomater Mol Nanotechnol 5. https://doi.org/10.4172/2324-8777.1000189

Vijayanandan AS, Balakrishnan RM (2018) Biosynthesis of cobalt oxide nanoparticles using endophytic fungus Aspergillus nidulans. J Environ Manag 218:442–450. https://doi.org/10.1016/J.JENVMAN.2018.04.032

Waris A, Din M, Ali A, Afridi S, Baset A, Khan AU, Ali AU (2021) Green fabrication of co and Co_3O_4 nanoparticles and their biomedical applications: a review. Open Life Sci 16:14–30. https://doi.org/10.1515/BIOL-2021-0003

Zhang X, He X, Wang K, Yang X (2011) Different active biomolecules involved in biosynthesis of gold nanoparticles by three fungus species. J Biomed Nanotechnol 7:245–254. https://doi.org/10.1166/JBN.2011.1285

Zomorodian K, Pourshahid S, Sadatsharifi A, Mehryar P, Pakshir K, Rahimi MJ, Monfared AA (2016) Biosynthesis and characterization of silver nanoparticles by Aspergillus species. Biomed Res Int 2016:435397. https://doi.org/10.1155/2016/5435397

Chapter 6
Fungal Endophytes and Their Role in Postharvest Disease Management: An Overview

Ramannagari Visweswara Maruthi Prasad, Kunuthuru Sivakumar, Charu Agnihotri, Shekhar Agnihotri, Ajit Kumar Passari, and Bhim Pratap Singh

Abstract The primary method for preventing postharvest fungal degradation of fruits and vegetables is the application of chemical fungicides. Increasing public concern regarding the lingering presence of fungicidal contamination of perishables and the emergence of resistant pathogen populations have been the main obstacles to the effective use of fungicides. Recent research has aimed to create and assess new substitute control methods to reduce dependency on synthetic fungicides. For the control of fungal deterioration, a number of nonchemical methods have been suggested. These methods have been found to lessen fruit and vegetable postharvest rots. However, they all have disadvantages that may make them less commercially viable. None of the nonchemical control approaches has consistently shown the ability to provide a reasonable level of disease control that supports acceptance as a replacement for synthetic fungicides. There has recently been a surge in interest in employing natural items to halt decomposition and extend the shelf life of perishables. Natural, biologically active compounds have the potential to replace synthetic fungicides. Significant genera of anamorphic postharvest infections include *Penicillium*, *Aspergillus*, *Geotrichum*, *Botrytis*, *Fusarium*, *Alternaria*, *Colletotrichum*, *Dothiorella*, *Lasiodiplodia*, and *Phomopsis*. Coordinated postharvest infection management strategies should be implemented to reduce the loss of fresh fruits and vegetables qualitatively and quantitatively during food supply chain.

Keywords Postharvest diseases · Chemical fungicides · Natural products · Synthetic fungicides

R. V. M. Prasad · K. Sivakumar · C. Agnihotri · S. Agnihotri · B. P. Singh (✉)
Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India
e-mail: bpsingh@niftem.ac.in

A. K. Passari (✉)
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Ciuded de Mexico, México

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1 Introduction

Fruit and vegetable postharvest losses are substantial due to fungal plant disease deterioration. Fruit is particularly susceptible to pathogenic fungus due to its low pH, greater moisture content, and nutritional makeup (Moss 2002). According to Eckert and Ratnayake (1983), plant pathogens account for less than 10% of the 100,000 fungal species, and less than 100 fungal species cause majority of postharvest illnesses. According to international organizations that monitor the world's food resources, reduced postharvest losses are one of the most likely options for fulfilling future food needs (Kelman 1984; FAO 2019).

Food loss and waste represent a significant global challenge, leading to an estimated annual loss of approximately one-third of total production, which amounts to 1.3 billion tons of food worldwide (Dos Santos et al. 2020; Lipinski et al. 2013). Remarkably, fruits and vegetables contribute to 40–50% of global losses, with 54% occurring during production, postharvest, handling, and storage stages, while 46% are attributed to processing, distribution, and consumption, resulting in an annual total loss of US$750 billion (FAO 2013; Gustavsson and Stage 2011; Ali et al. 2021).

The primary causes of postharvest losses in both developed and developing nations encompass inadequate packaging, insufficient quantity planning during procurement, and excessive handling by producers, traders, and consumers (Gustavsson and Stage 2011; Lebersorger and Schneider 2014; Mendonca et al. 2020). Furthermore, a significant deficiency in the adoption of good manufacturing practices is observed in numerous distribution establishments worldwide. This inadequacy is manifested through unsatisfactory hygienic conditions, increasing the risks of contamination during handling and exposure, consequently amplifying losses and fostering the incidence of food-borne diseases (Denis et al. 2016; Manzini and Accorsi 2013; Farber et al. 2021). The occurrence of postharvest decay in harvested fruit results in notable financial losses. To address this issue, fungicides have conventionally been employed as the primary means of control. Nevertheless, there is a rising public concern regarding food safety and environmental impacts associated with fungicide usage, alongside the emergence of pathogen-resistant populations. Consequently, there is a growing interest in exploring and developing alternative methods to fungicides to effectively manage and prevent postharvest fruit decay (Nunes 2012; Zhang et al. 2020).

It is common practice to use fungicides to treat postharvest diseases. The application of hot air, curative, and hot-water brushing lowers the frequency of illness and boosts antagonist effectiveness. To effectively manage the disease, for example, dipping in hot water (at 50 °C for 5–10 min, depending on the amount of produce in combination with the fungicide) is also utilized. Fungicides are frequently used to address postharvest infections; however, botanicals and biocontrol agents may reduce their need. When using stored goods, biological products and biopesticides have a huge potential to prevent postharvest illnesses of vegetable crops. The biopesticides Ecogen US (AspireTM), Azotobactor (Bio-SaveTM), and Anchor (Yield PlusTM) are used in conjunction with products containing a low amount of fungicide, salt solutions (calcium chloride or sodium bicarbonate at 1–2%), and

other food additives to increase the effectiveness against postharvest diseases. EcoTrol, Sporan (a fungicide), and Mataran (a weedicide) are three rosemary oil-based EcoSMART formulations certified as secure plant defenders. As a result, crop disease can be treated with environmentally friendly postharvest treatments.

2 What Are Endophytes?

The term endophyte (endo = within, phyte = plant) was coined by the father of modern plant pathology, Anton de Bary in 1866 for "any organism growing within plant tissues" (Collinge et al. 2022). Endophytic microorganisms live inside plant tissues throughout their lives or during certain life cycles without causing visible damage or morphological changes in their hosts (Gouda et al. 2016). Endophyte research has revealed potential applications in agriculture, forestry, and medicine. They can be employed as biofertilizers to boost agricultural yield, biocontrol agents to combat plant diseases, or as a source of novel bioactive chemicals for pharmaceutical applications (Arora and Ramawat 2017; Uzma et al. 2018; Passari et al. 2020).

Endophytes have a symbiotic connection with the plant, offering various benefits such as plant growth and development by improving nutrient intake, encouraging root growth, and creating growth-promoting substances like phytohormones (Mishra et al. 2016; Santoyo et al. 2016; Abdalla et al. 2020). Endophytes possess the ability to enhance a plant's resistance to various environmental stressors such as drought, heat, and infections. Additionally, they aid in the plant's defense mechanisms against herbivores and pathogens through the production of secondary metabolites with antibacterial or insecticidal properties (Schulz and Boyle 2014). Endophytes represent biological reservoirs of novel natural products, opening new avenues in the frontiers of drug discovery. The discovery of the well-known anticancer drug paclitaxel stimulated research into endophytic biology, yielding promising "drug candidates" with antibacterial, immunosuppressive, antioxidant, and anti-neurodegenerative properties (Uzma et al. 2018; Thirumurugan et al. 2018). Endophytes include fungi such as *Claviceps*, *Neotyphodium*, and *Piriformospora*, as well as bacteria such as *Bacillus* and *Pseudomonas*.

2.1 Bacterial Endophytes

Bacterial endophytes are the second most frequent, colonizing plant species locally or systemically residing within cells, intercellular gaps, or the vascular system (Zinniel et al. 2002). Endophytic bacteria invade plants' internal tissues without signs of illness or harmful effects on the host, and out of nearly 300,000 plant species on the earth, each plant is a host to one or more endophytes (Passari et al. 2016; Bangar et al. 2022). Endophytic bacteria can reduce or prevent the negative impacts of certain pathogens on host. They benefit their host plant through similar

mechanisms for rhizosphere-associated bacteria (Gray and Smith 2005). Endophytic bacteria trigger a phenomenon known as induced systemic resistance (ISR), which is phenotypically similar to systemic-acquired resistance (SAR). SAR arises when plants successfully engage their defense mechanism in response to primary pathogen infection, mainly when the pathogen triggers a hypersensitive reaction that limits it to a small necrotic lesion of brown desiccated tissues. ISR is efficient against a variety of diseases. However, unlike SAR, the inducing bacterium does not cause visible symptoms in the host plant (Kloepper and Ryu 2007).

Endophytes support plant growth through a variety of similar mechanisms. These include phosphate solubilization potential, indole acetic acid synthesis, and siderophore production (Lee et al. 2004). Endophytes have also been linked to various additional favorable impacts on plant development, including as osmotic regulation, stomatal regulation, root shape modification, increased mineral absorption, and changes in nitrogen buildup and metabolism (Compant et al. 2005). The recent areas where these plant growth-promoting bacterial endophytes are being used are in the developing areas of forest regeneration and phytoremediation of contaminated soils (Fig. 6.1). Many endophytes belong to bacterial genera found in soil, such as *Pseudomonas*, *Burkholderia*, and *Bacillus*. Antibiotics, anticancer chemicals, volatile organic compounds, antifungal, antiviral, insecticidal, and immunosuppressive substances are all secondary metabolic products. While many biologically active compounds have been isolated from

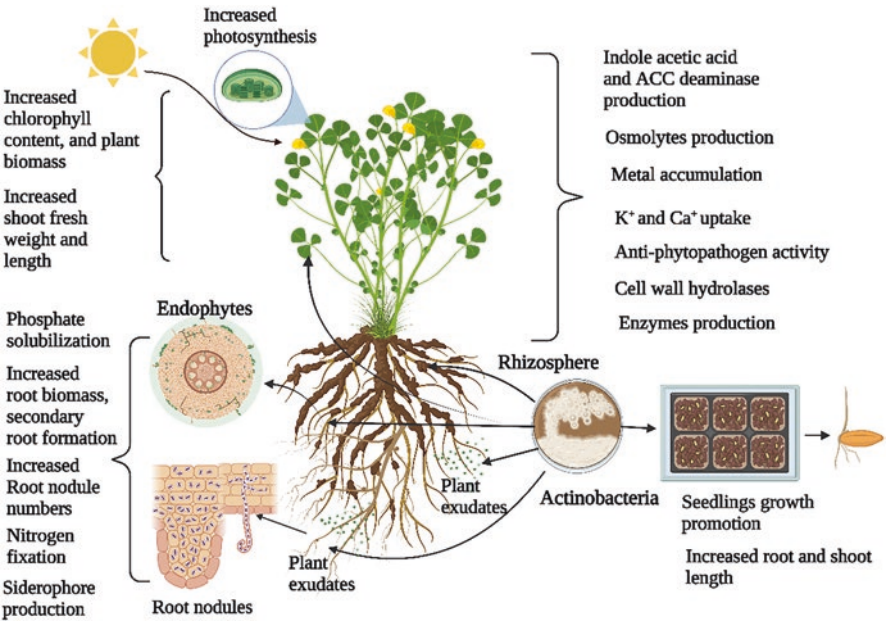

Fig. 6.1 Role of endophytic actinobacteria in enhancing the plant growth promotion of plants. (Adopted from: Narsing Rao et al. 2022, Open Access)

endophytic organisms, they remain a relatively untapped source of novel natural products (Christina et al. 2013).

2.2 Fungal Endophytes

Fungal endophytes are the most common, diversified, and well-studied endophytes for their function in plant stress resilience (Lugtenberg et al. 2016). Fungal endophytes are categorized into clavicipitaceous and non-clavicipitaceous groups based on their taxonomy, evolutionary relatedness, ecology, and host range (Santangelo et al. 2015). Calvicipitaceous endophytes include the genera *Balansia*, *Balansiopsis*, *Atkinsonella*, *Echinodothis*, *Epichloe*, *Myriogenospora*, *Neotyphodium*, and *Parepichloe*, which are commonly associated with grasses, which rely on their host throughout their life cycle as mutualists (Hardoim et al. 2015; De Silva et al. 2016). These endophytes develop in the intercellular gaps of aboveground plant tissues and are transmitted horizontally and vertically depending on the species (Santangelo et al. 2015). Non-clavicipitaceous endophytes such as *Fusarium*, *Colletotrichum*, *Phomopsis*, and *Xylaria* are found in most terrestrial plants and do not rely on plants to complete their life cycle (De Silva et al. 2016; Jayawardena et al. 2016).

Fungal endophytes are widely known for providing plant fitness benefits by allowing the plant host to adapt to biotic and abiotic challenges (Card et al. 2016). Endophytic fungi produce a wide range of secondary metabolites with various bioactive compounds expressed as defensive weapons to protect the host plant from pests and diseases and improve the plant's immune system. In contrast, some metabolites show specific interactions and communications with the plant host (Rodriguez et al. 2009). Specific fungal endophytes adapt to stress in a habitat-specific manner under different environmental conditions. The molecular and biochemical mechanisms behind how this habitat-adapted symbiotic interaction results in plant tolerance to high stress are unknown. Endophyte-enhanced plant growth promotion is another conferred beneficial trait that at least for the root-colonizing endophyte *Piriformospora indica* is likely achieved through enhanced nutrient uptake and translocation and by the modulation of phytohormones involved in growth and development (Singh et al. 2021).

Because of their tremendous biodiversity, fungal endophytes offer a largely untapped opportunity to discover novel natural products with distinct chemical structures tuned through (co-)evolution with higher plants. Recent screening technology advancements have revealed that fungal endophytes have enormous potential for creating novel physiologically active chemicals with interesting medical or agricultural applications (Aly et al. 2011; Peng et al. 2021). These chemicals have the potential to play a significant role in organism communication, plant protection, and plant adaptability to habitat and environmental changes. For many, synthetic agricultural agents have been and will be pulled from the market due to safety and environmental concerns. Secondary metabolites produced by fungal endophytes provide up fresh opportunities for pest and disease management (Qin et al. 1996).

Endophytic fungi have biotechnology potential in variety life science applications including anticancer drugs (taxol, L-asparaginase, L-glutaminase, tyrosinase, and methioninase) (El-Gendy et al. 2016, 2018b), antibacterial agents (essramycin, ayamycin, benzopyrones derivatives, and coumarine derivatives) (El-Gendy et al. 2018a, b), antifungal agents (saadamycin and prodigiosin) (El-Bondkly et al. 2012), antiviral agents (El-Gendy et al. 2014), and in the biological control of various plague and contaminants. Endophytes' distinctive products include steroids, alkaloids, terpenoids, quaternions, isocoumarin and quinones derivatives, phenolic, flavans, and xanthones compounds, and peptides with medicinal and biotechnology applications (Mohamed El-Bondkly et al. 2020) (Table 6.1).

2.3 Actinobacterial Endophytes

Actinobacteria are Gram-positive bacteria prevalent in soil and form one of the largest bacterial phyla (Barka et al. 2016; Passari et al. 2020). The taxonomy of actinobacteria has developed throughout time, with the most recent roadmap splitting the phylum into 6 classes, 46 orders, and 79 families, with 16 new orders and 10 new families added (Salam et al. 2020). Actinobacteria have a wide range of morphologies, including rod shape (*Acidiferrimicrobium*), coccoid (*Micrococcus*), rod-coccoid (*Arthrobacter*), and bent rods (*Sinomona*s) forms, as well as fragmenting hyphal forms (*Nocardia*) and forms with permanently differentiated branched mycelia (e.g., *Streptomyces* and *Frankia*). Some produce elongated filaments on the substrate but no true mycelium (Rhodococci), while others do not (Corynebacterium), and still others are distinguished by the production of branched substrate hyphae that break up into flagellated motile elements (Oerskovia) (Narsing rao et al. 2022). The effect of *Streptomyces* spp. isolated from the rhizosphere on five legumes (soybean, kidney bean, chickpea, lentil, and pea) indicated that soil microbial populations were increased, as were soil nutrients and organic matter content (AbdEglawad et al. 2020).

In salt stress-treated soils, actinobacteria predominated, particularly members of the family Nitriliruptoraceae, which was proposed as the most sensitive biomarker responding to high salinity (Wang et al. 2019). *Micrococcus yunnanensis*, *Corynebacterium* variabile, and *Arthrobacter nicotianae* were halotolerant actinobacteria with ACC deaminase activity that improved Canola plant development under salt stress (Siddikee et al. 2010). Because of their abundance in soil and the rhizosphere, their propensity to infect plant roots and surfaces, and their ability to create secondary metabolites, actinobacteria are regarded ideal candidates for plant growth promoters (Narsing Rao et al. 2022).

Table 6.1 Fungal endophytes as potential tool in control of postharvest losses

S. no.	Name of the fungal endophytes	Plant origin of fungus endophytes	Against pathogens	Conclusion	References
1.	Beauveria. bassiana	Coffee	*Hypothenemus hampei*	Pathogenic action verified	Vega et al. (2008)
		Banana	*Cosmopolites sordidus*	Reduction of larval survival	Akello et al. (2008)
		Tomato	*Helicoverpa armigera*	Reduced infestation	Qayyum et al. (2015)
		Sorghum	*Sesamia nonagrioides*	Reduced infestation	Mantzoukas and Grammatikopoulos (2020)
		Opium poppy	*Iraella luteipes*	Reduction of larval survival	Lopez and Sword (2015)
		Cotton	*Aphis gossypii*	Reduced reproduction	Gurulingappa et al. (2010)
			Chortoicetes terminifera	Reduced growth rate	Gurulingappa et al. (2010)
		Melon	*Aphis gossypii*	Reduced reproduction, no effect on natural enemies	González-Mas et al. (2019)
		Fava bean	*Helicoverpa armigera*	Reduction of larval survival	Vidal and jaber (2015)
			Liriomyza huidobrensis	Reduced population	Paper et al. (2015)
			Acyrthosiphon pisum	Reduced population	Juliet Akello and Sikora (2012)
			Aphis fabae	Reduced population	Jensen et al. (2019)
		Common bean	*Helicoverpa armigera*	Reduction of larval survival	Vidal and jaber (2015)
			Liriomyza huidobrensis	Reduced population	
		White jute	*Apion corchori*	Reduced infestation	Biswas et al. (2013)
		Soybean	*Aphis glycines*	Insignificant effect on pest population	Clifton et al. (2018)
			Helicoverpa gelotopoeon	Decreased larval food consumption	Russo et al. (2019)
		Grapevine	*Planococcus ficus*	Reduced infestation	Rondot and Reineke (2018)
			Empoasca vitis	Reduced infestation	Rondot and Reineke (2018)
		Pepper	*Myzus persicae*	Increased pest mortality Reduced development and fecundity	Mantzoukas and Lagogiannis (2019)
		Strawberry	*Myzus persicae*	Reduced feeding	Jaber and Araj (2018)
		Cauliflower	*Bemisia tabaci*	Reduced pest survival	Manoussopoulos et al. (2019)
		Pecan	*Melanocallis caryaefoliae*	Reduced pest population	Ramakuwela et al. (2020)
			Monellia caryella	Reduced pest population	Ramakuwela et al. (2020)
		Lemon	*Diaphorina citri*	Reduced reproduction and survival	Mantzoukas and Eliopoulos (2020)

(continued)

Table 6.1 (continued)

S. no.	Name of the fungal endophytes	Plant origin of fungus endophytes	Against pathogens	Conclusion	References
2.	*Lecanicillium lecanii*	Cotton	*Aphis gossypii*	Reduced reproduction	Gurulingappa et al. (2010)
3.	*Lecanicillium muscarium*	Cauliflower	*Plutella xylostella*	Increased larval mortality	Manoussopoulos et al. (2019)
4.	*Aspergillus parasiticus*	Cotton	*Chortoicetes terminifera*	Reduced growth rate	Gurulingappa et al. (2010)
5.	*Metarhizium anisopliae*	Fava bean	*Acyrthosiphon pisum*	Insignificant effect on pest population	Juliet Akello and Sikora (2012)
		Pepper	*Myzus persicae*	Increased pest mortality	Mantzoukas and lagogiannis (2019)
		Rape seed	*Aphis fabae*	Reduction of larval survival	Batta (2013)
			Plutella xylostella	Reduction of larval survival	Batta (2013)
		Strawberry	*Myzus persicae*	Reduced feeding	Jaber and Araj (2018)
6.	*Metarhizium brunneum*	Soybean	*Aphis glycines*	Increase of pest population	Clifton et al. (2018)
		Cauliflower	*Bemisia tabaci*	Reduced pest survival	Manoussopoulos et al. (2019)
		Melon	*Aphis gossypii*	Reduced reproduction and survival	González-mas et al. (2019)
7.	*Metarhizium robertsii*	Sorghum	*Sesamia nonagrioides*	Reduced infestation	Mantzoukas and Grammatikopoulos (2020)
8.	*Clonostachys rosea*	Coffee	*Hypothenemus hampei*	Pathogenic action verified	Vega et al. (2008)
9.	*Purpureocillium lilacinum*	Cotton	*Aphis gossypii*	Reduced reproduction	Gurulingappa et al. (2010)
10.	*Isaria fumosorosea*	Sorghum	*Sesamia nonagrioides*	Reduced infestation	Mantzoukas and Grammatikopoulos (2020)
		Pepper	*Myzus persicae*	Increased pest mortality	Mantzoukas and Lagogiannis (2019)
		Lemon	*Diaphorina citri*	Reduced reproduction and survival	Mantzoukas and eliopoulos (2020)

3 Postharvest Losses (PHL) of Fruits and Vegetables in Food Supply Chain (FSC)

The world's population is expected to reach 9.7 billion by 2050. To deal with this, worldwide edible crop production may need to expand by up to 119% (Berners-Lee et al. 2018). Alternatively, food waste and spoilage must be drastically reduced. As a result, the food industry faces serious challenges to meet current and projected demand. Aside from challenges relating to food transport and storage infrastructure, water resilience, and the impacts of climate change, there is too much food waste,

including livestock and crop disease. Fungi (molds and yeasts) are significant contributors to this, undermining the resilience of the food supply chain at critical stages from the infection of seeds and growing crops to spoilage postharvest and in processed foods, during processing, transport, and storage. Despite the favorable contributions of other fungi to food production, such as organic acids, mycoprotein, baking, brewing, and cheese production, these problems persist (Davies et al. 2021).

Globalization of the food trade is one of the reasons contributing to the rising incidence of foodborne outbreaks caused by microorganisms (Aung and Chang 2014). Bacterial pathogens are the most common contaminants in food products followed by viruses, pesticide residues, and mycotoxins (Van Boxstael et al. 2013). The presence of hazardous bacteria on food surfaces increases the potential of cross-contamination, which can result in food poisoning and/or food loss. Food safety in manufacturing and producing foods is important to protect the consumer from potential health risks and reduce food losses. Food safety and quality are critical in supply networks to ensure food security and allow food to flow from surplus to deficit areas in local, national, and global markets (Bryden 2012; Bosona and Gebresenbet 2013).

The Food and Agriculture Organization (FAO) estimates that the world generates enough food to feed everyone. However, over one in every four calories produced to feed people is not consumed yearly, and around one-third of all food production (1.3 billion tonnes) is either lost or discarded during production in the FSC. The FAO estimates that around 30% of grains, 20% of dairy products, 35% of fish and seafood, 45% of fruits and vegetables, 20% of meat, 20% of oilseeds and pulses, and 45% of roots and tubers are lost or wasted. The FSC is "the entire supply chain from agricultural production, harvest, or slaughter, to primary production and/or manufacturing, storage, and distribution, to retail sale or use in catering and by consumers" (Kuo and Chen 2010). Food waste and losses can occur at any point of the FSC, including agricultural production, postharvest, processing, distribution, and consumption. Food loss most typically refers to food products meant for human consumption that were lost during manufacturing, storage, shipping, and processing, primarily due to microbial infection and/or deterioration (Uyttendaele et al. 2006). Fruit and vegetable food losses and waste are higher in most nations other than products such as dairy and meat (Buzby and Hyman 2012). Fruit and vegetables suffered losses of up to 20–30% in the FSC in China, while meat and aquatic items suffered losses of more than 15% (Liu et al. 2013). According to FAO, fruit and vegetable losses in the FSC in Latin America exceeded 70%, whereas dairy and meat product losses were only 22% and 25%, respectively. Inadequate refrigeration facilities in the FSC, particularly during transit, have been identified as a significant contributor to these losses.

Microorganisms can be either free-living or parasitic, depending on their environment. Most microorganisms are unicellular, although some are multicellular, such as bacteria, fungus, archaea, protista, and viruses. Agricultural products that are to be stored for an extended period of time are exposed to a wide range of microorganisms, which cause degradation of the food product either

directly via rot or indirectly by creating a disagreeable taste. Microorganisms include prokaryotes (bacteria), which are single-celled organisms with no definite nucleus or other organelles, and eukaryotes, which can be single-celled (yeasts) or multicellular (moulds) (Bist and Bist 2020). Fruits and vegetables are especially sensitive to microbiological deterioration caused by fungi, bacteria, yeast, and molds due to their high moisture and nutrient content. During the postharvest phase, fungi and bacteria cause significant loss of fruits and vegetables (Yahaya 2019). Pathogens cause rots in plants and render them unfit for human consumption by producing mycotoxin. *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*, *Geotrichum*, *Phytophthora*, and *Botrytis* species have been identified as prevalent pathogens associated with postharvest illnesses of tomato fruits, accounting for up to a 10–30% loss in the yield of tomato crops (Etebu et al. 2013).

4 Postharvest Disease Management

Worldwide, postharvest vegetable losses are seen as a result of bacterial and fungal infestation. New challenges have been brought about by trade liberalization and globalization, and significant work is needed to reduce vegetable losses. Some of the majorly used methods adopted to control postharvest disease are chemical and biological methods.

4.1 Chemical Control

Chemical fungicides are widely used to treat vegetable postharvest disease. During the crop season, fungicides should be treated strategically as systemic fungicides or at the field level for postharvest diseases that infect products before harvest. Fungicides are widely used postharvest to prevent infections from emerging during storage and handling or to reduce infections that have already taken hold in produce surface tissues. Postharvest fungicides are typically fungistatic rather than fungicidal. Fungicides are applied to the product as waxes, coatings, fumigants, treated wraps, box liners, dips, sprays, and fumigants (Ampatzidis et al. 2017). Dip and spray techniques are widely used in postharvest treatments. Triazoles, such as prochloraz and imazalil, are commonly used by dipping or spraying to combat gray mold, benzimidazoles, such as benomyl and thiabendazole, to treat postharvest illness, and fumigants, such as sulfur dioxide. Dipping in hot water for 5–10 minutes at 50 °C, in addition to the fungicide, can effectively manage the illness, and the crop's size determines this. Sodium hypochlorite is used to disinfect produce, removing any disease spores on the surface (Waard et al. 1993).

4.2 Biological Control

Produce with unapproved pesticides, pesticide residues beyond allowable limits, and improper labelling and packing is rejected by international markets. Biological control of postharvest diseases has much potential, since postharvest environmental variables like temperature and humidity can be precisely adjusted to meet the needs of the biocontrol agent. The issues of developing commercial goods and postharvest biocontrol have garnered much attention (Droby et al. 2001). Biological control employs microbes such as fungi, bacteria, actinomycetes, and viruses (bacteriophages) to prevent postharvest loss in fresh fruits ad vegetables (Loganathan et al. 2016; Chaurasia et al. 2018). These bioagents, like chemicals, can regulate or inhibit disease. The Indian bioagent market is valued at Rs. 690 crores or 2.89% of the country's entire pesticide sector. It is expected to grow 2.3% annually in the coming years (Cheng et al. 2010). According to the Insecticide Act of 1968, only 18 different types of biopesticides have been registered in India thus far. The most promising bioagents include *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, which produce physiologically active compounds such as antibiotics and bacteriocin as well as induce and stimulate systemic resistance in plants. When given before to infection, biocontrol medications are predicted to suppress bacteria more efficiently. The yeast species *Pichia* and *Debaryomces* are antagonists against vegetable postharvest infections by limiting competition at wound sites. Chitosan, for example, has a direct fungicidal action on a wide range of postharvest illnesses while also improving host defense systems. *Trichoderma* has strong antifungal activity regarding *Botrytis cinerea*, *S. sclerotiorum*, *Cortictum rolfsii*, and other significant biotic stressors. The most widely employed biocontrol agents for postharvest diseases include microbial pesticide active components of *Streptomyces griseoviridis* K61 against bacterial soft rot, gray mold, and *Phytophthora*, *Trichoderma harzianum* against *Phytophthera*, *Botrytis*, and *Rhizoctonia*.

To adhere to the pathogen and parasitize its hyphae, antagonistic yeast creates a biofilm. According to Bar-Shimon et al. (2004), the ability of yeast to produce lytic enzymes and withstand high salt concentrations is connected to its efficiency as a biocontrol agent. The role of glucanases in the biocontrol activity of the yeast *C. oleophila* was investigated using molecular techniques, and the biocontrol activity was increased by overexpressing antimicrobial peptides. There were three postharvest biological products on the market in the year 2000: AspireTM (USA and Israel), Bio-SaveTM (USA), and Yield PlusTM (South Africa). However, Aspire was involved in the product's early composition, which incorporated various food additives, a small amount of postharvest fungicide, or salt solutions (1–2%) of calcium chloride or sodium bicarbonate. To increase effectiveness, these things were combined with physical treatments such as calcium pressure infiltration, hot air, curing, hot-water brushing, and combinations of the aforementioned. The antagonists may be coupled with a sugar analogue (2-deoxy-D-glucose) to increase bioefficacy. Natural materials such as acetic acid, jasmonates, glucosinolates, propolis

fusapyrone and deoxyfusapyrone, chitosan, essential oils, active components of various plants, and plant extracts are used to cure fruit deterioration caused by plant pathogenic fungus (Droby et al. 2003a, b).

Flavor Compounds

Many antimicrobial constituents and inducible compounds found in fruits and vegetables have yet to be thoroughly studied as biological control agents for postharvest disease (Culter et al. 1986). These elements could be isolated and employed in the production of other perishables. Flavor molecules are secondary metabolites with characteristics like volatility, fat, and water solubility. They are quite effective in postharvest protection due to their volatility, low water solubility, and quick absorption. Many of these substances are nontoxic to mammals, and treated perishables produce fewer objectionable odors. Several volatiles can have an effect despite their low quantities.

Certain fruit volatiles released by ripening peaches are particularly fungicidal, according to Wilson et al. (1987). To combat the green peach aphid, acetaldehyde has been sprayed on head lettuce as a fumigant (Stewart et al. 1980). Shaw (1969) postulated that the fruit's considerable synthesis of acetaldehyde and ethyl acetate in response to these circumstances gave strawberries resistance to rot in high CO2 storage. Prasad and Stadelbacher (1973) used acetaldehyde vapor to combat *Botrytis cinerea*. *B. cinerea* and *Rhizopus stolonifer*, two bacteria that cause strawberry fruit rot, have been tested using acetaldehyde (Avissar and Pesis 1991). Postharvest microorganisms such as *Erwinia carotovora*, *Pseudomonas fluorescens*, *Monilinia fructicola*, and various yeast species have also been found to be suppressed by acetaldehyde. In vitro experiments have revealed that the fruit and vegetable pathogens *P. digitatum*, *R. stolonifer*, *Colletotrichum musae*, and *Ervinia caratovora* are resistant to several plant volatiles such as acetaldehyde, benzaldehyde, cinnamaldehyde, ethanol, benzyl alcohol, nerolidol, and 2-nonanone. When peaches were fumigated in the lab to prevent *Rhizopus rot*, benzaldehyde entirely prevented spore germination of *B. cinerea* at 25 ll1 and *M. fructicola* at 125 gl1 (Wilson et al. 1987).

Jasmonates

Jasmonates are naturally occurring plant growth regulators that have been shown to influence plant development and stress responses (Sembdner and Parthier 1993). When fatty acids are oxidized using oxygenase, they produce jasmonates, an olypine subclass. According to various lines of evidence, jasmonates are crucial signal molecules in plant defense responses to pathogen attacks. Plant tissues or cell cultures exposed to plant defense system elicitors acquire jasmonic acid, according to Nojiri et al. (1996). Jasmonates have been found to activate several genes involved in the production of phytoalexins, including those that produce thionin (Andresen et al. 1992), osmotin (Xu et al. 1994), a new ribosomal inactive protein (RIP) (Chaudhry

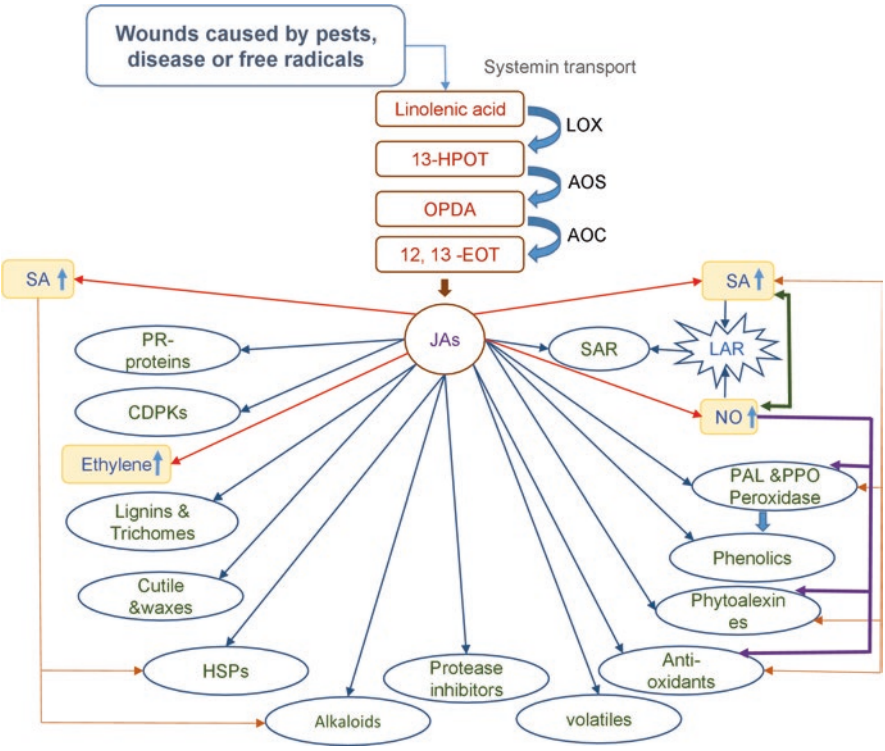

Fig. 6.2 Mode of action of jasmonates in controlling postharvest pathogens. (Adopted from Asghari et al. 2019, Open Access)

et al. 1994), and a variety of other antifungal proteins. According to a recent study, methyl jasmonate (MJ) vapors can be successfully utilized as a postharvest therapy in strawberries to prevent gray mold rot caused by *B. cinerea* (Moline et al. 1997). An overview of controlling postharvest pathogens using jasmonates is illustrated in Fig. 6.2.

Chitosan

Chitosan and its derivatives are antifungal and plant-protective. They can activate plant defense mechanisms against pathogenic attacks at very low dosages. Additionally, they have the potential to be employed in creating wettable coatings for seeds and fruits, whether in the form of a solution, powder, or wettable coatings themselves (Choi et al. 2002). Chitosan has been found to treat blue mold in harvested "Red Delicious" apple fruit effectively by promoting resistance instead of just inhibiting the illness (Capdeville et al. 2002). Importance of chitosan in controlling postharvest pathogens is projected in Fig. 6.3.

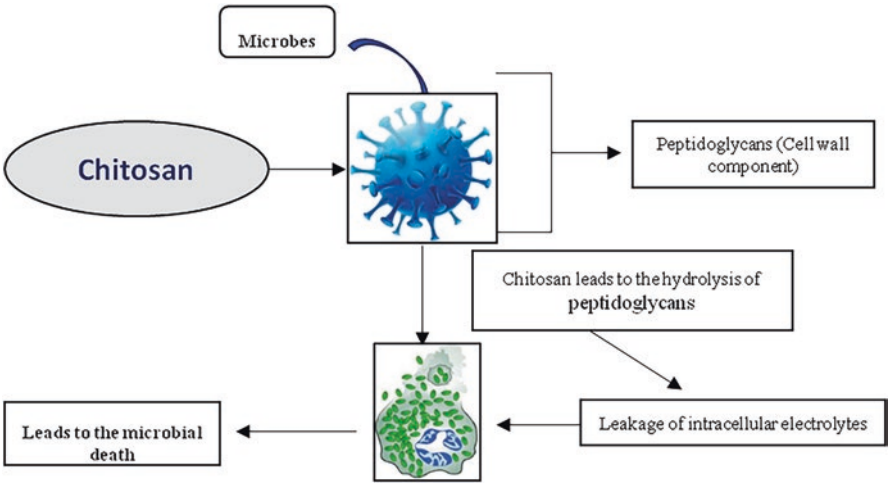

Fig. 6.3 Mode of action of chitosan in controlling postharvest pathogens. (Adopted from Sharif et al. 2018, open access)

Essential Oils

The antifungal action of essential oils is widely recognized, and investigations on the effects of essential oils on postharvest infections have been conducted (Meepagala et al. 2002). The advantage of essential oils lies in their biological activity in the vapor phase, making them desirable as potential fumigants for safeguarding stored goods. Several studies (Bellerbeck et al. 2001; Hidalgo et al. 2002) have shown that the majority of essential oils exhibit the ability to prevent postharvest fungal growth in vitro. However, only a limited number of essential oils have been thoroughly examined for their practical effectiveness in vivo. Some essential oils offer protection against biological deterioration of stored items. Moreover, experimental findings indicate that essential oils can prolong the shelf life of fruits and vegetables by inhibiting fungal decay. According to Dubey and Kishore (1988), essential oils extracted from *Melaleuca leucadendron*, *Ocimum canum*, and *Citrus medica* leaves may effectively hinder the production of biodeterioration by *Aspergillus flavus* and *Aspergillus versicolor* in various food products. The required quantities of these oils for action were observed to range between 500 and 2000 g/ml. Dixit et al. (1995) investigated the potential of essential oils in mitigating postharvest degradation in fruits and vegetables.Vegetable oils are a relatively new development in the control of plant diseases in plant pathology. Castor, linseed, mustard, sunflower, safflower, peanut, mineral oils, palmarosa, red thyme, and liquid paraffin are some of the antifungal oils that are applied to harvested fruits to stop disease penetration and lessen respiration. Composite coatings comprised of proteins (casein, soy), lipids (waxes, mineral oils), and polysaccharides (cellulose, pectin, starch, alginate, and chitosan) are frequently used to stop fruit and vegetable rot. Waxing particular fruits and

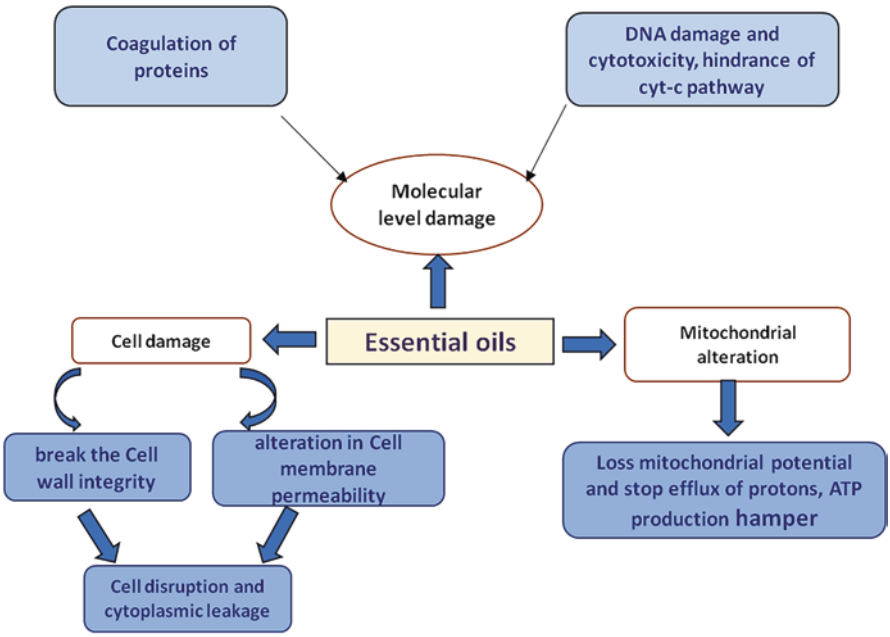

Fig. 6.4 Mode of action of essential oil in controlling postharvest pathogens. (Adopted from Anirban Sil et al. 2020, Open access)

vegetables is often used to improve look and reduce water loss by 30–40% when a product is exposed to less-than-ideal temperature and relative humidity conditions (Azizah et al. 2009). Antioxidants are occasionally employed to prevent oxidative rancidity, degradation, and discoloration. Probable mechanisms of how essential oil helps to combat postharvest pathogens are shown in Fig. 6.4.

5 Conclusion

Fruit and vegetable postharvest illness, which can be lethal, can arise from different factors affecting production of crops. Some of these illnesses harm the crops prior to harvest, while others result from surface damage during or after harvest. For effective management of postharvest diseases, it is crucial to assess the entire production and postharvest handling processes. Various preharvest factors can also be responsible for disease development, even after harvesting the crops. Traditionally, fungicides have been essential for controlling postharvest diseases, but as insecticide usage decreases in fruits and vegetables, researchers are exploring new treatment methods. Surveys indicate that about 27% of respondents ship their products directly to distributors without postharvest processing. By employing various postharvest management strategies to reduce respiration and halt the degradation of fruits and vegetables, we can effectively handle the postharvest losses. Moreover, farmers and sellers must have the necessary information to effectively prevent fruit and vegetable rotting (Fig. 6.5).

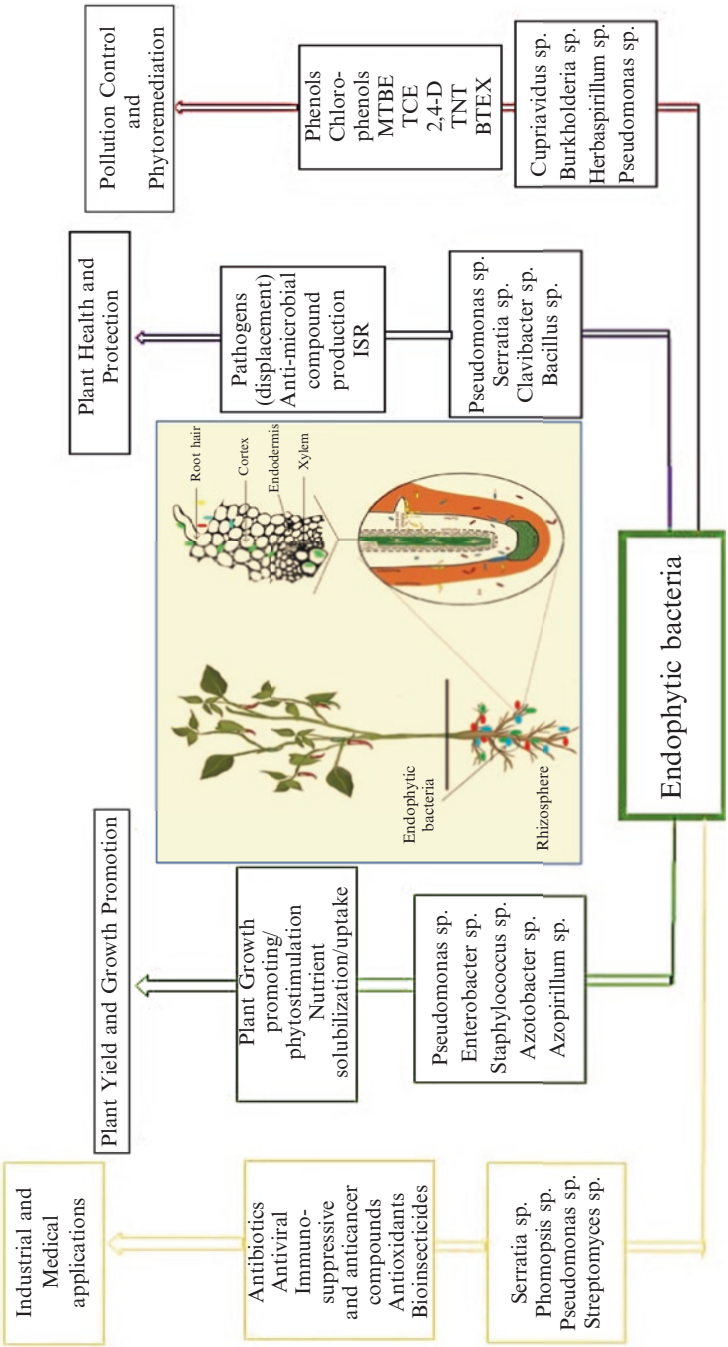

Fig. 6.5 Schematic diagram of bacterial endophytes applications (Eltabee Youghy 2014)

Natural fungicides show promising potential as safe alternatives to synthetic ones in the future. However, their use to prevent postharvest degradation of perishable crops is still in its early stages. The ongoing search for natural substances may yield safer alternatives to currently utilized pyrethroids and azadirachtin, which are known for their excellent fungicidal properties in various regions worldwide. Before making any recommendations, it is essential to conduct organoleptic testing to ensure that the product does not adversely affect the fruit's quality indicators, such as acidity, flavor, and fragrance. Since fruits have a limited shelf life after harvest, the treatment should be effective even for short-term use. Determining the lowest practical effective dose for the natural fungicide is crucial to optimize its application. To assess the practical potency of promising compounds identified in vitro investigations, safety limit profiles and other testing methods should be considered while acknowledging the benefits of botanicals as postharvest fungitoxicants.

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References

Abdalla MA, Aro AO, Gado D, Passari AK, Mishra VK, Singh BP, McGaw LJ (2020) Isolation of endophytic fungi from South African plants, and screening for their antimicrobial and extracellular enzymatic activities and presence of type I polyketide synthases. S Afr J Bot 134:336–342. https://doi.org/10.1016/j.sajb.2020.03.021

AbdElgawad H, Abuelsoud W, Madany MMY, Selim S, Zinta G, Mousa ASM, Hozzein WN (2020) Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomol Ther 10:1675

Akello J, Sikora R (2012) Systemic acropedal influence of endophyte seed treatment on Acyrthosiphon pisum and Aphis fabae offspring development and reproductive fitness. Biol Control 61(3):215–221. https://doi.org/10.1016/j.biocontrol.2012.02.007

Akello J, Dubois T, Coyne D, Kyamanywa S (2008) Endophytic Beauveria bassiana in banana (Musa Spp.) reduces banana weevil (Cosmopolites sordidus) fitness and damage. Crop Prot 27(11):1437–1441. https://doi.org/10.1016/j.cropro.2008.07.003

Ali A, Xia C, Mahmood I, Faisal M (2021) Economic and environmental consequences' of postharvest loss across food supply chain in the developing countries. J Clean Prod 323:129146

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90(6):1829–1845

Ampatzidis Y, DeBellisL LA, Luvisi A (2017) Pathology: robotic applications and management of plants and pant diseases. Sustainability 9(6):1010

Andresen I, Becker W, Schluter K, Burges J, Parthier B, Apel K (1992) The identification of leaf thionin as one of the main jasmonate induced proteins in barley (Hordeum vulgare). Plant Mol Biol 19:193–204

Arora J, Ramawat KG (2017) An introduction to endophytes. In: Endophytes: biology and biotechnology: volume 1, vol August 2022, pp 1–23

Asghari M (2019) Impact of jasmonates on safety, productivity and physiology of food crops. Trends in Food Science & Technology 91:169–183

Aung MM, Chang YS (2014) Traceability in a food supply chain: safety and quality perspectives. Food Control 39(1):172–184. https://doi.org/10.1016/j.foodcont.2013.11.007

Avissar I, Pesis E (1991) The control of postharvest decay in table grapes using acetaldehyde vapours. Ann Appl Biol 118:229–237
Azizah O, Saari N, Saleh R, Bakar J, Zainal ND, Yacob M (2009) Postharvest handling practices on selected local fruits and vegetables at different levels of the distribution chain. J Agribus Market 2:39–53
Bangar SP, Sharma N, Bhardwaj A, Phimolsiripol Y (2022) "Lactic acid bacteria." Quality assurance and safety of crops and foods. 14(2):13–31
Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-Kolthoff JP, Klenk HP, Clément C, Ouhdouch Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43
Bar-Shimon M, Yehuda H, Cohen L, Weiss B, Kobeshnikov A, Daus A et al (2004) Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent Candida oleophila. Curr Genet 45:140–148
Batta YA (2013) Efficacy of endophytic and applied Metarhizium anisopliae (Metch.) Sorokin (Ascomycota: Hypocreales) against Larvae of Plutella Xylostella L. (Yponomeutidae: Lepidoptera) infesting Brassica napus plants. Crop Prot 44:128–134. https://doi.org/10.1016/j.cropro.2012.11.001
Bellerbeck VG, De Roques CG, Bessiere JM, Fonvieille JL, Dargent R (2001) Effect of Cymbopogon nardus (L) W. Watson essential oil on the growth and morphogenesis of Aspergillus Niger. Can J Microbiol 47:9–17
Berners-Lee M, Kennelly C, Watson R, Hewitt CN (2018) Current global food production is sufficient to meet human nutritional needs in 2050 provided there is radical societal adaptation. Elementa 6(May):2023
Bist NS, Bist P (2020) Role of microorganisms in postharvest loss of agricultural products: a review. Sustain Food Agric 2(1):01–04
Biswas C et al (2013) Endophytic colonization of White Jute (Corchorus Capsularis) plants by different Beauveria bassiana strains for managing stem weevil (Apion Corchori). Phytoparasitica 41(1):17–21
Bosona T, Gebresenbet G (2013) Food traceability as an integral part of logistics management in food and agricultural supply chain. Food Control 33(1):32–48. https://doi.org/10.1016/j.foodcont.2013.02.004
Bryden WL (2012) Mycotoxin contamination of the feed supply chain: implications for animal productivity and feed security. Anim Feed Sci Technol 173(1–2):134–158. https://doi.org/10.1016/j.anifeedsci.2011.12.014
Buzby JC, Hyman J (2012) Total and per capita value of food loss in the United States. Food Policy 37(5):561–570. https://doi.org/10.1016/j.foodpol.2012.06.002
Capdeville G, De Wilson CL, Beer SV, Aist JR (2002) Alternative disease control agents induce resistance to blue mold in harvested Red Delicious apple fruit. Phytopathology 92:900–908
Card S, Johnson L, Teasdale S, Caradus J (2016) Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. FEMS Microbiol Ecol 92(8):1–19
Chaudhry B, Muller-Uri F, Cameron-Mills V, Gough S, Simpson D, Skriver K, Mundy J (1994) The barley 60 kDa jasmonate-induced protein (JIP60) is a novel ribosome-inactivating protein. Plant J 6:815–824
Chaurasia A, Meena BR, Tripathi AN, Pande KK, Rai AB, Singh B (2018) Actinomycetes: an unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops. World J Microbiol Biotechnol 34(9):132
Cheng XL, Liu CJ, Yao JW (2010) The current status, development trend and strategy of the biopesticide industry in China. Hubei Agric Sci 49:2287–2290
Choi WY, Park HJ, Ahn DJ, Lee J, Lee CY (2002) Wettability of chitosan coating solution on Fiji apple skin. J Food Sci 67:2668–2672
Christina A, Christapher V, Bhore SJ (2013) Endophytic bacteria as a source of novel antibiotics: an overview. Pharmacogn Rev 7(13):11

Clifton EH et al (2018) Effects of endophytic entomopathogenic fungi on soybean aphid and identification of Metarhizium isolates from agricultural fields. PLoS One 13(3):1–19
Collinge DB, Jensen B, Jørgensen HJL (2022) Fungal endophytes in plants and their relationship to plant disease. Curr Opin Microbiol 69:102177. https://doi.org/10.1016/j.mib.2022.102177
Compant S et al (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959. http://aem.asm.org/content/71/9/4951.short
Culter HG, Steverson RF, Cole PD, Jackson DM, Johnson AW (1986) Secondary metabolites from higher plants. Their possible role as biological control agents, ACS Symposium Series. American Chemical Society, Washington, DC, pp 178–196
Davies CR et al (2021) Evolving challenges and strategies for fungal control in the food supply chain. Fungal Biol Rev 36:15–26. https://doi.org/10.1016/j.fbr.2021.01.003
De Silva NI et al (2016) Mycosphere essays 9: defining biotrophs and hemibiotrophs. Mycosphere 7(5):545–559
Denis N, Zhang H, Leroux A, Trudel R, Bietlot H (2016) Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. Food Control 67:225–234
Dixit SN, Chandra H, Tiwari R, Dixit V (1995) Development of botanical fungicide against blue mold of mandarins. J Stored Prod Res 31:165–172
Dos Santos SF, Cardoso RDCV, Borges ÍMP, Almeida AC, Andrade ES, Ferreira IO, do Carmo Ramos L (2020) Post-harvest losses of fruits and vegetables in supply centers in Salvador, Brazil: analysis of determinants, volumes and reduction strategies. Waste Manag 101:161–170
Droby S, Cohen L, Wiess B, Daus A, Wisniewski M (2001) Microbial control of postharvest diseases of fruits and vegetables—Current status and future outlook. Acta Hortic 553:371–376
Droby S, Wisniewski M, El-Ghaouth A, Wilson C (2003a) Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast-based biocontrol product Aspire™. Postharvest Biol Technol 27:127–135
Droby S, Wisniewski M, El Ghaouth A, Wilson CL (2003b) Biological control of postharvest diseases of fruits and vegetables: current advances and future challenges. Acta Hortic 628:703–713
Dubey NK, Kishore N (1988) Exploitation of higher plant products as natural fumigants. In: Proceedings of the fifth international congress on plant pathology, Kyoto, Japan, p 423 (Abstract)
Eckert JW, Ratnayake M (1983) Host–pathogen interactions in postharvest disease. In: Lieberman M (ed) Postharvest physiology and crop preservation. Plenum Press, New York
El-Bondkly AMA, Mervat MMM, Bassyouni RH (2012) Overproduction and biological activity of prodigiosin-like pigments from recombinant fusant of endophytic marine streptomyces species. Anton Leeuw Int J Gen Mol Microbiol 102(4):719–734
El-Bondkly M, Ahmed A, El-Gendy MMAA, El-Bondkly EAM, Ahmed AM (2020) Biodiversity and biological activity of the fungal microbiota derived from the medicinal plants Salvia aegyptiaca L. and Balanties aegyptiaca L. Biocatal Agric Biotechnol 28(August):101720. https://doi.org/10.1016/j.bcab.2020.101720
El-Gendy MMA, El-Bondkly AMA, Yahya SMM (2014) Production and evaluation of antimycotic and antihepatitis C virus potential of fusant MERV6270 derived from mangrove endophytic fungi using novel substrates of agroindustrial wastes. Appl Biochem Biotechnol 174(8):2674–2701
El-Gendy MM, Ahmed A, Al-Zahrani HAA, El-Bondkly AMA (2016) Genome shuffling of mangrove endophytic aspergillus luchuensis MERV10 for improving the cholesterol-lowering agent lovastatin under solid state fermentation. Mycobiology 44(3):171–179
El-Gendy MM, Ahmed A, Yahya SMM et al (2018a) Phylogenetic analysis and biological evaluation of marine endophytic fungi derived from red sea sponge Hyrtios erectus. Appl Biochem Biotechnol 185(3):755–777
El-Gendy MM, Ahmed A, Mohamed ZK et al (2018b) Production of bioactive metabolites from different marine endophytic streptomyces species and testing them against Methicillin-Resistant Staphylococcus Aureus (MRSA) and cancer cell lines. Biotechnologia 99(1):13–35

Eltabee Youghy N (2014) Bacterial endophytes isolated from Capparis sinaica of sinai as a synthesizer of natural bioactive compound. Thesis

Etebu E et al (2013) Postharvest spoilage of tomato (Lycopersicon Esculentum Mill.) and control strategies in Nigeria. J Biol Agric Healthc 3(10):51–62

FAO (2019) The state of food and agriculture 2019. Moving forward on food loss and waste reduction. Rome. Licence: CC BY-NC-SA 3.0 IGO

Farber JM, Zwietering M, Wiedmann M, Schaffner D, Hedberg CW, Harrison MA et al (2021) Alternative approaches to the risk management of Listeria monocytogenes in low risk foods. Food Control 123:107601

Food and Agriculture Organization of the United Nations (FAO) (2013) Food Losses and Waste in Turkey, Country Report, 48 s. Available at: http://www.fao.org/3/a-au824e

González-Mas N, Cuenca-Medina M, Gutiérrez-Sánchez F, Quesada-Moraga E (2019) Bottom-up effects of endophytic Beauveria bassiana on multitrophic interactions between the cotton aphid, Aphis gossypii, and its natural enemies in melon. J Pest Sci 92(3):1271–1281. https://doi.org/10.1007/s10340-019-01098-5

Gouda S et al (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7(SEP):1–8

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37(3):395–412

Gurulingappa P, Sword GA, Murdoch G, McGee PA (2010) Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. Biol Control 55(1):34–41. https://doi.org/10.1016/j.biocontrol.2010.06.011

Gustavsson J, Stage J (2011) Retail waste of horticultural products in Sweden. Resour Conserv Recycl 55(5):554–556

Hardoim PR et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Hidalgo PJ, Ubera JL, Santos JA, LaFont F, Castelanos C, Palomino A, Roman M (2002) Essential oils in Culamintha sylvatica. Bromf. ssp. ascendens (Jorden) P.W. Ball wild and cultivated productions and antifungal activity. J Essential Oil Res 14:68–71

Jaber LR, Araj SE (2018) Interactions among endophytic fungal entomopathogens (Ascomycota: Hypocreales), the green peach aphid Myzus persicae Sulzer (Homoptera: Aphididae), and the aphid endoparasitoid Aphidius colemani viereck (Hymenoptera: Braconidae). Biol Control 116(March 2017):53–61. https://doi.org/10.1016/j.biocontrol.2017.04.005

Jayawardena RS et al (2016) Notes on currently accepted species of colletotrichum. Mycosphere 7(8):1192–1260

Jensen RE, Enkegaard A, Steenberg T (2019) Increased fecundity of Aphis fabae on Vicia faba plants following seed or leaf inoculation with the entomopathogenic fungus Beauveria bassiana. PLoS One 14(10):1–12

Kelman A (1984) In: Moline HE (ed) Postharvest pathology of fruits and vegetables: postharvest losses in perishable crops. University of California Agricultural Experimental Station Bulletin, pp 1–3

Kloepper JW, Ryu C-M (2007) Bacterial endophytes as elicitors of induced systemic resistance. Microbial Root Endophytes 9:33–52

Kuo JC, Chen MC (2010) Developing an advanced multi-temperature joint distribution system for the food cold chain. Food Control 21(4):559–566. https://doi.org/10.1016/j.foodcont.2009.08.007

Lebersorger S, Schneider F (2014) Food loss rates at the food retail, influencing factors and reasons as a basis for waste prevention measures. Waste Manag 34(11):1911–1919

Lee S et al (2004) Indole-3-acetic acid biosynthesis is deficient in gluconacetobacter diazotrophicus strains with mutations in cytochrome c biogenesis genes. J Bacteriol 186(16):5384–5391

Lipinski B, Hanson C, Waite R, Searchinger T, Lomax J (2013) Reducing food loss and waste

Liu J, Lundqvist J, Weinberg J, Gustafsson J (2013) Food losses and waste in China and their implication for water and land. Environ Sci Technol 47(18):10137–10144

Loganathan M, Rai AB, Pandey KK, Nagendran K, Tripathi AN, Singh B (2016) PGPR Bacillus subtilis for multifaceted benefits in vegetables. Indian Hortic 61(1):36–37

Lopez DC, Sword GA (2015) The endophytic fungal entomopathogens Beauveria bassiana and Purpureocillium lilacinum enhance the growth of cultivated cotton (Gossypium hirsutum) and negatively affect survival of the cotton bollworm (Helicoverpa zea). Biol Control 89:53–60. https://doi.org/10.1016/j.biocontrol.2015.03.010

Lugtenberg BJJ, Caradus JR, Johnson LJ (2016) Fungal endophytes for sustainable crop production. FEMS Microbiol Ecol 92(12):fiw194

Manoussopoulos Y et al (2019) Effects of three strawberry entomopathogenic fungi on the prefeeding behavior of the Aphid Myzus persicae. J Insect Behav 32(2):99–108

Mantzoukas S, Eliopoulos PA (2020) Endophytic entomopathogenic fungi: a valuable biological control tool against plant pests. Appl Sci (Switzerland) 10(1)

Mantzoukas S, Grammatikopoulos G (2020) The effect of three entomopathogenic endophytes of the sweet sorghum on the growth and feeding performance of its pest, Sesamia nonagrioides larvae, and their efficacy under field conditions. Crop Prot 127(June 2019):104952. https://doi.org/10.1016/j.cropro.2019.104952

Mantzoukas S, Lagogiannis I (2019) Endophytic colonization of Pepper (Capsicum Annum) controls Aphids (Myzus Persicae Sulzer). Appl Sci (Switzerland) 9(11)

Manzini R, Accorsi R (2013) The new conceptual framework for food supply chain assessment. J Food Eng 115(2):251–263

Meepagala KM, Sturtz G, Wedge DE (2002) Antifungal constituents of the essential oil fraction of Artemisia drancunculus L. var. dracunculus. J Agric Food Chem 50:6989–6992

Mendonca A, Thomas-Popo E, Gordon A (2020) Microbiological considerations in food safety and quality systems implementation. In: Food safety and quality systems in developing countries. Academic Press, pp 185–260

Mishra VK, Singh G, Passari AK, Yadav MK, Gupta VK, Singh BP (2016) Distribution and antimicrobial potential of endophytic fungi associated with ethnomedicinal plant Melastoma malabathricum L. J Environ Biol 37(2):229–237

Moline HE, Buta JG, Saftner RA, Maas JL (1997) Comparison of three volatile natural products for the reduction of post harvest diseases in strawberries. Adv Strawberry Res 16:43–48

Moss MO (2002) Mycotoxin review. 1. Aspergillus and penicillium. Mycologist 16:116–119

Narsing Rao MP, Lohmaneeratana K, Bunyoo C, Thamchaipenet A (2022) Actinobacteria plant interactionsin alleviating abiotic stress. Plan Theory 11:2976. https://doi.org/10.3390/plants11212976

Nojiri H, Sugimori M, Yamane H, Nishimura Y, Yamada A, Shibuya N, Kodama O, Murofushi N, Omori T (1996) Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-culture rice cells. Plant Physiol 110:387–392

Nunes CA (2012) Biological control of postharvest diseases of fruit. Eur J Plant Pathol 133:181–196

Paper C et al (2015) Fungal endophytes: isolation to application in pest management. October:1–2

Passari AK, Mishra VK, Leo VV, Gupta VK, Singh BP (2016) Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. Microbiol Res 193:57–73. https://doi.org/10.1016/j.micres.2016.09.006

Passari AK, Leo VV, Singh G, Samanta L, Ram H, Siddaiah CN, Hashem A, Al-Arjani A-BF, Alqarawi AA, Abd Allah EF, Singh BP (2020) In vivo studies of inoculated plants and in vitro studies utilizing methanolic extracts of endophytic Streptomyces sp. strain DBT34 obtained from *Mirabilis jalapa* L. exhibit ROS-scavenging and other bioactive properties. Int J Mol Sci 21:7364. https://doi.org/10.3390/ijms21197364

Peng XY et al (2021) Co-culture: stimulate the metabolic potential and explore the molecular diversity of natural products from microorganisms. Mar Life Sci Technol 3(3):363–374. https://doi.org/10.1007/s42995-020-00077-5

Prasad K, Stadelbacher GJ (1973) Control of post harvest decay of fresh raspberries by acetaldehyde vapor. Plant Dis Rep 57:795–797

Qayyum MA et al (2015) Infection of Helicoverpa armigera by endophytic Beauveria bassiana colonizing tomato plants. Biol Control 90:200–207. https://doi.org/10.1016/j.biocontrol.2015.04.005

Qin Z et al (1996) Effect of Eimeria tenella infection on the production of Salmonella enteritidis-contaminated eggs and susceptibility of laying hens to S. Enteritidis infection. Avian Dis 40(2):361–367

Ramakuwela T et al (2020) Establishment of Beauveria bassiana as a fungal endophyte in pecan (Carya Illinoinensis) seedlings and its virulence against pecan insect pests. Biol Control 140(August 2019):104102. https://doi.org/10.1016/j.biocontrol.2019.104102

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles: Tansley review. New Phytol 182(2):314–330

Rondot Y, Reineke A (2018) Endophytic Beauveria bassiana in grapevine Vitis vinifera (L.) reduces infestation with piercing-sucking insects. Biol Control 116:82–89. https://doi.org/10.1016/j.biocontrol.2016.10.006

Russo ML et al (2019) Effects of endophytic Beauveria bassiana (Ascomycota: Hypocreales) on biological, reproductive parameters and food preference of the soybean pest Helicoverpa gelotopoeon. J King Saud Univ Sci 31(4):1077–1082. https://doi.org/10.1016/j.jksus.2018.11.009

Salam N, Jiao J-Y, Zhang X-T, Li W-J (2020) Update on the classifification of higher ranks in the phylum Actinobacteria. Int J Syst Evol Microbiol 70:1331–1355

Santangelo JS, Turley NE, Johnson MTJ (2015) Fungal endophytes of Festuca rubra increase in frequency following long-term exclusion of rabbits. Botany 93(4):233–241

Santoyo G, Moreno-Hagelsieb G, del Carmen M, Orozco-Mosqueda, and Bernard R. Glick. (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. https://doi.org/10.1016/j.micres.2015.11.008

Schulz B, Boyle C (2014) "What are endophytes?" (January 2006). In: Microbial root endophytes. Springer, Berlin, Heidelberg, pp 1–13

Sembdner G, Parthier B (1993) The biochemistry and the physiological and molecular actions of jasmonates. Annu Rev Plant Physiol Plant Mol Biol 44:569–589

Shaw GW (1969) The effect of controlled atmosphere storage on the quality and shelf life of fresh strawberries with special reference to Botrytis cinerea and Rhizopus nigricans. Ph.D. thesis. University of Madison, 62 pp

Sharif R, Mujtaba M, Ur Rahman M, Shalmani A, Ahmad H, Anwar T., ... and Wang X (2018) The multifunctional role of chitosan in horticultural crops; a review. Molecules 23(4):872

Siddikee MA, Chauhan PS, Anandham R, Han GH, Sa T (2010) Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20:1577–1584

Sil A, Pramanik K, Samantaray P, Firoz M, Yadav V (2020) Essential oils: A boon towards eco-friendly management of phytopathogenic fungi. J Entomol Zool Stud 8:1884–1891

Singh N, Singh A, Dahiya P (2021) Plant growth-promoting endophytic fungi from different habitats and their potential applications in agriculture. February:69–87

Stewart JK, Aharoni Y, Hartsell PI, Young DK (1980) Acetaldehyde fumigation at reduced pressures to control the green peach aphids on wrapped and packed head lettuce. J Econ Entomol 73:149–152

Thirumurugan D, Cholarajan A, Raja SSS, Vijayakumar R (2018) An introductory chapter: secondary metabolites. In: Secondary metabolites - sources and applications (October)

Uyttendaele M et al (2006) Quantitative risk assessment of Campylobacter Spp. in poultry based meat preparations as one of the factors to support the development of risk-based microbiological criteria in Belgium. Int J Food Microbiol 111(2):149–163

Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Praveen V, Kamath PV, Singh BP, Mudili V, Gupta VK, Siddaiah CH, Chowdappa S, Alqarawi AA, Abd Allah EF (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:328484. https://doi.org/10.3389/fphar.2018.00309

Van Boxstael S et al (2013) Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. Food Control 32(1):190–197. https://doi.org/10.1016/j.foodcont.2012.11.038

Vega FE et al (2008) Entomopathogenic fungal endophytes. Biol Control 46(1):72–82

Vidal S, Jaber LR (2015) Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109(1):46–54

Waard D, Georgopoulos MA, Hollomon SG, Ishii DW, Leroux P (1993) Chemical control of plant diseases: problems and prospects. Annu Rev Phytopathol 31:403–421

Wang M, Chen S, Chen L, Wang D, Zhao C (2019) The responses of a soil bacterial community under saline stress are associated with Cd availability in long-term wastewater-irrigated fifield soil. Chemosphere 236:124372

Wilson CL, Franklin JD, Otto BE (1987) Fruit volatiles inhibitory to Monilinia fructicola and Botrytis cinerea. Plant Dis 71:316–319

Xu Y, Chang PL, Liu D, Narasimhan ML, Raghothma KG, Hasegawa PM, Bressan RA (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell 6:1077–1085

Yahaya SM (2019) Review of postharvest losses of fruits and vegetables. Biomed J Sci Technical Res 13(4):10192–10200

Zhang H, Godana EA, Sui Y, Yang Q, Zhang X, Zhao L (2020) Biological control as an alternative to synthetic fungicides for the management of grey and blue mould diseases of table grapes: a review. Crit Rev Microbiol 46(4):450–462

Zinniel DK et al (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68(5):2198–2208

Chapter 7
Fungal Endophytes and Their Bioactive Compounds: An Overview of Potential Applications

C. Lalbiaktluangi, Cherish Ranjan Mistry, Manveer Kaur, Navoneel Giri, Rashid Khalid Shaikh, Anish Nagar, Apurva Singh, Udita Sarkar, Sai Sravan K, Ojaswini Kalita, Richa Boro, Rahul Kumar, and Mukesh Kumar Yadav

Abstract Fungal endophytes are present in every species of living plant throughout the earth forming a balanced symbiotic relationship with the plants. They produce diverse range of bioactive compounds that protect the host plant from biotic and abiotic stress contributing to the plant's ecological success as well as their own. These bioactive compounds can be beneficial to not only the plants but also to human and the environment. A multitude of secondary metabolites produced by fungal endophytes showed antimicrobial activity against many plant and human pathogens including the antibiotic-resistant pathogens. They also produce antiparasitic compounds which can be used to eliminate deadly diseases including malaria. Many bioactive compounds produced by fungal endophytes also showed anticancer activity by disrupting the cell cycle, inducing apoptosis, and disrupting important pathways and gene expression. Furthermore, they also play a huge role in soil remediation. In this chapter, we discuss in detail fungal endophytes and their symbiotic relationship with their plant host. We also discuss the various bioactive compounds they produce and the different biological activities of these bioactive compounds.

Keywords Fungal endophytes · Secondary metabolites · Human pathogens · Malaria · Anticancer activity

C. Lalbiaktluangi · C. R. Mistry · M. Kaur · N. Giri · R. K. Shaikh · A. Nagar · A. Singh · U. Sarkar · S. Sravan K · O. Kalita · R. Boro · R. Kumar · M. K. Yadav (✉)
Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

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1 Introduction

Endophytes are a group of microorganisms that reside within the tissues of plants, forming a symbiotic relationship. They are primarily fungi that colonize the internal parts of plants without causing any visible harm or disease symptoms. Fungal endophytes are found in various plant species, including grasses, trees, shrubs, and herbs, and can be present in different plant tissues such as leaves, stems, and roots (Zhang et al. 2006). Microbial endophytes have formed a mutualistic relationship with their plant hosts where the host plant provides the endophytes with the necessary nutrients and a place to live. Endophytic compounds aid host plants in resisting various biotic and abiotic stresses and enabling them to endure harsh environments (Vyas and Bansal 2018). Fungal endophytes produce a wide range of bioactive compounds, including alkaloids, terpenoids, phenolics, and polyketides, which play a crucial role in their interaction with the host plant and other organisms. These bioactive compounds can contribute to the ecological success of both the endophyte and the host plant by deterring herbivores, inhibiting the growth of competing microorganisms, or providing a defense against pathogens (Rai et al. 2021). Many species of endophytic fungi found in plants have also been found to be capable of suppressing plant diseases and promotes growth of host plant (Passari et al. 2015).

The secondary metabolites of fungal endophytes are beneficial for both humans and plants due to their different biological activities including antimicrobial, antioxidant, anti-inflammation, anticancer activities. Due to their propensity to create substances that aid in plant growth, they increase soil fertility and stimulate plant growth. They have also drawn much interest because of their capacity to produce a variety of bioactive substances with potential medical applications. Bioactive substances having antibacterial, anticancer, cytotoxic, and insecticidal activities are known to be produced by these fungi. Endophytic fungi that produce bioactive substances might also be exploited commercially by the pharmaceutical industry (Mishra et al. 2016; Vyas and Bansal 2018).

The need for new antimicrobial drugs and interest in natural methods of pathogen control has grown due to pathogenic microorganisms' rising antibiotic resistance. The majority of microorganisms that produce secondary metabolites are fungi. Extracellular enzymes including cellulases, proteinases, lipases, and esterases are known to be produced by them. Metabolites produced by endophytes such as amines and amides have been proven harmful to insects but not to mammals. Fungal endophytes have been discovered in many plant species analyzed and are an essential part of plant micro-ecosystems. Auxins, cytokinins, and gibberellins, among other hormones that promote plant growth, have been observed to be produced along with the solubilization of insoluble phosphates. They also protect plants from diseases by creating antagonistic substances, triggering host defense mechanisms, or posing a threat to nutrients and colonization sites (Vyas and Bansal 2018)

2 Need to Explore Endophytic Fungi

Fungal endophytes play an important role in the growth and survival of their host plant. For example, they help plants establish abiotic stress tolerance and disease resistance (Rodriguez and Redman 2008; Ganley et al. 2008), improve nutrient uptake, inactivate the virulence of diseases, lessen the impact of competitive species (Wang et al. 2007), and promote plant growth and development (Bae et al. 2008). Utilization of the symbiotic relationship between these fungal endophytes and host could be a potential alternative for the production of improved crops that can withstand ever-changing climate.

A number of antimicrobial and anticancer compounds have been isolated from a wide range of fungal endophytes. It is estimated that almost all species of terrestrial plants will possibly contain at least one or more endophyte species (Fadiji and Babalola 2020); so plenty of fungal endophytes containing bioactive compounds that may be beneficial to human are left to be explored. A new era in the development of food, chemical, medicinal, and industrial units can be resulted from using fungal endophytes.

3 Classification of Fungal Endophytes

Endophytic fungi are a taxonomically complex and polyphyletic group, making categorization difficult. Fungal endophytes have been categorized using a various schemes based on one or more biological characteristics. The oldest categorization system for fungal endophytes divided them into two main groups: non-clavicipitaceous endophytes (NC-endophytes) and clavicipitaceous endophytes (C-endophytes), based on phylogeny, ecological functions, and life history strategies (Fig. 7.1). Clavicipitaceous endophytes, commonly known as grass endophytes or class 1 endophytes, are made up primarily of members of the Clavicipitaceae family (Hypocreales, Ascomycota). There are 27 identified genera in this group, which also include a number of free-living symbiotic organisms. On the other hand, non-clavicipitaceous endophytes are a hugely varied polyphyletic group with poorly

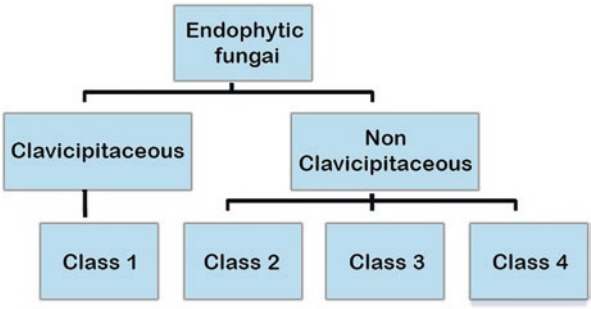

Fig. 7.1 Classification of fungal endophytes

defined taxonomical members. NC-endophytes are associated with both vascular and nonvascular plants, and the majority of them are Ascomycota or Basidiomycota members of the *Dikarya* subkingdom (Gakuubi et al. 2021)

Other classification criteria for endophytic fungi have arisen due to the continuing expansion of the study of endophyte fungal biology. These consist of categorization based on traits such as host range, route of infection and transmission, reproductive techniques, colonization sites for hosts, and the place of the fungi along the spectrum of interactions with their hosts. Some studies have concentrated on fungal endophytes from specific plants, such as medicinal plant endophytes, coniferous tree endophytes, mangrove endophytes, and fungal endophytes of grasses. For example, fungal endophytes have been classified according to the host plant types. There are two basic groups of fungal endophytes recognized in terms of means of transmission: those that are transferred vertically and those that are transmitted horizontally. The former comprises fungal strains that are mostly passed down by seeds from host generation to host generation. In contrast, the latter consists of fungi that are passed down through spores or other vegetative propagules between various members of a population (Gakuubi et al. 2021).

Similar classifications have been made for fungal endophytes based on their propensity to colonize particular host plant tissues. According to studies, fungal endophytes frequently exhibit selective tissue colonization, whereby particular fungal strains are more likely to be localized in particular locations on the host plant than to colonize the host systemically. Thus, there are foliar fungal endophytes, which are found in the leaves, bark, and stems of hosts, and root endophytic fungi, which are often isolated from the roots of the hosts. The distinction between symptomatic and asymptomatic fungal endophytes is based on the expression of infection, which is another popular criterion for classifying fungal endophytes. Other common classification criteria for fungal endophytes include their mode of reproduction, which can be either sexual or asexual (Gakuubi et al. 2021).

4 Bioactive Compounds Isolated from Endophytic Fungi

Endophytic fungi form captivating communities that live within the tissues of host organisms, either between or within cells. These fungi offer benefits to their hosts while also gaining advantages for themselves. They serve as a natural source of novel bioactive compounds with diverse structures, encompassing alkaloids, flavonoids, benzopyranones, phenolic acids, steroids, quinones, terpenoids, and more. These bioactive metabolites have a broad range of biological activities and find applications in various fields, including antimicrobial agents, agrochemicals, antibiotics, immunosuppressants, and antiparasitic drugs (Rai et al. 2021). Fungi can produce over 20,000 secondary metabolites, and a significant majority of these compounds, around 80%, exhibit bioactive properties (Lacerda et al. 2022). Popular fungal genera like *Fusarium*, *Penicillium*, *Aspergillus*, *Sclerotium*, *Alternaria*, *Colletotrichum*, *Curvularia*, *Cladosporium*, *Myxormia*, etc. are particularly valuable sources for obtaining bioactive compounds.

The bioactive compounds derived from fungal endophytes' extracts can undergo purification and characterization using various techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), electron spray ionization (ESI), and fast atom bombardment (FAB) (Rai et al. 2021).

Some of the well-studied secondary metabolites of endophytic fungi are briefly discussed below:

1. *Brefeldin-A (BFA)* is a compound produced by certain endophytic fungi that has a variety of biological activities, including tumor suppression, antibacterial action, and antiviral properties. BFA is thought to directly block the transport of glycoproteins from the endoplasmic reticulum to the Golgi apparatus, preventing the formation and release of viruses from infected cells (Lacerda et al. 2022).
2. *Betulinic acid* is a natural compound found in a variety of fungi, including *Phomopsis*, *Syncephalastrum*, *Botryosphaeriales*, and *Aspergillus*. It is a pentacyclic triterpenoid, which means it is a type of compound that is made up of five carbon rings. Betulinic acid has been shown to inhibit topoisomerase I, an enzyme that is important for DNA replication. This suggests that betulinic acid may be able to inhibit the growth of cancer cells. Additionally, betulinic acid has been shown to inhibit HIV, the virus that causes AIDS. This makes betulinic acid a potential candidate for the development of new antiviral drugs (Lacerda et al. 2022).
3. *Pestalotheol C* is a compound produced by an endophytic fungus *Pestalotiopsis theae*. It is a compound with multiple biological activities, including anti-HIV, antibacterial, and antifungal properties. It is thought to work by disrupting the HIV-1 virus's ability to replicate, which it does by binding to a specific protein on the surface of the virus, preventing it from entering the cell (Lacerda et al. 2022).
4. *Leucinostatin A* is a nonapeptide complex derived from a fungal endophyte of *Acremonium* sp. in liquid culture, isolated from the plant *T. baccata*. It shows a diverse range of biological activity, including anticancer, phytotoxic, antiviral, antimalarial, and antifungal properties. Leucinostatin A is also known to produce a glycosylated bioactive compound, leucinostatin A β-di-O-glucoside (Rai et al. 2021).
5. *Quinones* are natural compounds with a variety of biological activities, including cytotoxicity, antimicrobial activity, antiviral activity, and anti-inflammatory activity. Hinnuliquinone is a quinone that was isolated from the fungus *Nodulisporium hinnuleum*, which is associated with the plant Quercus coccifera. Hinnuliquinone has anti-HIV activity, and it inhibits the HIV-1 protease, an enzyme that is essential for the virus to replicate and mature. This inhibition can help to control the spread of HIV (Lacerda et al. 2022).
6. *Isoindolone derivatives* are a type of secondary metabolites that contains an isoindole ring produced by various microorganisms, including fungi, bacteria, and actinomycetes. They have a wide range of structures and biological activities, including phytotoxicity. Phytotoxins are compounds that are toxic to plants.

Zinnimidine, porritoxin, pyrimidine, and emeriphenolicins are some examples of isoindolone derivatives that are phytotoxins (Lacerda et al. 2022).

7. *Rugulosin*, a natural compound, exhibits cytotoxic properties. It belongs to the polyketide family, composed of multiple smaller molecules known as "ketides." Rugulosin induces cell necrosis, resulting in cell death. Additionally, it triggers degradation of fatty acids within cells, which can contribute to cellular damage and eventual cell death (Alam et al. 2021).
8. *Altersolanol A* is an anthraquinone derivative that was isolated from a fungus found in the plant Nyctanthes arbor-tristis. It has antibiotic properties and can induce cell death by apoptosis. Altersolanol A is a kinase inhibitor. By blocking the activity of kinases, altersolanol A can induce cell death by apoptosis. Altersolanol A also induces apoptosis by cleaving caspase-3 and caspase-9, which are enzymes that are involved in the apoptotic pathway. Altersolanol A can also decrease the expression of antiapoptotic proteins, which can further promote apoptosis (Alam et al. 2021).

5 Antimicrobial Activity of Endophytic Fungi and Associated Metabolites

Today, antimicrobial resistance is one of the most significant burdens in health care. The number of antimicrobial resistant strains isolated increased every year increasing the virulence and pathogenicity of many microbes. Secondary metabolites produced by secondary metabolites provide an attractive alternative to standard antibiotics currently in use. Different classes of compounds including phenols, piperazine, quinones, and quinolones have been isolated from endophytic fungi. A few of the compounds and their antimicrobial activity are briefly described below:

(a) *Crude Extracts*

Aqueous extracts of endophytic fungi can be prepared using different solvents including ethanol, methanol, ethyl acetate, etc. Multiple studies regarding the antimicrobial activity of endophytic crude extracts have been carried out; we have listed out a few examples of these studies in this section. The ethyl acetate and n-butanol extracts of two strains of fungi *Arthrinium arundinis* and *Papulaspora immersa* isolated from the roots of *Smallanthus sonchifolius* exhibit antimicrobial activity against different pathogens including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Kocuria rhizophila* (Ramos et al. 2010). Tayung et al. (2012) also found that *Curvularia* sp. 2 and *Fusarium* sp. 1 isolated from *Ipomoea carnea* exhibit antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*.

Endophytic fungi are also isolated from a number of medicinal plants in different parts of the world. Four strains of endophytic fungi, *Cochliobolus intermedius*, *Phomopsis* sp., G1-74 (non-identified – NI), and G23-100 (NI), isolated from a medicinal plant *Sapindus saponaria* sp., have been found to be capable

Table 7.1 Antimicrobial activity of various endophytic fungi crude extracts

Host plant	Endophytic Fungi	Solvent	Active against	References
Smallanthus sonchifolius	*Papulaspora immersa*	Ethyl acetate	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Kocuria rhizophila*	Ramos et al. (2010)
		n-Butanol	*Pseudomonas aeruginosa* and *Escherichia coli*	
	Arthrinium arundinis	Ethyl acetate	*Escherichia coli* and *Pseudomonas aeruginosa*	
		n-Butanol	*Escherichia coli* and *Pseudomonas aeruginosa*	
Ipomoea carnea	*Curvularia* sp. 2, *Fusarium* sp. 1	Ethyl acetate	*Bacillus subtilis* and *Escherichia coli*	Tayung et al. (2012)
Sapindus saponaria L.	*Cochliobolus intermedius*, G1-74 *Phomopsis* sp. and G23-100 NI	Ethyl acetate	*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus hirae*	Garcia et al. (2012)
Vochysia divergens	*Aeromicrobium ponti*	Ethyl acetate	Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*	Gos et al. (2017)

of eradicating five strains of pathogens (Garcia et al. 2012). Gos et al. (2017) also found that extracts of actinomycetes isolated from a medicinal plant, *Vochysia divergens*, exhibit antimicrobial activity against clinically important pathogens like methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Potential applications of crude extracts of endophytic fungi against major pathogens are illustrated in Table 7.1.

(b) *Alkaloids*

Alkaloids are a big class of organic compounds containing nitrogen atoms in their structure. Many endophytic compounds also contain a wide range of alkaloid compounds having antimicrobial activity. Fumigaclavine C and Pseurotin A, alkaloids produced by endophytic fungi *Aspergillus fumigatus* isolated from *Bauhinia guianensis*, have been found to inhibit the growth of various pathogens including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Pinheiro et al. 2013). In 2019, Zhang et al. (2019) isolated six prenylated indole/oxindole alkaloids which have antibacterial against *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Ralstonia solanacearum*. They also showed antifungal activity against fungal pathogens such as *Alternaria alternata*, *Cochliobolus heterostrophus*, *Botrytis*

cinerea, *Fusarium oxysporum*, etc. The alkaloids were obtained from the endophytic *Fusarium sambucinum* isolated from fresh leaves of *Nicotiana tabacum*. Mou et al. (2021) also isolated three new alkaloids, cytochrysins A–C from *Cytospora chrysosperma*, an endophytic fungus which is isolated from the plant *Hippophae rhamnoides*. Cytochrysins A and C were found to be capable of inhibiting multidrug-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* (MRSA), respectively. Lastly, chromenopyridin A, N-methoxy-1-pyridone alkaloid is also obtained from the endophytic fungus *Penicillium nothofagi* isolated from *Abies beshanzuensis*. This alkaloid has been found to exhibit antibacterial activity against *Staphylococcus aureus* (Zhu et al. 2022) (Table 7.2).

(c) *Phenolics*

Phenolic compounds consist of a wide range of organic compounds containing an aromatic ring with at least one hydroxyl group. Xanthoascin is a phenolic compound isolated from *Aspergillus* sp. which is obtained from the leaves of *Ginkgo biloba* plant. Xanthoascin showed better activity than streptomycin against the plant pathogen *Clavibacter michiganensis*. The mechanism of action was found to be disruption of cellular membrane followed by nucleic acid and protein leakage from the cell (Zhang et al. 2015). Another phenolic compound, 4-(2,4,7-trioxa-bicyclo[4.1.0]heptan-3-yl) phenol, also showed strong activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Escherichia coli*, and *Candida albicans*. This novel phenolic compound was isolated from the fungus *Pestalotiopsis mangiferae* found in *Mangifera indica* Linn (Subban et al. 2013) (Table 7.2).

(d) *Terpenoids*

Terpenoids are compounds which contain one or more 5-carbon isoprene units. A number of terpenoids having antimicrobial activity have been isolated from endophytic fungi. Six terpenoid compounds, namely, fusariumin A and B, agathic acid, asperterpenoid A, and trametenolic acid guignardone N were isolated from the fungus *Fusarium* sp. YD-2 found in the plant *Santalum album*. Fusariumin A showed potent activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while asperterpenoid A showed moderate activity against *Micrococcus luteus* and *Salmonella enteritidis* (Yan et al. 2018). Another terpenoid bipolarin E isolated from fungal endophyte *Bipolaris* sp. TJ403-B1 found in wheat showed antibacterial property against *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Meng-Ting et al. 2019). Li et al. (2020) isolated harzianol I from *Trichoderma atroviride* B7 which showed potent antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus subtilis*. The endophytic fungus was obtained from *Colquhounia coccinea* var. *Mollis* (Table 7.2).

(e) *Coumarin*

Coumarins are compounds composed of fused benzene and α-pyrone rings. Diaporone A, a dihydroisocoumarin derivative isolated from the fungus *Diaporthe* sp. an endophyte found in *Pteroceltis tatarinowii*, was found to

Table 7.2 Antimicrobial activity of different classes of compounds isolated from endophytic fungi

Name of compound	Class of compound	Endophytic fungi	Plant host	Active against	References
Fumigaclavine C and pseurotin A	Alkaloid	*Aspergillus fumigatus*	*Bauhinia guianensis*	*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*	Pinheiro et al. (2013)
Cytochrysins A and C	Alkaloid	*Cytospora chrysosperma*	*Hippophae rhamnoides*	*Enterococcus faecium and* MRSA	Mou et al. (2021)
Chromenopyridin A	Alkaloid	*Penicillium nothofagi*	*Abies beshanzuensis*	*Staphylococcus aureus*	Zhu et al. (2022)
Xanthoascin	Phenolics	*Aspergillus* sp.	*Ginkgo biloba*	*Clavibacter michiganensis*	Zhang et al. (2015)
4-(2,4,7-trioxa-bicyclo[4.1.0] heptan-3-yl) phenol	Phenolics	*Pestalotiopsis mangiferae*	*Mangifera indica* Linn	*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Escherichia coli* and *Candida albicans*	Subban et al. (2013)
Asperterpenoid A	Terpenoid	*Fusarium* sp. YD-2	*Santalum album*	*Micrococcus luteus* and *Salmonella enteritidis*	Yan et al. (2018)
Fusariumin A	Terpenoid	*Fusarium* sp. YD-2	*Santalum album*	*Pseudomonas aeruginosa* and *Staphylococcus aureus*	Yan et al. (2018)
Bipolarin E	Terpenoid	*Bipolaris* sp. TJ403-B1	Wheat	*Enterococcus faecalis* and *Pseudomonas aeruginosa*	Meng-Ting et al. (2019)
Harzianol I	Terpenoid	*Trichoderma atroviride* B7	*Colquhounia coccinea* var. *mollis*	*Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus subtilis*	Li et al. (2020)
Diaporone A	Coumarin	*Diaporthe* sp.	*Pteroceltis tatarinowii*	*Bacillus subtilis*	Guo et al. (2020)
Phomoisocoumarins C and D	Coumarin	*Phomopsis prunorum*	*Hypericum ascyron*	*Xanthomonas citri*	Qu et al. (2020)

(continued)

Table 7.2 (continued)

Name of compound	Class of compound	Endophytic fungi	Plant host	Active against	References
(+)-2,2′-epicytoskyrin A (epi) and (+)-1,1′-bislunatin (bis)	Quinones	*Diaporthe* sp. GNBP-10	*Uncaria gambir*	*Mycobacterium tuberculosis*	Oktavia et al. (2020)
3-hydroxy-6-hydroxymethyl-2,5-dimethylanthraquinone and 6-hydroxymethyl-3-methoxy-2,5-dimethylanthraquinone	Quinones	*Phomopsis* sp.	*Nicotiana tabacum* L	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Wu et al. (2021)
ω-hydroxyemodin	Quinones	*Penicillium restrictum*	*Silybum marianum*	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Graf et al. (2020)
Fusarnaphthoquinone B	Quinones	*Neocosmospora* sp.	*Rhizophora apiculata*	*Acidovorax citrulli*	Klomchit et al. (2021)

inhibit *Bacillus subtilis* growth (Guo et al. 2020). Phomoisocoumarins C and D are another example of coumarin produced by endophytic fungus *Phomopsis prunorum* found in *Hypericum ascyron*. They showed activity against a plant pathogen *Xanthomonas citri* pv. *phaseoli* var. *fuscan* (Qu et al. 2020) (Table 7.2).

(f) *Quinones*

Quinones are a group of cyclic organic compounds containing two carbonyl groups. Two bisanthraquinones, (+)-2,2′-epicytoskyrin A (epi) and (+)-1,1′-bislunatin (bis) isolated from *Diaporthe* sp. GNBP-10, an endophytic fungus of the plant *Uncaria gambir*, were found to inhibit *Mycobacterium tuberculosis* growth (Oktavia et al. 2020). Wu et al. (2021) purified two anthraquinones, 3-hydroxy-6-hydroxymethyl-2,5-dimethylanthraquinone and 6-hydroxymethyl-3-methoxy-2,5-dimethylanthraquinone from the fungus *Phomopsis* sp. isolated from *Nicotiana tabacum* L. The two compounds showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). ω-Hydroxyemodin, an anthraquinone isolated from *Penicillium restrictum* (strain G85), an endophyte of *Silybum marianum*, also showed anti-MRSA activity by inhibiting the quorum sensing system (Graf et al. 2020). Fusarnaphthoquinone B, isolated from *Neocosmospora* sp. MFLUCC 17-0253, an endophyte of *Rhizophora apiculata*, showed antibacterial and antibiofilm properties against a plant pathogen *Acidovorax citrulli* (Klomchit et al. 2021) (Table 7.2).

6 Antiparasitic Compounds Derived from Fungal Endophytes

Fungal endophytes are known to produce a wide range of bioactive compounds, including alkaloids, terpenoids, phenolics, and polyketides, which play a crucial role in their interaction with the host plant and other organisms (Martínez-Luis et al. 2011). A few examples of antiparasitic compounds are briefly discussed below:

1. *Beauvericin*
   Beauvericin is a cyclic peptide mycotoxin produced by various endophytic fungi. It has shown promising antiparasitic activity against a range of parasites, including *Trypanosoma brucei* (the causative agent of African sleeping sickness) and *Plasmodium falciparum* (the causative agent of malaria). Beauvericin exerts its antiparasitic effects through multiple mechanisms, including disruption of ion channels and inhibition of parasite mitochondrial respiration. Research studies have demonstrated its potential as an antimalarial and antitrypanosomal agent (Zhang et al. 2016).

2. *Cytochalasins*
   Cytochalasins are fungal metabolites that have shown promising antiparasitic activity. For example, cytochalasin D, derived from endophytic fungi, has demonstrated significant activity against *Plasmodium* spp. by inhibiting parasite growth and disrupting cytoskeletal structures. Cytochalasin D has also exhibited antiparasitic effects against *Trypanosoma* spp. and *Leishmania* spp. through disruption of microtubule assembly and inhibition of parasite proliferation (Zhang et al. 2016).

3. *Emodin*
   Emodin is a natural compound commonly found in various fungi, including *Aspergillus*, *Penicillium*, and *Fusarium* species. It exhibits antiamoebic activity against *Entamoeba histolytica*, the causative agent of amoebic dysentery. Emodin has been shown to inhibit the growth and adherence of *E. histolytica* trophozoites and induce apoptotic cell death in the parasite (Franco et al. 2017).

4. *Lactones*
   Fungal lactones have demonstrated antiamoebic activity against *E. histolytica*. For example, 8-O-methyl-maytenin, a natural lactone derivative produced by endophytic fungi, exhibits potent antiamoebic effects. It inhibits the growth and motility of *E. histolytica* trophozoites by affecting their cytoskeletal structure and disrupting cellular processes (Franco et al. 2017).

5. *Ergosterol Peroxide*
   Ergosterol peroxide, a steroidal compound produced by fungi, has also shown antiamoebic activity. It has been reported to inhibit the growth and motility of *E. histolytica* trophozoites by inducing apoptosis and affecting membrane permeability (Chen et al. 2007).

6. *Terpenoids*
   Terpenoids derived from fungal sources have demonstrated potential antiamoebic activity. For instance, β-caryophyllene oxide, a sesquiterpene oxide produced by various fungi, exhibits antiamoebic effects against *E. histolytica* by inhibiting parasite growth and inducing cell death (Khalid 2018).

   These examples represent some of the antiamoebic compounds derived from fungi. Further research is ongoing to explore the potential of fungal metabolites and identify new compounds with antiamoebic activity. These compounds hold promise for the development of novel therapeutics against amoebic infections.

7 Anticancer Activity of Endophytic Fungi

Earlier, chemotherapeutic agents were used in the treatment of cancer, but they cause cell toxicity. That's why an alternative method is discovered in which anticancer compounds are derived from endophytic fungi to treat various types of cancers such as Kaposi's sarcoma, lung, prostrate, breast, and ovarian cancer. Natural anticancer compounds obtained from endophytic fungi can stop the progression of development of cancer cells and induce apoptosis (Tiwari and Bae 2022; Dos Santos et al. 2022).

(a) *Paclitaxel*
   Paclitaxel, an anticancer drug, has been derived from many endophytic fungi like *Seimatoantlerium nepalense*, *Chaetomella raphigera*, *Alternaria alternata*, and *T. andreanae* (Sonaimuthu and Johnpaul 2010). Paclitaxel can be effectively used for the treatment of Kaposi's sarcoma which is a skin cancer, formed by the abundant increase of abnormal cells, ovarian cancer, prostate cancer, and lung cancer (Pandi et al. 2013). In the several events of cell cycle, the cytotoxicity shown by paclitaxel is high. Paclitaxel maintains the structure of microtubules during their polymerization and resists the depolymerization by binding with tubulin protein, which can affect the microtubule assembly. From previous studies, it has been concluded that on combining paclitaxel with cacl2 (calcium chloride) (4 mM), the process of depolymerization can be slowed down, because faster depolymerization can cause interference of unstable microtubules with the spindle network, formed during cell division in cell cycle. The disruption of the microtubule network brought on by elevated levels of Bax and Bcl-2, which result in the arrest of the cell cycle during the G2-M phase, is another important function of paclitaxel in cell signaling pathways, and during the mitotic G1 phase, these events triggered apoptosis in arrested cells (Weaver 2014; Pasquier et al. 2004; Kampan et al. 2015) (Fig. 7.2).

(b) *Podophyllotoxin*
   Podophyllotoxin drug is derived from *Dysosma veitchii* and *Sinopodophyllum hexandrum* endophytic fungi (Xianzhi et al. 2003). It could eliminate the metastatic cells at their premature level; thus, it is widely used in the treatment of testicular, lung, leukemia, ovarian, and prostate cancer (Uzma et al. 2018).

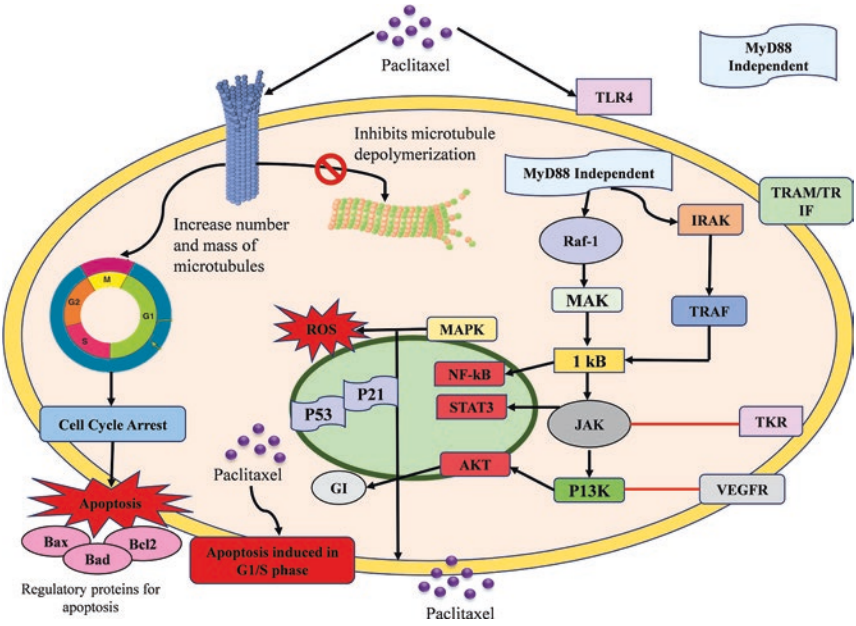

Fig. 7.2 Mechanism of action of paclitaxel. (Adapted from Kampan et al. (2015))

Podophyllotoxin can cause the death of cancer cells. Etoposide and teniposide are two precursors of podophyllotoxin which are toxic to topoisomerase II (enzyme used in DNA replication process), and they halt the replication process by binding tightly with topoisomerase II. Podophyllotoxin increases the levels of topoisomerase II by blocking their activities. Binding of podophyllotoxin with topoisomerase II can cleave the DNA duplex to a great extent which results in the breakage of double-stranded DNA that enhances the chance of DNA damage (Fig. 7.3). DNA alterations brought on by insertions, deletions, and genetic recombination resulted in a significant buildup of DNA damage that triggered cell death (Kumar et al. 2021).

(c) *Camptothecin*

During clinical trials, the camptothecin shows satisfactory results by inducing apoptosis in the cancerous cells of bladder, ovaries, liver, and lungs (Li et al. 2017). During DNA replication process, the topoisomerase I binds with the double-stranded DNA. Camptothecin shows anticancer properties by suppressing the catalytic activity of topoisomerase II through non-covalent binding. Consequently, p21, p53, and mTOR expression were all enhanced. However, the molecular events such as nuclear factor erythroid-related factor 2 and extracellular signal-regulated kinase that cause cell death due to apoptosis are enhanced by camptothecin (Beretta et al. 2013; Pommier 2006) (Fig. 7.4).

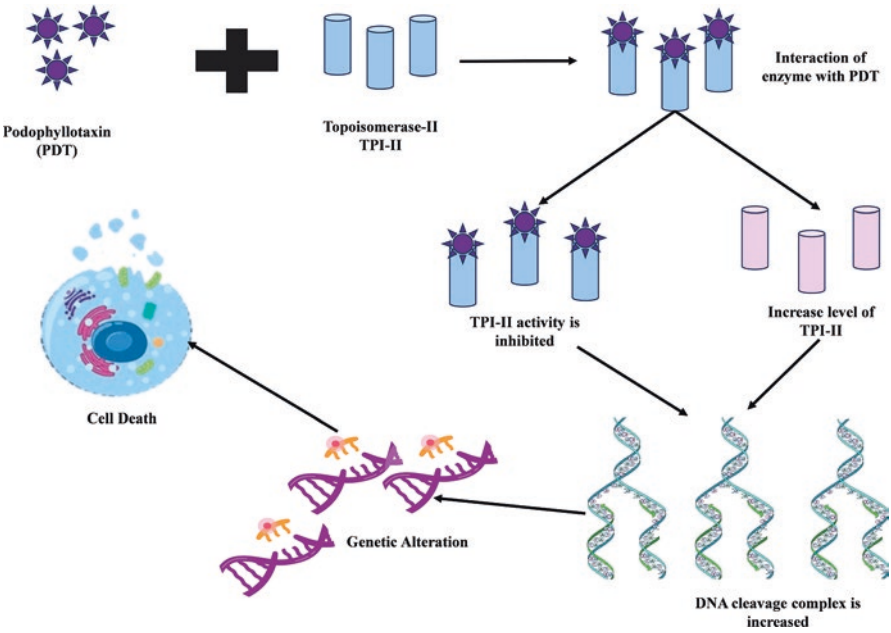

Fig. 7.3 Mechanism of action of podophyllotoxin. (Adapted from Kumar et al. (2021), open access and requested for permission to reproduce)

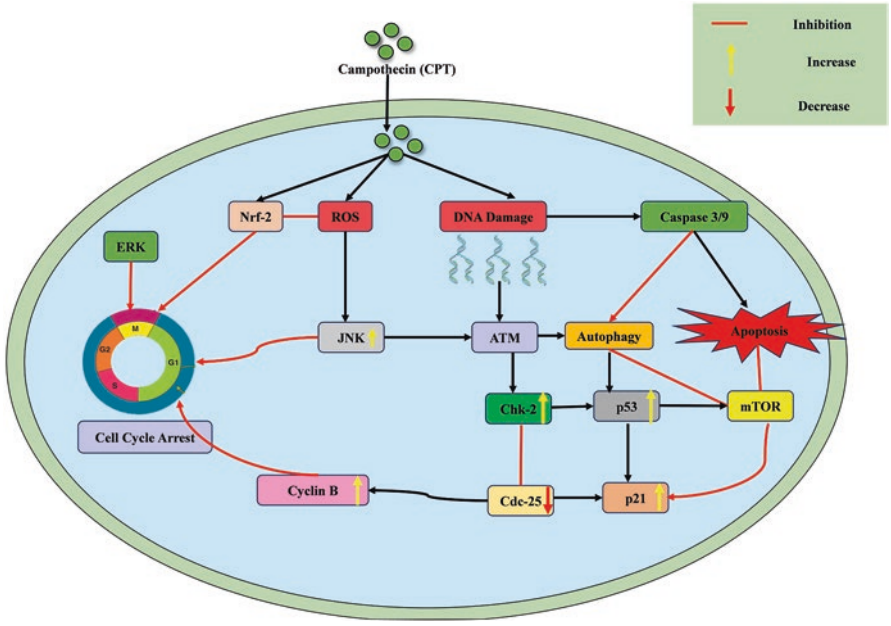

Fig. 7.4 Mechanism of action of camptothecin. (Adapted from Ghanbari-Movahed et al. (2021), open access and requested for permission to reproduce)

(d) *Vinblastine*

The endophytic fungi that produce vinblastine from their hosts are *Fusarium oxysporum* and *Catharanthus roseus* (Lee and Shim 2020; Lingqi et al. 2000). Vinblastine has been used in the treatment of lymphoblastic leukemia and the cancer cell lines (HepG-2) with the ICM (7.48 μg/mL) (Puri et al. 2018; El-Sayed 2021). The reason behind the antibiotic activity of vinblastine is that during the mitotic phase of cell cycle, the polymerization of spindle fibers by the tubulin proteins is inhibited. Vinblastine blocks the polymerization by binding with receptor's site of tubulin protein which results in assembly of microtubules and improper functioning of tubulin. This led to the death of cell due to its arrest in the anaphase stage for a longer period (Martino et al. 2018). PEGylated niosomal formulation of vinblastine (Pn-VB) produced via a thin-film hydration process (Amiri et al. 2018) was found stable, effective at encapsulation, and more potent at eliminating lung cancer TC-1 cells in comparison to vinblastine. The vinblastine molecule interacts with the dynamic equilibrium formed between α-tubulin and β-tubulin's association and dissociation to attain high activity in elimination of cancer cells. Vinblastine is composed of a vinblastine molecule and a catharanthine moiety molecule, both of which are the basis for causing toxicity to cancer cells (Zhou et al. 2019).

(e) *Hypericin*

Thielavia subthermophila endophytic fungi produce hypericin within their *H. perforatum* host plant (Kusari et al. 2012). Hypericin has been used as an anticancer drug against T-cell lymphoma by having an ability to inhibit several genes and activate the assembly of capases (Garnica et al. 2003). Hypericin can cause DNA damage by interacting with ROS. The molecular events that enhanced the expression of PARP are increase in the levels of p21, p27, cyclins B/D1/E, and activation of caspases -3,8 due to the release of cytochrome c from the inner protein of mitochondria which results in the cell arresting at the G-2 phase of cell cycle thus inducing apoptosis and cell death (Agostinis et al. 2002; Dong et al. 2021) (Fig. 7.5).

Hypericin is an effective anticancer compound and studies found that different amounts of hypericin (0.021, 0.2, and 0.02 μM) can promote apoptosis and cause cell death in breast cancer cell line (MCF-7), human lymphoma cancer cell line (U937), and human skin carcinoma cancer cell line (A431) and expressed metastatic invasion getting halt by 60–90% (Ferenc et al. 2010; Vandenbogaerde et al. 1997; Paba et al. 2001).

(f) *Diosgenin*

In preclinical trials, diosgenin is found to be effective against various cell lines of cancer such as breast, lung, hepatic carcinoma, chronic myeloid leukemia, prostate, and colon cancer by controlling the abnormal growth of metastatic cells, proliferation of tumor cells, and inducing apoptosis (Biswas et al. 2020). Anticancer effects of 50% and 36.18% were observed when varying doses of diosgenin such as 50 and 100 mg/kg were given to mice suffering from prostate and hepatic (HepG2) cancers (Chen et al. 2016). According to a different study,

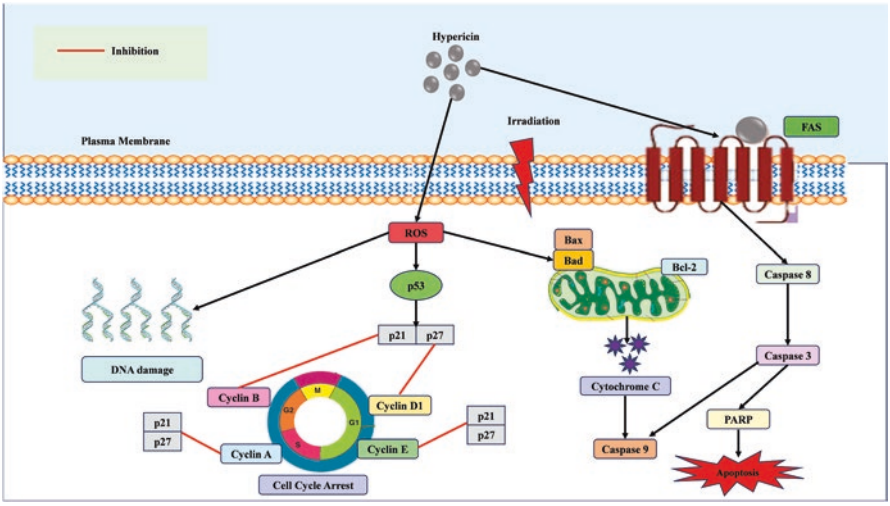

Fig. 7.5 Mechanism of action of hypericin. (Adapted from Dong et al. (2021), open access and requested for permission to reproduce)

administration of diosgenin to rats with colon cancer at a dosage of 0.1% for up to 48 weeks (about 11 months) resulted in positive anticancer effects, i.e., the incidence of both invasive and noninvasive colon cancer was greatly reduced by up to 60% (Lai et al. 2022). Another experimental investigation indicated that administration of diosgenin to mice at a dose of 10 mg/kg resulted in encouraging anticancer effects against breast cancer cell lines (MDA-231) that were 23 times greater than those seen in the control groups (Srinivasan et al. 2009).

In the Hep2 skin carcinomas, diosgenin triggered apoptosis via the AKT and JNK pathways, which were activated by caspases. In hypoxia-sensitive BGC-823 gastric cancer cells, it also reduced the expression of E-cadherin, integrin 5a and 6b, invasion, migration, and angiogenesis. Additionally, it was revealed that diosgenin binds to E-cadherin and is thus implicated in the angiogenesis of the BGC-823 gastric cancer cells (Mao et al. 2012). Recent investigations show that diosgenin has the capacity to connect with NF-kB triggered survival cyclin D1 and Cdk-2 and subsequently reduce their expressions in the breast metastatic/cancer cells (Liu et al. 2020). Diosgenin, a steroidal sapogenin, has been mentioned as a potential cancer treatment strategy. Specifically in cancer cells, it induces ROS-mediated autophagy, blocks the PI3K/Akt/mTOR pathway, and produces cytotoxicity (Bhardwaj et al. 2021) (Fig. 7.6).

Diosgenin works at a molecular and cellular levels in the NF-kB and STAT3 signaling pathways to stop the uncontrolled growth of cancer cells by inducing apoptosis. Through the NF-kB signaling pathway, diosgenin lowers the level of TNF-α, and through STAT3, it reduces the level of IL6 by angiogenesis and cell migration, functioning as an effective anticancer medication for the early treatment of malignancies (Chiang et al. 2007; Sethi et al. 2018).

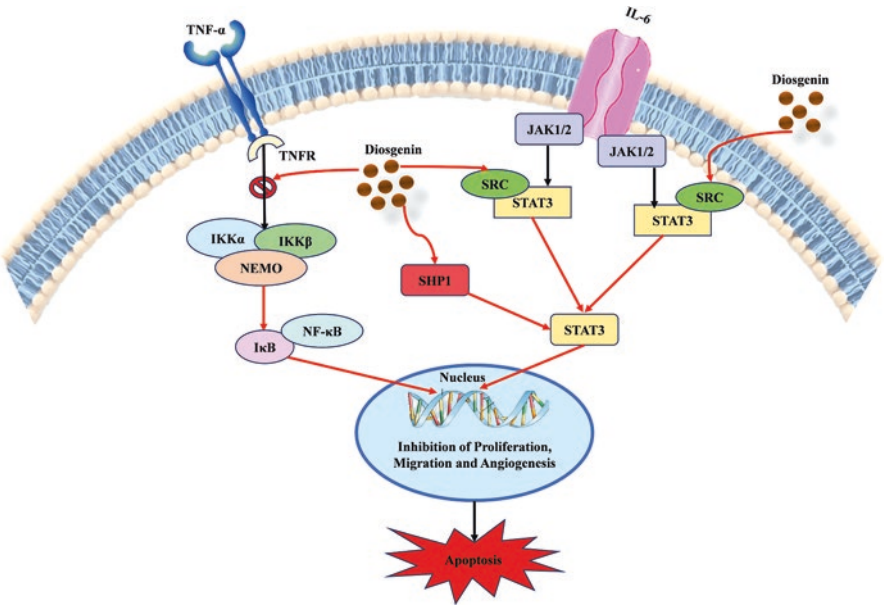

Fig. 7.6 Mechanism of action of diosgenin. (Adapted from Sethi et al. (2018), open access and requested for permission to reproduce)

(g) *Toosendanin*

Toosendanin (TSN) has unique biological properties and was highly valuable in both scientific research and therapeutic practice (Wu et al. 2013). Preclinical studies have gathered evidence that TSN has an anticancer impact on many cancer cells in vivo and in vitro (Zhang et al. 2019). The molecular mechanism behind the inhibition of the PI3K/AKt, MEK/Erk, and MAPK/JNK pathways in leukemia, colorectal cancer, hepatocellular carcinoma, prostate cancer, lymphoma, and breast cancer includes apoptosis and stopping of the initiation of cell cycle. According to a recent glioblastoma study, TSN prevented cancer cells in U87 and U6 from proliferating by triggering apoptosis through estrogen receptor-dependent machinery (Cao et al. 2016).

Several cancer cell lines are extremely cytotoxic to the triterpenoid TSN, and when it was applied to AGS and HGC-27 cells, the researchers discovered that it decreased the cell viability, halted the cell development by inducing G1/S arrest, and encouraged caspase-dependent apoptosis. The p38 MAPK pathway may play a role in TSN-induced cell death. In these trials, TSN showed therapeutic potential for the treatment of gastric cancer (Zhou et al. 2018).

In a study on cell viability, it was shown that TSN and isotoosendanin (ITSN) were cytotoxic to a variety of tumor cells, but they were particularly effective against triple-negative breast cancer cells such MDA-MB-231, BT549, and 4T1. In the presence of TSN (20 nM) or ITSN (2.5 nM), cell death was seen in MDA-MB-231 and 4T1 cells. After exposure to TSN (20 nM) or ITSN (2.5 nM),

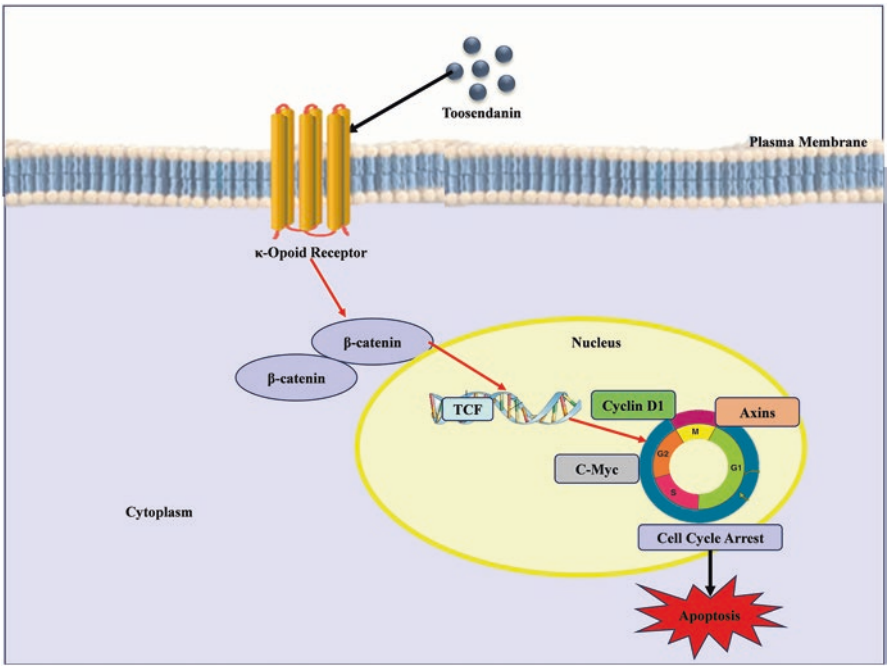

Fig. 7.7 Mechanism of action of toosendanin. (Adapted from Zhang et al. (2022), open access and requested for permission to reproduce)

pro-caspase-3 and Bcl-xL expression were likewise decreased in MDA-MB-231 and 4T1 cells. Natural substances TSN and ITSN inhibit the growth of TNBC by inducing necrosis, apoptosis, and autophagy, according to research findings (Zhang et al. 2022) (Fig. 7.7).

8 Endophytic Fungi in Soil Remediation

Remediating contaminated soils and (ground) water is a promising area to take advantage of plant-endophyte relationships. Numerous endophytes that promote plant growth can help their host plant overcome stress reactions brought on by contaminants, which leads to increased plant growth (Weyens et al. 2009). Endophytes with the proper degradation routes and metabolic skills can be of further assistance to plants during the phytoremediation of organic contaminants, resulting in more effective contaminant degradation and a decrease in both phytotoxicity and evapotranspiration of volatile contaminants (Weyens et al. 2009). Endophytes with a metal-resistance/sequestration system can reduce metal phytotoxicity and impact metal translocation to the aboveground plant parts in the phytoremediation of hazardous metals. Additionally, endophytes that can deal with or, much better, enhance

the extraction of the metals and degrade organic contaminants offer intriguing strategies to enhance the phytoremediation of mixed pollution (Weyens et al. 2009). Hazardous substances and carcinogens derived from industrial and agricultural sources have a significant negative influence on human health and are linked to toxicity and ecological imbalance. Utilizing a plant's metabolic system, phytoremediation is the process of removing harmful substances from the environment, such as heavy metals, xenobiotics, and toxic compounds (Khan et al. 2011). An in situ, solar-powered remediation method called phytoremediation (the use of plants and the associated microbes to clean up a site) causes little site disturbance and requires little upkeep, making it affordable and well-liked by the general public. Phytoremediation proves to be a viable alternative to currently available conventional remediation methods, because they are frequently expensive and environmentally invasive, especially for the treatment of large contaminated areas with diffuse pollution. The levels of contaminants, which are toxic for the organisms involved in remediation, the bioavailable fraction of the contaminants, which is too low, and, in some cases, evapotranspiration of volatile organic pollutants from soil or groundwater to the atmosphere are some of the challenges that large-scale applications of phytoremediation still face (Weyens et al. 2009).

By creating organic ligands through the microbial breakdown of soil organic matter, exudation of metal ions, and other processes, rhizosphere microorganisms can improve solubility and alter the speciation of metals and metalloids. Using ligand exchange, metabolites and microbial siderophores can compound cationic metals or desorb anionic species (Wenzel 2009). Siderophores made by microbes or plants may immobilize cadmium, copper, and zinc cations depending on the surface charge of soil minerals and pH levels below metal-specific levels. Modeling of copper solubility and transport in the root zone in the absence and presence of organic ligands has shown the complexity of interactions between ligands and metals; however, ligands do not always increase the solubility of metal, but pH is a key determining factor of mobilization and immobilization, together with metal bioavailability and bioavailability (Wenzel 2009).

Contaminants frequently change the structure and density of endophytic communities by disrupting the microbe-rhizosphere interface, which has a significant impact on the interactions between plants and microbes. As a result, the existence of metal- or xenobiotic-resistant endophytic fungal strains and subsequent phytoremediation positively affect a plant's ability to develop, survive under stress, and tolerate salinity and acidity, as well as other metabolic processes (Rajkumar et al. 2010).

Total petroleum hydrocarbons (TPHs) degrade at much greater rates in *Pharbitis nil* L. and its microbial community-driven plot-culture studies compared to the controls (controls) (27.63-67.42% vs. 10.20-35.61%). Due to the presence of remediation facilities, different TPH fractions are not significantly different, although saturated hydrocarbon removal is much higher than that of other components. When the amount of petroleum hydrocarbons in the soil was less than 2.0%, the biomass of *P. nil* did not significantly decrease (Zhang et al. 2010).

The endophytic fungi, along with the growth-promoting rhizobacteria and the arbuscular mycorrhizal fungi (AMF), have demonstrated an efficient mechanism in

heavy metal and contaminant tolerance and biosequestration, even though the role of endophytic bacteria in phytoremediation has been extensively studied (Pawlowska et al. 2000) (Fig. 7.8).

Tall fescue (*Lolium arundinaceum*) cultivated in greenhouses in polluted solutions was used to study the impact of endophyte infection on plant development, cadmium (Cd) uptake, and Cd translocation. Under both control and Cd-stress circumstances, endophyte infection markedly enhanced the host grass's tiller count and biomass.

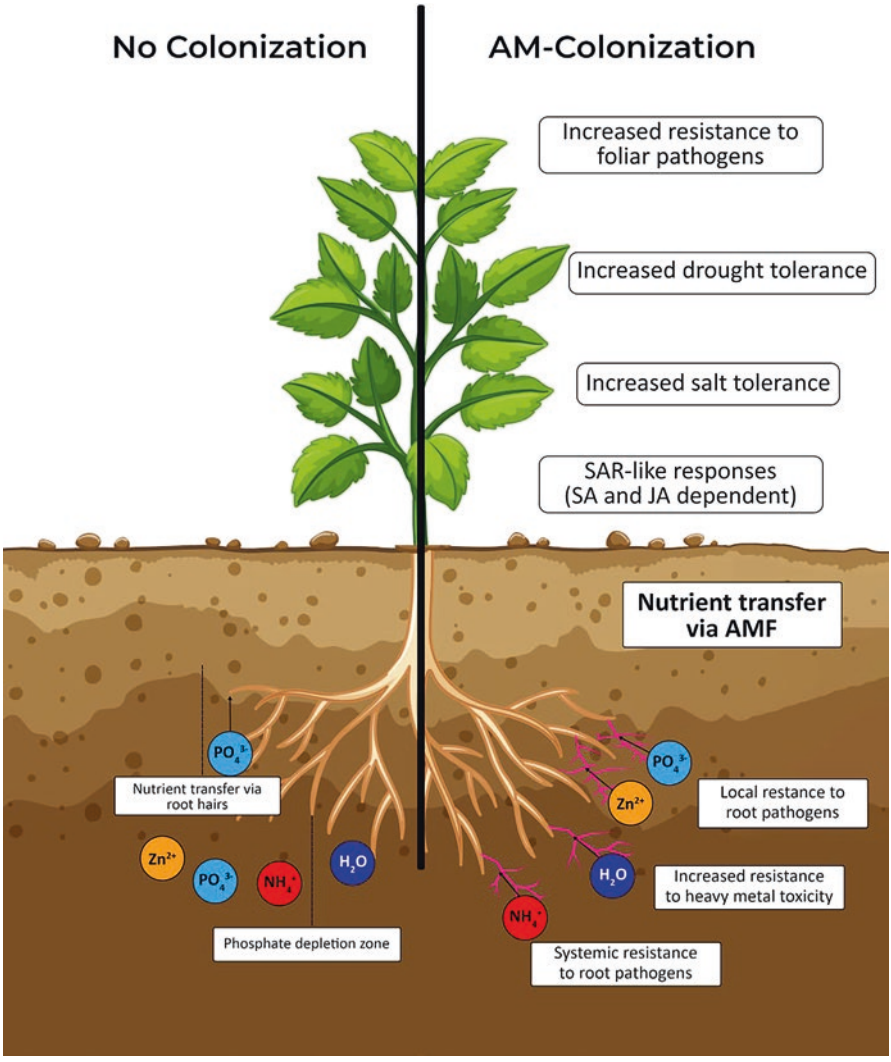

Fig. 7.8 Symbiotic relationship between plant and endophytic fungi. (Adapted from Jacott et al. (2017))

In tall fescue, endophyte infection boosted both Cd transport from the root to the shoot and Cd accumulation. Endophyte-infected (EI) tall fescue had a phytoextraction efficiency that was 2.41 times greater than endophyte-free plants under a 20 mg L^{-1} Cd stress. Endophyte/plant associations can be a model for endophyte-assisted phytoremediation of soils polluted with metals. Cd accumulation in EI tall fescue was insufficient for practical phytoextraction applications (Ren et al. 2011).

The idea of using mutualistic fungal endophytes to biologically improve plants to manage plant parasitic nematodes utilizing nonpathogenic strains of *Fusarium oxysporum* and, to a lesser extent, species of *Trichoderm*a has gained attention. Nonpathogenic strains of *Fusarium oxysporum* have been used extensively in research on the biological enhancement of plants with fungal endophytes (Sikora et al. 2008). Bananas, tomatoes, and rice have been the main crops researched thus far for biological enhancement utilizing endophytes. However, the idea of using nonpathogenic fungi that colonize the endorhiza for biological control is still very new (Sikora et al. 2008).

References

Agostinis P, Vantieghem A, Merlevede W, de Witte PA (2002) Hypericin in cancer treatment: more light on the way. Int J Biochem Cell Biol 34(3):221–241

Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S et al (2021) Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa? Front Plant Sci 12:3060

Amiri B, Ahmadvand H, Farhadi A, Najmafshar A, Chiani M, Norouzian D (2018) Delivery of vinblastine-containing niosomes results in potent in vitro/in vivo cytotoxicity on tumor cells. Drug Dev Ind Pharm 44(8):1371–1376

Bae H, Kim S, Sicher RC Jr, Kim MS, Strem MD, Bailey BA (2008) The beneficial endophyte, Trichoderma hamatum, delays the onset of drought stress in Theobroma cacao. Biol Control 46:24–35

Beretta GL, Gatti L, Perego P, Zaffaroni N (2013) Camptothecin resistance in cancer: insights into the molecular mechanisms of a DNA-damaging drug. Curr Med Chem 20(12):1541–1565

Bhardwaj N, Tripathi N, Goel B, Jain SK (2021) Anticancer activity of diosgenin and its semi-synthetic derivatives: role in autophagy mediated cell death and induction of apoptosis. Mini Rev Med Chem 21(13):1646–1665

Biswas D, Nazir R, Biswas P, Kumar V, Nandy S, Mukherjee A et al (2020) Endophytic sources of diosgenin, a natural steroid with multiple therapeutic values. S Afr J Bot 134:119–125

Cao L, Qu D, Wang H, Zhang S, Jia C, Shi Z et al (2016) Toosendanin exerts an anti-cancer effect in glioblastoma by inducing estrogen receptor β-and p53-mediated apoptosis. Int J Mol Sci 17(11):1928

Chen CJ et al (2007) Antiamibic and phytochemical investigation of the organic extract of cultured fungus Paecilomyces sp. Parasitol Res 101(3):559–564

Chen J, Zhang HF, Xiong CM, Ruan JL (2016) Inhibitory effect of diosgenin on experimentally induced benign prostatic hyperplasia in rats. J Huazhong Univ Sci Technolog Med Sci 36(6):806–810

Chiang CT, Way TD, Tsai SJ, Lin JK (2007) Diosgenin, a naturally occurring steroid, suppresses fatty acid synthase expression in HER2-overexpressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation. FEBS Lett 581(30):5735–5742

Dong X, Zeng Y, Zhang Z, Fu J, You L, He Y et al (2021) Hypericin-mediated photodynamic therapy for the treatment of cancer: a review. J Pharm Pharmacol 73(4):425–436

Dos Santos IR, Mohamed TA, Borges LL, Nahas HA, Abdel-Azeem MA, Carvalho CDC et al (2022) The global tendency in the research of biological activity in endophytic fungi: a scientometric analysis. Curr Res Environ Appl Mycol 12:1–14
El-Sayed ER (2021) Discovery of the anticancer drug vinblastine from the endophytic Alternaria alternata and yield improvement by gamma irradiation mutagenesis. J Appl Microbiol 131(6):2886–2898
Fadiji AE, Babalola OO (2020) Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi J Biol Sci 27(12):3622–3633
Ferenc P, Solár P, Kleban J, Mikeš J, Fedoročko P (2010) Down-regulation of Bcl-2 and Akt induced by combination of photoactivated hypericin and genistein in human breast cancer cells. J Photochem Photobiol B Biol 98(1):25–34
Franco CMM et al (2017) Antiamoebic activity of 8-O-methyl-maytenin obtained from cultures of an endophytic fungus of Celastraceae. Parasitol Int 66(3):262–266
Gakuubi MM, Munusamy M, Liang ZX, Ng SB (2021) Fungal endophytes: a promising frontier for discovery of novel bioactive compounds. J Fungi 7(10):786
Ganley RJ, Sniezko RA, Newcombe G (2008) Endophyte-mediated resistance against white pine blister rust in Pinus monticola. For Ecol Manag 255(7):2751–2760
Garcia A, Rhoden SA, Bernardi-Wenzel J, Orlandelli RC, Azevedo JL, Pamphile JA (2012) Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant Sapindus saponaria L. J Appl Pharm Sci 2(10):035–040
Garnica S, Weiß M, Oberwinkler F (2003) Morphological and molecular phylogenetic studies in South American Cortinarius species. Mycol Res 107(10):1143–1156
Ghanbari-Movahed M, Kaceli T, Mondal A, Farzaei MH, Bishayee A (2021) Recent advances in improved anticancer efficacies of camptothecin nano-formulations: a systematic review. Biomedicine 9(5):480
Gos FM, Savi DC, Shaaban KA, Thorson JS, Aluizio R, Possiede YM et al (2017) Antibacterial activity of endophytic actinomycetes isolated from the medicinal plant Vochysia divergens (Pantanal, Brazil). Front Microbiol 8:1642
Graf TN, Kao D, Rivera-Chávez J, Gallagher JM, Raja HA, Oberlies NH (2020) Drug leads from endophytic fungi: lessons learned via scaled production. Planta Med 86(13/14):988–996
Guo L, Niu S, Chen S, Liu L (2020) Diaporone A, a new antibacterial secondary metabolite from the plant endophytic fungus Diaporthe sp. J Antibiot 73(2):116–119
Jacott CN, Murray JD, Ridout CJ (2017) Trade-offs in arbuscular mycorrhizal symbiosis: disease resistance, growth responses and perspectives for crop breeding. Agronomy 7(4):75
Kampan NC, Madondo MT, McNally OM, Quinn M, Plebanski M (2015) Paclitaxel and its evolving role in the management of ovarian cancer. Biomed Res Int. 2015:413076
Khalid A (2018) Terpenoid production using Streptomyces reveromyceticus SN-593 (Doctoral dissertation, 埼玉大学)
Khan Z, Ahmad S, Joseph L, Chandy R, Theyyathel A (2011) Comparative in vitro susceptibility of clinical isolates of Candida parapsilosis complex and other Candida species to caspofungin and anidulafungin by etest. J Chemother 23(2):97–101
Klomchit A, Calderin JD, Jaidee W, Watla-Iad K, Brooks S (2021) Napthoquinones from Neocosmospora sp.—Antibiotic activity against acidovorax citrulli, the causative agent of bacterial fruit blotch in watermelon and melon. J Fungi 7(5):370
Kumar S, Aharwal RP, Jain R, Sandhu SS (2021) Bioactive molecules of endophytic fungi and their potential in anticancer drug development. Curr Pharmacol Rep 7:27–41
Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798
Lacerda ÍCDS, Polonio JC, Golias HC (2022) Endophytic fungi as a source of antiviral compounds–a review. Chem Biodivers 19(6):e202100971
Lai Z, Wang H, Tang X, Zhang L, Wang T, Cheng J (2022) Study on the mechanism of diosgenin targeting STAT3 to inhibit colon cancer proliferation and migration. Dis Markers 2022:7494887

Lee C, Shim SH (2020) Endophytic fungi inhabiting medicinal plants and their bioactive secondary metabolites. Nat Prod Sci 26(1):10–27

Li F, Jiang T, Li Q, Ling X (2017) Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer?. Am J Cancer Res 7(12):2350

Li WY, Liu Y, Lin YT, Liu YC, Guo K, Li XN et al (2020) Antibacterial harziane diterpenoids from a fungal symbiont Trichoderma atroviride isolated from Colquhounia coccinea var. mollis. Phytochemistry 170:112198

Lingqi Z, Bo G, Haiyan L, Songrong Z, Hua S, Su G, Rongcheng W (2000) Preliminary study on the isolation of endophytic fungus of Catharanthus roseus and its fermentation to produce products of therapeutic value. Zhong Cao Yao 31(11):805–807

Liu Y, Zhou Z, Yan J, Wu X, Xu G (2020) Diosgenin exerts antitumor activity via downregulation of Skp2 in breast cancer cells. BioMed Res Int 2020

Mao ZJ, Tang QJ, Zhang CA, Qin ZF, Pang B, Wei PK et al (2012) Anti-proliferation and anti-invasion effects of diosgenin on gastric cancer BGC-823 cells with HIF-1α shRNAs. Int J Mol Sci 13(5):6521–6533

Martínez-Luis S, Cherigo L, Higginbotham S, Arnold E, Spadafora C, Ibañez A et al (2011) Screening and evaluation of antiparasitic and in vitro anticancer activities of Panamanian endophytic fungi. Int Microbiol 14(2):95

Martino E, Casamassima G, Castiglione S, Cellupica E, Pantalone S, Papagni F et al (2018) Vinca alkaloids and analogues as anti-cancer agents: looking back, peering ahead. Bioorg Med Chem Lett 28(17):2816–2826

Meng-Ting LIU, Yan HE, Ling SHEN, Zheng-Xi HU, Zhang YH (2019) Bipolarins A–H, eight new ophiobolin-type sesterterpenes with antimicrobial activity from fungus Bipolaris sp. TJ403-B1. Chin J Nat Med 17(12):935–944

Mishra VK, Singh G, Passari, AK, Yadav MK, Gupta VK, Singh BP (2016) Distribution and antimicrobial potential of endophytic fungi associated with ethnomedicinal plant Melastoma malabathricum L. J Environ Biol 37(2):229

Mou QL, Yang SX, Xiang T, Liu WW, Yang J, Guo LP et al (2021) New cytochalasan alkaloids and cyclobutane dimer from an endophytic fungus Cytospora chrysosperma in Hippophae rhamnoides and their antimicrobial activities. Tetrahedron Lett 87:153207

Oktavia L, Krishna VS, Rekha EM, Fathoni A, Sriram D, Agusta A (2020) Anti-mycobacterial activity of two natural Bisanthraquinones:(+)-1, 1′-Bislunatin and (+)-2, 2′-Epicytoskyrin A. IOP Conf Ser Earth Environ Sci 591(1):012025. IOP Publishing

Paba V, Quarto M, Varriale L, Crescenzi E, Palumbo G (2001) Photo-activation of hypericin with low doses of light promotes apparent photo-resistance in human histiocytic lymphoma U937 cells. J Photochem Photobiol B Biol 60(2–3):87–96

Pandi M, SenthilKumaran R, Rajapriya P, Yogeswari S, Muthumary J (2013) Taxol, A potential drug for the treatment of cancer. Biores Bull 2(1):1–9

Passari AK, Mishra VK, Gupta VK, Yadav M. K, Saikia R, Singh BP (2015) In vitro and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. PLoS one 10(9):e0139468

Pasquier E, Carre M, Pourroy B, Camoin L, Rebaï O, Briand C, Braguer D (2004) Antiangiogenic activity of paclitaxel is associated with its cytostatic effect, mediated by the initiation but not completion of a mitochondrial apoptotic signaling pathway. Mol Cancer Ther 3(10):1301–1310

Pawlowska TE, Chaney RL, Chin M, Charvat I (2000) Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. Appl Environ Microbiol 66(6):2526–2530

Pinheiro EAA, Carvalho JM, dos Santos DCP, Feitosa ADO, Marinho PSB, Guilhon GMSP et al (2013) Antibacterial activity of alkaloids produced by endophytic fungus Aspergillus sp. EJC08 isolated from medical plant Bauhinia guianensis. Nat Prod Res 27(18):1633–1638

Pommier Y (2006) Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 6(10):789–802

Puri SK, Habbu PV, Kulkarni PV, Kulkarni VH (2018) Nitrogen containing secondary metabolites from endophytes of medicinal plants and their biological/pharmacological activities-a review. Syst Rev Pharm 9(1):22–30
Qu HR, Yang WW, Zhang XQ, Lu ZH, Deng ZS, Guo ZY et al (2020) Antibacterial bisabolane sesquiterpenoids and isocoumarin derivatives from the endophytic fungus Phomopsis prunorum. Phytochem Lett 37:1–4
Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S et al (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology 12(3):139–159
Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149
Ramos HP, Braun GH, Pupo MT, Said S (2010) Antimicrobial activity from endophytic fungi Arthrinium state of Apiospora montagnei Sacc. and Papulaspora immersa. Braz Arch Biol Technol 53:629–632
Ren A, Li C, Gao Y (2011) Endophytic fungus improves growth and metal uptake of Lolium arundinaceum Darbyshire ex. Schreb. Int J Phytoremediation 13(3):233–243
Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59(5):1109–1114
Sethi G, Shanmugam MK, Warrier S, Merarchi M, Arfuso F, Kumar AP, Bishayee A (2018) Pro-apoptotic and anti-cancer properties of diosgenin: a comprehensive and critical review. Nutrients 10(5):645
Sikora RA, Pocasangre L, zum Felde A, Niere B, Vu TT, Dababat AA (2008) Mutualistic endophytic fungi and in-planta suppressiveness to plant parasitic nematodes. Biol Control 46(1):15–23
Sonaimuthu V, Johnpaul M (2010) Taxol (anticancer drug) producing endophytic fungi: an overview. Int J Pharm Bio Sci 1(3):BS31
Srinivasan S, Koduru S, Kumar R, Venguswamy G, Kyprianou N, Damodaran C (2009) Diosgenin targets Akt-mediated prosurvival signaling in human breast cancer cells. Int J Cancer 125(4):961–967
Subban K, Subramani R, Johnpaul M (2013) A novel antibacterial and antifungal phenolic compound from the endophytic fungus Pestalotiopsis mangiferae. Nat Prod Res 27(16):1445–1449
Tayung K, Sarkar M, Baruah P (2012) Endophytic fungi occurring in Ipomoea carnea tissues and their antimicrobial potentials. Braz Arch Biol Technol 55:653–660
Tiwari P, Bae H (2022) Endophytic fungi: key insights, emerging prospects, and challenges in natural product drug discovery. Microorganisms 10(2):360
Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV et al (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:309
Vandenbogaerde AL, Cuveele JF, Proot P, Himpens BE, Merlevede WJ, de Witte PA (1997) Differential cytotoxic effects induced after photosensitization by hypericin. J Photochem Photobiol B Biol 38(2–3):136–142
Vyas P, Bansal A (2018) Fungal endophytes: role in sustainable agriculture. In: Fungi and their role in sustainable development: current perspectives, pp 107–120. https://doi.org/10.1007/978-981-13-0393-7_7
Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC (2007) Antimicrobial potentials of endophytic fungi residing in Quercus variabilis and brefeldin A obtained from Cladosporium sp. World J Microbiol Biotechnol 23:79–83
Weaver BA (2014) How Taxol/paclitaxel kills cancer cells. Mol Biol Cell 25(18):2677–2681
Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408
Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254
Wu JL, Leung ELH, Zhou H, Liu L, Li N (2013) Metabolite analysis of toosendanin by an ultrahigh performance liquid chromatography-quadrupole-time of flight mass spectrometry technique. Molecules 18(10):12144–12153
Wu F, Zhu YN, Hou YT, Mi QL, Chen JH, Zhang CM et al (2021) Two new antibacterial anthraquinones from cultures of an endophytic fungus Phomopsis sp. Chem Nat Compd 57:823–827

Xianzhi Y, Shiping G, Lingqi Z, Hua S (2003) Select of producing podophyllotoxin endophytic fungi from podophyllin plant. Nat Prod Res Dev 15(5):419–422

Yan C, Liu W, Li J, Deng Y, Chen S, Liu H (2018) Bioactive terpenoids from Santalum album derived endophytic fungus Fusarium sp. YD-2. RSC Adv 8(27):14823–14828

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771

Zhang Z, Zhou Q, Peng S, Cai Z (2010) Remediation of petroleum contaminated soils by joint action of Pharbitis nil L. and its microbial community. Sci Total Environ 408(22):5600–5605

Zhang W, Wei W, Shi J, Chen C, Zhao G, Jiao R, Tan R (2015) Natural phenolic metabolites from endophytic Aspergillus sp. IFB-YXS with antimicrobial activity. Bioorg Med Chem Lett 25(13):2698–2701

Zhang SP, Huang R, Li FF, Wei HX, Fang XW, Xie XS et al (2016) Antiviral anthraquinones and azaphilones produced by an endophytic fungus Nigrospora sp. from Aconitum carmichaeli. Fitoterapia 112:85–89

Zhang P, Yuan XL, Du YM, Zhang HB, Shen GM, Zhang ZF et al (2019) Angularly prenylated indole alkaloids with antimicrobial and insecticidal activities from an endophytic fungus Fusarium sambucinum TE-6L. J Agric Food Chem 67(43):11994–12001

Zhang J, Yang F, Mei X, Yang R, Lu B, Wang Z, Ji L (2022) Toosendanin and isotoosendanin suppress triple-negative breast cancer growth via inducing necrosis, apoptosis and autophagy. Chem Biol Interact 351:109739

Zhou Q, Wu X, Wen C, Wang H, Wang H, Liu H, Peng J (2018) Toosendanin induces caspase-dependent apoptosis through the p38 MAPK pathway in human gastric cancer cells. Biochem Biophys Res Commun 505(1):261–266

Zhou X, Xu Z, Li A, Zhang Z, Xu S (2019) Double-sides sticking mechanism of vinblastine interacting with α, β-tubulin to get activity against cancer cells. J Biomol Struct Dyn 37(15):4080–4091

Zhu YX, Peng C, Ding W, Hu JF, Li J (2022) Chromenopyridin A, a new N-methoxy-1-pyridone alkaloid from the endophytic fungus Penicillium nothofagi P-6 isolated from the critically endangered conifer Abies beshanzuensis. Nat Prod Res 36(8):2049–2055

Chapter 8
Therapeutic Potential of Fungal Endophyte-Derived Bioactive Compound in Protozoan Diseases

Vishal Kumar Singh, Rahul Tiwari, Awnish Kumar, Rajneesh, Rohit Prasad Gupta, and Rajiv Kumar

Abstract Endophytes are a group of microbes residing mutually within the tissues of their host plant without causing any overt symptoms. Secondary metabolites produced by fungal endophytes are used in the treatment of many diseases including protozoan diseases like malaria and visceral leishmaniasis (also known as kala-azar). These two parasitic diseases together cause mortality and morbidity to millions of people globally. There are drugs available to treat these diseases, but the increasing resistance among the protozoan parasite to present-day drugs has driven the scientific community in search of novel sources of pharmacologically active compounds of therapeutic value, and medicinal plants derived from fungal endophytes have shown substantial potential for this cause. Compounds like Taxol, quinine, cytochalasins, cyclodepsipeptide, fusaripeptide A, cochlioquinone A, purpureone, isocochlioquinone A, citrinin, terrenolide S, and curvulin derived from fungal endophytes have been used for their anti-plasmodial and antileishmanial property, respectively. This book chapter reviews natural bioactive compounds derived from fungal endophytes with various antiparasitic biological activities.

Keywords Endophytic fungi · Secondary metabolites · Anti-plasmodial and antileishmanial property

V. K. Singh · R. Tiwari · A. Kumar · Rajneesh · R. Kumar (✉)
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
e-mail: rajiv.kumar@bhu.ac.in

R. P. Gupta
Applied Microbiology, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

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1 Introduction

Endophytes are endosymbionts that live within the internal tissues of their host body (Stone et al. 2000). Endophytic microbes may belong to fungi, bacteria, or actinomycetes. They are ubiquitous in nature and present in all species of vegetation without causing any visible symptoms (Wilson 1995) and overt negative effect on their host (Carroll 2017). De Bary (1866) defined endophytes for the first time as "any organism that grows within host tissue" (Gouda et al. 2016). Later after years of research, more appropriate definition of endophytes was stated as "any organism which colonizes the inner tissues of host plant at some stage of their life cycle" (Gouda et al. 2016; Petrini 1991). A single host plant may have more than one microbe as endophytic species at an instant of time, and their colonization depends on several factors, such as genotype and physiological condition of the host plant, as well as the environmental conditions. Fungal endophytes thrive well inside the tissues of the plant as they derive nutrition and shelter from their host. The microenvironment within the host tissue is much more stable than outside as required for the survival of the endophytes, and in return, they assist the host by producing secondary metabolites like growth factors (Hermosa et al. 2012), comply with stress conditions such as heavy metal ions or edaphic stresses (Rodriguez et al. 2004; Worchel et al. 2013), and also provide competitive advantages to the host against various pathogens causing critical damage to plant (Arnold et al. 2003). Several studies have revealed that secondary metabolites produced by fungal endophytes resemble those of the host, probably because of horizontal transfer of genetic material between the host and the endophyte. Fungal endophytes produce several bioactive secondary metabolites such as terpenoids, tannins, saponins, phenolic compounds, resins, etc. which have therapeutic applications in the treatment of various ailments such as malaria, kala-azar, cancer, fever, etc. The advantage of using fungal endophytes over medicinal plant for mass production of these metabolites is that the secondary metabolite production time is shorter and can be cultivated in small area (culture media), and preservation of endogenous native medicinal plant species can also be achieved (Soares et al. 2017). Drugs like Taxol are derived from endophytic fungus *Taxomyces andreanae*, which resides within the bark of yew tree (*Taxus brevifolia*) (Stierle et al. 1993; Stierle et al. 1995; Zhang et al. 2009) and camptothecin from *Nothapodytes foetida* (Joseph and Priya 2011), which are pharmacologically active compounds as they are widely used in the treatment of cancer and antifungal infections, respectively. Few other examples of bioactive compounds are phomopsichalasin isolated from fungal endophyte *Diaporthe* sp., having antibacterial property (Soares et al. 2017). Similarly, cryptocandin is an antifungal drug obtained from fungi *Cryptosporidiosis* cf. *quercia* (G. A. Strobel et al. 1999), while cercosporin is an antiparasitic drug obtained from endophytic fungus *Mycosphaerella* spp. (Moreno et al. 2011).

Among the pool of diseases, protozoan diseases are causing major health concerns around the globe affecting people both socially and economically. Protozoan diseases like malaria (*Plasmodium* spp.), different forms of leishmaniasis

(*Leishmania* spp.) such as cutaneous and visceral leishmaniasis, amoebic dysentery (*Entamoeba* spp.), etc. are in an alarming situation and are majorly concentrated in developing countries among tropical and subtropical regions. Malaria and leishmaniasis are vector-borne parasitic diseases transmitted by female *Anopheles* mosquito and sand fly (*Phlebotomus*), respectively. The complexity of these diseases and the ever-evolving nature of parasites had put the researcher on their toes to deal effectively with increasing morbidity due to these infections. According to WHO, malaria and leishmaniasis together added up to 249 million new clinical cases and led to the death of around 687,000 people in the year 2021. Nowadays, scientists are focusing on natural bioactive secondary metabolites as potential drugs for treatment rather than chemosynthesis of drugs. The increasing resistance among microbes against already present drugs has attracted the attention of the scientific community toward the urgent need of bioactive compounds for management of several diseases. Coevolvement of the host along with the endophytes is necessarily important for the mutualistic functioning of this association (Lee et al. 2021).

In this book chapter, we will discuss about different fungal endophyte-derived bioactive secondary metabolites with potential novel anti-protozoan drug activities which could play a pivotal role in prevention/treatment of protozoan diseases like malaria and kala-azar.

2 Fungal Endophytes and Their Secondary Metabolites Having Antimalarial Properties

As mentioned above, endophytic fungi reside within the tissues of the host plant without causing any visible symptoms. Studies have revealed that no plants have been found without symbiotic association with endophytic fungi to date (Strobel and Daisy 2003). A large number of species diversity is one of the major characteristic properties of fungal endophytes which is evaluated by the fact that more than 30 fungal endophytes can be found to be associated with a plant at a time (Nisa et al. 2015). Medicinal plants have been exploited over the centuries for the treatment/prevention of various ailments, but the discovery of fungal endophytes associated with the medicinal plants secreting the secondary metabolites of similar medicinal values has changed the current focus of the natural bioactive chemists to these metabolites from fungal endophytes. Studies by WHO revealed that more than 80% of the world's population uses secondary metabolites from medicinal plants in some form or the other (Kaul et al. 2012).

The solemnity malaria brings in can be estimated by WHO's *World Malaria Report 2022* documenting 247 million cases in 2021 with an increased 2 million cases from last year. There are more than 120 species of *Plasmodium* that have been discovered to date, among which only six species, namely, *Plasmodium falciparum*, *P. infantum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*, are responsible for human infections (Varo et al. 2020). Among these six species, *P. falciparum* and

P. vivax together cause the majority of infections. Several fungal endophyte-derived secondary metabolites having antimalarial activity had been reported, and efforts are being made to obtain novel natural drugs with better efficacy to address the increasing resistance of *Plasmodium* species for already present drugs.

Previously used drugs in the treatment of malaria were mainly confined to three groups of compounds, which are quinolines, antifolates, and artemisinin derivatives. In the traditional system of medicine, Ch'ang shan was the oldest known remedy for the treatment of malaria since 200 BC (Anand and Sharma 1997). Grinded roots of *Dichroa febrifuga lout* or *hydrangea leaves* were used and the active ingredient was febrifugine (Anand and Sharma 1997), but unfortunately, this could not be implemented in clinical treatment due to its toxicity. A few of the novel secondary metabolites (crude extract) from the stem bark of *Morinda lucida* suppresses the growth of *P. falciparum* completely. The extract had seven natural compounds which worked synergistically to suppress the plasmodial growth. These compounds were asperuloside, asperulosidic acid, stigmasterol, β-sitosterol, cycloartenol, campesterol, and 5,15-O-dimethylmorindol, which alone did not have any antimalarial activity but collectively inhibits *P. falciparum* growth (Chithambo et al. 2017).

For the very first time, quinine was isolated from the bark of *Cinchona* spp. in the seventeenth century by Pelletier and Caventou (Maehara et al. 2011) which was the first successful plant extract used for malarial treatment. Thereafter, several alternatives such as chloroquine (CQ), mefloquine, and primaquine had been developed and used as antimalarial drugs. Afterward, it was reported that fungal endophytes of *Diaporthe* sp. associated with *Cinchona ledgeriana* produce similar alkaloid compounds like quinine, quinidine, cinchonidine, and cinchonine (Maehara et al. 2011). As shown in Fig. 8.1, quinine has gametocidal activity against malarial parasite as well as blood schizonticide. The theoretical mode of action for quinine and related antimalarial drugs states about the toxicity of these drugs to parasite. Specifically, they interfere with the parasite and prevent digestion of hemoglobin. Consequently, continued accumulation of partially degraded hemoglobin builds up toxicity and leads to parasite starvation and elimination.

Later, antifolates were discovered which contain combination of dihydrofolate reductase (DHFR) enzyme inhibitors, as proguanil, chlorproguanil, pyrimethamine, and dihydropteroate synthase (DHPS) enzyme inhibitors, such as dapsone and sulfadoxine (Luzzatto 2010). Artemisinin was discovered in 1970 from *Artemisia annua* (Tu 2011) and has changed the entire perspective of malarial treatment. Artemisinin and its derivatives (artesunate, artemether, dihydro-artemisinin) are fast-acting endoperoxide compounds and potent antimalarials in the elimination of *P. falciparum* in the areas where CQ resistance prevails (Mackinnon and Marsh 2010). In all countries where resistance to monotherapies is prevalent, artemisinin-based combination therapies (ACTs) are recommended by WHO since 2001, for treating *falciparum* malaria.

Experimentally, it has been found that a cyclopdepsipeptide compound fusaripeptide A derived from the endophytic fungi of *Fusarium* spp. associated with root tissues of *Mentha longifolia* (Labiatae) has antimalarial activity against

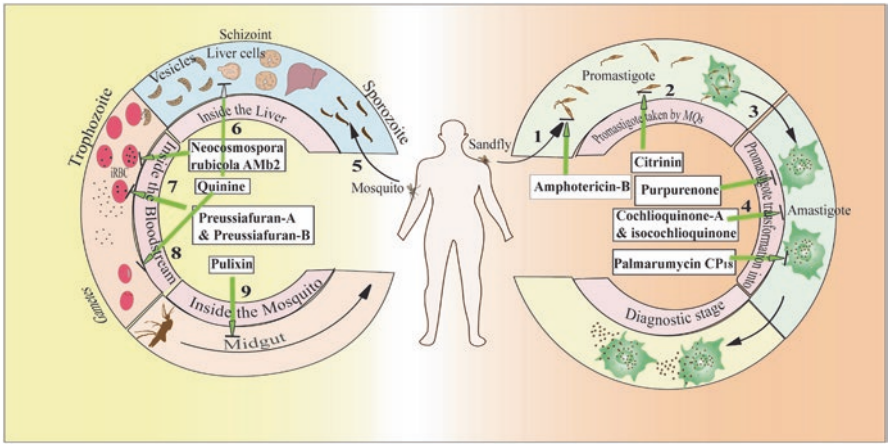

Fig. 8.1 Diagrammatic illustration of various compounds having antileishmanial and antimalarial propertiesy. Amphotericin B interacts with ergosterol formation on the membrane of protozoan parasite leading to porosity in the membrane (1); citrinin acts against promastigote stage of *L. mexicana* (2); promastigote transforms into amastigote stage inside macrophages which are targeted by group of compounds like purpureone, palmarumycin CP_{18}, and cochlioquinone A and isocochlioquinone A against *L. donovani* and *L. amazonensis*, respectively (3,4). Similarly, in malaria, the sporozoite introduced by *Anopheles* invades liver cells and transforms into schizont (5); quinine works against both gametes and schizont stage of parasitic development (6,8). Pulixin interferes with the interaction between FREP-1 (midgut protein) and parasite (9), while compounds such as crude extract of *Neocosmospora rubicola* AMb2 and preussiafuran A and preussiafuran B act against the promastigote stage of parasite (7)

chloroquine-sensitive *P. falciparum* D6 (Sierra Leone) (Ibrahim et al. 2018). Similarly, fungal endophytes associated with different organs of plants provide a wide range of antimalarial drugs belonging to different group of compounds such as mycotoxins, cytochalasins, and trichothecenes from different strains. Polyketides, Dicerandrol D, display antimalarial activity and low toxicity at nanomolar concentration (Calcul et al. 2013). Two dibenzofuran compounds, preussiafuran A and preussiafuran B isolated from *Preussia* spp., found associated with *Enantia chlorantha oliv* had shown to have an anti-plasmodial activity against the erythrocytic phase of chloroquine-resistant *P. falciparum* (Talontsi et al. 2014). Fungal endophytes *Neocosmospora rubicola* AMb2 isolated from medicinal plant *Annona muricata* produce secondary metabolite which have potent antimalarial activity against both *pf3*D7 (IC50 = 0.30 µg/ml) and *pf*Dd2 (IC50 = 0.42 µg/ml) after 4 hours of incubation, and complete clearance of parasite after 32 hours of incubation is observed in an experimental setup. This extract arrests the trophozoite stage of plasmodial infection (Fig. 8.1) (Toghueo et al. 2019).

Recently, a new anti-plasmodial compound named Pulixin has been reported from the fungus *Purpureocillium lilacinum* which prevents transmission of parasite by interfering with the interaction between fibrinogen-related protein-1 (FREP-1) of *Anopheles* and *Plasmodium* (Table 8.1) (Niu et al. 2021). Two compounds

Table 8.1 List of antimalarial and antileishmanial compounds with their mode of action

S. no	Name of compound	Mode of action	References
Antimalarial compounds			
1.	Pyrimethamine, proguanil, and chlorproguanil	Inhibits dihydrofolate reductase (DHFR) enzyme	Luzzatto (2010)
2.	Atovaquone	Interferes with mitochondrial electron transport and also blocks cellular respiration	Srivastava et al. (1997)
3.	Artemisinin	Inhibits PfATP6 of *Plasmodium falciparum*	Eckstein-Ludwig et al. (2003)
4.	Pulixin	Interferes with PREF-1 and *Plasmodium* interaction	Niu et al. (2021)
5.	Crude extract of *Neocosmospora rubicola* AMb2	Arrest of trophozoite stage	Toghueo et al. (2019)
Antileishmanial compounds			
1.	Amphotericin B	Binds to cell membrane and leads to formation of pores ion leakage and death	Stone et al. (2016)
2.	Citrinin	Effects of oxidative stress and altered enzymatic antioxidative responses	de Oliveira Filho et al. (2017)
3.	Miltefosine	Activation of the sphingosine-dependent plasma membrane Ca^{2+} channel	Pinto-Martinez et al. (2018)
4.	Paromomycin	Causes inhibition of protein synthesis by binding to 16S ribosomal RNA	Khan et al. (2013)

(flavasperone-1, aurasperone-A) extracted from *Aspergillus niger* an endophytic fungus from *Terminalia catappa* have been reported to have anti-plasmodial activity (Kouipou Toghueo et al. 2021), and various other organic extracts of this species are used as preventive measures against malaria.

3 Fungal Endophytes and Their Secondary Metabolites Having Antileishmanial Properties

Leishmaniasis comes within the group of neglected tropical diseases (NTD) as it is not listed in the global health agenda and has been ignored by international funding agencies as well national organizations. Leishmaniasis is caused by over 20 species of protozoan parasite *Leishmania*, and around 90 species of sand flies are responsible for transmitting leishmaniasis (https://www.who.int/news-room/fact-sheets/detail). Infected sand flies feed on blood for the development of their eggs during which it can infect their host with leishmanial parasite (Zhou et al. 2007). Depending upon the causative strain of parasite, there are three manifestations of leishmaniasis: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and the most

fatal one is visceral leishmaniasis (VL) in which parasites infect visceral organs of the body such as the bone marrow, liver, and spleen. Leishmaniasis mostly affects impoverished nations across the globe and is majorly confined to developing countries. Approximately around 700,000 to 1 million new cases are reported annually with a death toll of around 70,000. Not all those infected by the parasite end up acquiring leishmaniasis, but only a fraction of them will eventually develop the disease. The lack of effective vaccinations and safe, inexpensive medications for the prevention and treatment of human protozoan infections has worsened the disease's significant impact. Unfortunately, the effectiveness of currently available treatments is being exposed to risk as parasite resistance spreads.

As listed in Table 8.1, treatment options for leishmaniasis are limited to few of the drugs like pentavalent antimonial, amphotericin B, miltefosine, and paromomycin. But most of these drugs have some or other kind of side effects, and more alarmingly, there is increased resistance to them (Guerin et al. 2002). Pentavalent antimonials were the first drug used for the treatment of leishmaniasis, but the associated side effects such as nausea, vomiting, weakness, myalgia, abdominal colic, diarrhea, and skin rashes and complications like hepatotoxicity, cardiotoxicity, and nephrotoxicity can also occur (Jolliffe 1985). As shown in Fig. 8.1, second-line drug like amphotericin B has been used in the treatment of leishmaniasis as it inhibits the formation of ergosterol, thus causing membrane instability and parasite lysis. The main disadvantages associated with leishmanial drugs are their toxicity, and so to counteract the toxicity, several modifications have been performed on already available drugs such as lipid modification of amphotericin B which reduces its toxicity. To address the challenges of toxicity and increasing resistance toward available drugs, bioactive secondary metabolites from the fungal endophytes appear as an upstanding alternative. Two natural secondary metabolite, cochlioquinone A and isocochlioquinone A isolated from the fungal endophytes *Cochliobolus* spp. associated with plant *Piptadenia adiantoides* (Fabaceae), were found to be lethal against amastigote phase of *Leishmania amazonensis* with an inhibition concentration (IC_{50}) value of 1.71 and 4.09, respectively (Campos et al. 2008). The same bioactive secondary metabolite was isolated from the endophytes associated with leaves of *Vernonia polyanthes (*Do Nascimento et al. 2015*).*

Purpureone is a secondary metabolite isolated from *Purpureocillium lilacinum* from *Aspergillus* sp. strain F1544 and is highly effective against the amastigote form of *Leishmania donovani* with IC50 of 0.99 μM (Lenta et al. 2016). Another compound named citrinin isolated from *Penicillium janthinellum* resists the growth of promastigote stage of *Leishmania mexicana* with an IC50 value of 56.6 μM (Marinho et al. 2005).

A butanolide derivative compound called terrenolide S isolated from the fungal endophytes *Aspergillus terreus* associated within the roots of *Carthamus lanatus* (Asteraceae) along with few previously known compounds such as stigmasta-5,7,22-trien-3-*β*-ol and stigmast-4-en-3-one has been reported to show antileishmanial activity against *Leishmania donovani* with IC50 value of 11.24 μM, 15.32 μM, and 27.27 μM (Elkhayat et al. 2016). Similarly, three compounds, curvulin, spirostaphylotrichin U, and spirostaphylotrichin R isolated and identified from the extracts of

Eichhornia macrophyte species, have been documented to possess antileishmanial activity against *Leishmania amazonensis* and can be further explored as antileishmanial agent (de Almeida et al. 2018). A compound palmarumycin CP_{18}, identified from the extract of Panamanian fungal endophytes *Edenia* spp., showed the antileishmanial activity against *Leishmania donovani* in the macrophage stage of parasitic life cycle (Ortega et al. 2014). Many more similar compounds having in vitro antileishmanial activity are under investigation.

4 Future Perspective and Concluding Remarks

It had been a journey of more than a century since causative agents of these diseases had been identified. Over this period, there have been many scientific advancements in computational tools and scientific approaches capable of reducing diseases and associated mortality, majorly the discovery and development of drugs (antimalarial/antileishmanial) against these protozoan parasites and insecticides against vectors. To progress toward the complete eradication of these protozoan parasites, pharmaceutical sectors, government organizations, and academics altogether have to work in a coordinated way to maintain financial incentives. In recent years, recrudescence of resistance among the vectors and parasites for already present drugs diminishing their effectiveness is a major threat leading to increased mortality. Various organ-specific toxicities associated with drugs also limit the use of these drugs. To counteract the problem of developing resistance to parasites, various alternative drugs have been discovered from different sources. Once an isolated analyte is passed through computational tools for determination of its structure, it further has to pass through different in vitro and in vivo assays to confirm its high efficacy, minimum toxicity, and maximum safety. Isolation of secondary metabolite from fungal endophytes can provide us with a pool of drugs showing antimalarial and antileishmanial activity, respectively. Also, endophytes with their metabolites for disease protection have more advantages over chemical drugs and conventional bioformulations. Metabolites isolated from fungal endophytes have various properties such as it enhances defense against pathogens. Therefore, formulation of a cocktail of compounds derived from fungal endophytes may provide novel combinatorial drugs for treatment of protozoan disease. Recently, the field of endophytes has gained attractive popularity as a stage for pharmaceutical therapeutics which is more affordable, but a few of the odds associated, such as purity of fungal endophytes and low yields of metabolites, need to be addressed for optimum utilization, which can be overcome by advancement in scientific intervention and high-throughput method. Thereafter, for large-scale production of the isolated drug, different pharmaceutical firms can be approached to use large-scale cell culture bioreactors for mass production of drug extracted from fungal endophytes.

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References

Anand N, Sharma S (1997) Approaches to design and synthesis of antiparasitic drugs. Pharmachemistry Library 25, Elsevier, Amsterdam

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U S A 100(26):15649–15654. https://doi.org/10.1073/pnas.2533483100. Epub 2003 Dec 11. PMID: 14671327; PMCID: PMC307622

Calcul L, Waterman C, Ma WS, Lebar MD, Harter C, Mutka T et al (2013) Screening mangrove endophytic fungi for antimalarial natural products. Mar Drugs 11(12):5036–5050

Campos FF, Rosa LH, Cota BB, Caligiorne RB, Rabello AL, Alves TM, Rosa CA, Zani CL (2008) Leishmanicidal metabolites from Cochliobolus sp., an endophytic fungus isolated from Piptadenia adiantoides (Fabaceae). PLoS Negl Trop Dis 2(12):e348. https://doi.org/10.1371/journal.pntd.0000348. Epub 2008 Dec 16. Erratum in: PLoS Negl Trop Dis 2009;3(1). PMID: 19079599

Carroll FM (2017) Sources. In: A good and wise measure. University of Toronto Press, Toronto, pp 411–430

Chithambo B, Noundou XS, Krause RW (2017) Anti-malarial synergy of secondary metabolites from Morinda lucida Benth. J Ethnopharmacol 199:91–96

de Almeida TT, Ribeiro MADS, Polonio JC, Garcia FP, Nakamura CV, Meurer EC et al (2018) Curvulin and spirostaphylotrichins R and U from extracts produced by two endophytic Bipolaris sp. associated to aquatic macrophytes with antileishmanial activity. Nat Prod Res 32(23):2783–2790. https://doi.org/10.1080/14786419.2017.1380011

de Bary A. (1866) Morphologie und physiologie der pilze, flechten und myxomyceten. Engelmann

de Oliveira Filho JWG, Islam MT, Ali ES, Uddin SJ, de Oliveira Santos JV, de Alencar MVOB et al (2017) A comprehensive review on biological properties of citrinin. Food Chem Toxicol 110:130–141

Do Nascimento AM, Soares MG, da Silva Torchelsen FK, de Araujo JAV, Lage PS, Duarte MC et al (2015) Antileishmanial activity of compounds produced by endophytic fungi derived from medicinal plant Vernonia polyanthes and their potential as source of bioactive substances. World J Microbiol Biotechnol 31(11):1793–1800

Eckstein-Ludwig U, Webb R, Van Goethem I, East J, Lee A, Kimura M et al (2003) Artemisinins target the SERCA of Plasmodium falciparum. Nat Med 424(6951):957–961

Elkhayat ES, Ibrahim SR, Mohamed GA, Ross SA (2016) Terrenolide S, a new antileishmanial butenolide from the endophytic fungus Aspergillus terreus. Nat Prod Res 30(7):814–820

Gouda S, Das G, Sen SK, Shin H-S, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538

Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P et al (2002) Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect Dis 2(8):494–501

Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of Trichoderma and of its genes. Microbiology (Reading) 158(Pt 1):17–25. https://doi.org/10.1099/mic.0.052274-0. Epub 2011 Oct 13. PMID: 21998166

Ibrahim SR, Abdallah HM, Elkhayat ES, Al Musayeib NM, Asfour HZ, Zayed MF, Mohamed GA (2018) Fusaripeptide A: new antifungal and anti-malarial cyclodepsipeptide from the endophytic fungus Fusarium sp. J Asian Nat Prod Res 20(1):75–85

Jolliffe D (1985) Nephrotoxicity of pentavalent antimonials. Lancet 325(8428):584

Joseph B, Priya RM (2011) Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Biochem Mol Biol 1(3):291–309

Kaul S, Gupta S, Ahmed M, Dhar MK (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11(4):487–505

Khan W, Sharma SS, Kumar N (2013) Bioanalytical method development, pharmacokinetics, and toxicity studies of paromomycin and paromomycin loaded in albumin microspheres. Drug Test Anal 5(6):453–460

Kouipou Toghueo RM, Kemgne EAM, Sahal D, Yadav M, Kenou Kagho DU, Yang B et al (2021) Specialized antiplasmodial secondary metabolites from Aspergillus niger 58, an endophytic fungus from Terminalia catappa. J Ethnopharmacol 269:113672. https://doi.org/10.1016/j.jep.2020.113672

Lee K, Missaoui A, Mahmud K, Presley H, Lonnee M (2021) Interaction between grasses and Epichloë endophytes and its significance to biotic and abiotic stress tolerance and the rhizosphere. Microorganisms 9(11):2186

Lenta BN, Ngatchou J, Frese M, Ladoh-Yemeda F, Voundi S, Nardella F et al (2016) Purpureone, an antileishmanial ergochrome from the endophytic fungus Purpureocillium lilacinum. Z für Naturforsch B 71(11):1159–1167

Luzzatto L (2010) The rise and fall of the antimalarial Lapdap: a lesson in pharmacogenetics. Lancet 376(9742):739–741

Mackinnon MJ, Marsh K (2010) The selection landscape of malaria parasites. Science 328(5980):866–871. https://doi.org/10.1126/science.1185410

Maehara S, Simanjuntak P, Kitamura C, Ohashi K, Shibuya H (2011) Cinchona alkaloids are also produced by an endophytic filamentous fungus living in Cinchona plant. Chem Pharm Bull 59(8):1073–1074

Marinho AM, Rodrigues-Filho E, Moitinho M d LR, Santos LS (2005) Biologically active polyketides produced by Penicillium janthinellum isolated as an endophytic fungus from fruits of Melia azedarach. J Braz Chem Soc 16:280–283

Moreno E, Varughese T, Spadafora C, Arnold AE, Coley PD, Kursar TA et al (2011) Chemical constituents of the new endophytic fungus Mycosphaerella sp. nov. and their anti-parasitic activity. Nat Prod Commun 6(6):1934578X1100600620

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microb Pathog 82:50–59

Niu G, Wang X, Hao Y, Kandel S, Niu G, Raptis RG, Li J (2021) A novel fungal metabolite inhibits Plasmodium falciparum transmission and infection. Parasites vectors 14(1):1–11

Ortega HE, de Morais Teixeira E, Rabello A, Higginbotham S, Cubilla-Ríos L (2014) Anti-L. donovani activity in macrophage/amastigote model of palmarumycin CP18 and its large scale production. Nat Prod Commun 9(1):1934578X1400900128

Petrini O (1991) Fungal endophytes of tree leaves. In: Microbial ecology of leaves. Springer, New York, pp 179–197

Pinto-Martinez AK, Rodriguez-Durán J, Serrano-Martin X, Hernandez-Rodriguez V, Benaim G (2018) Mechanism of action of miltefosine on Leishmania donovani involves the impairment of acidocalcisome function and the activation of the sphingosine-dependent plasma membrane Ca2+ channel. Antimicrob Agents Chemother 62(1):e01614–e01617

Rodriguez RJ, Redman RS, Henson JM (2004) The role of fungal symbioses in the adaptation of plants to high stress environments. Mitig Adapt Strateg Glob Chang 9(3):261–272

Soares DA, Rosa LH, da Silva JFM, Pimenta RS (2017) A review of bioactive compounds produced by endophytic fungi associated with medicinal plants. Bol Mus Para Emílio Goeldi-Ciênc Nat 12(3):331–352

Srivastava IK, Rottenberg H, Vaidya AB (1997) Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. J Biol Chem 272(7):3961–3966

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Stierle A, Strobel G, Stierle D, Grothaus P, Bignami G (1995) The search for a taxol-producing microorganism among the endophytic fungi of the Pacific yew, Taxus brevifolia. J Nat Prod 58(9):1315–1324

Stone JK, Bacon CW, White JF Jr (2000) An overview of endophytic microbes: endophytism defined. In: Microbial endophytes. Marcel Dekker, New York, pp 17–44

Stone NR, Bicanic T, Salim R, Hope W (2016) Liposomal amphotericin B (AmBisome®): a review of the pharmacokinetics, pharmacodynamics, clinical experience and future directions. Drugs 76(4):485–500

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel GA, Miller RV, Martinez-Miller C, Condron MM, Teplow DB, Hess W (1999) Cryptocandin, a potent antimycotic from the endophytic fungus Cryptosporiopsis cf. quercina. Microbiology 145(8):1919–1926

Talontsi FM, Lamshöft M, Douanla-Meli C, Kouam SF, Spiteller M (2014) Antiplasmodial and cytotoxic dibenzofurans from Preussia sp. harboured in Enantia chlorantha Oliv. Fitoterapia 93:233–238

Toghueo RMK, Kemgne EAM, Eke P, Kanko MIM, Dize D, Sahal D, Boyom FF (2019) Antiplasmodial potential and GC-MS fingerprint of endophytic fungal extracts derived from Cameroonian Annona muricata. J Ethnopharmacol 235:111–121

Tu Y (2011) The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat Med 17(10):1217–1220

Varo R, Chaccour C, Bassat Q (2020) Update on malaria. Med Clin 155(9):395–402

Wilson D (1995) Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73(2):274–276. https://doi.org/10.2307/3545919

Worchel ER, Giauque HE, Kivlin SN (2013) Fungal symbionts alter plant drought response. Microb Ecol 65(3):671–678

Zhang P, Zhou P-P, Yu L-J (2009) An endophytic taxol-producing fungus from Taxus media, Cladosporium cladosporioides MD2. Curr Microbiol 59(3):227–232

Zhou G, Kohlhepp P, Geiser D, Frasquillo Mdel C, Vazquez-Moreno L, Winzerling JJ (2007) Fate of blood meal iron in mosquitoes. J Insect Physiol 53(11):1169–1178. https://doi.org/10.1016/j.jinsphys.2007.06.009

Chapter 9
Bioinformatics Approaches in Studying the Fungal Endophyte-Derived Bioactive Compounds with Pharmacological Relevance

Satyendra Singh, Ketan Kumar, Abhishek Rao, and Vijay Kumar Prajapati

Abstract Fungal endophytes (FEs) are endosymbionts that live inside the plant and produce compounds that protect the host from predators like grazing animals, pests, and insects. FEs are the repository of novel bioactive compounds, potentially treating various lifestyle and communicable diseases like microbial diseases, viral diseases, parasitic diseases, etc. Bioinformatics plays an essential role in the high-throughput screening of thousands and millions of compounds in a single click at a short time. Several tools and web servers are available to explore compounds, library preparation, protein and ligand preparation, grid generation for specific docking, ADME prediction to predict drug-like properties, docking for high-throughput ligand screening, and finally, molecular dynamics simulation to check the stability of docked protein-ligand complexes. Because of the occurrence of widespread drug resistance as a result of unprescribed and misused antibiotics, there is an urgent need for novel bioactive compounds, and bioinformatics is the first prominent choice for researchers in this strategy. Virtual screening through bioinformatics decreases the number of compounds. Hence, it will be very straightforward for researchers to test a limited number of compounds against specific diseases in in vitro and in vivo studies and reduce prerequisite validation.

Keywords Fungal endophyte · Phytochemicals · Computational biology · Pharmacology · Drug discovery

S. Singh · K. Kumar · A. Rao
Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India

V. K. Prajapati (✉)
Department of Biochemistry, University of Delhi, South Campus, New Delhi, India
e-mail: vkp@south.du.ac.in

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1 Introduction

Since after origin of life on earth, living together with the different organisms, a symbiotic lifestyle has been identified based on fitness benefits or impacts on the host. Considering plants in their natural habitats is symbiotic with a highly diverse group of bacteria and fungi as endophytes. Endophytes are a group of microorganisms that sporulate within plant tissues and develop fully within plant tissues, including roots, stems, and/or leaves (Rodriguez et al. 2009). With the decline in the breakthrough rate of new active novel chemical substance entities, it has become evident to explore plant sources extensively for various therapeutic purposes. Endophytic microorganisms play an important role in this lookup for natural bioactive compounds, with potential use in the health sector and in drug discovery (Lam 2007). Fungal endophytes (FEs) have been associated with plants for more than 400 million years (Krings et al. 2007), and these FE species vary in their symbiotic and ecological function. The *clavicipitaceous* endophytes (C-endophytes), which infect some grasses, and the *non-clavicipitaceous* endophytes (NC-endophytes), which can be recovered from asymptomatic tissues of nonvascular plants, ferns and allies, conifers, and angiosperms, have previously been recognized, reflecting differences in evolutionary relatedness, taxonomy, plant hosts, and ecological functions. For *clavicipitaceous* and *non-clavicipitaceous* endophytes, several colonization techniques have been identified, and based on colonization pattern, they have been differentiated into four functional classes, C-endophytes as class 1 and NC-endophytes into class 2, 3, and 4 (Rodriguez et al. 2009). C-endophytes are a small clique of phylogenetically associated *clavicipitaceous* species that are difficult to cultivate and only found on grasses (Bischoff and White 2005). They establish systemic intercellular infections that extend through the shoot meristem, populating the intercellular gaps of freshly formed shoots. NC-endophytes colonizing above- and belowground plant tissues, as well as aerial tissues (Gao and Mendgen 2006), and being horizontally and/or vertically transmitted (Redman et al. 2002) were classified as class 2, while those colonizing aerial tissues of various hosts, particularly trees, and being horizontally transmitted were classified as class 3 endophytes, and those restricted to roots' cortical cell layer (O'dell et al. 1993) and morphologically similar to mycorrhizal fungi were classified as class 4 endophytes. They are endosymbionts that colonize plants and provide a source of new bioactive secondary metabolites that might be used as antibacterial, anti-insect, and anticancer agents. The bioactive compounds that they can synthesize can be used by plants as growth enhancers, as a defense against pathogens, and be beneficial in developing new drugs, summarized in Fig. 9.1.

A very few of these compounds have been studied yet for their therapeutic application, and several groups of researchers around the globe are evaluating and exemplifying advanced biotechnological techniques for regimental applications (Joseph and Priya 2011). In contrast to the cost-effective and time-consuming conventional synthetic molecules used in drug discovery, there is a constant demand for promising potential wide-range therapeutic agents that are effective against life-threatening

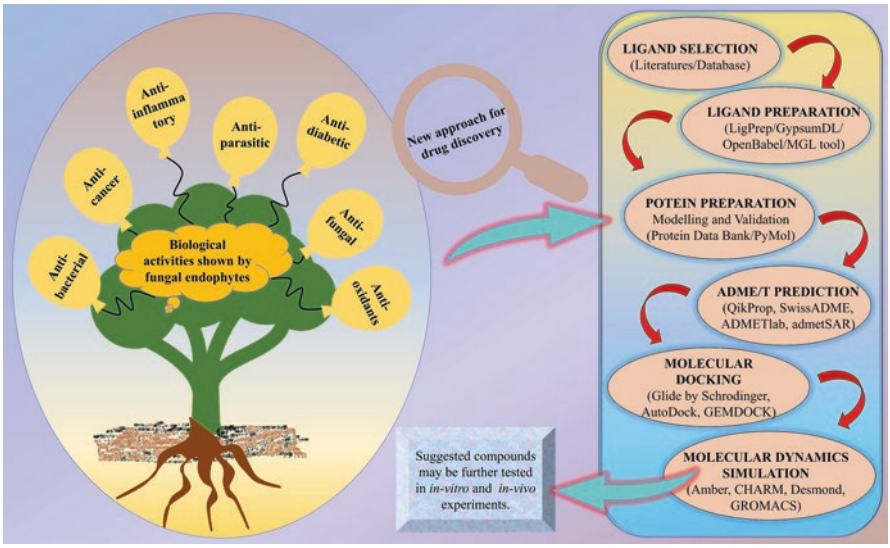

Fig. 9.1 Different biological activities (antibacterial, anticancer, anti-inflammatory, antiparasitic, antidiabetic, antifungal) have been shown by the fungal endophytes. Novel computational drug discovery approaches (high-throughput virtual screening, binding energy calculation, and molecular dynamics simulation) may lead to potential drug candidates for in vitro and in vivo experiments to tackle various diseases

viruses, have low toxicity, and have a promising future in human health and safety concerns. Novel bioactive isolates of FEs are molecules with many H-bond acceptors and donors, increased hydrophilicity, and higher molecular stiffness for dealing with protein-protein interaction (Atanasov et al. 2021).

2 Interaction Between Fungal Endophyte and Plant

FEs are the micromycetes that live inside living plant tissues, do not cause disease in the host, and remain asymptotically. Internally, they invade plant tissue such as stems, inflorescences, leaves, petioles, buds, fruits, seeds, and dead hyaline cells. They can also be present in plant roots without forming a mycorrhizal-type symbiotic association (Huang et al. 2019). Different fungi interact with higher plants, and genetic linkages between plant-arbuscular mycorrhizal fungus associations and root nodule symbioses have been established, suggesting coevolution of fungal endophytic populations with one another and with their hosts (Gherbi et al. 2008).

The successful colonization of endophytes in a plant relies on several factors, including the host species, developmental stage of the host, inoculum density, and environmental factors. FEs have a significant influence on plant evolution and communities and return for nourishment. This symbiotic endophyte relationship

benefits the host plant by improving fitness by bestowing abiotic and biotic stress tolerance, improving biomass while also reducing water usage or decreasing fitness by reallocating nutrients. Endophytes can be "obligate" that spend most of their lives, if not all of their lives, inside plants or "opportunistic" that thrive outside plant tissues and enter plants sporadically. Examples of such are *Balance*, *Epichlo*, and *Neotyphodium* and members of *Hypocrea* and *Trichoderma*, respectively. An intermediate of the above is the "facultative" group, which includes the great majority of endophytes and takes nutrients offered by the host plant, and its mutualist relationship is ambiguous and a source of discussion (Hardoim et al. 2015).

The interactions between host plants and endophytes in a natural habitat are poorly understood. It has been hypothesized that the endophyte relationship is characterized by a finely calibrated balance between fungal virulence and plant defense (Saikkonen et al. 1998; Schardl et al. 1991; Schulz et al. 2002). The relationship between the fungal endophyte and the plant host might be mutualistic if endophytic colonization resulted in higher phenolic defense metabolites than pathogenic infection (Schulz et al. 2002). Imbalances in nutrition exchange, as well as hereditary variables, describe the transition from a mutualistic to a parasitic relationship (Kogel et al. 2006). According to certain theories, miscommunication may also unwittingly begin disease processes under physiological stress and/or senescence (Rodriguez and Redman 2008). The preponderance of data suggests that culturable endophytes linked with woody plant leaf are more likely to be colonized from the outside (horizontal transmission) than vertical transmission (Arnold and Lutzoni 2007). Aboveground organs colonized locally and intercellularly by FEs tend to be antagonistic, whereas roots colonized systemically and inter- and intracellularly by FEs are more likely to be mutualistic (Schardl et al. 1991; Schulz et al. 2002). They produce exoenzymes necessary to penetrate and colonize their host using the apoplastic medium as the growth medium. Endophytes improve the host's stress and/or biotic stress tolerance, such as drought, disease, and metal toxicities by increasing reactive oxygen species generation versus increased antioxidant compound production by endophyte-infected plants, which denature host cell membranes, allowing nutrients to leak out of plant cells and be absorbed by fungal hyphae (Herrera-Carillo et al. 2009; White Jr and Torres 2010). Endophytic natural chemicals have antibacterial characteristics, and they have been related to protecting the host plant against phytopathogenic microorganisms, which improve disease tolerance in many cases (Gunatilaka 2006). Endophytes have also been shown to produce a wide range of antioxidant chemicals, including phenolic acids and their derivatives, isobenzofuranones, isobenzofurans, mannitol, and other carbohydrates. This antioxidant ability may contribute to their host's increased stress tolerance (Aly et al. 2011). Endophytes promote host fitness and competitiveness by boosting germination success and growth rate, by strengthening the host's ability to absorb nutritional materials, or by raising the yield of plant growth hormones such as indole-3-acetic acid, indole-3-acetonitrile, and cytokinins (Hartley and Gange 2009; Tan and Zou 2001; Zhang et al. 2006). Endophytes with appropriate degradation pathways, metal sequestration, or chelation systems were also able to increase host plant tolerance to heavy metal presence, assisting their hosts in surviving in contaminated soil (Weyens

et al. 2009). Plants give spatial structure, moisture protection, and nutrition to the following generation of hosts in exchange. The host plant may also give substances necessary for the endophyte's life cycle to be completed and for its development and self-defense (Rudgers et al. 2004).

3 Fungal Endophyte: Drug Discovery Approach Through Bioinformatics

The traditional drug discovery approach is expensive, laborious, and time-consuming. The process begins with identifying potential targets followed by drug molecules and their optimization, then in vitro and in vivo studies are also performed. After that, preclinical studies are conducted to decide whether these molecules are capable and can be developed as drug molecules or not (Malathi and Ramaiah 2018). Scientists have developed a wide range of bioinformatics software, tools, and web servers to deal with these issues to reduce time, money, and human resources. Computational tools help reduce potential drug candidates from hundreds of thousands to two-digit numbers; then, these potential compounds can be easily studied in in vitro and in vivo (Wooller et al. 2017).

3.1 Compound Library Preparation: Through Literature Survey or Structure Designing by Tools Like ChemBioDraw

A compound library is a collection of compounds used for high-throughput screening for drug development. In the case of fungal endophytes, the related library that contains bioactive compounds can be downloaded directly from the databases. If the fungal endophyte library is not present or has not been studied earlier for the screening process, then the compound library can be prepared by surveying the literature, including studies related to fungal-based endophytes. These bioactive compounds can be identified from the previously published research paper and downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the desired format, most probably in .sdf format. The PubChem database is freely accessible and maintained by the National Center for Biotechnology Information, comes under US National Institute of Health. Another method for library preparation is the generation of compound structures by using chemical structure development software like ChemBioDraw Ultra 14.0 (https://www.adeptscience.de/products/lab/chembiodraw/chembiodraw-ultra-suite.html), MarvinSketch (https://www.chemaxon.com/products/marvin/marvinsketch/), ChemDraw, etc. It contains all the tools required for drawing chemical structures and chemical reactions; chemists and biochemists widely use this application for databases, research, and publication purposes.

Published results or data from analytical techniques like HPLC, GC-MS, etc. sometimes provide the chemical structure. These structures can be developed by ChemBioDraw and can be exported in .sdf format for further studies.

3.2 Ligand Preparation

Prior to docking, ligand preparation is an important step. The compounds (ligands) downloaded from databases or designed with the help of tools are not in the correct order because of the wrong conformation, deprivation of hydrogen bonding, and atomic clashes. During ligand preparation, these blunders should be rectified with the help of computational tools. That can be done by removing atomic clashes, adding polar hydrogen, removing nonpolar hydrogen, optimizing hydrogen bonds, removing salts, and executing other calculations, like adding Kollman and Gasteiger charges. Ligand preparation through the LigPrep module of Schrodinger Suite requires input files in .sdf format, and the result should be exported in .maegz format. For ADT (AutoDock Tools) .pdb format as an input file and ligand should be exported in .pdbqt format. Ligands were converted in three-dimensional geometries during ligand preparation, also ionizing the molecules at physiological pH, i.e., pH = 7 ± 2, and a possible number of stereoisomers of these compounds were generated. Schrodinger uses one of its programs, Epik, to predict pKa values of the ionization groups present in the ligand by using Hammett and Taft's empirical equations (LigPrep_Schrödinger_2017 2017; Sastry et al. 2013; Shelley et al. 2007). Software and online tools used for ligand preparation for docking are listed in Table 9.1.

Table 9.1 Tools for ligand preparation

S. no.	Tools/ software	Websites/weblinks	References
1	LigPrep	https://www.schrodinger.com/products/ligprep	LigPrep (2018)
2	Gypsum-DL	https://durrantlab.pitt.edu/gypsum-dl/	Ropp et al. (2019)
3	OpenBabel	https://sourceforge.net/projects/openbabel/	O'Boyle et al. (2011)
4	MGL tools	http://mgltools.scripps.edu/downloads	Dallakyan (2010)
5	POAP	https://github.com/inpacdb/POAP	Samdani and Vetrivel (2018)
6	PyRx	https://pyrx.sourceforge.io/	Dallakyan and Olson (2015)
7	MarvinSketch	https://www.chemaxon.com/products/marvin/marvinsketch/	ChemAxon (2013)
8	Frog2	https://bioserv.rpbs.univ-paris-diderot.fr/services/Frog2/	Miteva et al. (2010)

3.3 Estimation of Druggable Property by ADME/T Prediction

Investigating druggable properties at the initial stage is crucial for drug discovery and development because drug development is very costly in terms of money and human resources. To reduce the failure of drug candidates in a later stage, it is essential to check ADME (absorption, distribution, metabolism, and excretion) and toxicity properties. In silico ADME analysis helps select potential drug candidates and reject the molecules with less probable ones, reducing the research and development failure in terms of efficacy and safety (Han et al. 2019; van de Waterbeemd and Gifford 2003; Xiong et al. 2021). ADME prediction by using computational tools also predicts human oral bioavailability by checking different parameters of the drug molecules that are already present in the databases and comparing them (Falcón-Cano et al. 2020). ADMETlab 2.0 (Xiong et al. 2021) (https://admetmesh.scbdd.com/) is the next version of the ADMETlab tool and is widely used to check the ADMET properties, i.e., pharmacokinetics and toxicity. ADMETlab 2.0 platform predicts seven different drug-like properties, for example, (1) absorption (Caco-2 permeability and MDCK permeability), (2) distribution (PPB, VD, and Fu), (3) metabolism, (4) excretion (CL), (5) toxicity (bioconcentration factor, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$), (6) pharmacology properties (Log S, Log D7.4, and Log P), and (7) physiochemical properties. ADME/T prediction tool of SwissADME (Daina et al. 2017), Accelrys Discovery studio software, admetSAR-2.0 (http://lmmd.ecust.edu.cn/admetsar2/), and preADMET (available at http://preadmet.bmdrc.org/) (Lee et al. 2003) can also be used to predict pharmacokinetics, physiochemical, lipophilicity, water solubility, toxicological, and drug-likeness properties. PAINS (pan-assay interference compounds) (Baell and Holloway 2010) is used for the removal of compounds having poor pharmacokinetic activity (https://www.cbligand.org/PAINS/). QikProp application of Schrödinger Suite has widely used software for ADME prediction. Therefore, QikProp is used worldwide in academic/research institutions (for educational purposes). Pharmaceutical companies predict different drug parameters by using QikProp at commercial levels based on which a drug candidate can be selected or rejected. Some important properties are MW (molecular weight of the compound), FISA (hydrophilic component of SASA), SASA (total solvent accessible surface area), FOSA (hydrophobic component), PISA (SASA's carbon and attached hydrogen component), QplogS (aqueous solubility prediction), QPlogHERG (IC50 value prediction of HERG K+ channel), QPlogo/w (partition coefficient of octanol and water prediction), QPPCaco (apparent Caco-2 cell permeability for gut-blood barrier), QPPMDCK (expected apparent MDCK cell permeability in nm/s), QPlogKp (skin permeability prediction), QPlogBB (blood/brain partition coefficient prediction), and QPlogKhsa (binding to human serum albumin prediction) (Naik et al. 2020; QikProp 2021). Table 9.2 shows the prediction tools for drug-like properties.

Table 9.2 Tools for absorption, distribution, metabolism, and excretion/toxicity (ADME/T) prediction

S. no.	Tools/software for ADME/T prediction	Websites/weblinks	References
1	ADMETlab	https://admet.scbdd.com/	Dong et al. (2018)
2	ADMET Predictor	https://www.simulations-plus.com/resource-center/?software-type=admet-predictor	Sohlenius-Sternbeck and Terelius (2022)
3	admetSAR	http://lmmd.ecust.edu.cn/admetsar2/	Yang et al. (2018)
4	KNIME	https://www.knime.com/download-previous-versions	Roy et al. (2015)
5	Molinspiration	https://www.molinspiration.com/docu/miscreen/druglikeness.html	Paramashivam et al. (2015)
6	ProTox-II	https://tox-new.charite.de/protox_II/	Banerjee et al. (2018)
7	QikProp	https://www.schrodinger.com/products/qikprop	QikProp. (2017)
8	SwissADME	http://www.swissadme.ch/	Daina et al. (2017)
9	ToxiM	http://metagenomics.iiserb.ac.in/ToxiM/index.html	Sharma et al. (2017)

3.4 Identification of Essential Drug Targets

Identification of a putative drug target is crucial, and several things should be considered during the drug target identification process. For example, drug targets should not have similarities with the host. While targeting the pathways, the same pathway should not appear in the host. BlastP can overcome this issue by checking the similarity between the query sequence and the protein structures previously deposited to the protein data banks. The search should be restricted to the host proteins, and a nonredundant database would be selected for better results. Some servers like KEGG Automatic Annotation Server (KAAS) (https://www.genome.jp/kegg/kaas/) predict functional annotation of the genes based on the BLAST or GHOST by comparing with manually arranged KEGG gene databases (Moriya et al. 2007). Another server named iDTI-ESBoost (http://farshidrayhan.pythonanywhere.com/iDTI-ESBoost/) is currently used to identify drug target based on structural and evolutionary information. The server analyzed several parameters to detect the interaction between the drug and targets like evolutionary features, structural information, and the molecular fingerprinting of the drug (Rayhan et al. 2017).

3.5 Protein (Drug Target) Preparation and Grid Generation

For docking, the protein's crystal structure downloaded from the protein data bank (PDB) may contain errors, like attachment of nonprotein parts, for example, heteroatoms (HETATM), water (SOL), and salts (Na^+, Cl^-). It is necessary to remove all these nonprotein parts from the pdb file during the protein preparation. The addition of missing hydrogen bonds and their optimization and removal of water molecules and cofactors are essential. Some protein structures may be present in a dimeric state or contain more than one chain. However, they may be functionally more active in their monomer form; dimer state and other chains may cause hindrance during the docking. Hence, depending on the functioning of the protein, it is essential to save the cleaned protein structure in monomeric form for further docking steps. Missing side chains and missing loops should also be added, and protein should be prepared in the presence of appropriate force fields (Sastry et al. 2013). Protein preparation wizard provided by Schrodinger LLC is a widely used application for protein preparation in academics and the industrial level (Friesner et al. 2006; Halgren et al. 2004).

In a protein, some specific residues act as the active site residues. These active site residues are responsible for the protein's folding, stability, and catalytic activity (Sun et al. 2001). During the molecular docking step, it is essential to specify the active site residues at the time of docking so that the ligand can be bound to the catalytic residues of the target. There are different computational tools like receptor grid generation tools of maestro provided by Schrödinger, discovery studio tool, autodock grid generation tool, etc. These tools define the particular area in the target

protein, surrounding active site residues. Coordinates surrounding active site residues in the x, y, and z axes must be provided (David et al. 2018). If the active site residues are not identified yet or available, then active site residues will be predicted using tools like site map for binding site prediction an application of Schrodinger Suite. Docking without providing specific active site residues is called blind docking.

3.6 Molecular Docking and Interaction Study

High-throughput screening or virtual screening is very effective in screening complete databases (containing hundreds of thousands of small molecules) compared to the traditional approach (Moitessier et al. 2008). Virtual screening is of mainly two types: ligand based and structure based. Ligand-based virtual screening is used when the active molecules are well known and very little or no information is available for the receptor. Structure-based virtual screening is preferred when the target or receptor is well known and the active molecules are not explicit. Molecular docking is an example of structure-based virtual screening. The main idea behind this approach is to find out the best-fitted molecule that can efficiently bind with the essential protein/target (Bailey and Brown 2001). Scores are obtained after molecular docking like docking score; glide score is used to screen the potential molecules from the library. The glide application of the Schrodinger Suite is widely used for protein-ligand docking studies. Softwares, online tools, and web servers that are commonly used for docking are listed in Table 9.3.

3.7 Estimation of Binding Energy

Potential compounds are selected to estimate binding free energy based on the scores obtained after molecular docking, like docking and glide scores. Prime application provided by the Schrodinger Incorporation is widely used for relative binding free energy calculation. Prime and other tools use MM-GBSA (Molecular Mechanics and Generalized Born Solvent Accessibility) to calculate binding free energy in biomolecular systems. MM-GBSA dG Bind is the complex energy minus the receptor and ligand energy (Pattar et al. 2020). The highest negative binding energy value shows the most stable compound, and the unit for binding free energy is kcal/mol. MM-GBSA dG Bind values are the relative binding energy, and on this value, further compounds are selected to check the stability of complexes at the atomic level (Jacobson et al. 2004; Naik et al. 2021).

Table 9.3 Tools for molecular docking

S. no.	Tools/software for ligand docking	Websites/weblinks	References
1	AADS	http://www.scfbio-iitd.res.in/dock/ActiveSite_new.jsp	Singh et al. (2011)
2	AMDock	https://github.com/Valdes-Tresanco-MS	Valdés-Tresanco et al. (2020)
3	AutoDock4	https://autodock.scripps.edu/	Morris et al. (2009)
4	AutoDock Vina	https://vina.scripps.edu/	Trott and Olson (2010)
5	DockIT	http://www.haptimol.co.uk/downloads.htm	Iakovou et al. (2020)
6	GEMDOCK	http://gemdock.life.nctu.edu.tw/	Bitencourt-Ferreira and de Azevedo Jr (2019)
7	Glide	https://www.schrodinger.com/products/glide	Halgren et al. (2004)
8	Molegro virtual docker	http://molexus.io/molegro-virtual-docker/#:~:text=Molegro%20Virtual%20Docker%20is%20an,binding%20modes%20of%20the%20ligands	Bitencourt-Ferreira and de Azevedo (2019)
9	Sanjeevini	http://www.scfbio-iitd.res.in/sanjeevini/sanjeevini.jsp	Jayaram et al. (2012)
10	SwissDock	http://www.swissdock.ch/	Grosdidier et al. (2011)

3.8 Molecular Dynamics Simulation (RMSD and RMSF Analysis)

Molecular dynamics simulation is the final step in selecting or dropping the compound for in vitro or in vivo experiments. Molecular dynamics verifies the stability of finalized compounds at the microscopic level. It tells about the different flexible regions of the biomolecule that undergo structural changes when the same solvents and conditions are added/provided as available in the physiological environment like temperature and pressure. Water molecules, salts, and ions are also added during the dynamics because they play an essential role in protein functioning and ligand binding. The biomolecular complex that shows stability during the dynamics seems more accurate than the unstable one (Hollingsworth and Dror 2018).

Root mean square deviation, commonly known as RMSD, is used to compare the docked molecules against the reference structure of the biomolecule. RMSD is the average deviation of the structure for the total period of dynamics period; continued increase in RMSD shows protein is not steady. Whereas RMSF is the root mean square fluctuation, it shows the average fluctuation of each particle (protein

Table 9.4 Tools for molecular dynamics simulation

S. no.	Tools/software	Websites/weblinks	References
1	Amber	https://ambermd.org/	Case et al. (2005)
2	CHARMM	https://www.charmm.org/	Brooks et al. (2009)
3	GROMACS	http://www.gromacs.org/	Abraham et al. (2015)
4	NAMD	https://www.ks.uiuc.edu/Research/namd/	Phillips et al. (2020)
5	Desmond	https://www.schrodinger.com/products/desmond	Zhu et al. (2014)

residues) present in the structure. Higher RMSF values mostly show the presence of loop regions in the structure with a high level of conformational flexibility that confirms the structure is not well defined (Barnett et al. 2021; Batut et al. 2018). Softwares and web servers for molecular dynamics simulation are shown in Table 9.4.

4 Importance of Fungal Endophytes in Medicine

Endophytic fungi are vital to medicinal host plants because they help them produce a variety of important chemicals, enzymes, and biopesticides while enduring biotic and abiotic stress. They also help in plant defense and growth and produce plant growth-promoting factors, natural compounds, and secondary metabolites from medicinal plants that help control plant pathogens (Al-Ani 2017; Egbuna and Sawicka 2019). Different bioactive compounds that possess antimicrobial, antitumor, antithrombotic, antioxidants, insecticides, and growth-promoting activity have been produced by these endophytic fungi of pharmaceutical or agricultural uses (Zhang et al. 2014). FEs can produce novel compounds, so researchers have isolated endophytic fungi from plants to produce phenols, quinones, flavonoids, terpenoids, peptides, alkaloids, and steroids (Kaul et al. 2012; Yu et al. 2010). Stierle and Strobel reported that the most famous drug Taxol has been isolated from the endophytic fungus *Taxomyces andreanae* from the bark of *Taxus brevifolia* (Stierle et al. 1993). Taxol is, commonly known as paclitaxel (PTX), is a natural source of cancer drug that shows antineoplastic activity and interferes with the growth and metastasis of cancer cells. US FDA has approved paclitaxel to treat ovarian and breast cancer (Cremasco et al. 2009). Vinblastine and vincristine are the two famous cancer drugs and are already in use against Hodgkin's disease and acute lymphoblastic leukemia, respectively. Both vinblastine and vincristine are isolated from the endophytic fungi *Fusarium oxysporum* from *Catharanthus roseus* plant and are structurally similar except for the presence of methyl group in vinblastine and aldehyde functional group attached to the nitrogen of indole (Agrawal 2007; Kumar et al. 2013). Infectious diseases caused or transmitted by parasites are called parasitic diseases. Two main agents responsible for parasitic diseases are protozoa and helminths. Endophytic fungi *Phomopsis archeri* produce the natural compound Pharmaceris A–C, exhibit antimalarial activity against the most severe and commonly found

species, *Plasmodium falciparum* which is highly fatal, and may cause cerebral malaria (Hemtasin et al. 2011). Leishmaniasis and trypanosomiasis are parasitic diseases caused by protozoa. Natural compounds cochlioquinone A and isocochlioquinone A extracted from *Cochliobolus* sp. of endophytic fungi have been reported to kill up to 90% amastigote form of *Leishmania amazonensis* (Campos et al. 2008). Moreno et al. isolated cercosporin and its analog from a new species of endophytic fungi *Mycosphaerella* sp. They also checked the effect of these compounds on different parasites like *Leishmania donovani*, *Trypanosoma cruzi*, and *Plasmodium falciparum*, which are causative agents of leishmania, Chagas disease, and malaria, respectively; and both compounds show great potency against these parasites (Moreno et al. 2011). Hinnuliquinone is a metabolite isolated from endophytic fungi that shows inhibitory activity against both wild-type and clinically resistant HIV-1 protease, which is an essential enzyme target for drug discovery and responsible for the replication of HIV (Singh et al. 2004).

5 Conclusion and Future Prospective

Fungal endophytes are the treasure of novel bioactive compounds with a wide range of biological activities like anticancer, antimicrobial, antiviral, antiparasitic, antitubercular, antioxidant, insecticidal, and immunomodulatory (Kaul et al. 2012; Yu et al. 2010). Bioactive compounds isolated from endophytic fungi also produce phytohormones, enzymes, plant growth promoters, defensin compounds, etc. that help in plants' growth, development, and defense and give rise to the production of novel secondary metabolites (Bamisile et al. 2018; Kaur 2020). Different research groups have tested compounds isolated from endophytic fungi against various life-threatening diseases. As we already know, the discovery of novel compounds is significant for drug discovery. In this approach, bioinformatics plays a crucial role in screening millions of compounds and reducing them to very little, particularly up to two digits. Researchers have developed many tools and web servers to predict compounds' drug-like properties that give insight into the selection or refusal of compounds as a drug candidate. The researchers use laboratory and clinical research data to develop bioinformatics tools and select compounds that follow these parameters. The compounds that show good scores against diseases have fewer failure chances, and in the future, it will be easy for researchers to test the limited number of compounds against specific diseases in in vitro, in vivo, and clinical trials. Bioinformatics reduces time, money, and resources that will be invested in the study (Fig. 9.1) (Bayat 2002).

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References

Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E (2015) GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1–2:19–25. https://doi.org/10.1016/j.softx.2015.06.001

Agrawal K (2007) Vinblastine. In: Enna SJ, Bylund DB (eds) xPharm: the comprehensive pharmacology reference. Elsevier, New York, pp 1–4

Al-Ani L (2017) Potential of utilizing biological and chemical agents in the control of Fusarium wilt of banana (School of Biology Science). Universiti Sains Malaysia Pulau, Pinang, p 259

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90(6):1829–1845

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88(3):541–549

Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT (2021) Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov 20(3):200–216

Baell JB, Holloway GA (2010) New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem 53(7):2719–2740. https://doi.org/10.1021/jm901137j

Bailey D, Brown D (2001) High-throughput chemistry and structure-based design: survival of the smartest. Drug Discov Today 6(2):57–59. https://doi.org/10.1016/s1359-6446(00)01596-8

Bamisile BS, Dash CK, Akutse KS, Keppanan R, Wang L (2018) Fungal endophytes: beyond herbivore management. Front Microbiol 9:544

Banerjee P, Eckert AO, Schrey AK, Preissner R (2018) ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 46(W1):W257–W263

Barnett C, Senapathi T, Bray S (2021) Analysis of molecular dynamics simulations (Galaxy Training Materials)

Batut B, Hiltemann S, Bagnacani A, Baker D, Bhardwaj V, Blank C et al (2018) Community-driven data analysis training for biology. Cell Syst 6(6):752–758. e751

Bayat A (2002) Science, medicine, and the future: bioinformatics. BMJ (Clin Res ed) 324(7344):1018–1022. https://doi.org/10.1136/bmj.324.7344.1018

Bischoff J, White J (2005) Evolutionary development of the Clavicipitaceae. Mycol Ser 23:505

Bitencourt-Ferreira G, de Azevedo WF (2019) Molegro virtual docker for docking. In: Docking screens for drug discovery. Springer, pp 149–167

Bitencourt-Ferreira G, de Azevedo WF Jr (2019) Docking with GemDock. Methods Mol Biol 2053:169–188. https://doi.org/10.1007/978-1-4939-9752-7_11

Brooks BR, Brooks CL III, Mackerell AD Jr, Nilsson L, Petrella RJ, Roux B et al (2009) CHARMM: the biomolecular simulation program. J Comput Chem 30(10):1545–1614

Campos FF, Rosa LH, Cota BB, Caligiorne RB, Teles Rabello AL, Alves TMA et al (2008) Leishmanicidal metabolites from Cochliobolus sp., an Endophytic fungus isolated from Piptadenia adiantoides (Fabaceae). PLoS Negl Trop Dis 2(12):e348. https://doi.org/10.1371/journal.pntd.0000348

Case DA, Cheatham TE III, Darden T, Gohlke H, Luo R, Merz KM Jr et al (2005) The Amber biomolecular simulation programs. J Comput Chem 26(16):1668–1688

ChemAxon L (2013) Marvinsketch. In: ChemAxon Cambridge

Cremasco M, Hritzko B, Linda Wang N-H (2009) Experimental purification of paclitaxel from a complex mixture of taxanes using a simulated moving bed. Braz J Chem Eng 26(1):207–218

Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7(1):42717. https://doi.org/10.1038/srep42717

Dallakyan, S. J. R. S. (2010). MGLTools

Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. In: Chemical biology. Springer, pp 243–250

David TI, Adelakun NS, Omotuyi OI, Metibemu DS, Ekun OE (2018) Molecular docking analysis of phyto-constituents from Cannabis sativa with pfDHFR. Bioinformation 14(9):574

Dong J, Wang N-N, Yao Z-J, Zhang L, Cheng Y, Ouyang D et al (2018) ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. J Chem 10(1):29. https://doi.org/10.1186/s13321-018-0283-x

Egbuna C, Sawicka B (2019) Natural remedies for pest, disease and weed control. Academic

Falcón-Cano G, Molina C, Cabrera-Pérez MÁ (2020) ADME prediction with KNIME: development and validation of a publicly available workflow for the prediction of human Oral bioavailability. J Chem Inf Model 60(6):2660–2667. https://doi.org/10.1021/acs.jcim.0c00019

Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA et al (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein−ligand complexes. J Med Chem 49(21):6177–6196

Gao K, Mendgen K (2006) Seed-transmitted beneficial endophytic Stagonospora sp. can penetrate the walls of the root epidermis, but does not proliferate in the cortex, of Phragmites australis. Can J Bot 84(6):981–988

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G et al (2008) SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankiabacteria. Proc Natl Acad Sci U S A 105(12):4928–4932. https://doi.org/10.1073/pnas.0710618105

Grosdidier A, Zoete V, Michielin O (2011) SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res 39(suppl_2):W270–W277

Gunatilaka AL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):509–526

Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL (2004) Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 47(7):1750–1759

Han Y, Zhang J, Hu CQ, Zhang X, Ma B, Zhang P (2019) In silico ADME and toxicity prediction of Ceftazidime and its impurities. Front Pharmacol *10*:434. https://doi.org/10.3389/fphar.2019.00434

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342

Hemtasin C, Kanokmedhakul S, Kanokmedhakul K, Hahnvajanawong C, Soytong K, Prabpai S, Kongsaeree P (2011) Cytotoxic Pentacyclic and tetracyclic aromatic Sesquiterpenes from Phomopsis archeri. J Nat Prod 74(4):609–613. https://doi.org/10.1021/np100632g

Herrera-Carillo Z, Torres MS, Singh AP, Vorsa N, Gianfagna T, Meyer W, White JF Jr (2009) Phenolic, flavonoid and antioxidant profiling for cool-season grasses with and without endophyte. Paper presented at the proceedings of the 18th annual Rutgers Turfgrass symposium

Hollingsworth SA, Dror RO (2018) Molecular dynamics simulation for all. Neuron 99(6):1129–1143

Huang G, Jin Q, Peng H, Zhu T, Ye H (2019) Effect of a fungus, Hypoxylon spp., on endophytes in the roots of asparagus. FEMS Microbiol Lett 366(16):fnz207

Iakovou G, Alhazzazi M, Hayward S, Laycock SD (2020) DockIT: a tool for interactive molecular docking and molecular complex construction. Bioinformatics 36(24):5698–5700. https://doi.org/10.1093/bioinformatics/btaa1059

Jacobson MP, Pincus DL, Rapp CS, Day TJ, Honig B, Shaw DE (2004) A hierarchical approach to all-atom protein loop prediction. Proteins 55(2):351–367

Jayaram B, Singh T, Mukherjee G, Mathur A, Shekhar S, Shekhar V (2012) Sanjeevini: a freely accessible web-server for target directed lead molecule discovery. BMC Bioinf 13(17):S7. https://doi.org/10.1186/1471-2105-13-S17-S7

Joseph B, Priya RM (2011) Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am J Biochem Mol Biol 1(3):291–309

Kaul S, Gupta S, Ahmed M, Dhar MK (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11(4):487–505. https://doi.org/10.1007/s11101-012-9260-6

Kaur T (2020) Fungal endophyte-host plant interactions: role in sustainable agriculture. Sustain Crop Prod 211. https://doi.org/10.5772/intechopen.92367

Kogel K-H, Franken P, Hückelhoven R (2006) Endophyte or parasite–what decides? Curr Opin Plant Biol 9(4):358–363

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007) Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol 174(3):648–657

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus Fusarium oxysporum isolated from Catharanthus roseus. PLoS One 8(9):e71805

Lam KS (2007) New aspects of natural products in drug discovery. Trends Microbiol 15(6):279–289

Lee S, Lee I, Kim H, Chang G, Chung J, No K (2003) The PreADME approach: web-based program for rapid prediction of physico-chemical, drug absorption and drug-like properties. In: EuroQSAR 2002 designing drugs and crop protectants: processes, problems and solutions. Blackwell Publishing, pp 418–420

LigPrep (2018) LigPrep. In: Schrödinger, LLC

LigPrep_Schrödinger_2017 (2017) 2: LigPrep. Schrödinger, LLC, New York

Malathi K, Ramaiah S (2018) Bioinformatics approaches for new drug discovery: a review. Biotechnol Genet Eng Rev 34(2):243–260

Miteva MA, Guyon F, Tufféry P (2010) Frog2: efficient 3D conformation ensemble generator for small compounds. Nucleic Acids Res 38(suppl_2):W622–W627

Moitessier N, Englebienne P, Lee D, Lawandi J, Corbeil CR (2008) Towards the development of universal, fast and highly accurate docking/scoring methods: a long way to go. Br J Pharmacol 153(S1):S7–S26

Moreno E, Varughese T, Spadafora C, Arnold AE, Coley PD, Kursar TA et al (2011) Chemical constituents of the new endophytic fungus Mycosphaerella sp. nov. and their anti-parasitic activity. Nat Prod Commun 6(6):1934578X1100600620

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res *35*(Web Server issue):W182–W185. https://doi.org/10.1093/nar/gkm321

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 30(16):2785–2791

Naik B, Gupta N, Ojha R, Singh S, Prajapati VK, Prusty D (2020) High throughput virtual screening reveals SARS-CoV-2 multi-target binding natural compounds to lead instant therapy for COVID-19 treatment. Int J Biol Macromol 160:1–17. https://doi.org/10.1016/j.ijbiomac.2020.05.184

Naik B, Mattaparthi VSK, Gupta N, Ojha R, Das P, Singh S, Prajapati VK, Prusty D (2021) Chemical system biology approach to identify multi-targeting FDA inhibitors for treating COVID-19 and associated health complications. J Biomol Struct Dyn 40:1–25

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open babel: An open chemical toolbox. J Chem 3(1):33. https://doi.org/10.1186/1758-2946-3-33

O'dell T, Massicotte H, Trappe J (1993) Root colonization of Lupinus latifolius AGARDH. and Pinus contorta DOUGL. by Phialocephala fortinii WANG & WILCOX. New Phytol 124(1):93–100

Paramashivam SK, Elayaperumal K, Bhagavan Natarajan B, Devi Ramamoorthy M, Balasubramanian S, Dhiraviam KN (2015) In silico pharmacokinetic and molecular docking studies of small molecules derived from Indigofera aspalathoides Vahl targeting receptor tyrosine kinases. Bioinformation 11(2):73

Pattar SV, Adhoni SA, Kamanavalli CM, Kumbar SS (2020) In silico molecular docking studies and MM/GBSA analysis of coumarin-carbonodithioate hybrid derivatives divulge the anti-

cancer potential against breast cancer. Beni-Suef Univ J Basic Appl Sci 9(1):36. https://doi.org/10.1186/s43088-020-00059-7
Phillips JC, Hardy DJ, Maia JD, Stone JE, Ribeiro JV, Bernardi RC et al (2020) Scalable molecular dynamics on CPU and GPU architectures with NAMD. J Chem Phys 153(4):044130
QikProp (2017) Schrödinger release 2017-1: QikProp. In: Schrödinger, LLC, New York
QikProp (2021) Schrödinger release 2021-4: QikProp. Schrödinger, LLC, New York
Rayhan F, Ahmed S, Shatabda S, Farid DM, Mousavian Z, Dehzangi A, Rahman MS (2017) iDTI-ESBoost: identification of drug target interaction using evolutionary and structural features with boosting. Sci Rep 7(1):17731. https://doi.org/10.1038/s41598-017-18025-2
Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298(5598):1581–1581
Rodriguez R, Redman RJ (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59(5):1109–1114
Rodriguez R, White J Jr, Arnold A, Redman AR (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330
Ropp PJ, Spiegel JO, Walker JL, Green H, Morales GA, Milliken KA et al (2019) Gypsum-DL: an open-source program for preparing small-molecule libraries for structure-based virtual screening. J Chem 11(1):34. https://doi.org/10.1186/s13321-019-0358-3
Roy S, Samant LR, Chowdhary A (2015) In silico pharmacokinetics analysis and ADMET of phytochemicals of Datura metel Linn. and Cynodon dactylon Linn. J Chem Pharm Res 7:385–388
Rudgers JA, Koslow JM, Clay K (2004) Endophytic fungi alter relationships between diversity and ecosystem properties. Ecol Lett 7(1):42–51
Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Annu Rev Ecol Syst 29(1):319–343
Samdani A, Vetrivel U (2018) POAP: a GNU parallel based multithreaded pipeline of open babel and AutoDock suite for boosted high throughput virtual screening. Comput Biol Chem 74:39–48
Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W (2013) Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des 27(3):221–234
Schardl CL, Liu J-S, White JF, Finkel RA, An Z, Siegel MR (1991) Molecular phylogenetic relationships of nonpathogenic grass mycosymbionts and clavicipitaceous plant pathogens. Plant Syst Evol 178(1):27–41
Schulz B, Boyle C, Draeger S, Römmert A-K, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004
Sharma AK, Srivastava GN, Roy A, Sharma VK (2017) ToxiM: a toxicity prediction tool for small molecules developed using machine learning and chemoinformatics approaches. Front Pharmacol 8:880
Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M (2007) Epik: a software program for pK a prediction and protonation state generation for drug-like molecules. J Comput Aided Mol Des 21(12):681–691
Singh SB, Ondeyka JG, Tsipouras N, Ruby C, Sardana V, Schulman M et al (2004) Hinnuliquinone, a C2-symmetric dimeric non-peptide fungal metabolite inhibitor of HIV-1 protease. Biochem Biophys Res Commun 324(1):108–113. https://doi.org/10.1016/j.bbrc.2004.08.234
Singh T, Biswas D, Jayaram B (2011) AADS – An automated active site identification, docking, and scoring protocol for protein targets based on physicochemical descriptors. J Chem Inf Model 51(10):2515–2527. https://doi.org/10.1021/ci200193z
Sohlenius-Sternbeck A-K, Terelius Y (2022) Evaluation of ADMET predictor in early discovery drug metabolism and pharmacokinetics project work. Drug Metab Dispos 50(2):95–104
Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Sun D, Jones LH, Mathews FS, Davidson VL (2001) Active-site residues are critical for the folding and stability of methylamine dehydrogenase. Protein Eng Des Sel 14(9):675–681. https://doi.org/10.1093/protein/14.9.675

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18(4):448–459

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31(2):455–461

Valdés-Tresanco MS, Valdés-Tresanco ME, Valiente PA, Moreno E (2020) AMDock: a versatile graphical tool for assisting molecular docking with Autodock Vina and Autodock4. Biol Direct 15(1):1–12

van de Waterbeemd H, Gifford E (2003) ADMET in silico modelling: towards prediction paradise? Nat Rev Drug Discov 2(3):192–204. https://doi.org/10.1038/nrd1032

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254

White JF Jr, Torres MS (2010) Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? Physiol Plant 138(4):440–446

Wooller SK, Benstead-Hume G, Chen X, Ali Y, Pearl FM (2017) Bioinformatics in translational drug discovery. Biosci Rep 37(4):BSR20160180

Xiong G, Wu Z, Yi J, Fu L, Yang Z, Hsieh C et al (2021) ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res 49(W1):W5–W14. https://doi.org/10.1093/nar/gkab255

Yang H, Lou C, Sun L, Li J, Cai Y, Wang Z et al (2018) admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. Bioinformatics 35(6):1067–1069. https://doi.org/10.1093/bioinformatics/bty707

Yu H, Zhang L, Li L, Zheng C, Guo L, Li W et al (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165(6):437–449. https://doi.org/10.1016/j.micres.2009.11.009

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771

Zhang J, Wang JD, Liu CX, Yuan JH, Wang XJ, Xiang WS (2014) A new prenylated indole derivative from endophytic actinobacteria Streptomyces sp. neau-D50. Nat Prod Res 28(7):431–437. https://doi.org/10.1080/14786419.2013.871546

Zhu K, Borrelli KW, Greenwood JR, Day T, Abel R, Farid RS (2014) Docking covalent inhibitors: a parameter free approach to pose prediction and scoring. J Chem Inf Model 54(7):1932–1940

Chapter 10
Omics-Based Approaches in Studying Fungal Endophytes and Their Associated Secondary Metabolites

Poorvi Saini, Ashish Verma, Harshita Tiwari, Vishnu Mishra, and Vibhav Gautam

Abstract The microbial communities that reside in the plant endosphere without exhibiting any apparent disease symptoms are known as endophytes, and they have the most distinct relationships in close proximity with their host plants in comparison to other plant-associated microbiota. The sturdy foundation for natural metabolic scaffolds with a wide range of applications is provided by bioactive secondary metabolites from endophytic fungus. The highly significant metabolites of these filamentous fungi are encoded by gene clusters, but many of these gene clusters are hidden in lab-like environments. Due to this reason, various approaches involving genetic modification(s) and/or metabolomic interventions are essential for stimulating such dormant gene clusters and enhancing metabolite production. In this chapter, chemical profiling of fungal endophyte-derived bioactive compounds has been discussed using omics-based approaches. The study about the association between plants and fungal endophytes using a variety of techniques, including genome sequencing, comparative genomics, microarray, next-generation sequencing, metagenomics, and metatranscriptomics, has also been covered in this topic.

P. Saini
Department of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad, India

A. Verma · V. Gautam (✉)
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
e-mail: vibhav.gautam4@bhu.ac.in

H. Tiwari
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

V. Mishra
Department of Plant and Soil Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA

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1 Introduction

Endophytes are endosymbionts that persist in plants asymptomatically for at least a portion of their life cycle, primarily consisting of bacteria, fungus, and actinomycetes (Rai et al. 2022a, b; Verma et al. 2022). They have evolved as a vital source of novel metabolites, industrially significant enzymes, and stress-relieving agents for the host plant, yet leaving many unexplored facets of endophytic life. The functions of individual endophytes arise due to their ongoing and intricate interactions with the host plant and other microorganisms (Kaul et al. 2016). They can be regarded as a promising source of natural metabolites with a wide range of possible medical benefits, for example, as antibacterial, antibiotics, antiviral compounds, anticancer agents, antioxidants, insecticides, etc. (Atri et al. 2020; Gupta et al. 2022; Kaul et al. 2012; Keshri et al. 2021; Rai et al. 2021, 2022a, b; Strobel and Daisy 2003). Bioactive and chemically unique substances are produced by endophytic fungus associated with medicinal plants (Verma et al. 2023). The bioactive compounds made by endophytic fungi come from many biosynthetic pathways and are classified into a wide range of structural classes, including terpenoids, steroids, quinones, phenols, and coumarins (Ranjan et al. 2019). Despite numerous publications on endophyte-derived bioactive chemicals, commercial manufacturing of these substances is still lagging behind. Additionally, endophytes can help the host plants by improving their nutrient uptake, promoting plant growth, and tolerating biotic and abiotic stress (Johnson et al. 2003).

Many aspects of these endophytes are yet to be discovered leading to the development of a need for integrative understanding of the capabilities of endophytes. In order to clarify the treacherous depths of endophytism, contemporary genomic investigations incorporating meta-omics and comparative studies may prove to be beneficial. A better understanding of the host infection process and the function of endophytes could be used to enhance agricultural management in terms of promoting plant growth, biocontrol, and bioremediation. Some of the approaches being utilized or that could be used to understand the interaction between plants and endophytes include genome sequencing, comparative genomics, microarray, next-generation sequencing, metagenomics, and metatranscriptomics. To study endophytes and their alleged function in host plant ecology, contemporary methods and approaches need to be investigated. With the turn of the twentieth century, research on fungal metabolites entered the “omics” era, and the sequencing of more and more fungal genomes allowed for high-throughput efforts to study the genes and biosynthetic pathways involved in the production of secondary metabolites (Salvi et al. 2022a, b). The organization of a wide variety of fungal secondary

metabolism genes in clusters was previously demonstrated (Keller et al. 2005) to characterize the genes and pathways involved in the biosynthesis of well-studied secondary metabolites, such as penicillin (Díez et al. 1990; Fierro et al. 1995; Smith et al. 1990 and cephalosporin (Gutiérrez et al. 1992). Fungal genome analysis validates these findings by using different omics-based techniques.

A significant advancement has been made in understanding the composition and abundance of plant-associated microbial diversity, as well as the function of the plant microbiome in influencing the host's responses, with the advent of omics technologies (primarily metagenomics, metatranscriptomics, metaproteomics, and microbial metabolomics) (Kaul et al. 2016). The "expressed" (RNA and protein components, respectively) portion of the microbial genome (Guo et al. 2021; Liao et al. 2019) is revealed by metatranscriptomic and metaproteomic techniques, whereas metagenomic investigations of plant materials give an overview of the quantity and functional potential of the plant-associated microbiota (Mishra et al. 2021; Sessitsch et al. 2012). However, since metabolites represent the final stage of gene expression, high-throughput quantitative analysis of metabolites would offer the most accurate depiction of an organism's physiological state. Typically, there are different levels of metabolite analysis that can be carried out, including (i) target analysis (precise measurement of the concentration of a small number of known metabolites), (ii) metabolite profiling (nontargeted measurement of metabolite levels), (iii) metabolic fingerprinting (generates a total profile or fingerprint, representing a snapshot of the metabolism, without precise quantification of metabolites), and (iv) detection and quantification of thousands of metabolites defining the phenotype of the biological system (Porzel et al. 2014; Shulaev et al. 2008).

The integration of omics into this field is relatively new, even though most breakthroughs and findings about endophytic fungi-plant mutualism are based on conventional scientific methodologies. The conventional methods included toxicity and bioactivity evaluations by performing in vitro and in vivo assays. Most of the findings in this field have been largely influenced by these methods. A workflow for omics-based study of fungal endophytes and their bioactive compounds is shown in (Fig. 10.1). There are relatively new techniques significantly emerging in the field of omics, particularly high-throughput techniques like whole genome sequencing, RNA-Seq (transcriptomics), metagenomics, two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) for proteomics, and microfluidics-coupled mass spectrometry devices for metabolomics (Fig. 10.2) (Gupta et al. 2021). The omics-based insights can be useful in several ways and be utilized to develop multiresistant plants that are crucial to the economy.

2 Genome Analysis

The endophyte-host interaction on a basic physiological level is still unexplored, and therefore in order to effectively manipulate the mutualistic link between them, it is crucial to identify, isolate, and characterize the genes involved in such beneficial interactions. A novel approach for closely examining endophytism and

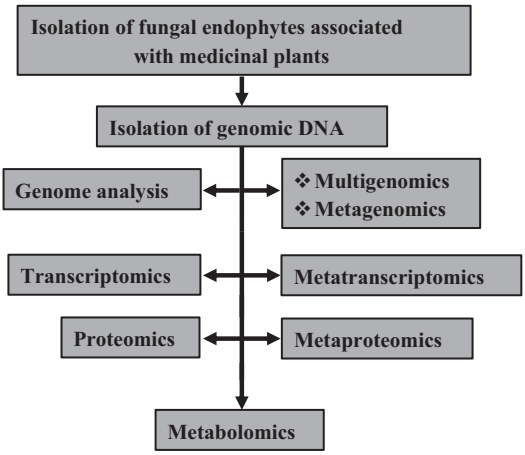

Fig. 10.1 A procedural framework for conducting an omics-based investigation of the fungal endophyte genome and the bioactive compounds isolated from it

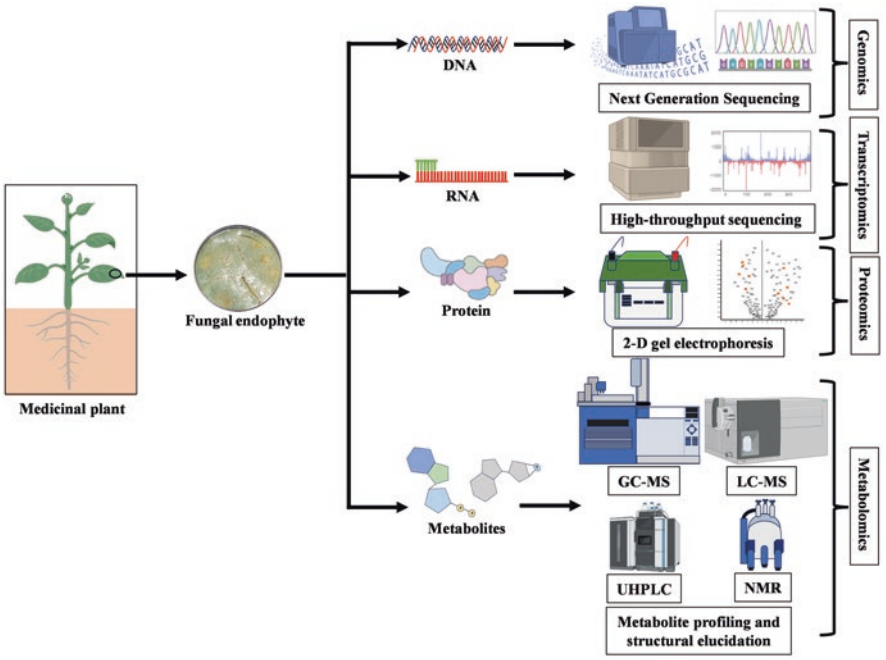

Fig. 10.2 The visual depiction illustrates an overview of omics-based technologies explored in this chapter. These methods leverage state-of-the-art platforms focused on fungal endophytes and their metabolites, enabling advanced profiling at the molecular level, encompassing genomics (genes), transcriptomics (transcripts), proteomics (proteins), and metabolomics (metabolites)

revealing the characteristics needed to harbor plants as a habitat has been made available through endophyte genome analysis. This has indicated the genes essential for the endophytic lifestyle that are found frequently in the genomes of endophytes, including genes for nitrogen fixation, the production of phytohormones

(IAA, GA, etc.), the acquisition of minerals (Fe, P, etc.), stress tolerance, adhesion, and other genes pertaining to colonization (Firrincieli et al. 2015; Fouts et al. 2008; Martínez-García et al. 2015). Whole genome analysis can unravel otherwise hidden information that is inaccessible by conventional characterization techniques. Many examples of such studies have revealed significant information to uplift the research in this field, which are as follows: the fungal endophyte of *Brassica* crops, *Leptosphaeria maculans* associated with the host plant by switching between different modes of life including parasitic, saprotrophic, necrotrophic, and endophytic. Its genome has an AT-rich block, a genomic survival mechanism unique to fungal genomes, which contains effector genes and transposable element families scattered with point mutations, allowing rapid sequence diversification and adaptability to host-directed limitations (Rouxel et al. 2011). In order to acquire knowledge of endophyte-associated bio-saprotrophism, the genomes of *Piriformospora indica* and other fungi with various lifestyles were examined (Zuccaro et al. 2011). Research on the genome of *Empetrum nigrum* has demonstrated its capacity of producing metabolites that are destructive to host plant pathogens such as *Xanthomonas albilineans*, *Fusarium verticillioides*, *Ceratocystis paradoxa*, and *Colletotrichum falcatum* as well as increasing the biomass of the sugarcane root system, concluding the evidences for *Epicoccum nigrum* to serve as a biocontrol agent (Fávaro et al. 2012).

To comprehend the biology of *Ascocoryne sarcoides* (an endophyte of *Picea mariana*), amalgamation study of genomes, transcriptomics, and metabolomics was applied to explore the formation of C8 volatiles, a class of unusual secondary metabolites (Gianoulis et al. 2012). The identification of genes for paclitaxel production, which most likely developed independently of the host plant metabolic pathway, was made possible by whole genome sequencing and study of *Penicillium aurantiogriseum* (Yang et al. 2014). One such species is *Pestalotiopsis fici*, an endophyte of *Camellia sinensis* (tea), which generates significant secondary metabolites like pestaloficiol, pestalofones, pestalodiols, chloropupukeananin, chloropestolides, chloropupukeanone, and chloropupukeanolides and is thought to be responsible for the inhibition of HIV-1 replication (Wang et al. 2015). With a high capacity for natural product synthesis and evidence that these may have helped it adapt to an endophytic lifestyle, its genome study revealed an abundance of carbohydrate-active enzymes, including pectinases and a rich set of secondary metabolite production genes (Wang et al. 2015).

The whole genome of fungal endophytes like *Piriformospora indica* (Zuccaro et al. 2011), *Rhodotorula graminis* (Firrincieli et al. 2015), *Harpophora oryzae* (Xu et al. 2014), and *Xylona heveae* (Gazis et al. 2016) of economically significant plants have been analyzed deciphering the genes necessary for their nutrient intake, colonization, biotic and abiotic stress tolerance, etc. *Gaeumannomyces* sp., an endophytic fungus isolated from *Phragmites communis*, has a unique genome sequence indicating its potential to synthesize a variety of anti-inflammatory secondary metabolites (Kim et al. 2017; Lee et al. 2017). It has also been discovered that host genomes with slight genetic changes influence the outcomes of symbiotic interactions (Rodriguez et al. 2008). Various genotype-specific interactions may increase,

decrease, or have no influence on the host plant's ability to withstand stress (Cheplick et al. 1989). An early horizontal transfer of a gene encoding the fungal enzyme β-1,6-glucanase, which is capable of dissolving cell walls, has been demonstrated by the assembly of the perennial ryegrass' genome and transcriptome. It has been suggested that this enzyme enables certain *Epichloe* species to withstand both biotic and abiotic stress. The Loliinae and Dactylidinae families of plants both include orthologs of this gene (Shinozuka et al. 2017). Such thorough genomic research can considerably aid in the discovery of features with agronomic value, as evidenced by the identification of this class of critical endophytic enzymes linked to stress tolerance. The symbiotic interactions of *Piriformospora indica* (order Sebacinales) have also been studied using this approach to unravel its potential as a plant probiotic (Qiang et al. 2012).

2.1 Multigenome Analysis

Comparison of multiple genomes termed as multigenome analysis is extremely beneficial in understanding the genetic and metabolic diversity of comparable microorganisms that participate in various sorts of interactions with plants and animals. A narrow line appears to separate the colonization of a host plant by a bacterium as asymptomatic endophyte or a pathogen (Kaul et al. 2016). Zuccaro et al. (2011) analyzed the genome of *Piriformospora indica*, comparing it with those of fungi belonging to various classes which indicated the existence of genes relevant to both saprotrophic and biotrophic behaviors (Zuccaro et al. 2011).

When comparing the genomes of endophytic isolates and their non-endophytic counterparts, characteristics that are expected to be unavoidable for initiating and sustaining plant microbial relationships are revealed. The research is also useful for understanding the genetic underpinnings of niche adaptation (Lòpez-Fernàndez et al. 2015). Genomes of endophytic species that are closely related but have various functional roles in the host plant can also be compared to learn how adaptable they are and how their evolution has progressed. The genomes of three different *P. ananatis* strains have been compared by the researchers, and each of the three strains displayed a different approach to interact with the host plant after being separated as maize seed endophytes. Genome comparisons showed variations between the strains for the genes associated with secretory proteins, integrases, transposons, and phages (Sheibani-Tezerji et al. 2015).

Endophytic isolates' genomes were compared to non-endophytic isolates' genomes, revealing traits that are likely to be indispensable for establishing and maintaining plant microbial relationships. A multigenome comparative examination of more than ten Clavicipitaceae family members for gene clusters of four classes of alkaloids revealed that changes in the alkaloid loci's peripheral genes are accountable for their pharmacological specificities (Schardl et al. 2013). Thus, understanding the metabolic diversity among the constituents of a microbiome can be achieved

through comparative genome analysis of endophytes for various metabolite gene clusters, and this insight can then be utilized in metabolic engineering.

2.2 Metagenomics

The analysis of sequencing information from microbial members of distinct ecological communities is known as metagenomics which eliminates the necessity for individual species isolation and cultivation. There is a significant need to reveal the metabolic potential and beneficial qualities of endophytes in order to comprehend and regulate their contribution to the host plants. The inability of many endophytes to be cultured makes determining endophytic microbial functions challenging wherein metagenomic approach could be helpful in unleashing the endophytic potential of such microorganisms (Dinsdale et al. 2008). High-throughput metagenomic analysis of microbial communities has improved the understanding of the structure, functions, and community dynamics. These approaches, however, have only been used for fungal endophyte screening since late 2013. High-throughput sequencing, often known as next-generation sequencing (NGS), has made metagenomic investigations accessible and has stimulated the rapid, remarkable characterization of microbiomes (Akinsanya et al. 2015; Barik et al. 2020). These next-generation technologies, together with systems biology, have enabled researchers to investigate these endophytic residents while also investigating their interactions with the host. Simultaneously, data mining has made it exceedingly simple to identify biochemical routes for novel metabolites. High-throughput investigations have allowed for the unbiased identification of metabolites across the genomes and transcriptomes of newly discovered endophytes, as well as the acquisition of new sources of already commercialized natural bioactive metabolites. For the first time, a study published in late 2013 described the use of next-generation sequencing for large-scale analysis of the internal transcribed spacer (ITS) region of resident endophytic species in *Eucalyptus grandis* from South Africa (Kemler et al. 2013). The genomes of filamentous fungi such as *Aspergillus* spp. have indicated that they have a higher proportion of secondary metabolite biosynthetic gene clusters than expected, and it has also been evidenced that *Aspergillus nidulans* can synthesize a variety of polyketides, non-ribosomal peptides, and indole alkaloids (Brakhage et al. 2008). Illumina sequencing was used in a separate study in order to uncover a network exhibiting co-occurrence trend of symbiotic connections in fungi linked with the roots of a monodominant forest (Toju et al. 2016). Using 454 pyrosequencing techniques, they also established the cohabitation of mycorrhizal fungi and endophytic fungi in the roots of many plant species which would almost certainly include intricate interactions between the two ecotypes, which may be researched further employing metaproteomics, metatranscriptomics, or metaproteogenomics (Toju et al. 2013). A comprehensive examination of endophytic fungi articles from the earliest available literature through March 2018 revealed that the frequency of the traditional approach-based studies on endophytic fungi is around ten times

more than that of omics-based studies. ITS region sequencing was used for a limited number of samples in early research on fungal species for identification of endophytic fungus prevailing in *Aquilaria malaccensis*, a medicinal tree, the majority of which belonged to the phylum Ascomycota (Premalatha and Kalra 2013).

3 Transcriptomics and Metatranscriptomics

Transcriptomics has been identified as a viable tool for studying the microbial communities related to various plants since it can reveal novel information on the alterations in gene expression linked with fungal-plant mutualism. It entails comparing transcriptomes of groups of interacting species and aids in understanding the response of microbial communities to variable environments. Understanding the interactive factors responsible for endophytism, production of secondary metabolites, and substances that promote plant growth can be aided by comparative expression analysis of the transcriptome of plants with and without endophyte infestation as well as of endophytes inside and outside of the host.

To learn more about endophyte biology and how it relates to plant health, researchers conducted a number of experiments. "*Epulorhiza* sp." transcriptome report is one of the earliest reports and observation of overexpression of genes involved in pyrimidine metabolism which was identified from the roots of an orchid called *Anoectochilus roxburghii* (Li et al. 2012a, b). Transcriptomes can be analyzed using existing whole genome sequences or built from scratch; both procedures are known to obtain a very accurate snapshot of gene expression status, and both exist on endophytic fungi (Chetia et al. 2019). While genomic and metagenomic investigations identify the presence or absence of certain genes, expression studies of individual genes in diverse microenvironments are required to fully comprehend the endophytic phenomena. A thorough examination of the differentially expressed genes in both the host plant and the symbiotic microorganisms would shed light on the fundamental nature and mechanism of the two mutualistic connections. Dual RNA-seq transcriptional profiling provides a deeper understanding of the gene expression in both symbiotic partners at the same time (Kaul et al. 2012). Several experiments were carried out in order to discover more about endophyte biology and its relationship to plant health. SOLID-SAGE transcriptomic analyses of endophyte-free and *Epichloe festucae* (a fungal endophyte)-infected *Festuca rubra* have revealed approximately 200 plant-associated genes (including antifungal gene) that are expressed differently in the two plant samples. They reasoned that the homologous gene product, which is secreted in nature, could play a role in giving "disease resistance," which is a hallmark property of endophyte-infected fescue (Ambrose and Belanger 2012). Transcriptomics has also been employed to identify genes involved in secondary metabolite production with potential commercial use. One study used integrated genomes, transcriptomics, and metabolomics to implicate the cellulose biodegradation and potential biofuel production capability of *Ascocoryne sarcoides* which is one of the significantly characterized endophytic

fungal species (Gianoulis et al. 2012; Wang et al. 2019) identified many putative gene clusters involved in endophyte-derived bioactive chemical production by *P. fici*. Transcriptional changes may occur in the endophyte genome owing to plant stress tolerance, for example, in the transcriptome of heavy metal tolerant *Exophiala pisciphila*, multiple glutathione-S-transferase genes were obtained (Shen et al. 2015). In one of the studies, it was discovered that the expression of 200 genes connected to the endophyte *Festuca rubra* differed from that of *Epichloe festucae*, which was infesting the host plant. However, the correlation between the data from the various genomes and the transcriptomes can complete our comprehension of the facts (Ambrose and Belanger 2012).

Transcripts or RNAs are directly isolated from the environment or community for the metatranscriptomic analysis. By profiling the expressed transcripts and connecting them to the current ecophysiological settings, this sort of analysis establishes a direct relationship between the genetic makeup of the community and the corresponding functionality in situ. In wheat roots infected with the bacterial endophyte *A. brasilense*, differential regulation of genes for nutrient availability was discovered using dual RNA sequencing method for comparative transcriptional profiling (Li et al. 2012a, b). This made it easier for the team to understand the fundamental mutualism they had. By comparing metatranscriptomic analyses, it was possible to determine whether soybean host plants had endophytes and free-living microorganisms infesting them. This allowed researchers to identify the source of the infestation (Molina et al. 2012). These are some of the recent approaches to community RNA analysis that offer a significant amount of knowledge and insight, however, they also have some limitations. It is frequently difficult to directly extract the RNA with frequently low concentration from an environmental sample. For this reason, extra amplification stages have been utilized in earlier research to boost the concentration of initial transcripts (Frias-Lopez et al. 2008).

With the examples explained above, we can conclude that genome-based studies provide a solid foundation for effective transcriptomics making the combined genomic and transcriptomic analysis more reliable in decoding symbiont endophytic lifestyles. These transcriptomic analysis reports, generated using microarray, serial analysis of gene expression, and more recent forms of second-generation sequencing, have provided us with beneficial sequence data with derived putative functions inferred from homology, enabling the estimation of endophytic traits and metabolic pathways ranging from nutrient acquisition and storage, quorum sensing, reactive species detoxification, and biosynthesis.

4 Proteomics and Metaproteomics

Post-genomic analysis, such as proteomics and metaproteomics, is gaining popularity as researchers realize that genomic and metagenomic analysis is still unable to decipher the real-time in situ functional information about the community. The process of directly identifying and evaluating the predominant functioning of the

microbial population in an environmental sample is known as metaproteomics. It evaluates the microbial functional profile directly. The advancement of bioinformatics and computational techniques also offers a more reliable source for protein identification (Schneider and Riedel 2010). A limited number of stand-alone experiments on proteomic profiling of fungal endophytes are also documented in literature, in addition to the potential protein sequence data generated by transcriptomic analysis. The widespread endophyte *P. indica* from the Sebacinales order, which colonizes the roots of practically all known terrestrial plants, has been the subject of two proteome-based investigations. This species is recognized for encouraging plant growth both under pressure and typical circumstances. It has been shown how this endophyte affects barley plants under various levels of drought stress (Ghabooli et al. 2013). The sample protein in metaproteomics must be a distinctive one in terms of both quality and quantity. In order to understand the impact of any parameter on the production of secondary metabolites, among other things, metaproteomic analysis of endophytes has been carried out by direct lysis method, which involves extracting the total protein of the endosphere (the microenvironment where the plant and endophyte association is established) (Maron et al. 2007).

Through host adaptation to environmental stress, plant mutualistic symbionts provide long-term abiotic stress tolerance. Under both well-watered and water-stressed situations, *P. indica* increased root and shoot biomass of colonized plants. They claimed that *P. indica*-mediated drought stress tolerance is barely achieved via photosynthetic acceleration, energy release, and improved antioxidative capacity in colonized plants as a consequence of proteomic and mass spectrometry analysis. An in-depth understanding of the relationship between plants and endophytes is made possible by proteomics. The fungal endophyte *Serendipita indica*, formerly known as *Piriformospora indica*, which colonizes the root tissues of the majority of terrestrial plants, is the subject of a proteome-based study that has been published. Mass spectrometry has been used to identify a total of 45 differentially expressed proteins that are involved in ROS scavenging, photosynthesis, signal transduction, metabolism, and plant defense response (Yadava et al. 2015). They indicated that *P. indica* increases the foliar potassium (K^+)/sodium (Na^+) ratio, which is considered as a reliable predictor of salt stress tolerance along with calcium (Ca^{2+}) buildup that could have altered stress signal transduction by employing proteomic analysis. An efficient approach for extracting cellular proteins from endophytic fungi was described in another work by Yadava et al. (2015) for two-dimensional gel electrophoresis to acquire proteomic data of *P. indica*. With a focus on its probable role in symbiosis and the general growth and development of the plant (Shrivastava et al. 2018), comprehensive proteomic analysis was used to define the connection between endophyte *Piriformospora indica* and *Brassica napus* plant. Another group of investigators showed that *P. indica* induces a systemic response to salt stress by altering the physiological and proteomic responses of the plant host (Alikhani et al. 2013). Such findings enhance the minimal information on plant-endophyte connections, and additional combinatorial investigations utilizing a four-way approach (genome, transcriptome, proteome, and metabolome) will offer a better understanding.

Metaproteomics is the study of the metabolic processes that occur within a community at the time of sampling and the determination of the functional expression of the metagenome. It is also referred to as whole-community proteomics. The four critical phases of any metaproteomic analysis are as follows: (i) protein extraction and purification; (ii) denaturation and reduction; (iii) protein separation, digestion, and MS analysis; and (iv) protein identification based on spectroscopic data. To strengthen the reliability of an environmental proteomic analysis, the collected data must be validated using supplementary approaches, such as transcriptomic analyses or phenotypic testing. This approach must be able to overcome additional problems inherent in natural environment samples, such as high organism/protein complexity, over- or underrepresentation of certain organisms/proteins, heterogeneity of organic and inorganic pollutants, and so on (Schneider and Riedel 2010). A group of researchers discovered that the fungal endophyte *Gilmaniella* sp. AL12 decreased the plant immunological response of *A. lancea*, possibly contributing to the favorable plant-endophyte interaction (Yuan et al. 2019). Furthermore, endophyte-plant interaction increased the production (biomass and sesquiterpenoid content) in *A. lancea* by boosting the source (photosynthesis), extending the sink (glycolysis and the TCA cycle), and improving metabolic flux (sesquiterpenoids biosynthesis-related genes). The transcriptional and translational regulation of *Gilmaniella* sp. AL12 on physiological functions and associated regulatory mechanisms in *A. lancea* shoots was discovered in this work which provides a theoretical foundation for therapeutic herb cultivation and contributes to a better understanding of plant-endophyte interactions. The proteomic analysis of *Undifilum oxytropis* using 2-DE was carried out to find enzymes involved in the swainsonine production pathway (Li et al. 2012a, b). Furthermore, this protein data can also be used in a reverse genetics strategy to generate primers for searching the genes in several pathways. The expression levels of proteins between pathogenic plant and mutualistic *Undifilum* species, as in the case of pathogenic and saprophytic *Alternaria* species, may also provide information about the nature of plant disease (Lawrence et al. 2008). Multivariate analysis based on liquid chromatography or gas chromatography mass spectrometry is used in most of the large-scale proteomic research (Chetia et al. 2019). After isolation of bioactive compounds, proteomic analysis, and structural characterization with the aid of devices like mass spectrometry, disease-specific stem cells like cancer stem cells (CSCs) are used for targeted therapy of cancer (Rai et al. 2023a, b; Rai et al. 2020). Using GC/MS analysis of the ethyl acetate (EA) extract of the fungus *Colletotrichum gloeosporioides*, it was possible to determine the molecular modifications driven by the anticancerous bioactive chemicals produced by the fungal endophyte in the tumor microenvironment of human breast cancer cell lines (Rai et al. 2023a, b).

Many other such proteomics- and metaproteomics-based research have helped to explore the in-depth evaluation of endophyte-plant relationship in order to delve deeper into the field for leveraging the potential of endophytes as plant probiotics.

5 Metabolomics

Metabolomics examines metabolites, such as small components found in cells, tissues, or other living things, as well as biofluids. The metabolome is the collective term for this minute subset of chemicals and how they interact with biological systems. The remarkable expansion of the metabolomic field is crucial for the researcher to gather data on the production and characterization of fungal secondary metabolites. Furthermore, it offers chances to investigate new fungal ecological niches with a variety of untapped secondary metabolite diversities and their production and functional characteristics. In order to categorize distinct sample groups and the distribution of metabolites when subjected to various experimental settings, the huge metabolomic findings received from analytical techniques require multivariate analysis. As a result, improvements in analytical technologies combined with multivariate analysis provide a summary of the reported metabolites that are supported by visual depiction (Nagarajan et al. 2021).

The ability to detect many unidentified compounds has been expanded by the development of numerous technologies, including high-resolution mass spectrometry, liquid chromatography with tandem mass spectrometry (LC-MS/MS), nuclear magnetic resonance (NMR), and metabolite mass spectrometry imaging (MSI). Ultra-high performance liquid chromatography (UHPLC), liquid chromatography with ultraviolet (LC-UV), liquid chromatography with mass spectrometry (LC-MS/MS), and gas chromatography with mass spectrometry (GC-MS) and high-performance thin layer chromatography (HPTLC) fingerprinting are highly sensitive detector machines with exceptional selectivity (like co-eluting separation of analytes through m/z ratio of an ionized molecule and physical compound separation by chromatographic technique). These machines can identify hundreds of metabolic compounds present in fungal extract (El-Elimat et al. 2013; Klitgaard et al. 2014; Salvi et al. 2022a, b).

Analytical reproducibility is regarded as one of the most crucial factors in metabolomic studies. Among the most common studies used for this function are principal component analysis (PCA), partial least square discriminant analysis (PLS-DA), and orthogonal partial least square discriminant analysis (OPLS-DA) which have been employed in order to identify novel proline-containing cyclic non-ribosomal peptides from *Xylaria ellisii* which is a leaf and stem endophyte of *Vaccinium angustifolium* (Ibrahim et al. 2020) and investigate the fungal endophytes connected to the well-known antimalarial herb *Artemisia annua* for their capacity to produce additional antimalarial drugs (Alhadrami et al. 2021). Another study used untargeted metabolomic analysis to investigate the effect of histone deacetylase (HDAC) inhibition on the production of a bioactive metabolite by an endophytic *Aspergillus nidulans*. Studies using quantitative and differential methods revealed that 61 and 47 metabolites, respectively, were upregulated and downregulated by a factor of more than 100. Transcriptomic elucidation shows that HDAC inhibitors usually result in an upregulation of the biosynthetic machinery. Their findings, however, suggest that secondary metabolome responses are much more complex than an

increase in the diversity of bioactive metabolites (Keshri et al. 2021). Solamargine, assumed to be one of the plant's unique metabolites, was discovered to be produced by *Aspergillus flavus* as a result of recent studies that examined the endophytes that are associated with the medicinal herb *Solanum nigrum* (El-Hawary et al. 2016). Numerous instances of fungi acting as endophytes have been documented, and they have been shown to synthesize different bioactive metabolites that resemble those that were initially produced by the host plant. The discovery of the paclitaxel-producing endophytic fungus *Taxomyces andreanae*, which was isolated from the Pacific yew *Taxus brevifolia*, is the best-known illustration of this. Another anticancer medication is vincristine, which was first discovered in cultures of *Catharanthus roseus*' endophytic fungus, *Fusarium oxysporum*. A comparative metabolomic analysis using 11 distinct growing conditions has been published on *Aspergillus terreus*, an endophyte of *Opuntia ficus-indica* (Adpressa and Loesgen 2016). They demonstrated that their LC-MS-based analytical method can be used to easily track the environment-modulated variations in metabolite production for the discovery of novel bioactive compounds along with the identification of a novel compound, 7-desmethyl citreoviridin, in addition to 16 other known fungal metabolites. Another study indicated that vincamine and its analogues can be produced alternatively by using the fungal endophyte *Geomyces* sp. from *Nerium indicum* via LC-MS-based metabolomics (Na et al. 2016).

In order for endophytes to create bioactive chemicals, a variety of plant species and components as well as environmental conditions must coexist (Giauque and Hawkes 2013; Guevara-Araya et al. 2020; Harrison and Griffin 2020). Environmental factors have been shown to play a significant role in determining the endophytic communities in plants, including cultivation history (Correa-Galeote et al. 2018), climate, and season (Hosseyni-Moghaddam and Soltani 2014; Oita et al. 2021; L. Wang et al. 2019; Zimmerman and Vitousek 2012). The mechanics of the plant-fungi interactions have evolved in entirely new perspectives as a result of some metabolomic research. For example, the use of global metabolomic analysis by GC-MS analysis demonstrated that *P. indica* suppresses the formation of reactive species in the host plant to encourage more favorable conditions for symbiotic association (Hua et al. 2017). From the experiment with Chinese cabbage, it was revealed that the presence of the endophyte may prepare the host plant for biotic or abiotic stress by considerably increasing the expression of gamma-aminobutyric acid (GABA) in its roots.

It will be feasible to determine the biological underpinnings and diversity of symbiont endophytic fungi and forecast various results of plant-fungal interactions based on genomic or environmental variations owing to the benefits afforded by the various types of biological analysis. These findings have implications for agricultural methods, particularly for the management of stressful circumstances without owing to chemical alternatives in an environmentally beneficial manner (Chetia et al. 2019). The reliability of the database and the quality of the raw data therefore determine the validity and success of the experiment in metabolomics, where the detection and identification of metabolites involve the comparison of experimental MS/MS spectra to library spectra (a procedure known as dereplication). A unified

procedure for the detection and quantification of all kinds of metabolites does not exist due to the chemical diversity of metabolites. Small laboratories cannot use this technology since it is expensive and necessitates specialized equipment and facilities. The uses of metabolomics are constrained by the absence of standards and reference libraries for metabolites. Another issue is that it is impossible to identify a particular microbial species as being responsible for the production of metabolites in a natural community (biological source and biosynthetic pathway). Lastly, the findings are susceptible to impurities and experimental artifacts. Nevertheless, metabolomics has expanded our knowledge of how plants and microbes interact, and these problems can be solved with additional technical research and development (Mishra et al. 2022).

6 Conclusion

To achieve the employment of endophytes as plant probiotics, a thorough understanding of the endophyte-host relationships is needed which can be achieved using a multidisciplinary approach by understanding the variables necessary for both the formation and sustenance of their symbiotic relationship. Such research studies are also crucial for establishing the ability of endophytes to help their host plants develop more resilient to stress and grow efficiently. In order to develop models to anticipate and understand endophyte-mediated activities, complementing data from contemporary "omics" investigations in conjunction with other system biology methodologies is crucial. But a detailed scrutiny of such studies included above reveals that the species that are being investigated are spread disproportionately.

Additionally, this will be very helpful in illuminating and better comprehending the network of the intricate connections between endophytes and the host plant as well as other connected microorganisms. The relationship between plants and endophytes can be understood using research on plant-pathogen interactions as a starting point. To accurately expose the genetic and metabolic capabilities, ecology, and evolution of endophytes, advanced approaches can be applied to fungal endophytes. As the third generation of sequencers can produce more accurate sequences with better length, next-generation sequencing technologies have dramatically advanced in terms of data accuracy. Even though there are many outstanding instances of multidimensional studies on fungal endophytes, it is found that most genomes or transcriptomes have poor coverage and intermediate sequencing depths. Re-sequencing and analysis can be used to enhance these assemblies. In order to utilize their biotechnological potential more effectively and sustainably, this can assist in understanding the role of such varied microbial communities in both the plant microbiome and the natural ecosystem. Lastly, the information currently known about fungal endophytes connected with plants can be used to create plant probiotics, a more environmentally conscious approach, and explore their potential utilization in the areas of drug development and agriculture.

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References

Adpressa DA, Loesgen S (2016) Bioprospecting chemical diversity and bioactivity in a marine derived Aspergillus terreus. Chem Biodivers 13(2):253–259

Akinsanya MA, Goh JK, Lim SP, Ting ASY (2015) Metagenomics study of endophytic bacteria in Aloe vera using next-generation technology. Genomics data 6:159–163

Alhadrami HA, Sayed AM, El-Gendy AO, Shamikh YI, Gaber Y, Bakeer W et al (2021) A metabolomic approach to target antimalarial metabolites in the Artemisia annua fungal endophytes. Sci Rep 11(1):1–11

Alikhani M, Khatabi B, Sepehri M, Nekouei MK, Mardi M, Salekdeh GH (2013) A proteomics approach to study the molecular basis of enhanced salt tolerance in barley (Hordeum vulgare L.) conferred by the root mutualistic fungus Piriformospora indica. Mol BioSyst 9(6):1498–1510

Ambrose KV, Belanger FC (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PLoS One 7(12):e53214

Atri N, Rai N, Singh AK, Verma M, Barik S, Gautam V, Singh SK (2020) Screening for endophytic fungi with antibacterial efficiency from Moringa oleifera and Withania somnifera. J Sci Res 64(1):127–133

Barik S, Rai N, Mishra P, Singh SK, Gautam V (2020) Bioinformatics: how it helps to boost modern biological research. Curr Sci 118:698–699

Brakhage AA, Schuemann J, Bergmann S, Scherlach K, Schroeckh V, Hertweck C (2008) Activation of fungal silent gene clusters: a new avenue to drug discovery. Nat Compd Drug:1–12

Cheplick G, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses Lolium perenne and Festuca arundinacea. New Phytol 111(1):89–97

Chetia H, Kabiraj D, Bharali B, Ojha S, Barkataki MP, Saikia D et al (2019) Exploring the benefits of endophytic fungi via omics. In: Advances in endophytic fungal research. Springer, pp 51–81

Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize endophytic bacterial diversity as affected by soil cultivation history. Front Microbiol 9:484

Díez B, Gutiérrez S, Barredo J, van Solingen P, Van Der Voort L, Martin J (1990) The cluster of penicillin biosynthetic genes. Identification and characterization of the pcbAB gene encoding the alpha-aminoadipyl-cysteinyl-valine synthetase and linkage to the pcbC and penDE genes. J Biol Chem 265(27):16358–16365

Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM et al (2008) Functional metagenomic profiling of nine biomes. Nat Rev Microbiol 452(7187):629–632

El-Elimat T, Figueroa M, Ehrmann BM, Cech NB, Pearce CJ, Oberlies NH (2013) High-resolution MS, MS/MS, and UV database of fungal secondary metabolites as a dereplication protocol for bioactive natural products. J Nat Prod 76(9):1709–1716

El-Hawary S, Mohammed R, AbouZid S, Bakeer W, Ebel R, Sayed A, Rateb M (2016) Solamargine production by a fungal endophyte of Solanum nigrum. J Appl Microbiol 120(4):900–911

Fávaro LCDL, Sebastianes FLDS, Araújo WL (2012) Epicoccum nigrum P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PLoS One 7(6):e36826

Fierro F, Barredo JL, Diez B, Gutierrez S, Fernandez FJ, Martin J (1995) The penicillin gene cluster is amplified in tandem repeats linked by conserved hexanucleotide sequences. Proc Natl Acad Sci 92(13):6200–6204

Firrincieli A, Otillar R, Salamov A, Schmutz J, Khan Z, Redman RS et al (2015) Genome sequence of the plant growth promoting endophytic yeast Rhodotorula graminis WP1. Front Microbiol 6:978

Fouts DE, Tyler HL, DeBoy RT, Daugherty S, Ren Q, Badger JH et al (2008) Complete genome sequence of the N2-fixing broad host range endophyte Klebsiella pneumoniae 342 and virulence predictions verified in mice. PLoS Genet 4(7):e1000141

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, DeLong EF (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci 105(10):3805–3810

Gazis R, Kuo A, Riley R, LaButti K, Lipzen A, Lin J et al (2016) The genome of Xylona heveae provides a window into fungal endophytism. Fungal Biol 120(1):26–42

Ghabooli M, Khatabi B, Ahmadi FS, Sepehri M, Mirzaei M, Amirkhani A et al (2013) Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by Piriformospora indica in barley. J Proteome 94:289–301

Gianoulis TA, Griffin MA, Spakowicz DJ, Dunican BF, Alpha CJ, Sboner A et al (2012) Genomic analysis of the hydrocarbon-producing, cellulolytic, endophytic fungus Ascocoryne sarcoides. PLoS Genet 8(3):e1002558

Giauque H, Hawkes CV (2013) Climate affects symbiotic fungal endophyte diversity and performance. Am J Bot 100(7):1435–1444

Guevara-Araya M, Vilo C, Urzúa A, González-Teuber M (2020) Differences in community composition of endophytic fungi between above-and below-ground tissues of Aristolochia chilensis in an arid ecosystem. Rev Chil Hist Nat 93(1):1–9

Guo L, Yu H, Wang B, Vescio K, DeIulio GA, Yang H et al (2021) Metatranscriptomic comparison of endophytic and pathogenic Fusarium–Arabidopsis interactions reveals plant transcriptional plasticity. Mol Plant-Microbe Interact 34(9):1071–1083

Gupta P, Verma A, Rai N, Singh AK, Singh SK, Kumar B et al (2021) Mass spectrometry-based technology and workflows for studying the chemistry of fungal endophyte derived bioactive compounds. ACS Chem Biol 16(11):2068–2086

Gupta P, Rai N, Verma A, Saikia D, Singh SP, Kumar R et al (2022) Green-based approach to synthesize silver nanoparticles using the fungal endophyte Penicillium oxalicum and their antimicrobial, antioxidant, and in vitro anticancer potential. ACS omega, vol 7, p 46653

Gutiérrez S, Velasco J, Fernandez FJ, Martín J (1992) The cefG gene of Cephalosporium acremonium is linked to the cefEF gene and encodes a deacetylcephalosporin C acetyltransferase closely related to homoserine O-acetyltransferase. J Bacteriol 174(9):3056–3064

Harrison JG, Griffin EA (2020) The diversity and distribution of endophytes across biomes, plant phylogeny and host tissues: how far have we come and where do we go from here? Environ Microbiol 22(6):2107–2123

Hosseyni-Moghaddam MS, Soltani J (2014) Bioactivity of endophytic Trichoderma fungal species from the plant family Cupressaceae. Ann Microbiol 64(2):753–761

Hua MD-S, Senthil Kumar R, Shyur L-F, Cheng Y-B, Tian Z, Oelmüller R, Yeh K-W (2017) Metabolomic compounds identified in Piriformospora indica-colonized Chinese cabbage roots delineate symbiotic functions of the interaction. Sci Rep 7(1):1–14

Ibrahim A, Tanney JB, Fei F, Seifert KA, Cutler GC, Capretta A et al (2020) Metabolomic-guided discovery of cyclic nonribosomal peptides from Xylaria ellisii sp. nov., a leaf and stem endophyte of Vaccinium angustifolium. Sci Rep 10(1):1–17

Johnson LJ, Johnson RD, Schardl CL, Panaccione DG (2003) Identification of differentially expressed genes in the mutualistic association of tall fescue with Neotyphodium coenophialum. Physiol Mol Plant Pathol 63(6):305–317

Kaul S, Gupta S, Ahmed M, Dhar MK (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11(4):487–505

Kaul S, Sharma T, Dhar K, M. (2016) "Omics" tools for better understanding the plant–endophyte interactions. Front Plant Sci 7:955

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3(12):937–947

Kemler M, Garnas J, Wingfield MJ, Gryzenhout M, Pillay K-A, Slippers B (2013) Ion torrent PGM as tool for fungal community analysis: a case study of endophytes in Eucalyptus grandis reveals high taxonomic diversity. PLoS One 8(12):e81718

Keshri PK, Rai N, Verma A, Kamble SC, Barik S, Mishra P et al (2021) Biological potential of bioactive metabolites derived from fungal endophytes associated with medicinal plants. Mycol Prog 20(5):577–594

Kim JA, Jeon J, Kim K-T, Choi G, Park S-Y, Lee H-J et al (2017) Draft genome sequence of an endophytic fungus, Gaeumannomyces sp. strain JS-464, isolated from a reed plant, Phragmites communis. Genome Announc 5(31):e00734–e00717

Klitgaard A, Iversen A, Andersen MR, Larsen TO, Frisvad JC, Nielsen KF (2014) Aggressive dereplication using UHPLC–DAD–QTOF: screening extracts for up to 3000 fungal secondary metabolites. Anal Bioanal Chem 406(7):1933–1943

Lawrence CB, Mitchell TK, Craven KD, Cho YR, Cramer RA Jr, Kim KH (2008) At deaths door: alternaria pathogenicity mechanisms. Plant Pathol J 24(2):101–111

Lee C, Kim S, Li W, Bang S, Lee H, Lee H-J et al (2017) Bioactive secondary metabolites produced by an endophytic fungus Gaeumannomyces sp. JS0464 from a maritime halophyte Phragmites communis. J Antibiot 70(6):737–742

Li H, Holguin O, Wang J, Schaub T, Wang J, Hao C et al (2012a) Proteomic analysis of the endophytic fungus Undifilum oxytropis. Afr J Biotechnol 11(46):10484–10493

Li B, Tang M, Tang K, Zhao L, Guo S (2012b) Screening for differentially expressed genes in Anoectochilus roxburghii (Orchidaceae) during symbiosis with the mycorrhizal fungus Epulorhiza sp. Sci China Life Sci 55(2):164–171

Liao H-L, Bonito G, Rojas JA, Hameed K, Wu S, Schadt CW et al (2019) Fungal endophytes of Populus trichocarpa alter host phenotype, gene expression, and rhizobiome composition. Mol Plant-Microbe Interact 32(7):853–864

Lòpez-Fernàndez S, Sonego P, Moretto M, Pancher M, Engelen K, Pertot I, Campisano A (2015) Whole-genome comparative analysis of virulence genes unveils similarities and differences between endophytes and other symbiotic bacteria. Front Microbiol 6:419

Maron P-A, Ranjard L, Mougel C, Lemanceau P (2007) Metaproteomics: a new approach for studying functional microbial ecology. Microb Ecol 53(3):486–493

Martínez-García PM, Ruano-Rosa D, Schilirò E, Prieto P, Ramos C, Rodríguez-Palenzuela P, Mercado-Blanco J (2015) Complete genome sequence of Pseudomonas fluorescens strain PICF7, an indigenous root endophyte from olive (Olea europaea L.) and effective biocontrol agent against Verticillium dahliae. Stand Genomic Sci 10(1):1–7

Mishra S, Goyal D, Phurailatpam L (2021) Targeted 16S rRNA gene and ITS2 amplicon sequencing of leaf and spike tissues of Piper longum identifies new candidates for bioprospecting of bioactive compounds. Arch Microbiol 203(7):3851–3867

Mishra S, Bhardwaj P, Sharma S (2022) Metabolomic insights into endophyte-derived bioactive compounds. Front Microbiol 416

Molina LG, Cordenonsi da Fonseca G, Morais GL, de Oliveira LFV, Carvalho JB, Kulcheski FR, Margis R (2012) Metatranscriptomic analysis of small RNAs present in soybean deep sequencing libraries. Genet Mol Biol 35:292–303

Na R, Jiajia L, Dongliang Y, Yingzi P, Juan H, Xiong L et al (2016) Indentification of vincamine indole alkaloids producing endophytic fungi isolated from Nerium indicum, Apocynaceae. Microbiol Res 192:114–121

Nagarajan K, Ibrahim B, Ahmad Bawadikji A, Lim J-W, Tong W-Y, Leong C-R et al (2021) Recent developments in metabolomics studies of Endophytic fungi. Journal of Fungi 8(1):28

Oita S, Ibáñez A, Lutzoni F, Miadlikowska J, Geml J, Lewis LA et al (2021) Climate and seasonality drive the richness and composition of tropical fungal endophytes at a landscape scale. Commun Biol 4(1):1–11

Porzel A, Farag MA, Mülbradt J, Wessjohann LA (2014) Metabolite profiling and fingerprinting of Hypericum species: a comparison of MS and NMR metabolomics. Metabolomics 10(4):574–588

Premalatha K, Kalra A (2013) Molecular phylogenetic identification of endophytic fungi isolated from resinous and healthy wood of Aquilaria malaccensis, a red listed and highly exploited medicinal tree. Fungal Ecol 6(3):205–211

Qiang X, Weiss M, Kogel KH, Schäfer P (2012) Piriformospora indica—a mutualistic basidiomycete with an exceptionally large plant host range. Mol Plant Pathol 13(5):508–518

Rai N, Singh AK, Singh SK, Gaurishankar B, Kamble SC, Mishra P et al (2020) Recent technological advancements in stem cell research for targeted therapeutics. Drug Deliv Transl Res 10(4):1147–1169

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S et al (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology 12(3):139–159

Rai N, Gupta P, Keshri PK, Verma A, Mishra P, Kumar D et al (2022a) Fungal endophytes: an accessible source of bioactive compounds with potential anticancer activity. Appl Biochem Biotechnol 194(7):3296–3319

Rai N, Keshri PK, Gupta P, Verma A, Kamble SC, Singh SK, Gautam V (2022b) Bioprospecting of fungal endophytes from Oroxylum indicum (L.) Kurz with antioxidant and cytotoxic activity. PLoS One 17(3):e0264673

Rai N, Gupta P, Verma A, Singh SK, Gautam V (2023a) Isolation and characterization of N-(2-hydroxyethyl) hexadecanamide from Colletotrichum gloeosporioides with apoptosis-inducing potential in breast cancer cells. Biofactors 49:663

Rai N, Gupta P, Verma A, Tiwari RK, Madhukar P, Kamble SC et al (2023b) Ethyl acetate extract of colletotrichum gloeosporioides promotes cytotoxicity and apoptosis in human breast cancer cells. ACS Omega 8(4):3768–3784

Ranjan A, Singh RK, Khare S, Tripathi R, Pandey RK, Singh AK et al (2019) Characterization and evaluation of mycosterol secreted from endophytic strain of Gymnema sylvestre for inhibition of α-glucosidase activity. Sci Rep 9(1):1–13

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2(4):404–416

Rouxel T, Grandaubert J, Hane JK, Hoede C, Van de Wouw AP, Couloux A et al (2011) Effector diversification within compartments of the Leptosphaeria maculans genome affected by repeat-induced point mutations. Nat Commun 2(1):1–10

Salvi P, Kumar G, Gandass N, Verma A, Rajarammohan S, Rai N, Gautam V (2022a) Antimicrobial potential of essential oils from aromatic plant Ocimum sp.; a comparative biochemical profiling and in-Silico analysis. Agronomy 12(3):627

Salvi P, Mahawar H, Agarrwal R, Gautam V, Deshmukh R (2022b) Advancement in the molecular perspective of plant-endophytic interaction to mitigate drought stress in plants. Front Microbiol 3407

Schardl CL, Young CA, Hesse U, Amyotte SG, Andreeva K, Calie PJ et al (2013) Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the Clavicipitaceae reveals dynamics of alkaloid loci. PLoS Genet 9(2):e1003323

Schneider T, Riedel K (2010) Environmental proteomics: analysis of structure and function of microbial communities. Proteomics 10(4):785–798

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T et al (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25(1):28–36

Sheibani-Tezerji R, Naveed M, Jehl M-A, Sessitsch A, Rattei T, Mitter BJF, i. m. (2015) The genomes of closely related Pantoea ananatis maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. Front. Microbiol 6:440

Shen M, Zhao D-K, Qiao Q, Liu L, Wang J-L, Cao G-H et al (2015) Identification of glutathione S-transferase (GST) genes from a dark septate endophytic fungus (Exophiala pisciphila) and their expression patterns under varied metals stress. PLoS One 10(4):e0123418

Shinozuka H, Hettiarachchige IK, Shinozuka M, Cogan NO, Spangenberg GC, Cocks BG et al (2017) Horizontal transfer of a ß-1, 6-glucanase gene from an ancestral species of fungal endophyte to a cool-season grass host. Sci Rep 7(1):1–11

Shrivastava N, Jiang L, Li P, Sharma AK, Luo X, Wu S et al (2018) Proteomic approach to understand the molecular physiology of symbiotic interaction between Piriformospora indica and Brassica napus. Sci Rep 8(1):1–13

Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Plant 132(2):199–208

Smith DJ, Burnham MK, Edwards J, Earl AJ, Turner G (1990) Cloning and heterologous expression of the penicillin biosynthetic gene cluster from Penicillium chrysogenum. Bio/Technology 8(1):39–41

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Molecul Biol Rev 67(4):491–502

Toju H, Yamamoto S, Sato H, Tanabe AS, Gilbert GS, Kadowaki K (2013) Community composition of root-associated fungi in a Q uercus-dominated temperate forest: "codominance" of mycorrhizal and root-endophytic fungi. Ecol Evol 3(5):1281–1293

Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs in plant-root fungal biomes. J R Soc Interface 13(116):20151097

Verma A, Gupta P, Rai N, Tiwari RK, Kumar A, Salvi P et al (2022) Assessment of biological activities of fungal endophytes derived bioactive compounds isolated from Amoora rohituka. J Fungi 8(3):285

Verma A, Rai N, Kamble SC, Mishra P, Barik S, Kumar R et al (2023) Beyond the synthetic drugs: fungal endophytes derived bioactive compounds in the management of neurodegenerative disorders. Tradit Med Neurl Health 82

Wang X, Zhang X, Liu L, Xiang M, Wang W, Sun X et al (2015) Genomic and transcriptomic analysis of the endophytic fungus Pestalotiopsis fici reveals its lifestyle and high potential for synthesis of natural products. BMC Genomics 16(1):1–13

Wang L, Ren L, Li C, Gao C, Liu X, Wang M, Luo Y (2019) Effects of endophytic fungi diversity in different coniferous species on the colonization of Sirex noctilio (hymenoptera: Siricidae). Sci Rep 9(1):1–11

Xu X-H, Su Z-Z, Wang C, Kubicek CP, Feng X-X, Mao L-J et al (2014) The rice endophyte Harpophora oryzae genome reveals evolution from a pathogen to a mutualistic endophyte. Sci Rep 4(1):1–9

Yadava PK, Bhuyan SK, Bandyopadhyay P (2015) Extraction of proteins for two-dimensional gel electrophoresis and proteomic analysis from an endophytic fungus

Yang Y, Zhao H, Barrero RA, Zhang B, Sun G, Wilson IW et al (2014) Genome sequencing and analysis of the paclitaxel-producing endophytic fungus Penicillium aurantiogriseum NRRL 62431. BMC Genomics 15(1):1–14

Yuan J, Zhang W, Sun K, Tang M-J, Chen P-X, Li X, Dai C-C (2019) Comparative transcriptomics and proteomics of Atractylodes lancea in response to endophytic fungus Gilmaniella sp. AL12 reveals regulation in plant metabolism. Front Microbiol 10:1208

Zimmerman NB, Vitousek PM (2012) Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc Natl Acad Sci 109(32):13022–13027

Zuccaro A, Lahrmann U, Güldener U, Langen G, Pfiffi S, Biedenkopf D et al (2011) Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont Piriformospora indica. PLoS Pathog 7(10):e1002290

Chapter 11
Untapped Bioactive Compounds from Endophytic Fungi with Potential Antioxidant Activity

Pranav Bhaskar, Divya Jain, and Reetesh Srivastava

Abstract Endophytic fungi (EF) have attracted a lot of attention over the past century because of their capacity to produce novel bioactive chemicals with a variety of biological properties, which are then used for medical, pharmacological, and agricultural purposes. EF maintain the ecological and physiological characteristics of the host plant by living inside the plant tissues without displaying any disease symptoms. Innovative lead chemicals created by EFs, such as paclitaxel and penicillin, cleared the path for the investigation of new bioactive compounds for industrial application. Despite this, not much research has been done in this important and special field. The current chapter is concerned with the importance of EFs in the synthesis of new bioactive metabolites with a range of biological activities, such as antibacterial, antiviral, antifungal, antiprotozoal, antiparasitic, antioxidant, immunosuppressive, and anticancer activities. Special attention is given to the antioxidant activity of the metabolites produced by EFs in light of the significant application of these substances as potential therapeutics for major, life-threatening illnesses, thereby driving the necessity to escalate research into novel, more potent, and reasonably priced antioxidants medications. The potential obstacles in the large-scale

Pranav Bhaskar and Divya Jain contributed equally with all other contributors.

P. Bhaskar (✉)
Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, VA, USA

Integrative Centre for Research & Innovation in Biology, Braj Mohan Jha Science Research & Innovation Foundation, Chandigarh, India

D. Jain
Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali, Rajasthan, India

Integrative Centre for Research & Innovation in Biology, Braj Mohan Jha Science Research & Innovation Foundation, Chandigarh, India

R. Srivastava
Faculty of Agricultural Sciences, Institute of Applied Sciences & Humanities, GLA University, Mathura, UP, India

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bioprospecting in vitro production of antioxidants were also highlighted in this chapter, as were the strategies to overcome these hurdles.

Keywords Endophytes · Phytochemicals · Antioxidants · Secondary metabolites · Symbiosis · Bioactive compounds · Endophytic fungi · Biotransformation

Abbreviations

BGCs	Biosynthetic gene clusters
CPT	Camptothecin
CRISPR	Clustered regularly interspaced short palindromic repeats
DAM	Deacetylmycoepoxydiene
DPPH	2,2-diphenyl-1-picrylhydrazyl
EF	Endophytic fungus
EFs	Endophytic fungi
GGPP	Geranylgeranyl diphosphate
GlcNAc	N-acetyl-D-glucosamine
HDACs	Histone deacetylases
IPP	Isopentenyl pyrophosphate
LC	Liquid chromatography
LC-MS	Liquid chromatography mass spectroscopy
NPs	Natural products
OSMAC	One strain-many compounds
ROS	Reactive oxygen species
SABA	Suberohydoxamic acid
SAHA	Suberoylanilide hydroxamic acid
SM	Secondary metabolite
VOC	Volatile organic compounds

1 Introduction

Endophytes are microorganisms that inhabit the internal tissues of living plants without inflicting any apparent harm (Bacon and White 2000) but can become dangerous as the host senesces (Rodriguez and Redman 2008). They develop parasitic or symbiotic associations with host plants throughout their life cycle. Endophytes appear to be able to imitate the chemistry of their hosts, thanks to horizontal gene transfer from the host plant. Most endophytes are spread horizontally by airborne spores to their host plants. Some endophytes, however, might be vertically transferred on to succeeding plant generations through seeds (Hartley and Gange 2009). Once they enter into the host tissue, they remain dormant for the host plant's life or a more extended period until environmental conditions favor the fungus or the host's

ontogenetic condition changes to benefit the fungus (Sieber 2007). Endophytes synthesize chemicals inside the plants (Nisa et al. 2015). The possibility of utilizing endophytes as an alternate and continual source of these molecules has increased because they can synthesize bioactive metabolites compared to the hosts. Recent advancements in screening methods have opened up the immense potential of endophytes as a producer of bioactive chemicals with prospective medical or agronomic applications (Zhang et al. 2006). Thus, there is enormous potential for discovering novel natural compounds from previously understudied endophytic microbes that live in various niches and environments (Guo et al. 2008).

Endophytic fungi (EFs) are prospective sources of natural compounds. They are the most thoroughly explored group among endophytic microorganisms for biotechnological research and applications. Hundreds of new EFs have been isolated, identified, and systematically researched in the past ten years. They produce more than 200 distinct bioactive substances with a wide range of biological functions and structural variations. They are more efficient and discerning in modern biology owing to their distinctiveness. Chemists discovered the importance of fungi as a great source of bioactive chemicals post-penicillin discovery from *Penicillium notatum* in 1928. With the discovery of widely used prescription drugs like penicillin from the fungus *P. notatum*, griseofulvin from the fungus *P. griseofulvum*, lovastatin from the fungus *Aspergillus terreus*, and cyclosporin A from the fungus *Tolypocladium inflatum*, researchers realized that fungi are another alternative natural source for the isolation of novel chemicals with potential for medicinal use. It contains a broad range of bioactive substances that can be converted into various pharmacological activities, including antibacterial, anticancer, anti-inflammatory, antidiabetic, antitubercular, and immunosuppressive agents. As a result, scientists have considered EFs for the past 20 years, examining their diversity, interactions with hosts, and potential bioactive byproducts. Owing to their precision, affordability, and low toxicity, these bioactive molecules significantly impact modern medicine and have substantial therapeutic potential for severe diseases such as cancer, diabetes, and neurological disorders. These EFs may be more effective for humans with the aid of contemporary technologies, such as genetic engineering and microbial fermentation.

2 Diversity and Distribution of Fungal Endophytes in Nature

The geographic distribution of the biota based on biodiversity across several levels, including species, function, and phylogenesis, is described as a continuous gradient distribution of attributes. Even though little is known about the mechanisms of biodiversity growth in a particular geographic area, fungi mediate a variety of processes and may be quite important in their habitats (Vandenkoornhuyse et al. 2002). A variety of endophytic bacteria can live naturally in a space and time-supportive refuge created by many layers of plant tissue. According to a well-established estimate of a 1:4 or 1:5 ratio of vascular plants to fungal strains, there may be more than one million EF strains yet to be found (Sun and Guo 2012). These ratios are skewed

because of our incomplete knowledge of the diversity of EFs and the inability of rare species and those categorized as non-sporulating, non-cultivable, or aseptic to be thoroughly studied in current laboratory isolation and fermentation efforts (Alvear-Daza et al. 2021).

The Clavicipitaceous/Balansiaceous group (C-group), and the non-Clavicipitaceous/non-Balansiaceous group are the two categories into which EF communities can be categorized. Based on the reproductive pattern and host occurrence, these categories can be divided (NC-group). C-group EFs propagate vertically from parents to offspring by infecting host seeds, which then infect host ovules. The target tissues for their colonization are active rhizomes and shoots of the host plants, and the host range is restricted to grass species (Poaceae). According to Carroll (1988), C-group species are prototypical obligatory endophytes that release protective or supportive bioactive substances to either defend their hosts from attacks by herbivores or to aid the hosts in thriving in situations that are prone to drought (Poveda 2021). Non-grass-host-related NC-group EFs (Ascomycota and Basidiomycota) are found all over the world, from the tropics to the poles, and they have a wide variety of hosts, including nonvascular, vascular, and woody plant groups. By creating spores or conidia, which aid in horizontal proliferation, or the induction of symbiosis, they spread sexually or asexually. These EFs are not directly connected with the host plants because of their capacity to exist in a dormant state until they notice the chemical changes brought on by host plants suffering wounds, injuries, or other environmental stresses (Mishra et al. 2021a, b). The NC colonization of roots or the rhizosphere is extensive, planned, systematic, intercellular, and intracellular, in contrast to the aerial organs, which are frequently localized, limited, and largely intercellular. Several mycobiomes from the NC-endophytic category include dark septate mycobiota, *Piriformospora indica*, and *Fusarium* species (Schulz et al. 2002).

3 Fungal Endophyte Interactions with Host Plants

From the Arctic to tropical rain forests, practically every plant species examined to date has been confirmed to possess endophytes (Tan and Zou 2001). According to the discovery of plant-associated microorganisms in fossilized plant organs like stems or leaves, host-endophyte connections may have existed since the emergence of higher plants on Earth millions of years ago (Gutierrez et al. 2012). Endophytes have been found in a variety of plant tissues, including leaves, stems, roots, fruits, seeds, and bark (Zhang et al. 2006). Although it has been hypothesized that bacteria and fungi are the most prevalent plant-associated microorganisms, endophytic fungi appear to be more numerous than their bacterial counterparts (Spiteller 2015). On Earth, there are more than 300,000 different species of higher plants. According to certain theories, a single plant species may harbor a large number of endophytic bacterial and fungal strains (Kharwar et al. 2011). Therefore, it is clear that endophytes constitute a crucial component of microbial biodiversity when considering the intricate community of endophytic species that exist in plants (Zhang et al. 2006).

It has been hypothesized that endophytes that colonize the interior organs of plants receive nourishment and/or defense from their host plant. In exchange, endophytes may increase their hosts' resistance to various abiotic challenges including low pH, heavy metals, or temperature fluctuations (Jia et al. 2016; Tan and Zou 2001). For instance, endophyte-infected grasses have been demonstrated to be significantly more resilient to environmental pressures than their endophyte-free counterparts (Zhang et al. 2006). In addition, reports suggest that endophytes may aid in the growth of plants. Some endophytes can create phytohormones like indole-3-acetic acid and/or a variety of chemicals that aid in plant growth, including vitamins and siderophores, which may contribute to this impact (Tan and Zou 2001; Zhang et al. 2006). Additionally, endophytes have been shown to improve the uptake of nutrients like nitrogen and phosphorus, demonstrating the critical function that these microbial communities play in the health and environmental adaptability of plants (Jia et al. 2016; Ludwig-Müller 2015).

Fungi can colonize the intercellular or intracellular sections of the same plants, but systematic and extensive colonization is more likely to occur in the roots than in the aerial leaves or stems of plants. To maintain the correct reproduction of EFs in aerial organs, colonization in aerial organs predominantly relies on the host's apoplastic fluid (Schulz et al. 2002). Depending on the physiological state or particular conditions that host plants undergo, fungi create three forms of interactions with hosts: beneficial symbiotic (mutualistic endophytes), non-beneficial commensalistic (virulent endophytes), and pathogenic (virulent pathogens) (Fig. 11.1). Fungal strains can raise, have no apparent effects on, or decline host fitness following these three means of association (Kogel et al. 2006).

3.1 Symbiotic Relationship with Host Plants

In a symbiotic relationship, EFs and host plants both experience this advantageous mutualistic continuity and finally succeed in terms of evolution and ecology (Jia et al. 2016). According to several studies, EFs change the way that host plants use nutrients, improve their ability to withstand metals and drought, and boost growth (MejÃa et al. 2014; Poveda 2021). They also improve the effectiveness of the host plants' defenses against pests, herbivorous animals, and infections from pathogenic microbes (Cui et al. 2021). Due to these advantages, host plants offer symbiotic housings and extra accommodations for proliferation, such as a sufficient source of nutrients and guard, to help fungi safely continue their life cycles all through colonization (Fig. 11.1a). Although the precise mechanism of the collaboration between EFs and plants has yet to be determined, EFs may provide host plants with these potential benefits in many different ways. Enhancing the plant's immune system by generating a large number of bioactive SMs as defense mechanisms is one of these methods. The host plant's physiological alterations brought on by an increase in SMs are thought to further activate the plant defense system (Poveda et al. 2020a).

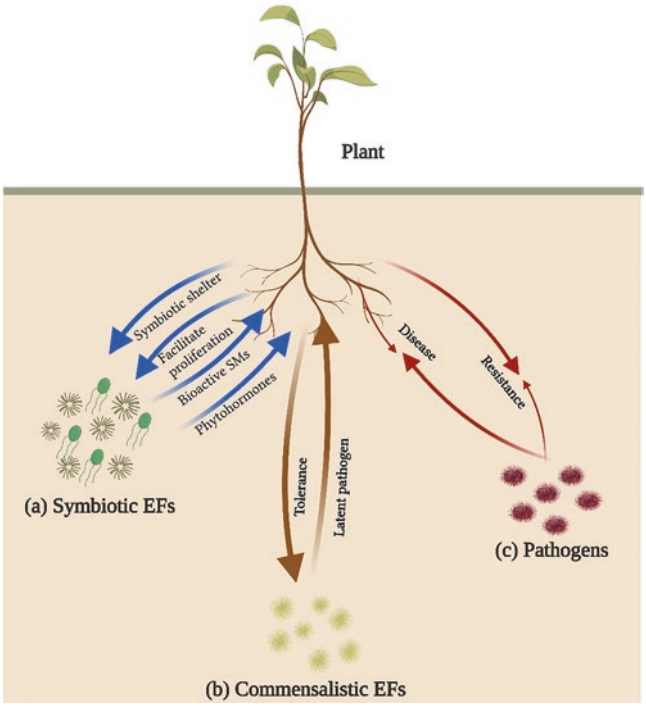


Fig. 11.1 A schematic representation of relationship between plant and endophytic fungi (EF) including interaction between (**a**) symbiotic EFs and plants, (**b**) commensalistic EFs and plants, and (**c**) pathogens and plants. [Created with BioRender]

According to numerous studies comparing the impact of pathogenic and endophytic fungi on host plants, EFs help host plants' defensive mechanisms by producing bioactive SMs and herbicidal compounds in their hosts (Figueiredo et al. 2008; Kaur and Kaur 2020; Rai et al. 2021). According to reports, endophytes secrete bioactive secondary metabolites that increase host plant resilience to herbivores and hinder the colonization of microbial plant diseases (Gutierrez et al. 2012; Tan and Zou 2001). This increases the host plant's tolerance to biotic stressors. For instance, it has been shown that the presence of fungal endophytes on the ryegrass *Lolium perenne* increased the production of hydroxycinnamic acids and glycosylated flavonoids, including chlorogenic acid, which is the primary chemical responsible for the antioxidant activity of the plant extract (Ludwig-Müller 2015; Qawasmeh et al. 2012). In addition, several antimicrobial metabolites generated by plant-associated microorganisms have been found and/or identified, including ergot alkaloids, aflatoxin, patulin, and trichothecenes (Spiteller 2015; Zhang et al. 2006). These substances are thought to be crucial in the competition between endophytes and other microbes in the host plant's microenvironment.

During the rapid destruction of *Arabidopsis thaliana* plants by *Fusarium oxysporum*, an in vitro tripartite interaction investigation demonstrated that

Paraconiothyrium variabile dramatically reduced plant death by up to 85% (Bärenstrauch et al. 2020). The following experiments supported this theory. Big bluegrass (*Poa ampla* Merr.) ethanol extracts were tested on mosquito larvae, and the outcomes showed that only extracts from plants that had been inoculated with *Neotyphodium typhonium* were successful in repelling the insect, while extracts from plants that hadn't been exposed to the fungus were not active (Ju et al. 1998). An additional method that an EF benefits the host plant is by promoting growth of the plant through the production and provision of plant hormones such as auxins, cytokines, and gibberellins, supported by the identification of a similar mechanism for the manufacture of gibberellin in higher plants and fungi (Kumar et al. 2013). Studies have shown that endophytes can enhance plants' growth and reproduction by interacting with ethylene-targeted transcription factors or by enhancing their defense mechanisms, similar to what ethylene and jasmonic acid do (Di et al. 2016; Forni et al. 2017; Van der Ent et al. 2009; Yang et al. 2019). An EF called *Neotyphodium* settles in tall fescue ryegrass, where it offers the host plants resilience and protection in harsh environments. In exchange, ryegrass supplies the conditions for the growth of fungi through ryegrass seeds that are infected with fungal hyphae (Tan and Zou 2001).

3.2 Commensalistic Association with the Host

EFs quickly sporulate and interact with host plants in a commensalistic or latent pathogenic association, by means of or without any appreciable positive benefits on plant physiology (Hiruma et al. 2016). According to studies, these endophytes live in hosts as latent pathogens under normal circumstances (Cui et al. 2021; Górzyńska et al. 2018; Photita et al. 2004; Zakaria et al. 2016; Fig. 11.1b), whereas other studies have described various species and genera of EFs from host plants as active pathogens under unusual physiological stresses (Photita et al. 2004; Fig. 11.1c). *Fusarium*, *Cladosporium*, *Nigrospora*, *Colletotrichum*, *Periconiella*, *Cordana*, *Verticillium*, *Curvularia*, *Deightoniella*, *Phoma*, and *Guignardia* are examples of endophytic fungi that have been discovered as the potential pathogens (Cui et al. 2021; Photita et al. 2004).

Long before the onset of disease symptoms, these EFs remain dormant or latent in the host plants' tissue. The dormancy phase is crucial in these situations because it establishes when the fungus is virulent as a pathogen and when it is innocuous as an endophyte. During the virulent phase, EFs manifest clear symptoms and alter the physiology and morphology of the host plant in unfavorable circumstances. Malnutrition, disruption of the ontogenetic state (Sieber 2007), biotic stresses, abrupt climate changes (like high temperatures and high humidity), and senescence are just a few of the hostile circumstances that cause EFs to lose their equilibrium with their hosts and transform from latent to active virulent pathogens, although there are no obvious disease symptoms before transition (Photita et al. 2004; Poveda et al. 2020b). Effectors, enzymes, nutrient status, and secondary metabolites are

thought to work in concert to decide whether an interaction is pathogenic or endophytic (Di et al. 2016; Hiruma et al. 2016; Poveda et al. 2020b).

4 Endophytic Fungi-Mediated Biotransformation of Secondary Metabolites

Less adaptable microorganisms are less likely to get sufficient resources and proliferate in abundance when the available resources are insufficient to meet the needs of the community. As a result, the former might not be able to withstand challenging circumstances. Organisms have developed two distinct competing survival strategies. One tactic is to create allelochemicals that stop their rivals from growing and eliminate whatever negative side effects they might have left behind (Poveda 2021). The other is to synthesize allelochemicals that aid in the establishment of relationships between their producers and symbiotic hosts or symbionts. Even in severely unfavorable settings, both partners can survive and reproduce securely, thanks to these symbiotic interactions (Macías-Rubalcava et al. 2014). This symbiotic orientation of EFs and their plant hosts results into allowing each EF to accumulate in a certain eukaryotic host group, under the notion of enduring coevolution in biotic communities (Ji et al. 2009).

Biotransformation describes unique chemical changes or alterations in molecules caused by biocatalysts or biotic (microbial, plant, or animal) systems (Smitha et al. 2017; Kozłowska et al. 2018). In addition to chemical processes (including oxidation, reduction, hydrogenation, hydrolysis, hydroxylation, and glycosyl conjugation), bioconversion may also entail stereoselective procedures that allow the creation of enantiomerically different molecules. Microorganisms, especially endophytes, are chosen for bioconversion because of their high surface-to-volume ratio, rapid growing rate and faster metabolism, and lower toxicity and endurance (the latter of which is crucial for the food and pharmaceutical industries) (Hegazy et al. 2015; Yildirim et al. 2020). There have been reports of biotransformation reactions being carried out by both bacterial and fungal endophytes (Ekiz et al. 2018; Tian et al. 2021).

The majority of these allelochemicals are SMs, a class of typically low-molecular-weight and strikingly diverse molecules that were previously thought to have no apparent physiological impact on the growth, evolution, and reproductivity of the organisms that synthesize them (Fox and Howlett 2008; Yu and Keller 2005). Volatile organic compounds (VOCs), a diverse family of chemicals, allow their producers, such as plants and microorganisms, to defend themselves from pest or pathogen attacks or to send warnings intra- or interspecifically during such attacks (Poveda 2021). It has been shown that these natural metabolites play significant functions in the growth and development of organisms in unintended ways despite the term “secondary” previously being used to denote their “inessentiality” (Deepika et al. 2016). By limiting competition from other species, SMs act as agents to aid

endophytic fungi in competing and surviving under challenging circumstances. Additionally, they might injure members of the same species. Examples of these SMs include those that aid organisms in accessing scarce resources, surviving in a particular hostile niche, and impeding rivals (Xie et al. 2019). For instance, thioridazine, a phenothiazine neuroleptic medication, underwent stereoselective biotransformation by some endophytic fungi, which includes *Glomerella cingulata*, *Phomopsis* sp., *Diaporthe phaseolorum*, and *Aspergillus fumigatus* (Borges et al. 2007). By hydroxylating cyclocanthogenol, the EF *Alternaria eureka* 1E1BL1 serves as a biocatalyst (Ekiz et al. 2018). In a different investigation, it was shown that the uncommon chemical ginsenoside Rg3 can be produced by the endophytic bacterium *Flavobacterium* sp. from *Panax ginseng* (Fu 2019). Artemisinic acid, the precursor to the antimalarial medicine artemisinin, has recently been shown to undergo biotransformation by the fungus *Penicillium oxalicum*. Both human colon cancer cell lines and promyelocytic leukemia cell lines are significantly cytotoxically affected by these metabolites (Tian et al. 2021).

Endophytes have received a lot of attention as possible lead structures for drug discovery because of their ecological significance as a rich source of secondary metabolites with distinct biological activity (Kaur and Kaur 2020; Ludwig-Müller 2015). At the present, terpenoids, alkaloids, peptides, and polyketides are only a few of the many chemically unique secondary metabolites from endophytes that have been found and identified (Spiteller 2015). The growing number of patents that have been registered in the last ten years explaining the possible uses of these fungi in many fields, including the manufacture of bioactive natural products (Gokhale et al. 2017), serves as another example of the impact of endophytes.

5 Endophytic Fungi Are a Natural Source of Antioxidants

Reactive oxygen species (ROS, such as O^{2-} and OH^{-}) and free radical-meditated reactions have been shown to damage biomolecules (such as lipids, proteins, and DNA) through oxidation, leading to conditions like aging, coronary heart disease, diabetes, cancer, atherosclerosis, Alzheimer's, and other neurodegenerative diseases (Chen et al. 2018a, b; Collin 2019; Finkel and Holbrook 2000; Hensley et al. 2000; Sharifi-Rad et al. 2020). As free radical scavengers, antioxidants—which also include vitamins, minerals, and enzymes—are the substances that safeguard cells from oxidative damage by preventing or lowering ROS and free radicals. When pathologies or diseases occur, these free radicals are reduced by cellular processes that act to impede normal structure and function. Antioxidant medicines are used to treat, prevent, and combat these diseases, and these treatments are highly efficient against ROS-related diseases. It has been suggested that ROS strengthens the immune system by facilitating cell signaling. Antioxidant substances are used for good in many industries, including the food, pharmaceutical, and agricultural sectors. Despite the health risks linked to oxidative stress, there is a significant need for safer, more effective, and affordable natural antioxidants. Numerous investigations

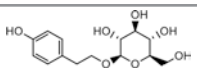
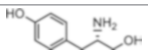
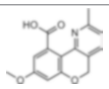
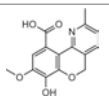
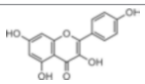
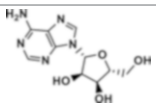
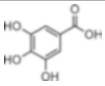
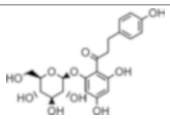
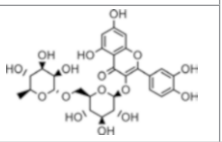
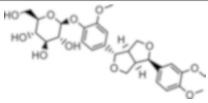
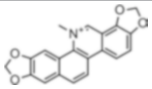
have shown that compounds with antioxidant activity include flavonoids, phenolic acids, phenylpropanoids, tannins, melanin, and lignin (Patipong et al. 2019; Smith et al. 2015). There is strong evidence that the production of various antioxidant chemicals by EFs is what gives host plants the ability to withstand stress. After 5 minutes of treatment, the endophyte *Fusarium oxysporum* from the leaves of *Otoba gracilipes* demonstrated antioxidant activity with a maximum scavenging effect of 51.5% on 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Caicedo et al. 2019). Among other substances, flavonoids and phenolics have been demonstrated to have very potent antioxidant activities (Liu and Liu 2018). *Alternaria alternata* AE1, an EF isolated from *Azadirachta indica*, was recently the subject of investigations that demonstrated it produced secondary metabolites with strong antioxidant characteristics (Chatterjee et al. 2019). Investigation of new chemicals from fungal endophytes offers an alternative source because many antioxidants are not approved for clinical use due to health concerns. For instance, the flavonoid kaempferol is produced by the endophytic *Mucor fragilis* fungus in the host plant *Sinopodophyllum hexandrum* (Huang et al. 2014). Some of the antioxidants released by endophytic fungi species are listed in Table 11.1.

6 Challenges in Endophytic-Fungi-Based Large-Scale Production of Antioxidants

When new pharmacological compounds with complex implications are discovered through the bioprospecting of EF, the pharmaceutical sector could be greatly affected. However, little is understood about plant-endophyte dynamics, poor production, and yield loss since subculturing and scaling-up cultures constitute some significant bottlenecks. Global trends, taking into account the dwindling supply of antibiotic arsenals, are defined by rising demand and consistent supply of innovative, high-value metabolites. Less knowledge about EF interactions with other bacteria further hinders endophyte utility, even though the plant-endophyte relationship is still poorly known. The use of tissue culture techniques has declined as a result of genetic instability in plant tissue culture and slow fungal development (Charlwood and Rhodes 1990). In this sense, fermentation technologies offer a compelling method for obtaining valuable compounds from fungi. These techniques have specific advantages in terms of lower costs, quick development, and optimum growth conditions for the successful biosynthesis of the desired SMs as well as sustainable yield.

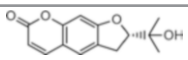
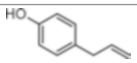
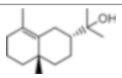
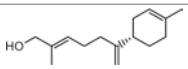
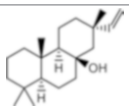
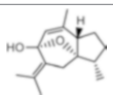
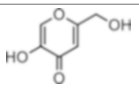
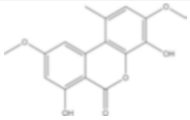
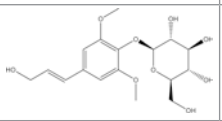
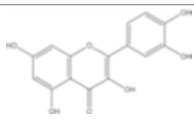
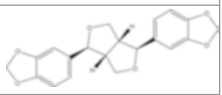
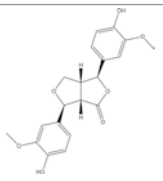
The procedure is complicated by the factors in increased operations, such as medium viscosity, pH, temperature, oxygen solubility, time of cultivation, their optimization, and others. For the large-scale targeted synthesis of a desired metabolite, the culture conditions must be appropriately tuned and controlled (Pan et al. 2019). Although there has been very little documented research on endophyte chassis, genetic manipulation approaches yet need further refinement for the activation

Table 11.1 Compounds having antioxidant properties isolated from fungal endophytes

Fungal endophytes	Host plants	Compounds	Structure	References
Phialocephala fortinii	*Rhodiola rosea*	Salidroside		Cui et al. (2016)
		p-tyrosol		
Phomopsis sp.	*Rhizophora stylosa*	Phochrodine C		Chen et al. (2018a, b)
		Phochrodine D		
Mucor fragilis	*Sinopodophyllum hexandrum*	Kaempferol		Huang et al. (2014)
Fusarium chlamydosporum	*Tylophora indica*			Chaturvedi et al. (2014)
Penicillium sp. YY-20	*Ginkgo biloba*	Adenosine		Yuan et al. (2014)
Fusarium sp.	*Fritillaria unibracteata*	Gallic acid		F. Pan et al. (2017)
		Phloridzin		
		Rutin		
Colletotrichum gloeosporioides	*Forsythia suspensa*	Phillyrin		Zhang et al. (2012) and Chen et al. (2016)
Fusarium proliferatum	*Macleaya cordata*	Sanguinarine		Wang et al. (2014)
Pseudocercospora sp.	*Elaeocarpus sylvestris*	Terreic acid		Prihantini and Tachibana (2017)
		6-Methylsalicylic acid		

(continued)

Table 11.1 (continued)

Fungal endophytes	Host plants	Compounds	Structure	References
Fusarium sp. ZZF41	*Kandelia candel*	Marmesin		Huang et al. (2010)
Nigrospora MFLUCC16–0605	*Ocimum basilicum*	Chavicol		Atiphasaworn et al. (2017)
		10-epi-γ-eudesmol		
		Z-lanceol		
		Nezukol		
		Curcumenol		
Aspergillus minisclerotigenes	*Mangifera casturi* Kosterm	Dihydropyran		Nuraini et al. (2019)
Aspergillus oryzae		Kojic acid		
Cephalosporin sp.	*Trachelospermum jasminoides*	Graphislactone-A		Song et al. (2005)
Pestalotiopsis microspora	*Terminalia morobensis*			
Xylaria sp.	*Scapania verrucosa*	Syringin (eleutheroside B)		Li et al. (2016)
Annulohypoxylon ilanense	*Cinnamomum cassia*	Quercetin		Nicoletti and Fiorentino (2015)
		Sesamin		
		4-ketopinoresinol		

of BGCs for enhanced production. Combinational synthesis of complex metabolites draws attention to related issues that can be solved by using elicitors to boost metabolite production along a specific metabolic pathway. The intricate structure of

metabolites and undesirable pathway intermediates, which limit enzyme action, are further difficulties. The negative impacts of endophytic fungi in the management of diseases are currently a cause for worry. For instance, because endophytes are thought of as latent pathogens capable of generating diseases, they may be harmful to the host plant and cause infection (Eaton et al. 2011). To prevent negative consequences and improve biocontrol techniques, it is essential to have a thorough awareness of the risk factors connected to EFs.

6.1 Unculturable EFs

The majority of endophytic fungi are unculturable under in vitro conditions (Staley and Konopka 1985). The dearth of knowledge of the precise growth needs (culture media, pH, temperature, and growth rate) of these bacteria is the main cause of their unculturability. However, by using the proper growth medium, reducing the inoculum amount, and allowing for longer incubation periods of up to two to three months, the wide variety of the bacterial and fungal communities acquired in culture might be amplified (Davis et al. 2005). The search for ideal culture media and growth conditions to guarantee the retrieval of a variety of culturable endophytes is a frequently overlooked facet of endophyte research. In other words, the inability of many common culture mediums to accurately mimic the in planta environment of endophytes presents a considerable barrier to the ability to cultivate native endophytes. Using a variety of culture media (Czapek Dox, malt extract, corn meal extract, Sabouraud dextrose, and potato dextrose media), Murphy et al. (2015) examined the advent of root EFs from *Hordeum murinum* and discovered that the variety of the isolated EFs and their biomass are significantly influenced by the type of media used. The bulk of experimental research, however, certainly fall short of isolating the largest number of the potential EFs present in host tissues because they do not employ different and diverse mediums for endophyte isolation.

Furthermore, several other problems make it difficult to characterize EF isolates with accuracy. First of all, it can be challenging to distinguish between endophytic and epiphytic fungi and tissues (Sessitsch et al. 2012). Second, the numerous stages during culture-dependent techniques require a lot of time and resources, which places significant limitations on the ability to find bioactive chemicals in EFs. Third, the ambiguity and misunderstanding surrounding fungal nomenclature might cause contradicting reports in the literature by causing inaccurate taxonomic and species identification. In the past, naming species relied on physical characteristics including the size and form of conidia; the presence or absence of setae, acervuli, and/or appressoria; as well as other elements like colony growth, color, and texture (Hyde et al. 2009).

Low sensitivity of (plant or microbial) metabolite is another factor to take into account. False-negative results from detection methods can prevent the discovery of promising endophytic bacteria. For instance, one analysis using the HPLC technique revealed that not any of the EFs isolated from the black pepper blooming

plant (*Piper nigrum*) produced piperine, the alkaloid chemicals in black pepper that give it its pharmacological qualities and biting quality. However, reanalysis of the same EF using high-sensitive LC-MS/MS methods showed that *Mycosphaerella* sp.-related fungi were responsible for producing the plant metabolite (Chithra et al. 2014).

6.2 Inadequate and Unstable Yields

Many endophytes display substantial disparity in the production of the "plant" metabolites that they harvest. The formation of the natural products of EFs, especially SMs, requires the induction of stimulus from host plants or symbiosis. In artificial axenic culture, EFs might not be able to create the same substances they can in a symbiotic continuum due to the absence of these plant-mediated stimuli or signals. Under axenic monoculture conditions, attenuation, a phenomenon that results from the sequential subculturing of fungal isolates, leads to the decline in the SM production of the successfully isolated and cultured EFs and has an extensive negative influence on the impending commercial production efforts (Cánovas et al. 2017; Strobel and Daisy 2003). Some potential causes of this attenuation include the absence of host signals and/or biosynthetic precursors, the absence of other coexisting or stimulating microbial endophytes, the absence of favorable environmental conditions or selection pressures necessary to maintain metabolite production, and/or the silencing of the SM gene clusters. Li et al. (1998) confirmed the absence of a host stimulus as the cause of attenuation by stating that adding host extract to the fungal growth media could increase paclitaxel yield in attenuated *Periconia* cultures (from 118 to 350 ng/l). Furthermore, Kusari et al. (2014) found that the expression levels of BGCs encoding for SMs may be impacted by the buildup of mutations in the microbial genome throughout multiple rounds of subculturing. By the seventh generation of subculturing, the researchers discovered arbitrary, non-synonymous changes in the open reading frames of multiple *F. solani* (endophyte) genes, which led to a decline of the enzyme activity necessary for camptothecin (CPT) production. An intriguing case is presented in a recent study on CPT generation by an EF isolated from *Nothapodytes nimmoniana* (Mohinudeen et al. 2021). While the highest CPT-producing strain displayed severe attenuation upon subculturing in the axenic state (after an initial decline in CPT levels from 403 to 200 g/g dry weight), the second-highest CPT-producing strain (*Alternaria burnsii* NCIM1409) demonstrated sustained production of the metabolite even after 12 rounds of subculturing (Mohinudeen et al. 2021). The degradability of SMs extracted from target EFs must also be addressed. The in vitro or axenic cultured compounds that are taken out of a symbiotic continuum may be quite unstable. As a result, creating these essential natural components in an artificial medium is difficult. These results underline the importance of maintaining the strain and passage difficulties as well as suggest that interactions between the environment and either the plant host, and perhaps, other components could be essential for maintaining the

translation of the BGCs that produce desired metabolites. As a result, maintaining the "competency" of successfully isolated EFs in culture while monitoring their in vivo stimulation under quasi-natural circumstances is difficult.

6.3 Intricate Plant-Endophyte Cross Talk

The final result/physiological response of the holobiont is controlled by the interactions among plant endophyte and endophyte-endophyte under natural circumstances (Mishra et al. 2021a, b). When microorganisms are isolated from their host and cultivated in a monoculture environment (in vitro axenic monocultures), the natural communication network within plants is disturbed. The proportional participation of the two symbiotic partners for the generation of metabolites must be investigated because we currently possess a relatively inadequate knowledge of plant-endophyte cross-talk mechanisms.

7 Strategies for Bioprospecting EFs for In Vitro Production of Antioxidant

The target metabolite produced undesirable/low yields despite intensive attempts to isolate and select endophytes for high-value products. Even if new chemical compounds like camptothecin, paclitaxel, kaempferol, quercetin, podophyllotoxin, and huperzine A were discovered when EF were isolated, there are still significant production and commercialization constraints. Scientific instruments and methods are being developed to define the dynamics of host-EF interactions and increase the supply of pharmacologically significant compounds as the field of endophytes gains prominence in a variety of environmental applications. To successfully target and produce high yields of the required metabolites, co-integration of genetic/metabolomics strategies and bioprocess methods may be used.

7.1 Stimulation of Cryptic Pathway Genes in EFs by Genome Mining

To induce the expression of silent BGCs, several techniques have been devised. This is because EF can create a wide range of structurally diverse and physiologically active secondary metabolites. Strong bioinformatic techniques and the availability of the genomic sequences of numerous endophytic bacteria have made it possible to predict and identify potential genes involved in SM production. The microbial genes encoding for SMs are often organized in the genome and are known as "cryptic" BGCs due to the absence of functional information on the metabolites produced by

these clusters (Katz and Baltz 2016). Consequently, the genes that encode for SM are either suppressed or expressed at extremely low rates in a typical lab setting. However, scientists have shown that by using techniques like changing culture conditions, coculturing with other bacteria, adding certain elicitors, and genetic modifications, it is possible to make otherwise "cryptic" BCGs express more strongly or more actively.

7.2 EF Strain Improvement

Various techniques, such as mutagenesis (Zhou et al. 2005), genetic engineering (Wei 2012), genome shuffling (Zhang et al. 2018), and genome editing (Mishra 2019), could be used to genetically modify endophytes for increased output of metabolites. Expression of silent BGCs has been observed to be induced by several genetic engineering techniques, including the overexpression of transcription factors, promoter engineering, and the generation of gene knockouts. Various strain improvement strategies have been applied to get enhanced SM yield from EFs.

Metabolic Engineering of EFs

Researches on the genetic manipulation of EFs focus on the endophyte chassis to increase the production of specific metabolites. By adding important pathway genes via genetic transformation to increase the yield of high-value SMs in axenic cultures, genetic manipulation of EFs specifies potential future outcomes. Gene overexpression and genome reorganization with mutagenesis have both been studied concerning the genetic modification of taxol-biosynthesizing endophytes (Ahamed and Ahring 2011; El-Gendy et al. 2016). For a genetic change of the EF genome, induction of SMs, and screening of mutants, random mutagenesis techniques are employed (El-Gendy et al. 2016). Through the protoplast fusion of two strains—UL50-6 and UV40-19—taxol production was boosted (endophyte strain HDF-68), resulting in a 20–25% upsurge in taxol yield (Zhao et al. 2014). Deacetylmycoepoxydiene (DAM), an anticancer metabolite, is generated by the endophytic fungus *Phomopsis* sp. Wang et al. (2016) demonstrated this, and the genome shuffle of eight parental protoplasts produced a high-yielding strain (made >200-fold DAM) in the transgenic endophytic strain. Similar to this, earlier research on EF *Pestalotiopsis microspores* sought to unravel the protoplast transformation-based taxol synthesis route (Long et al. 1998). Extrachromosomal DNA was found in the transformants as a result of these surprising findings, pointing to a crucial role in growth and adaptation.

As a result of the commercialization and translational success of taxol, numerous studies on taxol-producing endophytes have been conducted, with the use of genetic manipulation defining a crucial stage in the endophyte machinery. In this course, a variety of methods for altering the endophyte's genome have been tried to intensify

the metabolic flow, either by restricting competitive pathways or by reorienting the supply of precursors, and strain improvement techniques that increase the yield of specified metabolites. In an interesting study of pathway restoration, the inhibition of the sterol biosynthesis pathway in *Alternaria alternata* TPF6 produced a consistent amount of taxadiene (Bian et al. 2017). The taxadiene-biosynthetic cluster was heterologously overexpressed in mutant *Escherichia coli* T2 and T4 by engineering the mevalonate pathway followed by an *Agrobacterium tumefaciens*-mediated transformation of the taxadiene-producing platform into *A. alternata* TPF6. The drastically increased taxol yield caused by the pathway restoration raises the possibility of using genetic engineering to increase the yield of metabolites. With the advent of the CRISPR-Cas system, when used to modify filamentous fungi's genomes, the potential for how natural products is made has transformed. The CRISPR-Cas9 tool attempts to transport the Cas9 gene direct DNA in cassettes and forms an active Cas9/guide RNA complex. By using the CRISPR-Cas genome manipulation method for the conversion of EF, it may be possible to increase the validation of BGCs and the production of specific metabolites.

Mutagenesis of EFs

Understanding and utilizing the metabolic potential of EFs to improve the production of high-value SMs is crucial if endophytes are to be exploited on a commercial scale. Strain enhancement procedures may be used to improve yield and other features, such as the use of nitrogen/carbon sources, changes in shape, and decrease in undesirable products, to address the problem of low-yielding endophyte strains (Fidan and Zhan 2019). In addition to genetic engineering, endophyte mutagenesis through protoplast fusion or random screening has many advantages and promising futures. Employing mutagens, either chemical (nitrosoguanidine, diethyl sulfate, ethyl methyl sulfonate) or physical (X-rays, γ-rays, microwave, etc.), changes in the genetic makeup of microbial strains were examined a specific relevant genotype using random approaches. Using two or more mutagens or one mutagen repeatedly can also cause mutations (Zhou et al. 2010). Although the process has to be characterized, endophytic fungal mutations result in regulatory gene alterations that modify phenotypic traits and increase the synthesis of secondary metabolites (Adrio and Demain 2006). Kai et al. (2009) detailed the mutagenesis in endophytes and the selection of hygromycin-resistant strains for increased taxol production. Mutagenesis in EFs is tried using mycelia, spores, or protoplasts and was found useful with exceptional success for the mutation of taxol-producing endophytes (Pan et al. 2017; Paramanantham et al. 2019). Although mutagenesis provides a prospective approach, endophyte mycelium mutation marks the drawback of genetic deviation in the progeny, and it is challenging to optimize situations for EF spore mutagenesis, signifying that protoplast fusion is the most effective means to trigger mutagenesis (Kai et al. 2009) highlighting the advantages of this approach in mutagenesis of EF strains and in increasing the yield of the targeted metabolites (Zhao et al. 2014; Zhou et al. 2005). These conditions included the right amount of time and culture

conditions. The amalgamation of two mutant protoplasts of *Nodulisporium sylviforme* led to the development of an enhanced strain of high-yielding taxol producer (HDF-68) (Zhao et al. 2014), pointing to mutagenesis as a potent method for yield improvement with some caveats.

7.3 Simulation and Optimization of the In Vitro Conditions

The dearth of information regarding the circumstances in which a BGC that encodes for SM is expressed is a significant barrier to the finding of bioactive substances originating from endophytes. Diverse microbial communities naturally coexist in the same plant endosphere niche, engaged in substantial cross talk between plant and endophyte as well as between endophyte and endophyte (Mishra et al. 2021a, b). As a result, one key factor in the in vitro cultured endophytes' failure to synthesize plant metabolites may be their axenic monoculture. Numerous studies have demonstrated the importance of interactions between microbes in starting or promoting the establishment of BGCs in endophytes. For instance, coculturing the endophytes *Fusarium tricinctum* and *Bacillus subtilis* significantly boosted the synthesis of enniatin A1 and B1 (Ola et al. 2013). Similar results were obtained when the taxol-producing endophytic fungus *Paraconiothyrium* SSM001 was cocultured with two other endophytic species from the genera *Alternaria* and *Phomopsis* (Soliman and Raizada 2013). Therefore, it could be necessary to coculture fungi with fungi and bacteria with bacteria, under in vitro conditions in order to simulate the microbe-microbe interactions that take place in plants. In addition to coculturing endophytic microorganisms, adding plant extracts to microbial cultures is another method for simulating the natural environment of endophytes. By virtue of the proper pH, cofactors, enzymes, etc., the addition of host plant extract gives the endophyte a "nearly native environment" that encourages the creation of the necessary chemical.

One strain-many compounds (OSMAC) are streamlined but effective method that entails comprehensive modification of various cultivation variables (such as growth media composition, pH, incubation time, optimum temperature, aeration, and addition of enzyme inhibitors) to allow the expression of numerous bioactive compounds from a single microbial strain (Bode et al. 2002). This approach has emphasized the amazing synthetic variability of endophytic strains and the variation in metabolic parameters seen with slight modifications in cultivation characteristics.

7.4 Use of Elicitors

Elicitors, or substances that cause the synthesis of SMs, can be classed as either biotic (such as microorganisms or extracts like chitin or pectin) or abiotic (such as heavy metals, UV radiation, salicylic acid, methyl jasmonate, etc.) (Thakur et al. 2019). It has been discovered that adding elicitors to microbe culture media causes them to express BGCs in an in vitro setting. For instance, the output of taxol was significantly increased by including sodium acetate as a precursor, copper sulfate as an inducer of oxidase activity, and salicylic acid as an elicitor to taxol (Qiao et al. 2017). Likewise, it was discovered that utilizing methyl jasmonate as an elicitor and optimizing fermentation conditions led to a 50- to 75-fold increase in the endophyte-mediated synthesis of the plant product CPT (Pu et al. 2013). A quick and clever technique to increase the production of the desired metabolite is to add the necessary precursor(s) to the axenic cultures of endophytes. Enhancing the endophyte-mediated synthesis of CPT and taxol has been done so with success. For instance, Venugopalan et al. (2016) showed that adding 0.5 mM tryptamine as a precursor and optimizing the fermentation conditions increased CPT synthesis by almost 150 times. According to Soliman and Riazada, (Soliman and Raizada 2013), adding the precursors, geranylgeranyl diphosphate (GGPP) and isopentenyl pyrophosphate (IPP), to the culture of the endophytic fungus *Paraconiothyrium* SSM001 increased the production of taxol by many folds.

Epigenetic modifiers are a particular class of elicitors that impact the degree of gene expression by changing the heterochromatin and euchromatin architecture. In fungi, the BGCs are heterochromatin present, and epigenetic mechanisms, such as DNA methylation and histone deacetylation, control the expression and silencing of genes (Pettit 2011). Epigenetic modifiers such as 5-aza-2′-deoxycytidine (azacytidine), hydralazine hydrochloride, suberoylanilide hydroxamic acid (SAHA), suberohydoxamic acid (SBHA), N-acetyl-D-glucosamine (GlcNAc), trichostatin A, valproic acid, etc., may instigate the translation of BGCs by preventing DNA methyltransferases and histone deacetylases (HDACs). These epigenetic modifiers are capable to start chromatin remodeling and activate BGCs for bioactive secondary metabolite production. To develop novel approaches for isolating secondary metabolites from fungi, a comprehensive understanding of SM biosynthesis and control must first be established (Venugopalan and Srivastava 2015). BGC-specific transcriptional upregulation, promoter exchange, or using inducible promoters for regulation of gene expression are potential methods for finding or isolating high-value metabolites. These methods can also be applied to EF to transform natural product networks.

7.5 Deep Learning and High-Throughput Approaches

Chemoinformatics, the discovery of anticancer and antibiotic drugs, and metabolomics have all benefited from the development of machine learning and deep learning techniques (Lusci et al. 2013; Mitchell 2014). These methods have been particularly helpful for exploring organic chemicals (Mitchell 2014), predicting bioactivity on the basis of chemical structure, and mapping BGC combinations to chemical groups. The next critical step would be the creation of chemoinformatics and bioactivity-focused informatics that support and complement bioprospecting. Research on systematic computational learning techniques for predicting chemical structural diversity from EFs based on integrated comparative metabolomics and chemical compound analysis, coupled with biotic interaction network analysis, could lead to the development of a model of correlations between planta biochemistry and plant microbiome ecology. These frameworks can also be changed to achieve certain goals. A different deep learning framework might focus on bioactivities (such antiviral, antifungal, antibacterial, anti-protozoan, or anticancer) or the most complex synthesis of structures, like list chemical forms, bonds, or chirality groups. Given that the strength of these techniques lies in their capacity to handle unidentified interactions, recent thinking on the subject holds that it is crucial to avoid reductionism (Martinez-Mayorga et al. 2020). Therefore, we suggest that researchers begin by becoming familiar with databases of encoded natural product chemical structures combined with libraries of synthetic organic chemistry and organismal metadata, particularly from habitat and metagenomic data.

But what about uncultivable EFs, considering that the idea that endophytes are more easily cultivated than plants is the driving force behind a lot of research on endophyte NPs (Gangadevi and Muthumary 2009)? Chemical structure prediction based on computational learning will be especially useful for uncultivatable endophytes to avoid the requirement for isolation and synthesis, but such methods can also focus the search for targets for subsequent experimental (and computational) unsilencing. Hidden or muted biosynthetic capacities seem to be the norm rather than the exception in plant microbiomes, according to the bioinformatic finding of BGCs. This important scientific issue has been addressed by the use of cocultivation, OSMAC experiments, heterologous expression experiments, high-throughput elicitor screening, transcription factor decoys, and in planta approaches. However, there hasn't been much of a concentrated attempt to use computational learning techniques to address this issue. This may come as a surprise given that tools for genome data mining are available to uncover various regulatory signaling processes, metabolic flux, metabolic pathway regulation, and holobiont metabolic relationships including pathway complementation. Computational learning techniques could make advantage of the training data that is already accessible from high-throughput elicitor or expression experiments, OSMAC arrays, as well as in planta or coculture holometabolic and holoregulomic data.

8 Conclusion and Future Outlook

Antioxidants are biological molecules that stop chemical compounds from oxidizing. Certain foods and plant materials have a high amount of antioxidants. Reactive oxygen species (ROS) and free radicals can induce pathological consequences that are detrimental and pronounced, such as DNA damage, cancer, and degenerative disorders like Alzheimer's disease. Antioxidants shield cells from these effects. The investigation of antioxidants in medicinal plants shows promise as an alternate remedy for treating human diseases caused by reactive oxygen species. Antioxidants are emerging as a potential and different natural biological therapy in contemporary medicine for the treatment of human ailments. To treat reactive oxygen species (ROS) linked to diseases like cancer, hypertension, cardiovascular diseases, neurodegenerative diseases, diabetes mellitus, atherosclerosis, rheumatoid arthritis, ischemia/reperfusion injury, and aging, new antioxidants from plants and microorganisms are effective and safe chemopreventive therapies. Pestacin, coumarin, isopestacin, phloroglucinol, salidroside, p-tyrosol, borneol, and rutin are few examples of molecules from EFs having antioxidant activity. These molecules are excellent at preventing damage from oxygen-derived free radicals and ROS. Even though research on antioxidants has been done, few of them have received approval for usage in clinical settings; as a result, the necessity for ongoing research for a unique and effective antioxidant became crucial.

Due to the significant potential for the compounds released by EF-host symbiotic interactions in sustainable agriculture and biomedicine, endogenous biology is currently attracting more and more attention. Secondary metabolites are crucial to facilitate multidimensional biochemical communication between EFs, host plants, and pathogens in their community. Several genetic mechanisms precisely regulate the production of SMs during symbiosis, including gene clustering, transcription factors, the alteration of the host's genetic makeup in the presence of EFs, and horizontal gene transfer (HGT) between EFs and plants. These control systems might have started to develop at the same time as EF-host symbiosis. The biological and ecological functions of endophytism, tremendous potential for the development of modern medications, sustainable agriculture, and industry and its underlying mechanisms recently gained a lot of research attention. Even while EF research is of interest to scientists, there are still a lot of obstacles to overcome in the next few decades. The current "omics" era's tools, such as genomics, epigenomics, transcriptomics, and proteomics, as well as their related meta-omics (metagenomics, metatranscriptomics, and metaproteomics), will be incredibly helpful in enlightening the murky areas of myco-endophytisms and in addressing the difficulties to disclose additional evidence on these symbionts and their relations within the core niches of host plants. Additionally, building new molecular models of these EF-host interactions will be more efficient and fruitful through concerted research involving omics techniques and other fields, such as combinatorial chemistry. For effectively discovering the concealed genes involved in the manufacture of SMs and novel chemicals in axenic culture, as well as for a deeper understanding of the endophyte-host

relationship in natural environments, extensive molecular research may still be required. Therefore, research on the bioprospecting of endophytic fungi as biological agents that will ensure sustained human health as well as the identification of the pharmacological roles of fungal endophytes can both benefit from the screening of fungal endophytes for potential bioactive metabolite synthesis.

References

Adrio JL, Demain AL (2006) Genetic improvement of processes yielding microbial products. FEMS Microbiol Rev 30(2):187–214. https://doi.org/10.1111/j.1574-6976.2005.00009.x

Ahamed A, Ahring BK (2011) Production of hydrocarbon compounds by endophytic fungi Gliocladium species grown on cellulose. Bioresour Technol 102(20):9718–9722. https://doi.org/10.1016/j.biortech.2011.07.073

Alvear-Daza JJ, García-Barco A, Osorio-Vargas P, Gutiérrez-Zapata HM, Sanabria J, Rengifo-Herrera JA (2021) Resistance and induction of viable but non culturable states (VBNC) during inactivation of E. Coli and Klebsiella pneumoniae by addition of H_2O_2 to natural well water under simulated solar irradiation. Water Res 188:116499. https://doi.org/10.1016/j.watres.2020.116499

Atiphasaworn P, Monggoot S, Gentekaki E, Brooks S, Pripdeevech P (2017) Antibacterial and antioxidant constituents of extracts of Endophytic fungi isolated from Ocimum basilicum var. thyrsiflora leaves. Curr Microbiol 74(10):1185–1193. https://doi.org/10.1007/s00284-017-1303-1

Bacon C, White J (2000) Microbial endophytes. In: Bacon C, White J (eds) Microbial endophytes. Dekker, pp 341–388

Bärenstrauch M, Mann S, Jacquemin C, Bibi S, Sylla O-K, Baudouin E, Buisson D, Prado S, Kunz C (2020) Molecular crosstalk between the endophyte Paraconiothyrium variabile and the phytopathogen Fusarium oxysporum – modulation of lipoxygenase activity and beauvericin production during the interaction. Fungal Genet Biol 139:103383. https://doi.org/10.1016/j.fgb.2020.103383

Bian G, Yuan Y, Tao H, Shi X, Zhong X, Han Y, Fu S, Fang C, Deng Z, Liu T (2017) Production of taxadiene by engineering of mevalonate pathway in Escherichia coli and endophytic fungus Alternaria alternata TPF6. Biotechnol J 12(4):1600697. https://doi.org/10.1002/biot.201600697

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. Chem Bio Chem 3(7):619. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9

Borges KB, Borges W, Pupo M, Bonato PS (2007) Endophytic fungi as models for the stereoselective biotransformation of thioridazine. Appl Microbiol Biotechnol 77:669–674. https://doi.org/10.1007/s00253-007-1171-x

Caicedo NH, Davalos AF, Puente PA, Rodríguez AY, Caicedo PA (2019) Antioxidant activity of exo-metabolites produced by Fusarium oxysporum: an endophytic fungus isolated from leaves of Otoba gracilipes. Microbiology Open 8(10):e903. https://doi.org/10.1002/mbo3.903

Cánovas D, Studt L, Marcos AT, Strauss J (2017) High-throughput format for the phenotyping of fungi on solid substrates. Sci Rep 7(1):4289. https://doi.org/10.1038/s41598-017-03598-9

Carroll G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69(1):2–9. https://doi.org/10.2307/1943154

Charlwood BV, Rhodes MJ (1990) Secondary products from plant tissue culture. Clarendon Press

Chatterjee S, Ghosh R, Mandal NC (2019) Production of bioactive compounds with bactericidal and antioxidant potential by endophytic fungus Alternaria alternata AE1 isolated from Azadirachta indica A. Juss. PLoS One 14(4):e0214744. https://doi.org/10.1371/journal.pone.0214744

Chaturvedi P, Gajbhiye S, Roy S, Dudhale R, Chowdhary A (2014) Determination of Kaempferol in extracts of Fusarium chlamydosporum, an endophyticfungi of Tylophora

indica (Asclepeadaceae) and its anti-microbial activity. IOSR 9(1):51–55. https://doi.org/10.9790/3008-09155155

Chen J, Chen Q, Yu F, Huang H, Li P, Zhu J, He X (2016) Comprehensive characterization and quantification of phillyrin in the fruits of Forsythia suspensa and its medicinal preparations by liquid chromatography-ion trap mass spectrometry. Acta Chromatogr 28(1):145–157. https://doi.org/10.1556/AChrom.28.2016.1.11

Chen H, Huang M, Li X, Liu L, Chen B, Wang J, Lin Y (2018a) Phochrodines A–D, first naturally occurring new chromenopyridines from mangrove entophytic fungus Phomopsis sp. 33#. Fitoterapia 124:103–107. https://doi.org/10.1016/j.fitote.2017.10.013

Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L (2018b) Reactive oxygen species: key regulators in vascular health and diseases. Br J Pharmacol 175(8):1279–1292. https://doi.org/10.1111/bph.13828

Chithra S, Jasim B, Anisha C, Mathew J, Radhakrishnan EK (2014) LC-MS/MS based identification of piperine production by endophytic Mycosphaerella sp. PF13 from piper nigrum. Appl Biochem Biotechnol 173(1):30–35. https://doi.org/10.1007/s12010-014-0832-3

Collin F (2019) Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. Int J Mol Sci 20(10):2407. https://doi.org/10.3390/ijms20102407

Cui J, Guo T, Chao J, Wang M, Wang J (2016) Potential of the Endophytic fungus Phialocephala fortinii Rac56 found in Rhodiola plants to produce Salidroside and p-Tyrosol. Molecules 21(4):502. https://doi.org/10.3390/molecules21040502

Cui R, Lu X, Chen X, Malik WA, Wang D, Wang J, Wang S, Guo L, Chen C, Wang X, Wang X, Dai M, Ye W (2021) A novel raffinose biological pathway is observed by symbionts of cotton≡ Verticillium dahliae to improve salt tolerance genetically on cotton. J Agron Crop Sci 207(6):956–969. https://doi.org/10.1111/jac.12556

Davis KER, Joseph SJ, Janssen PH (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Appl Environ Microbiol 71(2):826–834. https://doi.org/10.1128/AEM.71.2.826-834.2005

Deepika VB, Murali TS, Satyamoorthy K (2016) Modulation of genetic clusters for synthesis of bioactive molecules in fungal endophytes: a review. Microbiol Res 182:125–140. https://doi.org/10.1016/j.micres.2015.10.009

Di X, Takken FLW, Tintor N (2016) How phytohormones shape interactions between plants and the soil-borne fungus Fusarium oxysporum. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00170

Eaton CJ, Cox MP, Scott B (2011) What triggers grass endophytes to switch from mutualism to pathogenism? Plant Sci 180(2):190–195. https://doi.org/10.1016/j.plantsci.2010.10.002

Ekiz G, Duman S, Bedir E (2018) Biotransformation of cyclocanthogenol by the endophytic fungus Alternaria eureka 1E1BL1. Phytochemistry 151:91–98. https://doi.org/10.1016/j.phytochem.2018.04.006

El-Gendy MMAA, Al-Zahrani HAA, El-Bondkly AMA (2016) Genome shuffling of mangrove endophytic aspergillus luchuensis MERV10 for improving the cholesterol-lowering agent lovastatin under solid state fermentation. Mycobiology 44(3):171–179. https://doi.org/10.5941/MYCO.2016.44.3.171

Fidan O, Zhan J (2019) Discovery and engineering of an endophytic pseudomonas strain from Taxus chinensis for efficient production of zeaxanthin diglucoside. J Biol Eng 13(1):66. https://doi.org/10.1186/s13036-019-0196-x

Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008) Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr J 23(4):213–226. https://doi.org/10.1002/ffj.1875

Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408(6809):239–247. https://doi.org/10.1038/35041687

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410(1–2):335–356. https://doi.org/10.1007/s11104-016-3007-x

Fox EM, Howlett BJ (2008) Secondary metabolism: regulation and role in fungal biology. Curr Opin Microbiol 11(6):481–487. https://doi.org/10.1016/j.mib.2008.10.007

Fu Y (2019) Biotransformation of ginsenoside Rb1 to Gyp-XVII and minor ginsenoside Rg3 by endophytic bacterium Flavobacterium sp. GE 32 isolated from Panax ginseng. Lett Appl Microbiol 68(2):134–141. https://doi.org/10.1111/lam.13090

Gangadevi V, Muthumary J (2009) A novel endophytic taxol-producing fungus Chaetomella raphigera isolated from a medicinal plant, Terminalia arjuna. Appl Biochem Biotechnol 158(3):675–684. https://doi.org/10.1007/s12010-009-8532-0

Gokhale M, Gupta D, Gupta U, Faraz R, Sandhu SS (2017) Patents on endophytic fungi. Recent Pat Biotechnol 11(2):120–140. https://doi.org/10.2174/1872208311666170215151834

Górzyńska K, Ślachetka M, Ryszka P, Turnau K, Płachno BJ, Lembicz M (2018) Incidence, identification, and mycoparasitic activity of Clonostachys epichloë, a Hyperparasite of the fungal endophyte Epichloë typhina. Plant Dis 102(10):1973–1980. https://doi.org/10.1094/PDIS-02-18-0320-RE

Guo B, Wang Y, Sun X, Tang K (2008) Bioactive natural products from endophytes: a review. Appl Biochem Microbiol 44:136–142

Gutierrez RMP, Gonzalez AMN, Ramirez AM (2012) Compounds derived from endophytes: a review of phytochemistry and pharmacology. Curr Med Chem 19(18):2992–3030. https://doi.org/10.2174/092986712800672111

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342. https://doi.org/10.1146/annurev.ento.54.110807.090614

Hegazy M-EF, Mohamed TA, ElShamy AI, Mohamed A-E-HH, Mahalel UA, Reda EH, Shaheen AM, Tawfik WA, Shahat AA, Shams KA, Abdel-Azim NS, Hammouda FM (2015) Microbial biotransformation as a tool for drug development based on natural products from mevalonic acid pathway: a review. J Adv Res 6(1):17–33. https://doi.org/10.1016/j.jare.2014.11.009

Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA (2000) Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 28(10):1456–1462. https://doi.org/10.1016/S0891-5849(00)00252-5

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root endophyte Colletotrichum tofieldiae confers plant fitness benefits that are phosphate status dependent. Cell 165(2):464–474. https://doi.org/10.1016/j.cell.2016.02.028

Huang Z, Yang J, She Z, Lin Y (2010) Isoflavones from the mangrove endophytic fungus Fusarium sp. (ZZF41). Nat Prod Commun 5(11):1934578X1000501. https://doi.org/10.1177/1934578X1000501114

Huang J-X, Zhang J, Zhang X-R, Zhang K, Zhang X, He X-R (2014) Mucor fragilis as a novel source of the key pharmaceutical agents podophyllotoxin and kaempferol. Pharm Biol 52(10):1237–1243. https://doi.org/10.3109/13880209.2014.885061

Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H (2009) Colletotrichum: a catalogue of confusion. Fungal Divers 39(March 2001):1–17

Ji H, Li X, Zhang H (2009) Natural products and drug discovery. EMBO Rep 10(3):194–200. https://doi.org/10.1038/embor.2009.12

Jia M, Chen L, Xin H-L, Zheng C-J, Rahman K, Han T, Qin L-P (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 7:906. https://doi.org/10.3389/fmicb.2016.00906

Ju Y, Sacalis JN, Still CC (1998) Bioactive flavonoids from endophyte-infected blue grass (Poa ampla). J Agric Food Chem 46(9):3785–3788. https://doi.org/10.1021/jf980189m

Kai Z, Qingshen S, Yanjun Z, Wenxiang P, Tao J, Dongpo Z (2009) Screening and characterization of a high taxol producing fungus by protoplast mutagenesis. High Technol Lett 15:220–226. https://doi.org/10.3772/j.issn.1006-6748.2009.02.020

Katz L, Baltz RH (2016) Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol 43(2–3):155–176. https://doi.org/10.1007/s10295-015-1723-5

Kaur P, Kaur G (2020) Microbial endophytes: an untapped resource with antitumor and antimicrobial properties. Appl Biol Chem J 9–20:9. https://doi.org/10.52679/tabcj.2020.0003

Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28(7):1208–1228. https://doi.org/10.1039/c1np00008j

Kogel K-H, Franken P, Hückelhoven R (2006) Endophyte or parasite – what decides? Curr Opin Plant Biol 9(4):358–363. https://doi.org/10.1016/j.pbi.2006.05.001

Kozłowska E, Hoc N, Sycz J, Urbaniak M, Dymarska M, Grzeszczuk J, Kostrzewa-Susłow E, Stępień Ł, Pląskowska E, Janeczko T (2018) Biotransformation of steroids by entomopathogenic strains of Isaria farinosa. Microb Cell Factories 17(1):71. https://doi.org/10.1186/s12934-018-0920-0

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from Endophytic fungus Fusarium oxysporum isolated from Catharanthus roseus. PLoS One 8(9):e71805. https://doi.org/10.1371/journal.pone.0071805

Kusari S, Singh S, Jayabaskaran C (2014) Biotechnological potential of plant-associated endophytic fungi: Hope versus hype. Trends Biotechnol 32(6):297–303. https://doi.org/10.1016/j.tibtech.2014.03.009

Li JY, Sidhu RS, Ford EJ, Long DM, Hess WM, Strobel GA (1998) The induction of taxol production in the endophytic fungus – Periconia sp from Torreya grandifolia. J Ind Microbiol Biotechnol 20(5):259–264. https://doi.org/10.1038/sj.jim.2900521

Li F, Zhang N, Wu Q, Yuan Y, Yang Z, Zhou M, Zhu J, Tang Q (2016) Syringin prevents cardiac hypertrophy induced by pressure overload through the attenuation of autophagy. Int J Mol Med 39:199. https://doi.org/10.3892/ijmm.2016.2824

Liu J, Liu G (2018) Analysis of secondary metabolites from plant Endophytic fungi. Method Molecul Biol (Clifton, N.J.) 1848:25–38. https://doi.org/10.1007/978-1-4939-8724-5_3

Long DM, Smidansky ED, Archer AJ, Strobel GA (1998) In vivo addition of telomeric repeats to foreign DNA generates extrachromosomal DNAs in the taxol-producing fungus Pestalotiopsis microspora. Fungal Genet Biol 24(3):335–344. https://doi.org/10.1006/fgbi.1998.1065

Ludwig-Müller J (2015) Plants and endophytes: equal partners in secondary metabolite production? Biotechnol Lett 37(7):1325–1334. https://doi.org/10.1007/s10529-015-1814-4

Lusci A, Pollastri G, Baldi P (2013) Deep architectures and deep learning in chemoinformatics: the prediction of aqueous solubility for drug-like molecules. J Chem Inf Model 53(7):1563–1575. https://doi.org/10.1021/ci400187y

Macías-Rubalcava ML, Ruiz-Velasco Sobrino ME, Meléndez-González C, Hernández-Ortega S (2014) Naphthoquinone spiroketals and organic extracts from the Endophytic fungus Edenia gomezpompae as potential herbicides. J Agric Food Chem 62(16):3553–3562. https://doi.org/10.1021/jf500965k

Martinez-Mayorga K, Madariaga-Mazon A, Medina-Franco JL, Maggiora G (2020) The impact of chemoinformatics on drug discovery in the pharmaceutical industry. Expert Opin Drug Discovery 15(3):293–306. https://doi.org/10.1080/17460441.2020.1696307

MejÃa LC, Herre EA, Sparks JP, Winter K, GarcÃ MN, Van Bael SA, Stitt J, Shi Z, Zhang Y, Guiltinan MJ, Maximova SN (2014) Pervasive effects of a dominant foliar endophytic fungus on host genetic and phenotypic expression in a tropical tree. Front Microbiol 5. https://doi.org/10.3389/fmicb.2014.00479

Mishra S (2019) Targeted genome editing tools in plants. In: Sinha RP, Pandey S, Ghoshal N (eds) Innovations in life science research. NOVA Science Publisher, pp 129–145

Mishra S, Bhattacharjee A, Sharma S (2021a) An ecological insight into the multifaceted world of plant-endophyte association. Crit Rev Plant Sci 40(2):127–146. https://doi.org/10.1080/07352689.2021.1901044

Mishra Y, Sharma L, Dhiman M, Sharma MM (2021b) Endophytic fungal diversity of selected medicinal plants and their bio-potential applications. In: Fungi bio-prospects in sustainable agriculture, environment and nano-technology. Elsevier, pp 227–283. https://doi.org/10.1016/B978-0-12-821394-0.00010-X

Mitchell JBO (2014) Machine learning methods in chemoinformatics. WIREs Computational Molecular Science 4(5):468–481. https://doi.org/10.1002/wcms.1183

Mohinudeen IAHK, Kanumuri R, Soujanya KN, Shaanker RU, Rayala SK, Srivastava S (2021) Sustainable production of camptothecin from an Alternaria sp. isolated from Nothapodytes nimmoniana. Sci Rep 11(1):1478. https://doi.org/10.1038/s41598-020-79239-5

Murphy BR, Batke SP, Doohan FM, Hodkinson TR (2015) Media manipulations and the culture of beneficial fungal root endophytes. Int J Biol 7(3). https://doi.org/10.5539/ijb.v7n3p94

Nicoletti R, Fiorentino A (2015) Plant bioactive metabolites and drugs produced by endophytic fungi of Spermatophyta. Agriculture 5(4):918–970. https://doi.org/10.3390/agriculture5040918

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microb Pathog 82(April):50–59. https://doi.org/10.1016/j.micpath.2015.04.001

Nuraini FR, Setyaningsih R, Susilowati A (2019) Antioxidant activity of bioactive compound produced by endophytic fungi isolated from endemic plant of South Kalimantan Mangifera casturi Kosterm. AIP Conference Proceedings 2120. https://doi.org/10.1063/1.5115751

Ola ARB, Thomy D, Lai D, Brötz-Oesterhelt H, Proksch P (2013) Inducing secondary metabolite production by the endophytic fungus Fusarium tricinctum through Coculture with Bacillus subtilis. J Nat Prod 76(11):2094–2099. https://doi.org/10.1021/np400589h

Pan F, Su TJ, Cai SM, Wu W (2017) Fungal endophyte-derived Fritillaria unibracteata var. wabuensis: diversity, antioxidant capacities in vitro and relations to phenolic, flavonoid or saponin compounds. Sci Rep 7(211):1–14. https://doi.org/10.1038/srep42008

Pan R, Bai X, Chen J, Zhang H, Wang H (2019) Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: a literature review. Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.00294

Paramanantham P, Pattnaik S, Siddhardha B (2019) Natural products from Endophytic fungi: synthesis and applications. Springer, pp 83–103. https://doi.org/10.1007/978-3-030-03589-1_5

Patipong T, Hibino T, Waditee-Sirisattha R, Kageyama H (2019) Induction of antioxidative activity and antioxidant molecules in the halotolerant cyanobacterium Halothece sp. PCC7418 by temperature shift. Nat Prod Commun 14(7):1934578X1986568. https://doi.org/10.1177/1934578X19865680

Pettit RK (2011) Small-molecule elicitation of microbial secondary metabolites. Microb Biotechnol 4(4):471–478. https://doi.org/10.1111/j.1751-7915.2010.00196.x

Photita W, Lumyong S, Lumyong P, McKenzie EHC, Hyde KD (2004) Are some endophytes of Musa acuminata latent pathogens? Fungal Divers 16:131–140

Poveda J (2021) Beneficial effects of microbial volatile organic compounds (MVOCs) in plants. Appl Soil Ecol 168:104118. https://doi.org/10.1016/j.apsoil.2021.104118

Poveda J, Abril-Urias P, Escobar C (2020a) Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: trichoderma, mycorrhizal and endophytic fungi. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.00992

Poveda J, Zabalgogeazcoa I, Soengas P, Rodríguez VM, Cartea ME, Abilleira R, Velasco P (2020b) Brassica oleracea var. acephala (kale) improvement by biological activity of root endophytic fungi. Sci Rep 10(1):20224. https://doi.org/10.1038/s41598-020-77215-7

Prihantini AI, Tachibana S (2017) Antioxidant compounds produced by Pseudocercospora sp. ESL 02, an endophytic fungus isolated from Elaeocarpus sylvestris. Asian Pac J Trop Biomed 7(2):110–115. https://doi.org/10.1016/j.apjtb.2016.11.020

Pu X, Qu X, Chen F, Bao J, Zhang G, Luo Y (2013) Camptothecin-producing endophytic fungus Trichoderma atroviride LY357: isolation, identification, and fermentation conditions optimization for camptothecin production. Appl Microbiol Biotechnol 97(21):9365–9375. https://doi.org/10.1007/s00253-013-5163-8

Qawasmeh A, Obied HK, Raman A, Wheatley W (2012) Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction between Lolium perenne and different strains of Neotyphodium lolii. J Agric Food Chem 60(13):3381–3388. https://doi.org/10.1021/jf204105k

Qiao W, Ling F, Yu L, Huang Y, Wang T (2017) Enhancing taxol production in a novel endophytic fungus, Aspergillus aculeatinus Tax-6, isolated from Taxus chinensis var. mairei. Fungal Biol 121(12):1037–1044. https://doi.org/10.1016/j.funbio.2017.08.011

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S, Kumar Singh S, Gautam V (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology 12(3):139–159. https://doi.org/10.1080/21501203.2020.1870579

Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59(5):1109–1114. https://doi.org/10.1093/jxb/erm342

Schulz B, Boyle C, Draeger S, Römmert A-K, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004. https://doi.org/10.1017/S0953756202006342

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T, Sarkar A, Bodrossy L, van Overbeek L, Brar D, van Elsas JD, Reinhold-Hurek B (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25(1):28–36. https://doi.org/10.1094/MPMI-08-11-0204

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, El Beyrouthy M, Polito L, Iriti M, Martins N, Martorell M, Docea AO et al (2020) Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol 11. https://doi.org/10.3389/fphys.2020.00694

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biol Rev 21(2–3):75–89. https://doi.org/10.1016/j.fbr.2007.05.004

Smith H, Doyle S, Murphy R (2015) Filamentous fungi as a source of natural antioxidants. Food Chem 185:389–397. https://doi.org/10.1016/j.foodchem.2015.03.134

Smitha M, Singh S, Singh R (2017) Microbial biotransformation: A process for chemical alterations. J Bacteriol Mycol 4(2):47–51. https://doi.org/10.15406/jbmoa.2017.04.00085

Soliman SSM, Raizada MN (2013) Interactions between co-habitating fungi elicit synthesis of taxol from an endophytic fungus in Host Taxus plants. Front Microbiol 4. https://doi.org/10.3389/fmicb.2013.00003

Song YC, Huang WY, Sun C, Wang FW, Tan RX (2005) Characterization of graphislactone A as the antioxidant and free radical-scavenging substance from the culture of Cephalosporium sp. IFB-E001, an endophytic fungus in Trachelospermum jasminoides. Biol Pharm Bull 28(3):506–509. https://doi.org/10.1248/bpb.28.506

Spiteller P (2015) Chemical ecology of fungi. Nat Prod Rep 32(7):971–993. https://doi.org/10.1039/C4NP00166D

Staley JT, Konopka A (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol 39(1):321–346. https://doi.org/10.1146/annurev.mi.39.100185.001541

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003

Sun X, Guo LD (2012) Endophytic fungal diversity: review of traditional and molecular techniques. Mycology 3(1):65–76. https://doi.org/10.1080/21501203.2012.656724

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18(4):448–459. https://doi.org/10.1039/b100918o

Thakur M, Bhattacharya S, Khosla PK, Puri S (2019) Improving production of plant secondary metabolites through biotic and abiotic elicitation. Journal of Applied Research on Medicinal and Aromatic Plants 12:1–12. https://doi.org/10.1016/j.jarmap.2018.11.004

Tian H, Li XP, Zhao J, Gao HW, Xu QM, Wang JW (2021) Biotransformation of artemisinic acid to bioactive derivatives by endophytic Penicillium oxalicum B4 from Artemisia annua L. Phytochemistry 185:112682. https://doi.org/10.1016/j.phytochem.2021.112682

Van der Ent S, Van Wees SCM, Pieterse CMJ (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70(13–14):1581–1588. https://doi.org/10.1016/j.phytochem.2009.06.009

Vandenkoornhuyse P, Baldauf SL, Leyval C, Straczek J, Young JPW (2002) Extensive fungal diversity in plant roots. Science 295(5562):2051–2051. https://doi.org/10.1126/science.295.5562.2051

Venugopalan A, Srivastava S (2015) Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol Adv 33(6):873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Venugopalan A, Potunuru UR, Dixit M, Srivastava S (2016) Reprint of: effect of fermentation parameters, elicitors and precursors on camptothecin production from the endophyte Fusarium solani. Bioresour Technol 213:311–318. https://doi.org/10.1016/j.biortech.2016.05.023

Wang X-J, Min C-L, Ge M, Zuo R-H (2014) An Endophytic sanguinarine-producing fungus from macleaya cordata, fusarium proliferatum BLH51. Curr Microbiol 68(3):336–341. https://doi.org/10.1007/s00284-013-0482-7

Wang M, Zhang W, Xu W, Shen Y, Du L (2016) Optimization of genome shuffling for high-yield production of the antitumor deacetylmycoepoxydiene in an endophytic fungus of mangrove plants. Appl Microbiol Biotechnol 100(17):7491–7498. https://doi.org/10.1007/s00253-016-7457-0

Wei Y (2012) Engineering taxol biosynthetic pathway for improving taxol yield in taxol-producing endophytic fungus EFY-21 (Ozonium sp.). Afr J Biotechnol 11(37). https://doi.org/10.5897/AJB10.1896

Xie W, Hao Z, Yu M, Wu Z, Zhao A, Li J, Zhang X, Chen B (2019) Improved phosphorus nutrition by arbuscular mycorrhizal symbiosis as a key factor facilitating glycyrrhizin and liquiritin accumulation in Glycyrrhiza uralensis. Plant Soil 439(1–2):243–257. https://doi.org/10.1007/s11104-018-3861-9

Yang H-R, Yuan J, Liu L-H, Zhang W, Chen F, Dai C-C (2019) Endophytic Pseudomonas fluorescens induced sesquiterpenoid accumulation mediated by gibberellic acid and jasmonic acid in Atractylodes macrocephala Koidz plantlets. PCTOC 138(3):445–457. https://doi.org/10.1007/s11240-019-01640-4

Yildirim K, Kuru A, Küçükbaşol E (2020) Microbial transformation of androstenedione by Cladosporium sphaerospermum and Ulocladium chartarum. Biocatal Biotransformation 38(1):7–14. https://doi.org/10.1080/10242422.2019.1604690

Yu J-H, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43(1):437–458. https://doi.org/10.1146/annurev.phyto.43.040204.140214

Yuan Y, Tian J-M, Xiao J, Shao Q, Gao J-M (2014) Bioactive metabolites isolated from Penicillium sp. YY-20, the endophytic fungus from Ginkgo biloba. Nat Prod Res 28(4):278–281. https://doi.org/10.1080/14786419.2013.850686

Zakaria L, Jamil MIM, Anuar ISM (2016) Molecular characterisation of endophytic fungi from roots of wild banana (Musa acuminata). Trop Life Sci Res 27(1):153–162. http://www.ncbi.nlm.nih.gov/pubmed/27019688

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771. https://doi.org/10.1039/b609472b

Zhang Q, Wei X, Wang J (2012) Phillyrin produced by Colletotrichum gloeosporioides, an endophytic fungus isolated from Forsythia suspensa. Fitoterapia 83(8):1500–1505. https://doi.org/10.1016/j.fitote.2012.08.017

Zhang P, Lee Y, Wei X, Wu J, Liu Q, Wan S (2018) Enhanced production of tanshinone IIA in endophytic fungi Emericella foeniculicola by genome shuffling. Pharm Biol 56(1):357–362. https://doi.org/10.1080/13880209.2018.1481108

Zhao K, Xiao Y, Wang C, Liu D, Zhang Y, Wang X, Li X, Jin T (2014) Screening of taxol biosynthesis-related genes in taxol produced from Nodulisporium sylviforme HDF-68 by mRNA differential display. Ann Microbiol 64(4):1633–1642. https://doi.org/10.1007/s13213-014-0807-1

Zhou D, Zhao K, Ping W, Ge J, Ma X, Jun L (2005) Study on the mutagensis of protoplasts from taxol-producing fungus nodulisporium sylviforme. The Journal of American Science 1(1):55–62

Zhou X, Zhu H, Liu L, Lin J, Tang K (2010) A review: recent advances and future prospects of taxol-producing endophytic fungi. Appl Microbiol Biotechnol 86(6):1707–1717. https://doi.org/10.1007/s00253-010-2546-y

Chapter 12
Genetic, Epigenetic, and Physicochemical Strategies to Improve the Pharmacological Potential of Fungal Endophytes

Mayur G. Naitam, Viabhav Kumar Upadhyay, A. K. Lavanya, Aman Jaiswal, Geeta Kumari, M. N. Jha, Himanshu Mahawar, and Rajeev Kaushik

Abstract Endophytic fungi are ubiquitous microorganisms that reside within healthy plant tissues without causing any apparent harm to the host. The myriad of diverse pharmacological properties makes these fungi a valuable source of bioactive compounds. Research on fungal endophytes has gained significant momentum in recent years, with an increasing focus on their potential for pharmaceuticals. The present scenario of endophytic fungi's pharmacological potential involves identifying novel compounds, elucidating their mechanisms of action, and evaluating their efficacy and safety. Numerous studies have documented the discovery of endophytic fungi bioactive metabolites with potent immunomodulatory, antimicrobial, anticancer, and antioxidant properties. The demand for such compounds in the pharmaceutical industry is high owing to their therapeutic potential and low toxicity. Improving the yield and quality of bioactive compounds from endophytic fungi is crucial for meeting market demand. Various approaches, including genetic, epigenetic, and physicochemical methods, have been employed to enhance the production of bioactive metabolites. Genetic manipulation of fungal endophytes using techniques such as CRISPR/Cas9 and RNA interference has shown promising results in increasing the yield of biologically active compounds. Epigenetic modifications and physicochemical treatments such as elicitation and fermentation optimization have also been explored to improve bioactive metabolite production. Endophytic fungi have

M. G. Naitam · V. K. Upadhyay · A. Jaiswal · G. Kumari · M. N. Jha
Department of Microbiology, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

A. K. Lavanya
ICAR-Central Research Institute for Jute and Allied Fibers, Barrackpur, Kolkata, West Bengal, India

H. Mahawar (✉)
ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh, India
e-mail: himanshu.mahawar@icar.gov.in

R. Kaushik
Division of Microbiology, ICAR- Indian Agricultural Research Institute, New Delhi, India

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enormous pharmacological potential, which has substantial implications for drug research and development. The market need for such compounds can be met by increasing the production of bioactive substances from endophytic fungi utilizing a variety of methods.

Keywords Endophytes · Immunomodulatory potential · Antimicrobial potential · Genetic manipulation · Pharmacological potential

1 Introduction

Fungal endophytes are fungi that thrive in the actively growing plant tissues of different parts of plants without harming the host. These fungi form mutualistic relationships with their host plants, providing them several benefits such as protection from herbivores, pathogens, and environmental stresses. In exchange, the plant provide nutrients and a safe and stable niche for the fungal endophytes to grow and reproduce (Jeewon et al. 2019). Endophytic fungi have been found in virtually all plant species studied to date and can be found in various plant tissues such as leaves, stems, roots, and fruits. Additionally, they can be found in many different ecosystems, including forests, grasslands, wetlands, and deserts. *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma* are among the genera most frequently identified to contain endophytic fungus (El-Gendy et al. 2018). These microorganisms are found in various plant species, including medicinal plants, and are known to produce a wide variety of bioactive substances with pharmacological applications. A wide variety of secondary metabolic by-products with significant pharmacological activities, including antibacterial, antiviral, anticancer, and anti-inflammatory properties, are produced by endophytic fungi (Gouda et al. 2016). These metabolites have the potential to be drug candidates for the treatment of a variety of diseases.

Endophytic fungal metabolites' pharmacological potential has received increased attention in recent years. Many of these substances have been found to have strong biological effects, and researchers are investigating their potential for use in the development of novel medications (Gouda et al. 2016). The need for new drugs is growing, and endophytic fungi are a promising source of novel bioactive compounds. As a result, there is an increasing need to investigate and capitalize on the potential of these microorganisms for the development of new drugs (Jeewon et al. 2019; Gouda et al. 2016). Fungal endophytes have been used to create a number of drugs, including the anticancer drug Taxol, the immunosuppressant cyclosporin A, and the cholesterol-lowering drug lovastatin (Demain 2014). These medications have demonstrated the potential of endophytic fungus as a source of novel medications. Fungal endophytes produce diverse range of bioactive substances, such as alkaloids, terpenoids, flavonoids, and polyketides. Many of these compounds have

demonstrated antimicrobial, antiviral, antiparasitic, anticancer, antidiabetic, and anti-inflammatory properties (Demain 2014; Demain and Martens 2017). The global pharmaceutical market is expected to reach $1.5 trillion by 2023, with demand for new drugs expected to rise further (Singh et al. 2023; Kakkar 2021). Endophytic fungi may be a source of new drugs to meet this demand. However, the production potential of these microorganisms remains largely untapped, and methods for large-scale cultivation of these microorganisms must be explored and optimized.

The endophytic fungal secondary metabolites have drawn attention of pharmaceutical industry owing to their novel pharmaceutical properties. Furthermore, endophytic fungal secondary metabolites have several advantages over other sources. They are frequently more diverse and complex, with greater specificity and selectivity for their biological targets (Kusari and Spiteller 2011; Kusari et al. 2012). In addition, compared to other sources, such as aquatic organisms or terrestrial plants, fungal endophytes produce much higher yields of these metabolites. Endophytic fungal secondary metabolites have shown promise in the therapy of cancer, infectious diseases, metabolic disorders, and inflammatory conditions. Taxol, a powerful anticancer drug, was first isolated from the endophytic fungus *Taxomyces andreanae*, which was discovered in the bark of the Pacific yew tree (Stierle et al. 1995). Similarly, cyclosporine, an immunosuppressive drug used to prevent organ rejection in transplant patients, was discovered in the soil by the endophytic fungus *Tolypocladium inflatum* (Adeleke and Babalola 2021). Cordycepin, a nucleoside analogue with antiviral and anticancer properties; griseofulvin, an antifungal agent for treating ringworm infections; and epothilones, a group of anticancer drugs with mechanisms of action similar to taxemes, are other examples of metabolites from fungal endophytes with significant pharmacological potential (Adeleke and Babalola 2021; Aly et al. 2011a, b).

The global demand for new drugs to treat various diseases is rapidly increasing, with a market size of more than $1 trillion expected by 2025. The current drug development pipeline, however, is insufficient to meet this demand, with high costs, lengthy development times, and high failure rates limiting the count of novel drugs that reach the market. As a result, alternative sources of biologically active compounds must be investigated in an effort to provide a more sustainable and efficient approach to drug discovery. However, the prospects of endophytic fungi remain largely untapped, and more investigation and development are required to fully exploit their pharmacological potential. Advances in genetics, epigenetics, and physicochemical strategies have opened up new avenues for improving endophytic fungi bioactive compound production, which will be discussed in detail in the following sections of this review.

2 Present Scenario of Pharmacological Drugs from Fungal Endophytes

Possibilities for fungal endophytes as a formidable tool in pharmaceuticals are brightened by their capacity to create a variety of bioactive chemicals as prospective candidates for medication development. Numerous drugs of endophytic fungal origin are already being for treatment of several diseases. Probably, the most notable example is the immunosuppressant drug cyclosporine from *Tolypocladium inflatum*, which is used in organ transplant patients (Koster and Uges 2015). Another example is lovastatin, a drug developed from *Aspergillus terreus*, which is used to lower cholesterol levels in the blood (Goswami et al. 2013). Griseofulvin, an antibiotic produced by *Penicillium griseofulvum*, is used for treating skin, hair, and nail fungal infections (Deore et al. 2019). These drugs have proven to be extremely effective and have completely transformed the medical industry. Many fungal endophytic bioactive compounds have been identified having therapeutic potential and are broadly categorized as follows:

Antibacterial: Fungal endophytes have been found to produce a diverse range of antibacterial compounds. Beauvericin, for example, a compound produced by the fungus *Beauveria bassiana*, has been shown to have antibacterial activity against a variety of bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Narasimha et al. 2010; Jakubczyk and Dussart 2020). Similarly, the fungus *Bacillus brevis*'s compound gramicidin S has been shown to be effective against a variety of gram-positive bacteria (Berditsch et al. 2007).

Antiviral: Fungal endophytes have also been found to produce antiviral compounds. For instance, *Penicillium* sp. produces a compound called arugosin C that inhibits the replication of human immunodeficiency virus (HIV) (Kharwar et al. 2011). Similarly, the fungus *Chaetomium globosum*'s compound chaetoglobosin A has been shown to have antiviral activity against herpes simplex virus type 1 (HSV-1) (Rajamanikyam et al. 2017).

Antiparasitic: Several endophytic fungi have also been reported to produce antiparasitic compounds. For example, the fungus *Aspergillus* sp.-produced compound emodin has been shown to have antiparasitic activity against *Plasmodium falciparum*, the parasite responsible for malaria (Chinnasamy et al. 2023; Gong et al. 2022). Similarly, the fungus *Penicillium citrinum*'s compound citrinin has been shown to be effective against the parasite *Leishmania donovani*, which causes leishmaniasis (Meng et al. 2015).

Antimicrobials: Fungal endophytes have also been found to produce antimicrobial compounds with a broad spectrum of activity. Fusaric acid, for example, a compound produced by the fungus *Fusarium oxysporum*, has been shown to have antimicrobial activity against a variety of microorganisms, including bacteria, fungi, and yeast (Poleto et al. 2021). Similarly, isofusidienol, a compound produced by *Aspergillus fumigatus*, has been shown to inhibit the growth of several gram-positive and gram-negative bacteria (Hussain et al. 2017; Zhao et al. 2011).

Anticancer: Endophytic fungal metabolites have shown promising anticancer properties owing to their capacity to trigger cell cycle arrest, promote apoptosis, and inhibit angiogenesis. Several endophytic fungal metabolites, including paclitaxel, camptothecin, and griseofulvin, have been discovered to be effective anticancer agents (Li et al. 2018). Paclitaxel, a well-known anticancer drug derived from *Taxus brevifolia*, an endophytic fungus, is another example (Agung et al. 2019). It functions by binding to microtubules and preventing their breakdown during cell division, which results in cell death. Similarly, camptothecin, which is produced by a variety of endophytic fungi, inhibits the activity of topoisomerase I, an enzyme required for DNA replication, causing apoptosis (Singh et al. 2021). Another endophytic fungal metabolite with anticancer properties is griseofulvin. It works by inhibiting mitosis and interfering with microtubule formation, resulting in cell cycle arrest and cell death (Singh et al. 2008). Aside from these compounds, many other endophytic fungal metabolites have demonstrated anticancer activity, making them a promising stockpile of new cancer therapeutics. Endophytic fungal metabolites, in general, represent a vast and largely untapped source of natural compounds with potential therapeutic applications in cancer treatment. In order to fully understand their mechanisms of action and maximize their efficacy and safety, more investigations are required.

Antidiabetics: Endophytic fungal metabolites have shown potential as antidiabetic agents attributing their ability to regulate blood glucose levels by stimulating insulin secretion and improving insulin sensitivity. Some examples of endophytic fungi-derived metabolites having antidiabetic properties are berberine, beauvericin, and cytochalasin D (Kaul et al. 2017).

Anti-inflammation: Endophytic fungal metabolites can also reduce inflammation by inhibiting pro-inflammatory cytokines and enzymes. Compounds with potent anti-inflammatory effects, such as cordycepin, emodin, and curcumin, have been encountered as potential candidates for treating inflammatory disorders (Kaul et al. 2017).

Antitumor: Endophytic fungal metabolites that induce cell cycle arrest, promote apoptosis and inhibit angiogenesis have all been shown to have antitumor properties (Kumar et al. 2021). Paclitaxel, camptothecin, and griseofulvin are examples of such compounds (Kumar et al. 2021; Evidente et al. 2014).

In addition to the aforementioned bioactive compounds, endophytic fungi also produce an array of metabolites with substantial pharmaceutical potential. Endophytic *Aspergillus*, for example, produces a diverse range of bioactive compounds, including secondary metabolites such as alkaloids, terpenoids, and polyketides (Orfali et al. 2021), and among them, compounds such as fumagillin, have shown anticancer activity (Guruceaga et al. 2019). Furthermore, *Aspergillus*-derived compounds like gliotoxin and viriditoxin have anti-inflammatory and immunosuppressive properties, making them potential treatments for autoimmune diseases (Orfali et al. 2021). Several in vitro and in vivo studies have shown that Pestaloficiolide A produced by *Pestalotiopsis fici* is another promising endophytic fungal metabolite with antitumor potential (Sui et al. 2016). Similarly, endophytic

Fusarium produces secondary metabolites like fusaric acid, which has anticancer potential (Ahmed et al. 2023). Endophytic fungi also produce antimicrobial substances having potential to be used for the development of new antibiotics. For example, the endophyte *Phomopsis* sp. has been found to produce antibacterial metabolites against a variety of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Lechner et al. 2004). *Alternaria*, another fungal endophyte, produces a variety of secondary metabolites including alternariol and alternariol monomethyl ether, which have antimicrobial activity (Qader et al. 2021). Along with these substances, endophytic fungi also produce a wide range of antioxidants, insecticides, and neuroprotective substances. These compounds could be used in a variety of fields, including food preservation, agriculture, and medicine (Rai et al. 2021).

Endophytic fungal metabolites have a vast and largely untapped pharmaceutical potential. Many bioactive compounds have been identified, but many more remain undiscovered. It is possible to identify new endophytic fungal strains and their associated metabolites with potential pharmaceutical applications using advanced screening and analytical techniques. Finding new drugs to treat severe conditions like cancer, bacterial and viral infections, diabetes, and inflammation may be facilitated by novel methods for enhancing the therapeutic potential of endophytic fungal strains.

3 Present Production Potential of Pharmacologically Important Endophytic Fungal Metabolites

The global market for microbial natural products, including endophytic fungi, has been expanding rapidly due to significant therapeutic benefits. The increasing rate of long-lasting illnesses such as diabetes, heart disease, and cancer necessitates the development of new and effective treatment options for these natural products (Singh et al. 2011). The global market for natural products was valued at $207.6 billion in 2017 and is anticipated to grow at a compound annual growth rate (CAGR) of 6.3% from 2018 to 2025 (PFPM 2020). Though the commercialization is gaining importance, there are several constraints in the production of endophytic fungal metabolites due to several factors such as low metabolite yields from wild-type strains, difficulty of cultivating some fungal endophytes in the laboratory, and a lack of understanding of many secondary metabolite biosynthetic pathways (Kusari et al. 2012; Aly et al. 2011a, b). The present production potential of some of the pharmacological endophytic fungal metabolites is presented in Table 12.1.

To circumvent these barriers, several strategies for upscaling the potential to produce endophytic fungal metabolites have been developed. To boost the yield of target metabolites, one approach is to optimize cultivation conditions such as culture medium, pH, temperature, and aeration. Another approach is to use genetic and epigenetic strategies to engineer fungal strains to upregulate the expression of

Table 12.1 Present production potential of endophytic fungal metabolites having pharmacological properties

Metabolite	Fungal species	Production	Pharmacological use	References
Taxol	*Taxomyces andreanae*	11.5 g/L	Anticancer	Andrea et al. (1993)
Camptothecin	*Camptotheca acuminata*	2.5 mg/g	Anticancer	Kusari et al. (2009)
Podophyllotoxin	*Podophyllum peltatum*	0.1–1.5 mg/g	Anticancer, antiviral	Giri and Lakshmi Narasu (2000)
Ergot alkaloids	*Claviceps purpurea*	Up to 150 mg/L	Migraine treatment	Králová et al. (2021)
Asperlicin	*Aspergillus alliaceus*	7 mg/L	Antifungal	Kusari and Spiteller (2011)
Mycophenolic acid	*Penicillium brevicompactum*	220 mg/L	Immunosuppressive	Dong et al. (2015)
Griseofulvin	*Xylaria* sp.	129 mg/L	Antifungal	Li et al. (2018)
Cordycepin	*Cordyceps militaris*	30 mg/L	Anticancer, antiviral	Luthra et al. (2015)
Lovastatin	*Aspergillus terreus*	14.9 mg/g	Cholesterol lowering	Ravuri and Shivakumar (2020)
Terpestacin	*Aspergillus terreus*	15.8 mg/L	Antifungal, antitumor	Jiang et al. (2020)
Verruculogen	*Aspergillus fumigatus*	3.0 mg/L	Neurotoxin	Uzma et al. (2019)
Penicillic acid	*Penicillium crustosum*	5.4 mg/L	Antibacterial	Kusari et al. (2012)
Mycotoxin T-2	*Fusarium sporotrichioides*	120 µg/g	Toxin, antitumor	Bae et al. (2009)
Citrinin	*Penicillium citrinum*	20.3 mg/L	Nephrotoxic, antimicrobial	Kusari et al. (2012)

biosynthetic genes or activate silent gene clusters for secondary metabolite production (Kusari et al. 2012; Shweta et al. 2010). In addition, physicochemical methods such as UV irradiation, chemical elicitors, and physical stress have been employed to induce secondary metabolite production in endophytic fungi. Despite significant advances in the fabrication of endophytic fungal metabolites, commercialization of these natural products remains limited. The production of natural products from endophytic fungi is difficult and often involves time-consuming and costly processes. Furthermore, regulatory issues such as intellectual property rights and quality control standards may have an impact on the commercialization of these products (Aly et al. 2011a, b). Despite these obstacles, the growing demand for natural products derived from fungal endophytes suggests that these products have a high commercialization potential. The development of new strategies for the efficient and cost-effective production of these natural products, as well as the establishment of

appropriate regulatory frameworks, will be crucial in realizing the full potential of endophytic fungal metabolites in the pharmaceutical industry.

4 Approaches for Improving Fungal Strains for Pharmacological Properties

Fungi are attributed as significant sink of biologically active compounds with therapeutic potential. However, many fungal strains produce these compounds in meager quantity or do not produce them at all. To address this issue, various approaches have been established to improve the pharmacological properties of fungal strains. Genetic approaches involve the manipulation of genes responsible for the synthesis of bioactive metabolites, such as through gene deletion or overexpression. Epigenetic methods such as histone modification or DNA methylation alter the expression of genes without interfering the genetic code. Physicochemical approaches involve the manipulation of environmental variables, such as nutrient availability or temperature, to augment the synthesis of bioactive compounds. These approaches have demonstrated great promise in enhancing the pharmacological characteristics of fungal strains that may be combined to accomplish even greater enhancements. By enhancing the production of biologically active compounds in fungal strains, these approaches have the promising prospects to provide new sources of drugs for various therapeutic applications (Fig. 12.1).

4.1 Genetic Approach

For improving the pharmacological potential of fungal endophytes, genetic engineering has emerged as a powerful tool. This method entails modifying the fungal genome to upsurge the specific secondary metabolite production or to introduce new biosynthetic pathways. Gene overexpression, gene knockout, and heterologous expression are examples of genetic engineering strategies. Genetic approaches can significantly improve the pharmacological properties of endophytic fungi. Researchers can introduce new traits, modify existing ones, and escalate the production of target compounds by manipulating the genetic material of these organisms (Kusari and Spiteller 2011). We will discuss about the genetic strategies that are deployed to improve fungal strains for pharmacological applications.

(i) *Gene editing*: CRISPR/Cas9 gene-editing technologies enable researchers to meticulously modify the genetic material of fungal endophytes (Song et al. 2019). Using this technique, particular genes associated with the biosynthesis of pharmacological important chemicals can be deleted, added, or modified. For example, in *Aspergillus nidulans*, researchers used CRISPR/Cas9 to delete

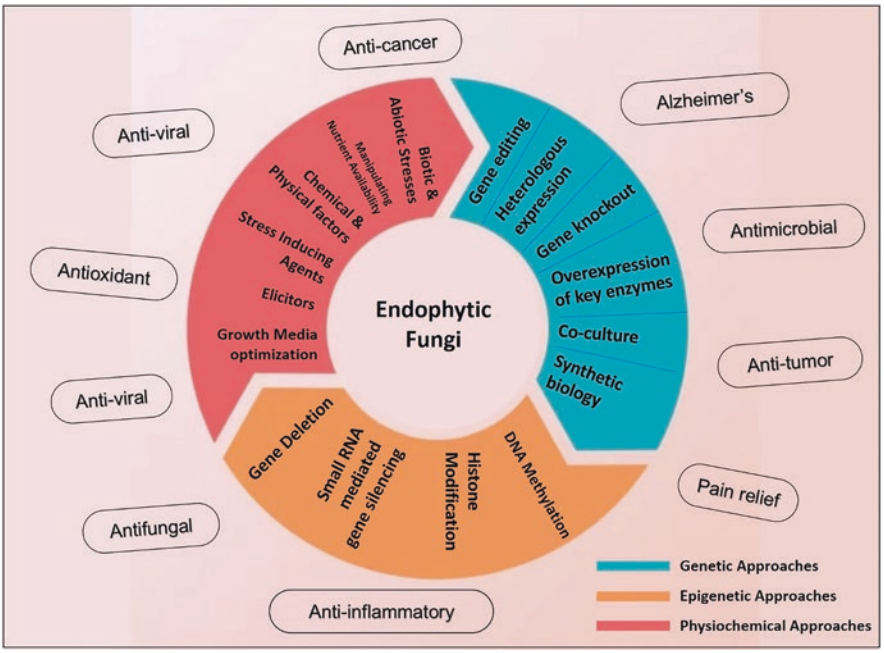

Fig. 12.1 Different approaches for improving pharmacological properties in fungal strains

a gene involved in the biosynthesis of a pigment, resulting in increased production of lovastatin, a cholesterol-lowering drug (Shi et al. 2019; Zou et al. 2020).

(ii) *Heterologous expression*: Heterologous expression transfers genes from one organism to another to synthesize a specific chemical and has been utilized to increase a range of pharmacologically active chemical compounds in fungal endophytes (Khan et al. 2014). For instance, to develop vinblastine, a potent anticancer drug, researchers transferred DNA from *Catharanthus roseus* (plant) to *Colletotrichum gloeosporioides* (fungal endophyte) (Chandra 2012).

(iii) *Overexpressing the key enzymes*: The production of target substances in fungal endophytes can be increased by overexpressing essential enzymes involved in their production. This method has been applied to boost the synthesis of a variety of compounds such as lovastatin, Taxol, and camptothecin (El-Sayed et al. 2020). For example, the production of lovastatin was significantly increased when a crucial enzyme involved in its synthesis was overexpressed in the *Aspergillus terreus* (Zhgun et al. 2018).

(iv) *Gene knockout*: The procedure of deleting a target gene to eliminate or reduce the synthesis of a specific compound or enzyme is known as gene knockout. This method is useful when the target gene is involved in a negative regulatory pathway or encodes an enzyme that competes with the target enzyme for the same substrate. The production of fungi-derived substances, including secondary metabolic products, antibiotics, and mycotoxins, has been enhanced using

gene knockout technology. Successful application of gene knockout in fungi is the improvement of mycotoxin aflatoxin B1 production by *Aspergillus flavus*. Aflatoxin B1 production was completely eliminated by deleting the regulatory gene aflR, which controls the expression of the genes involved in aflatoxin biosynthesis (Yu et al. 2004).

(v) *Coculture:* Coculture refers to the growing of two or more organisms together to promote the synthesis of particular compounds, and this technique is used to increase the production of pharmacologically active compounds. The coculturing of *Penicillium* sp. (endophytic fungus) with *Streptomyces* sp. (the bacterium) enhanced the synthesis of a unique polyketide with potent antimicrobial properties (Peng et al. 2021).

(vi) *Synthetic biology*: The design and construction of novel genetic circuits or metabolic pathways to achieve specific changes in gene expression or function are known as synthetic biology (Tucker and Zilinskas 2006). This method genetically modifies the fungi to promote the production of novel compounds or enzymes with improved properties or to upsurge the yield of existing compounds. The application of synthetic biology in fungi for establishing a synthetic pathway by *Saccharomyces cerevisiae* for the synthesis of the opioid alkaloid thebaine is one such example (Walker and Pretorius 2018). A yeast strain capable of producing thebaine was created by introducing multiple genes from the opium poppy *Papaver somniferum* (Galanie et al. 2015).

Genetic approaches can be way forward to enhance the therapeutic potential of fungal endophytes, introducing new traits, modifying existing ones, and increasing the synthesis of target compounds, making them a potential sink of novel drugs.

4.2 Epigenetic Approach

The term “epigenetic” was invented by Waddington and refers to cellular changes in target gene expression levels in response to certain stimuli that occur through alterations in the chromosome rather than modifications in the DNA sequences or protein coding but instead regulate or affect the expression of target genes (Waddington 1942). The epigenetic regulation of gene function has reached pivotal importance in life sciences in the last decades. These modifications are broadly categorized into three different mechanisms, vize., DNA methylation, histone modification, and noncoding RNA (ncRNA)-associated gene silencing (Berger et al. 2009) (Table 12.2). These mechanisms have been studied extensively in *Aspergillus nidulans* and *Neurospora crassa*, and can be used as a tool for fungal strain improvement. Epigenetic modifications can be heritable, but they are also transient and rapidly reversible. Thus, they are more flexible than direct changes to the genetic code. Epigenetic modifications serve as a bet-hedging strategy by which the pharmacological property of the fungi can be improved. The studies of epigenetic

Table 12.2 Production of pharmacologically significant chemicals/compounds stimulated by epigenetic modification of fungi

Fungi	Epigenetic approach	Effect(s)	References
Hygromycin resistance genes in *Schizophyllum commune* and *Neurospora crassa;* phleomycin resistance gene in *Phanerochaete chrysosporium*	DNA methyltransferase (DNMT) inhibitors such as 5-azacytidine and 5-aza-2′-deoxycytidine	Ability to reduce the DNA methylation-mediated silencing of antibiotic resistance	Mooibroek et al. (1990), Birch et al. (1998), and Cheng et al. (2003)
Nuclear localized protein LaeA coding gene *laeA* from *Aspergillus* spp.	Putative S-adenosylmethionine binding motif presence in protein LaeA might serve as a histone methylating agent	Transcriptional control of several secondary metabolite families, lovastatin (effective in lowering cholesterol and the risk of atherosclerotic cardiovascular disease), penicillin, and gliotoxin (antifungal and as an antiviral agent)	Luger and Hansen, (2005) and Happel and Doenecke (2009)
Aspergillus nidulans	Inactivation of *hdaA* gene encoding histidine deacetylase inhibitors (HDAC)	Increased norsolorinic acid and penicillin production	Cichewicz (2010)
Aspergillus spp.	Physical (UV-255 nm), cyclic mutagenesis and chemical (ethyl methane sulphonate) mutagenesis-based strain improvement	Increased lovastatin production (3912 mg/l). Effective in cancer treatment, hypercholesterolemia, Parkinson's disease, Alzheimer's disease, atherosclerosis, and lower bad cholesterol and fats in the blood	Kaur et al. (2009), Jaivel and Marimuthu (2010), and Upendra and Khandelwal (2016)
Xylaria psidii	Using DNA methyltransferases and histone deacetylase (HDAC) inhibitors such as suberoylanilide hydroxamic acid (SAHA) and dMNTs 5-azacytidine (AZA)	Enhanced resveratrol production (52.32 µg/ml). Effective against heart diseases, anticancer, anti-inflammatory, antidiabetic, and antiaging	Kundu and Surh (2008) and Dwibedi et al. (2019)
Aspergillus nidulans	Proteasome inhibitors when treated with SAHA, an HDACi	Overexpress the genes for fellutamides A-D (potent inhibitor of the *mycobacterium tuberculosis* proteasome)	Albright et al. (2015)

(continued)

Table 12.2 (continued)

Fungi	Epigenetic approach	Effect(s)	References
Botryosphaeria rhodina	Chemical epigenetic manipulation: DNA methyltransferase, cultured in the presence of 5-azacytidine	Enhanced the production of camptothecin (CPT) as compared to wild type. CPT was reported to have anticancer activity	Vasanthakumari et al. (2015)
Marine-derived fungus *Cochliobolus lunatus*	Chemical epigenetic manipulation: DNA methyltransferase, 5-azacytidine	Stimulated the production of diethylene glycol phthalate ester monomers and oligomers (used as a plasticizer in tablet and skin care preparation)	Chen et al. (2016)
Penicillium brevicompactum	Use of HDAC inhibitors, nicotinamide during growth	Tenfold increase in the production of bioactive phenolic metabolites, syringic acid, sinapic acid, and acetosyringone exhibited potent in vitro free radical scavenging, and antiproliferative activities against liver carcinoma cells (HepG2)	El-Hawary et al. (2018)
Five species of *Talaromyces* and *Penicillium janthinellum*	Different combinations of SBHA, procainamide, and hydralazine	Increased antibacterial activity against *Listeria monocytogenes*. Inhibition of the acetylcholinesterase activity	Lima et al. (2018)
Drechslera sp.	Suberohydroxamic acid (SAHA), VPA, octanoylhydroxamic acid (OHA)	Increased production of benzophenone, used to treat skin diseases	Siless et al. (2018)

mechanisms involved in the regulation of secondary metabolites' (SMs) production by fungi are gaining importance (Gacek and Strauss 2012; Aghcheh and Kubicek 2015).

(i) *DNA methylation*: DNA is posttranslationally modified in fungi by DNA methyltransferases (DNMTs), resulting in the conversion of cytosine to its corresponding 5-methylcytosine (5-mC) product. Typical methylation levels ranging from 0.25% to 1.5% of cytosines in *Aspergillus flavus* and *Neurospora crassa*, respectively, have been reported in metabolically active fungi (Chen et al. 2017; Parashar et al. 2018).

(ii) *Histone modifications*: Histones are highly abundant chromatin-associated proteins having important roles, wherein it functions as scaffolds for nucleosome assembly by providing a substrate for DNA binding. It also participates in the control of transcriptional regulation, which is essential for proper cell function. Histones act as substrates for several posttranslation modifications (PTMs) which include methylation, acetylation, and phosphorylation. Histone

modifications can alter gene expression levels by allowing or restricting binding of transcription factors, enhancers, or chromatin remodeling proteins. These modifications lead to changes in chromatin organization that allow selective accessibility for transcription factors to specific genomic regions while restricting the binding of transcription machinery to other genomic locations (Bannister and Kouzarides 2011).

(iii) *RNA-based pathways include RNA interference (RNAi) and noncoding RNAs*: RNAi is a mechanism mediated by small RNAs (sRNAs) produced through a core RNAi pathway involving RNA-dependent RNA polymerases and the endonuclease Dicer (Villalobos-Escobedo et al. 2016). Processed sRNAs selectively target complementary RNAs and either induce degradation or inhibit translation of the target RNA. In some instances, the RNAi machinery can also recruit heterochromatin proteins to target genes, thus inhibiting gene expression (Martienssen and Moazed 2015). Other noncoding RNAs are long noncoding RNAs (lncRNAs) that are generally larger than 200 bp, which distinguishes them from small nuclear RNAs. lncRNAs are mainly found in the nucleus and are subjected to degradation by exosomes, are considered as transcriptional noise, and hence, play roles in epigenetic gene regulation (Moran et al. 2012; Dimond and Fraser 2013).

(iv) *Chemical epigenetic – using small molecules to manipulate the fungal epigenome*: Selectively or semi-selectively inhibiting DNA and histone posttranslational modifying proteins and creating probe inhibitors that are effective at altering human disease-related processes.

(v) *Molecular methods for altering the epigenome*: The application of molecular techniques to manipulate the performance of fungal genes (e.g., deletion or promotion), resulting in novel opportunities for directly probing the genetic basis by which fungi control secondary metabolite production.

4.3 Physicochemical Approach

Physicochemical strategies are used to improve the pharmacological potential of fungal endophytes by optimizing growth conditions, manipulating nutrient availability, and using elicitors or stress-inducing agents. In addition to genetic and epigenetic approaches, these strategies seek to improve the physical and chemical conditions of fungal growth and metabolism to increase the production of bioactive compounds (Taritla et al. 2021).

Optimizing culture conditions such as temperature, pH, nutrient availability, and aeration can have a significant impact on the biosynthesis of secondary metabolites in fungi. High temperatures and an acidic pH, for example, have been shown to increase bioactive compound production in some fungal endophytes (Ramos and Said 2011; Taritla et al. 2021). Elicitors, which are compounds that induce stress responses in fungi and trigger the production of secondary metabolites, are another physicochemical approach. Plant hormones, heavy metals, and various chemical

compounds are examples of elicitors that regulate the synthesis of bioactive substances in fungal endophytes. Furthermore, coculturing and biotic stress can be used as a physicochemical strategy for increasing the synthesis of fungal bioactive metabolites (Ramos and Said 2011). Stressors, such as pathogens and other microbes, stimulate the secondary metabolite production by inducing defense mechanism in fungi. Besides these, genetic engineering techniques such as genome editing can be used to improve the pharmacological potential of fungal endophytes. Overall, physicochemical methods play a significant role in enhancing the pharmacological prospects of endophytic fungi and, when combined with genetic and epigenetic methods, can result in the development of novel therapeutic metabolites.

(i) *Optimization of media composition*: The composition of the culture medium can have a significant impact on fungi growth and secondary metabolite production. To maximize the yield of the desired compound, the media composition must be optimized by adjusting the levels of nutrients, carbon and nitrogen sources, vitamins, and minerals (Ramos and Said 2011). *Aspergillus terreus* improved its production of lovastatin, a cholesterol-lowering drug, as an example of a successful application of media optimization in fungi. The production of lovastatin was increased by up to ninefold by optimizing the media composition, which included the addition of soybean meal (Luthra et al. 2015; Lai et al. 2002).

(ii) *Physical treatments*: Various physical treatments, including ultrasound, electric fields, and radiation, have been shown to stimulate secondary metabolite production in fungi. These treatments can cause fungi to respond to stress, resulting in increased production of valuable compounds (Deng et al. 2020). The improvement of the production of the antifungal compound cordycepin by *Cordyceps militaris* is one example of the successful application of physical treatments in fungi. When the fungus was subjected to ultrasound treatment, its capacity to produce cordycepin increased by 2.5-fold with respect to untreated control (Wellham 2022).

(iii) *Manipulation of environmental factors*: The physical and chemical conditions of a fungal culture such as temperature, pH, and aeration, etc. can be manipulated to enhance the production of valuable compounds (Shu-Ting and Miles 2004). By adjusting the culture's temperature and pH, *Taxus chinensis*' production of the anticancer agent Taxol was enhanced (Wang et al. 2001).

5 Conclusion

In conclusion, endophytic fungi have the potential to produce a wide range of bioactive compounds with potent biological activities. Genetic, epigenetic, and physicochemical approaches have emerged as powerful tools for improving the pharmacological potential of endophytic fungi. However, more study and development are required to improve these methods and explore endophytic fungi's full ability to be the source of novel bioactive substances. Therefore, further research

into the biology of endophytic fungi is required, as well as the development of more effective and sustainable techniques for their large-scale cultivation as well as generation of pharmacologically pertinent molecules. In the end, the possibility of discovering new medicines from endophytic fungi opens up a fascinating new field of drug discovery and has important ramifications for the future of healthcare.

6 Future Prospects

The potential of fungal endophytes in producing novel bioactive compounds for pharmaceutical use is vast, and research in this area is expected to continue growing in the future. Deployment of genetic, epigenetic, and physicochemical strategies for improving the pharmacological potential of endophytic fungi is a promising avenue for advancing this field. Discussed below are some potential outcomes for these tactics in the future:

Identification and characterization of new fungal endophytes: The range of potential pharmaceutical applications must be expanded by unearthing new fungal endophytes with distinctive bioactive substances. With advancement of more complex methods for isolating and identifying fungal endophytes, more untapped endophytes are anticipated to be revealed.

Synthetic biology: Synthetic biology has revolutionized genetic engineering by allowing for the genetic modification of endophytic fungi to enhance bioactive compound production. As synthetic biology techniques advance, new biosynthetic pathways will be introduced into fungal endophytes, producing novel and distinctive bioactive compounds.

Epigenetic modifications: A promising strategy for increasing the potential of therapeutically important fungal endophytes is epigenetic modification. New possibilities for altering expression levels without altering the fundamental gene makeup have been made possible by advancements in our understanding of epigenetic mechanisms.

Metabolic engineering: It is a powerful approach for the generation of high-value metabolites, modifying the metabolic pathways of an organism for increased biosynthesis of the target compound. Recent advances have shown promise in enhancing the bioactive compound production from fungal endophytes.

Microbial cocultures: Coculturing fungal endophytes with other microorganisms can have synergistic effects and lead to the production of unique compounds that are not synthesized by the endophyte alone, as well as higher yields and improved pharmacological properties.

These genetic, epigenetic, and physicochemical strategies for improving the pharmacological potential of fungal endophytes offer exciting prospects for the discovery of new bioactive compounds, and with continued research and development, yields of vital and rare targeted compounds of pharmacological significance can be generated.

References

Adeleke BS, Babalola OO (2021) Pharmacological potential of fungal endophytes associated with medicinal plants: A review. J Fungi 7(2):1–16

Aghcheh RK, Kubicek CP (2015) Epigenetics as an emerging tool for improvement of fungal strains used in biotechnology. Appl Microbiol Biotechnol 99(15):6167–6181

Agung SD, Febrina AS, Ridesti R (2019) Characterization of seeds of Peltophorum pterocarpum (DC.) K. Heyne and Spondias mombin L. and effect of storage conditions on seed viability and seedling growth. Biosci Res 16(2):2272–2281

Ahmed AM, Mahmoud BK, Millán-Aguiñaga N, Abdelmohsen UR, Fouad MA (2023) The endophytic *Fusarium* strains: a treasure trove of natural products. RSC Adv 13(2):1339–1369

Albright JC, Henke MT, Soukup AA, McClure RA, Thomson RJ, Keller NP, Kelleher NL (2015) Large-scale metabolomics reveals a complex response of *Aspergillus nidulans* to epigenetic perturbation. ACS Chem Biol 10(6):1535–1541

Aly AH, Debbab A, Proksch P (2011a) Fifty years of drug discovery from fungi. Fungal Divers 50:3–19

Aly AH, Debbab A, Proksch P (2011b) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90(6):1829–1845

Andrea S, Gary S, Donald S (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60(11):3279–3295

Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21(3):381–395

Berditsch M, Afonin S, Ulrich AS (2007) The ability of *Aneunnibacillus migulanus* (*Bacillus brevis*) to produce the antibiotic gramicidin S is correlated with phenotype variation. Appl Environ Microbiol 73(20):6620–6628

Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A (2009) An operational definition of epigenetics. Genes Dev 23(7):781–783

Birch PRJ, Sims PFG, Broda P (1998) A reporter system for analysis of regulatable promoter functions in the basidiomycete fungus *Phanerochaete chrysosporium*. J Appl Microbiol 85(3):417–424

Chandra S (2012) Endophytic fungi: novel sources of anticancer lead molecules. Appl Microbiol Biotechnol 95(1):47–59

Chen D, Meng L, Pei F, Zheng Y, Leng J (2017) A review of DNA methylation in depression. J Clin Neurosci 43:39–46

Chen M, Zhang W, Shao CL, Chi ZM, Wang CY (2016) DNA methyltransferase inhibitor induced fungal biosynthetic products: diethylene glycol phthalate ester oligomers from the marine-derived fungus *Cochliobolus lunatus*. Mar Biotechnol 18:409–417

Cheng JC, Matsen CB, Gonzales FA, Ye W, Greer S, Marquez VE, Jones PA, Selker EU (2003) Inhibition of DNA methylation and reactivation of silenced genes by zebularine. J Natl Cancer Inst 95(5):399–409

Chinnasamy R, Govindasamy B, Venkatesh M, Magudeeswaran S, Dhanarajan A, Devarajan N, Willie P, Perumal V, Mekchay S, Krutmuang P (2023) Bio-efficacy of insecticidal molecule emodin against dengue, filariasis, and malaria vectors. Environ Sci Pollut Res Int 30(22):61842–61862

Cichewicz RH (2010) Epigenome manipulation as a pathway to new natural product scaffolds and their congeners. Nat Prod Rep 27(1):11–22

Demain AL (2014) Valuable secondary metabolites from fungi. In: Biosynthesis and molecular genetics of fungal secondary metabolites, fungal biology. Springer, New York, pp 1–15

Demain AL, Martens E (2017) Production of valuable compounds by molds and yeasts. J Antibiot 70(4):347–360

Deng H, Bai Y, Fan T, Zheng X, Cai Y (2020) Advanced strategy for metabolite exploration in filamentous fungi. Crit Rev Biotechnol 40(2):180–198

Deore AB, Dhumane JR, Wagh R, Sonawane R (2019) The stages of drug discovery and development process. Asian J Pharm Res Dev 7(6):62–67

Dimond A, Fraser P (2013) Long noncoding RNAs exist in three dimensions. Science 341(6147):720–721

Dong Y, Xu R, Wang L, Zhang J, Bai C, Sun A, Wei D (2015) A combined feeding strategy for enhancing mycophenolic acid production by fed-batch fermentation in *Penicillium brevicompactum*. Process Biochem 50(3):336–340

Dwibedi V, Kalia S, Saxena S (2019) Isolation and enhancement of resveratrol production in *Xylaria psidii* by exploring the phenomenon of epigenetics: using DNA methyltransferases and histone deacetylase as epigenetic modifiers. Mol Biol Rep 46:4123–4137

El-Gendy MMAA, Yahya SMM, Hamed AR, Soltan MM, El-Bondkly AMA (2018) Phylogenetic analysis and biological evaluation of marine endophytic fungi derived from Red Sea sponge *Hyrtios erectus*. Appl Biochem Biotechnol 185(3):755–777

El-Hawary SS, Sayed AM, Mohammed R, Hassan HM, Zaki MA, Rateb ME, Mohammed TA, Amin E, Abdelmohsen UR (2018) Epigenetic modifiers induce bioactive phenolic metabolites in the marine-derived fungus *Penicillium brevicompactum*. Mar Drugs 16(8):253–265

El-Sayed ASA, El Sayed MT, Rady A, Zein N, Enan G, Shindia A, El-Hefnawy S, Sitohy M, Sitohy B (2020) Exploiting the biosynthetic potency of taxol from fungal endophytes of conifers plants; genome mining and metabolic manipulation. Molecules 25(13):1–21

Evidente A, Kornienko A, Cimmino A, Andolfi A, Lefranc F, Mathieu V, Kiss R (2014) Fungal metabolites with anticancer activity. Nat Prod Rep 31(5):617–627

Gacek A, Strauss J (2012) The chromatin code of fungal secondary metabolite gene clusters. Appl Microbiol Biotechnol 95:1389–1404

Galanie S, Thodey K, Trenchard IJ, Filsinger Interrante M, Smolke CD (2015) Complete biosynthesis of opioids in yeast. Science 349(6252):1095–1100

Giri A, Lakshmi Narasu M (2000) Production of podophyllotoxin from *Podophyllum hexandrum*: A potential natural product for clinically useful anticancer drugs. Cytotechnology 34(1–2):17–26

Gong L, Wu Y, Qiu X, Xin X, An F, Guo M (2022) Adsorption characteristics and enrichment of Emodin from marine-derived *Aspergillus flavipes* HN4-13 extract by macroporous resin XAD-16. Mar Drugs 20(4):231

Goswami S, Vidyarthi AS, Bhunia B, Mandal T (2013) A review on lovastatin production. J Biochem Technol 4(1):581–587

Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: A treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1–8

Guruceaga X, Perez-Cuesta U, De Cerio AAD, Gonzalez O, Alonso RM, Hernando FL, Ramirez-Garcia A, Rementeria A (2019) Fumagillin, a mycotoxin of *Aspergillus fumigatus*: biosynthesis, biological activities, detection, and applications. Toxins 12(1):7

Happel N, Doenecke D (2009) Histone H1 and its isoforms: contribution to chromatin structure and function. Gene 431(1–2):1–12

Hussain H, Abdullah A-S, M., Schulz, B., Michael Steinert, A. K., & I. R. G., & Ahmed, I. (2017) A fruitful decade for fungal polyketides from 2007 to 2016: antimicrobial activity, chemotaxonomy and chemodiversity. Future Med Chem 14(9):1631–1648

Jaivel N, Marimuthu P (2010) Strain improvement of *Aspergillus terrus* for increased lovastatin production. Int J Eng Sci Technol 2(7):2612–2615

Jakubczyk D, Dussart F (2020) Selected fungal natural products with antimicrobial properties. Molecules 25(4):1–18

Jeewon R, Luckhun AB, Bhoyroo V, Sadeer NB, Mahomoodally MF, Rampadarath S, Puchooa D, Sarma VV, Durairajan SSK, Hyde KD (2019) Pharmaceutical potential of marine fungal endophytes. Reference series in phytochemistry, April, 283–305. Springer, Cham

Jiang M, Wu Z, Guo H, Liu L, Chen S (2020) A review of terpenes from marine-derived fungi: 2015–2019. Mar Drugs 18(6):2015–2019

Kakkar AK (2021) Pharmaceutical price regulation and its impact on drug innovation: mitigating the trade-offs. Expert Opin Ther Pat 31(3):189–192
Kaul S, Gupta S, Sharma S, Dhar MK (2017) The fungal endobiome of medicinal plants: a prospective source of bioactive metabolites. Recent Adv Res Dev, Medicinal plants and fungi, pp 167–228
Kaur H, Kaur A, Saini H, Chadha B (2009) Screening and selection of lovastatin hyper-producing mutants of *Aspergillus terreus* using cyclic mutagenesis. Acta Microbiol Immunol Hung 56(2):169–180
Khan AA, Bacha N, Ahmad B, Lutfullah G, Farooq U, Cox RJ (2014) Fungi as chemical industries and genetic engineering for the production of biologically active secondary metabolites. Asian Pac J Trop Biomed 4(11):859–870
Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28(7):1208–1228
Koster RA, Uges ECFDDRA (2015) Robust, high-throughput LC-MS/MS method for therapeutic drug monitoring of cyclosporin A, tacrolimus, everolimus and sirolimus in whole blood. Ther Drug Monit 31(1):116–125
Králová M, Frébortová J, Pěnčík A, Frébort I (2021) Overexpression of Trp-related genes in *Claviceps purpurea* leading to increased ergot alkaloid production. New Biotechnol 61:69–79
Kumar S, Aharwal RP, Jain R, Sandhu SS (2021) Bioactive molecules of endophytic fungi and their potential in anticancer drug development. Curr Pharm Rep 7(2):27–41
Kundu JK, Surh YJ (2008) Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett 269(2):243–261
Kusari S, Spiteller M (2011) Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes? Nat Prod Rep 28(7):1203–1207
Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798
Kusari S, Zühlke S, Spiteller M (2009) An endophytic fungus from *Camptotheca acuminata* that produces camptothecin and analogues. J Nat Prod 72(1):2–7
Lai LT, Pan C, Tzeng B (2002) The influence of medium design on lo v astatin production and pellet formation with a high-producing mutant of *Aspergillus terreus* in submerged cultures. Process Biochem 38(9):1317–1326
Lechner BE, Wright JE, Albertó E (2004) The genus *Pleurotus* in Argentina. Mycologia 96(4):845–858
Li SJ, Zhang X, Wang XH, Zhao CQ (2018) Novel natural compounds from endophytic fungi with anticancer activity. Eur J Med Chem 156:316–343
Lima MTNS, Santos LBD, Bastos RW, Nicoli JR, Takahashi JA (2018) Antimicrobial activity and acetylcholinesterase inhibition by extracts from chromatin modulated fungi. Braz J Microbiol 49:169–176
Luger K, Hansen JC (2005) Nucleosome and chromatin fiber dynamics. Curr Opin Struct Biol 15(2):188–196
Luthra U, Singh N, Tripathi A, Vora S, Bhosle V (2015) Media optimization for lovastatin production by statistical approach using *Aspergillus terreus* by submerged fermentation. J Med Sci Clin Res 3(2):4520–4528
Martienssen R, Moazed D (2015) RNAi and heterochromatin assembly. Cold Spring Harb Perspect Biol 7(8):1–20
Meng LH, Liu Y, Li XM, Xu GM, Ji NY, Wang BG (2015) Citrifelins A and B, Citrinin adducts with a tetracyclic framework from cocultures of marine-derived isolates of *Penicillium citrinum* and *Beauveria felina*. J Nat Prod 78(9):2301–2305
Mooibroek H, Kuipers AG, Sietsma JH, Punt PJ, Wessels JG (1990) Introduction of hygromycin B resistance into *Schizophyllum commune*: preferential methylation of donor DNA. Mol Gen Genet MGG 222:41–48
Moran VA, Perera RJ, Khalil AM (2012) Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. Nucleic Acids Res 40(14):6391–6400

Narasimha RP, Akbar AP, Sarayu B (2010) Antibacterial efficacy of secondary metabolites from entomopathogenic fungi *B. bassiana*. Int J Chem Anal Sci 1(5):94–96

Orfali R, Aboseada MA, Abdel-Wahab NM, Hassan HM, Perveen S, Ameen F et al (2021) Recent updates on the bioactive compounds of the marine-derived genus Aspergillus. RSC Adv 11(28):17116–17150

Parashar S, Cheishvili D, Mahmood N, Arakelian A, Tanvir I, Khan HA, Kremer R, Mihalcioiu C, Szyf M, Rabbani SA (2018) DNA methylation signatures of breast cancer in peripheral T-cells. BMC Cancer 18(1):1–9

Peng XY, Wu JT, Shao CL, Li ZY, Chen M, Wang CY (2021) Co-culture: stimulate the metabolic potential and explore the molecular diversity of natural products from microorganisms. Mar Life Sci Technol 3(3):363–374

Poleto L, da Rosa LO, Fontana RC, Rodrigues E, Poletto É, Baldo G, Paesi S, Sales-Campos C, Camassola M (2021) Production of antimicrobial metabolites against pathogenic bacteria and yeasts by *Fusarium oxysporum* in submerged culture processes. Bioprocess Biosyst Eng 44(6):1321–1332

Qader MM, Hamed AA, Soldatou S, Abdelraof M, Elawady ME, Hassane ASI, Belbahri L, Ebel R, Rateb ME (2021) Antimicrobial and antibiofilm activities of the fungal metabolites isolated from the marine endophytes *Epicoccum nigrum* m13 and *Alternaria alternata* 13a. Mar Drugs 19(4)

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S, Kumar Singh S, Gautam V (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology 12(3):139–159

Rajamanikyam M, Vadlapudi V, Amanchy R, Upadhyayula SM (2017) Endophytic fungi as novel resources of natural therapeutics. Braz Arch Biol Technol 60(December):1–26

Ramos HP, Said S (2011) Modulation of biological activities produced by an endophytic fungus under different culture conditions. Adv Biosci Biotechnol 2011(December):443–449

Ravuri M, Shivakumar S (2020) Bioprospecting medicinal plants for the isolation and screening of lovastatin producing endophytic fungi. Indian J Nat Prod Resour (IJNPR) [Formerly Natural Product Radiance (NPR)] 11(1):46–51

Shi TQ, Gao J, Wang WJ, Wang KF, Xu GQ, Huang H, Ji XJ (2019) CRISPR/CAS9-based genome editing in the filamentous fungus *Fusarium fujikuroi* and its application in strain engineering for gibberellic acid production. ACS Synth Biol 8(2):445–454

Shu-Ting C, Miles PG (2004) Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC Press, pp 159–185

Shweta S, Zuehlke S, Ramesha BT, Priti V, Mohana Kumar P, Ravikanth G, Spiteller M, Vasudeva R, Uma Shaanker R (2010) Endophytic fungal strains of *Fusarium solani*, from Apodytes dimidiata E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71(1):117–122

Siless GE, Gallardo GL, Rodriguez MA, Rincón YA, Godeas AM, Cabrera GM (2018) Metabolites from the dark septate endophyte *Drechslera* sp. evaluation by LC/MS and principal component analysis of culture extracts with histone deacetylase inhibitors. Chem Biodivers 15(8):1–23

Singh A, Singh DK, Kharwar RN, White JF, Gond SK (2021) Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: insights, avenues, and challenges. Microorganisms 9(1):1–42

Singh B, Ahuja A, Verma D, Sharma D, Kumar A, Sharma A (2023) Top Indian pharmaceutical companies: a review. J Innov Pharm 1:37

Singh P, Rathinasamy K, Mohan R, Panda D (2008) Microtubule assembly dynamics: an attractive target for anticancer drugs. IUBMB Life 60(6):368–375

Singh SK, Strobel GA, Knighton B, Geary B, Sears J, Ezra D (2011) An endophytic Phomopsis sp. possessing bioactivity and fuel potential with its volatile organic compounds. Microb Ecol 61(4):729–739

PFPM (2020) Size, share, and trends analysis report by product (intumescent coatings, cementitious materials), by application (construction, warehousing), and segment forecasts, 2020–2027. Market Analiz Rep 115

Song R, Zhai Q, Sun L, Huang E, Zhang Y, Zhu Y, Guo Q, Tian Y, Zhao B, Lu H (2019) CRISPR/Cas9 genome editing technology in filamentous fungi: Progress and perspective. Appl Microbiol Biotechnol 103(17):6919–6932

Stierle A, Strobel G, Stierle D, Grothaus P, Bignami G (1995) The search for a taxol-producing microorganism among the endophytic fungi of the pacific yew, *Taxus brevifolia*. J Nat Prod 58(9):1315–1324

Sui Y, Zheng Y, Li X, Li S, Xie B, Sun Z (2016) Characterization and preparation of oligomeric procyanidins from *Litchi chinensis* pericarp. Fitoterapia 112:168–174

Taritla S, Kumari M, Kamat S, Bhat SG, Jayabaskaran C (2021) Optimization of physicochemical parameters for production of cytotoxic secondary metabolites and apoptosis induction activities in the culture extract of a marine algal – derived endophytic fungus. Front Pharmacol 12:542891

Tucker JB, Zilinskas RA (2006) The promise and perils of synthetic biology. New Atlantis (Washington, D.C.) 12:25–45

Upendra RS, Khandelwal P (2016) Physical mutagenesis based strain improvement of Aspergillus sp. for enhanced production of lovastatin. Int J Pharm Pharm Sci 8(7):163–167

Uzma F, Mohan CD, Siddaiah CN, Chowdappa S (2019) Endophytic fungi: promising source of novel bioactive compounds. In: Advances in endophytic fungal research: present status and future challenges. Springer, Cham, pp 243–265

Vasanthakumari MM, Jadhav SS, Sachin N, Vinod G, Shweta S, Manjunatha BL et al (2015) Restoration of camptothecine production in attenuated endophytic fungus on re-inoculation into host plant and treatment with DNA methyltransferase inhibitor. World J Microbiol Biotechnol 31:1629–1639

Villalobos-Escobedo JM, Herrera-Estrella A, Carreras-Villaseñor N (2016) The interaction of fungi with the environment orchestrated by RNAi. Mycologia 108(3):556–571

Waddington CH (1942) The epigenotype. Int J Epidemiol 41(1):10–30

Walker RSK, Pretorius IS (2018) Applications of yeast synthetic biology geared towards the production of biopharmaceuticals. Genes 9(7). https://doi.org/10.3390/genes9070340

Wang C, Wu J, Mei X, Mei CWX (2001) Enhancement of taxol production and excretion in *Taxus chinensis* cell culture by fungal elicitation and medium renewal. Appl Microbiol Biotechnol 55:404–410

Wellham PA (2022) Cordycepin and the entomopathogenic fungus *Cordyceps militaris*. Doctoral dissertation, University of Nottingham

Yu J, Chang PK, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, Payne GA, Linz JE, Woloshuk CP, Bennett JW (2004) Clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol 70(3):1253–1262. https://doi.org/10.1128/AEM.70.3.1253-1262.2004

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Mini-Rev Med Chem 11(2):159–168. https://doi.org/10.2174/138955711794519492

Zhgun AA, Dumina MV, Voinova TM, Dzhavakhiya VV, Eldarov MA (2018) Role of acetyl-CoA synthetase and LovE regulator protein of polyketide biosynthesis in lovastatin production by wild-type and overproducing *Aspergillus terreus* strains. Appl Biochem Microbiol 54(2):188–197. https://doi.org/10.1134/S0003683818020138

Zou G, Meili Xiao SC, Zhihua Zhu YW, Z. Z. (2020) Microbial biotechnology – 2020 – Zou – efficient genome editing in filamentous fungi via an improved CRISPR-Cas9.pdf. Microb Biotechnol 14(6):2343–2355. https://doi.org/10.1111/1751-7915.13652

Chapter 13
Fungal Endophytes as Biocontrol Agents of Plant Pathogens: Recent Developments and Prospects

Felicia Lalremruati, Nancy Lalhriatpuii, Awmpuizeli Fanai, Beirachhitha Bohia, Lalrokimi, Rosie Lalmuanpuii, Zirlianngura, Mukesh Kumar Yadav, Prashant Kumar Singh, and Zothanpuia

Abstract Biotic stresses caused by plant pathogens like bacteria, viruses, and fungi have been a significant problem globally. Many chemically derived products have been used for decades to mitigate these problems. While these agrochemicals have been proven to be effective, the indiscriminate use of these toxic chemicals has adversely affected the environment leading to health risks in humans and animals and also results in the emergence of pathogens with chemical resistance. This calls for an urgent need to develop more ecofriendly products from natural sources. Fungal endophytes with the potential to colonize internal tissues of plants without causing diseases are the most suitable alternative to mitigate plant pathogens since they are known to inhabit a wide variety of bioactive compounds that have been proven to possess advantageous features like antibacterial, nematocidal, antifungal, and herbicidal properties. This chapter highlights the different types of fungal endophytes that can be used as biocontrol agents against phytopathogens, the methods of their isolation, and their bioactive compounds with their prospects to be formulated as sustainable natural biocontrol agents.

F. Lalremruati · N. Lalhriatpuii · A. Fanai · B. Bohia · P. K. Singh · Zothanpuia (✉)
Department of Biotechnology/Life Sciences Pachhunga University College, Mizoram University, Aizawl, Mizoram, India
e-mail: zothanpuia@pucollege.edu.in

Lalrokimi
Department of Biotechnology, Mizoram University, Aizawl, Mizoram, India

R. Lalmuanpuii
Department of Botany, Mizoram University, Aizawl, Mizoram, India

Zirlianngura
Department of Chemistry, Govt. Zirtiri Residential Science College, Aizawl, Mizoram, India

M. K. Yadav
Department of Microbiology, Central University of Punjab, Bathinda, Punjab, India

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1 Introduction

Plants are homes to vast communities of microbial populations that may reside above or below the organs embedded in the soil. These microbial populations have been reported to act as controlling agents against phytopathogens (plant pathogens) (Bruisson et al. 2019). Extensive and uncontrolled use of chemically derived pesticides has exponentially led to the exaggeration of complications such as resistance against synthetic chemicals used for disease control, subsequent nutrient loss, and release of harmful residues in the soil resulting in the decrease of their water holding capacity, fertility, and loss of microflora (Sharma et al. 2022). Humans bear this consequence as a cycle as these chemicals eventually reach our food through biomagnification and crop bioaccumulation (Najam and Alam 2023). Therefore, there is an urgent need to tap into safer natural sources for obtaining bioactive compounds for controlling phytopathogens/manufacturing of biopesticides (Chouhan et al. 2022). Fungal endophytes are the richest untapped reservoirs of novel metabolites in healthy plant tissues. Exploring these endophytic fungi for their biocontrol potential against plant pathogens can greatly relieve the need to use harmful pesticides that could further harm the environment (Chaudhary et al. 2022).

Globally, phytopathogens like bacteria, viruses, and fungi are the biotic stressors that cause adverse effects on crops. It has continued to be a problem with the increased resistance against major pesticides used commercially (Kim et al. 2004). The indiscriminate use of these agrochemicals has resulted in pathogens exhibiting multiple resistance against several agrochemicals (Talibi et al. 2014). This has also posed a threat to many of the animals, both terrestrial and aquatic, since the overuse of toxic chemicals in high concentrations has accumulated in the soils and reached the rivers, in turn affecting the populations of organisms that feed or live in these environments (Bai et al. 2013). Along with the decrease in crop productivity, health, and subsequent decrease in the yield of the crop plants, the biotic stresses causing diseases to plants have also resulted in the excessive production of reactive oxygen species that adversely affect the physiological and molecular functioning of the plants. Endophytes will soon prove to be the safest and most effective weapons for combatting plant diseases due to their ability to colonize the tissues of plants without causing any disease in them (Shi et al. 2017).

Endophytic fungi are of wide varieties and are present in nearly all plants. They reside within the internal tissues of host plants and are reported to form complex relationships with the host plants, including mutualism, commensalism, and, very rarely, parasitism (Jia et al. 2016). Besides providing tolerance against biotic and abiotic stresses and promoting the plant's growth and nutrition intake, they provide

a wide range of bioactive metabolites that protect their hosts against pests and pathogenic microorganisms (Gouda et al. 2016). This indicates the importance of endophytic fungi as rich sources of bioactive compounds to protect against plant pathogens in the agricultural sector. Different metabolites that may be used to produce agrochemicals, such as alkaloids, terpenoids, isocoumarins, peptides, etc., are reported to be inhabited by these fungal endophytes. The metabolites possess several mitigating attributes, such as antibacterial, nematocidal, antifungal, herbicidal, and insecticidal properties (Yamazaki et al. 2020).

2 Fungal Endophytes

Endophytes are prevalent and can form associations with different groups of organisms in plants. They can also protect against pests (Hartley and Gange 2009). They mostly live in mutualistic relationships within the plants in roots and different parts of the plant without causing detrimental effects such as chlorosis, necrosis, and lesions in the host plants (Hardoim et al. 2015). While a few fungal endophytes have been reported to cause harm to their host plants after incubation, fungal endophytes have been generally known to have mutualistic relationships with their hosts. Some also exist commensally with their hosts (Sikora et al. 2007). According to Petrini (1991), the pathogenicity of some fungal endophytes may be suppressed or dormant and activated in certain environmental conditions under stress or aging of the host plants. Some cases also support this statement when the hosts are infected but do not expose symptoms until environmental changes occur and lead to the exposure of these symptoms under stresses or maturity of either pathogens or host plants (Alvarez-Loayza et al. 2011).

Many studies have reported the benefits of endophytic fungi to the host plants, including the detrimental effects to the pests that feed on the plants inhabited by the endophytes (Rodriguez et al. 2009). The results of several studies have shown that plants that are colonized by endophytic fungi have been protected from extensive damage and the pests that prey on these plants lose their productivity (Gurulingappa et al. 2011; Gathage et al. 2016; Rodriguez et al. 2009). The substantial decrease in the developmental rate of pests, obstruction in their feeding habits, and growth retardation of the pests leading to the increase in mortality of the pests feeding on these plants are some of the mechanisms that are incorporated by endophytic fungi (Akello and Sikora 2012; Lacey and Neven 2006; Martinuz et al. 2013).

Since endophytes reside within the tissues of host plants, they create barriers against phytopathogens that may colonize the host plants (Moy et al. 2000). Endophytes are rich sources of secondary bioactive metabolites like tannins, terpenoids, saponins, steroids, and phenolic acids. Besides these, they also act as antagonists of insect pests, anticancer, anti-inflammatory, and antimicrobial properties (Gouda et al. 2016). Endophytes also produce chemicals that hinder the maturation of other antagonists, including the progression of pathogens, and endure biotic stresses like rhizospheric nematodes and other insects and abiotic stresses like

drought, saline conditions, and high-temperature changes (Khan et al. 2012). An investigation conducted by Knoch et al. (1993) stated that endophytic fungi are involved in the indirect dispersal of seeds through ants. The findings were that *Neotyphodium coenophialum*-infected seeds of *Festuca arundinacea* L. (Schreb) were sheltered against two types of seed-invading ants – *Pogonomyrmex rugosus* and *Pogonomyrmex occidentalis.* The seeds infected with *Neotyphodium coenophialum* were then collected by these two types of ants, thus helping in the dispersal of seeds periodically.

3 Isolation of Fungal Endophytes

Khalil et al. (2021) isolated endophytic fungi from the leaves and stems of *Ephedra pachyclada* by first washing the plant segments with running water and cutting the leaves in equal sizes from the middle portions of the leaf to include the midrib. The leaf segments were then subjected to surface sterilization by using double-distilled water for 60 s, 70% alcohol (ethanol) for 60 s, and 2.5% sodium hypochlorite for 4 min and finally rinsing with distilled water three times. 100 uL from the final water used for rinsing was then inoculated by using spread plate technique on Malt Extract Agar (MEA) medium and incubated for 24 h to check the effectiveness of the sterilization. The sterile plant parts were then cut into five pieces each (approx. 5 mm), and twenty segments of the leaf were inoculated or placed on the MEA plate (five leaf segments per plate), enhanced with 0.05 g of streptomycin sulfate per 100 ml of the medium to inhibit the growth of unwanted bacteria, and incubation was done at 28 °C ± 2 °C. The Petri plates were checked daily for fungal growth, and single isolates were then reinoculated on fresh MEA media plates to purify the isolates. Deepthi et al. (2018) isolated fungal endophytes from the leaves of *Elaeocarpus sphaericus* (Gaertn.) K. Schum. and *Myristica fragrans* Houtt. The leaves were rinsed with tap water for 10 min, dipped in 1% sodium hypochlorite for 3–4 min, and then washed with 70% ethanol for 1 min. Lastly, double-distilled water was used to wash the leaf segments three times. The excess water of the surface of the leaves was then blotted using sterile filter paper, and the water from the final rinse was streaked onto PDA (potato dextrose agar) media plates to check the efficacy of the sterilization process. The sterilized leaf segments were then cut into 1cm each and inoculated in Petri dishes with PDA media (HiMedia, Mumbai, India) enhanced with 100mg/ml chloramphenicol and incubated at 28 °C ± 2 °C. Individual tips of hyphae emerging from each plant segment were then transferred to fresh PDA media.

The *Uvaria grandiflora* plant leaf discs were used as sources for isolating fungal endophytes- *Colletotrichum* spp. and *Nigrospora* spp. following modified steps. The leaf discs of the plant were sterilized using immersion in 70% ethanol followed by immersion in 0.5% sodium hypochlorite for 1 min and 30 s for the latter. The leaves were then rinsed with sterilized distilled water, and sterilized tissue paper was used to blot the excess moisture from the surface of the leaves. These explants

were then placed in Petri plates containing half-strength Malt Extract Agar (MEA) (Hi-Media, Mumbai, India) amended with 450 μg/mL of streptomycin (Sigma Aldrich, USA). On each MEA plate, six explants were inoculated and the imprints of the explants were also taken on the media plates. Two uninoculated media plates were also exposed to ensure the sterility of the environment in which the work is being carried out. The Petri plates were then incubated at room temperature for 7–14 days to allow the growth of endophytic fungi. Spore touch method was employed to obtain pure fungal cultures from the colonies that arise from the incubated explants and subcultured in freshly prepared Malt Extract Agar (MEA). MEA was also prepared in test tube slants and utilized to prepare stock cultures of pure fungal colonies (Notarte et al. 2019).

Fresh leaves, stems, and fruits of healthy *Solanum mauritianum* were used for the isolation of culturable endophytic fungi to investigate their antimicrobial and antimycobacterial activities against three pathogenic bacteria: *M. bovis*, *M. smegmatis*, and *K. pneumoniae.* Surface sterilization of the plant parts was performed by rinsing them with running water followed by immersing them in 70% ethanol (250 ml) for 60 s and 5% sodium hypochlorite (250 ml) for 5 min each. Then, the plant parts were rinsed three times separately using 3 L of sterile water. The sterilized plant parts were then cut into 5 mm each using a sterile scalpel and placed onto Petri plates with three different nutritional media: potato dextrose agar (PDA), nutrient agar (NA), and Sabouraud dextrose agar (SDA) to obtain maximum diversity of endophytic fungi. Incubation was done at 25 °C for 1–2 weeks in the dark. After 1 week, emerging hyphae were subcultured in fresh media for purification (Pelo et al. 2020).

Mishra et al. (2016) isolated fungal endophytes from healthy leaves, stems (twigs), and the bark of *Schima wallichii* plant which was collected from Dampa Tiger Reserve Forest, Mizoram. The plant parts were first washed with running water to remove dirt and dust particles and cut into 2–3 cm pieces each. Seventy percent ethanol was firstly used for surface sterilization for 1 min and then 3% sodium hypochlorite was used for 3 min of immersion of the plant parts, and then immersed in 70% ethanol again for 30 s followed by a rinse with sterile distilled water. A sterile scalpel was used to remove the outer layer of the plant segments. The stem and bark parts were cut 1cm each, while the leaves were sliced 5 mm each. The water used for the last rinse of the plant tissues were spread onto Petri plates containing nutritional media to check the effectiveness of the surface sterilization methods, and for the presence of epiphytic fungal growth, the plant tissues were imprinted on PDA media. Different types of nutritional media such as potato dextrose agar (PDA) media, Czapek Dox agar (CDA) media, and malt yeast extract agar (MYA) media were employed, which were enhanced with 50 mg/L of streptomycin to inhibit the growth of bacteria. The culture plates were incubated for 3–5 days at 27 °C for three to five days under 12 h white light/12 h of darkness. To check the growth of emerging hyphae from the plant tissues, the culture plates were observed for 2–3 weeks. The emerging tips of hyphae from the sterile plant tissues were transferred to freshly prepared culture media without antibiotics. Stock cultures

were prepared by preserving the pure fungal cultures in 30% glycerol stored at −80 °C.

4 Antagonism of Fungal Endophytes

"Biological control" is a term that is used to describe the suppression of pathogenic microbial activities in disease development using organisms with antagonistic potential (Fig. 13.1) (Stangarlin et al. 2011). Different pests like nematodes, bacteria, fungi, and viruses are some examples that may cause diseases or recur the development of plants and their growth (Segaran and Sathiavelu 2019). Inhibition of pathogens by other microbes is mostly due to the various antimicrobial or bioactive compounds they produce and is most extensively investigated (Ting et al. 2010). Plant pathogens are sensitive toward various antibiotics which are produced by different strains of biocontrol agents such as alkaloids, terpenoids, sesquiterpenoids, and polypeptide compounds. 3,11,12-trihydroxycadalene is a sesquiterpenoid derivative which was isolated from a fungal endophyte *Phomopsis cassia* with antifungal property toward *Cladosporium* sp. (Gao et al. 2010). The utilization of biocontrol agents such as biopesticides has increased exponentially in the global market. The biopesticide market has increased by 0.2% among the overall use of pesticides since the year 2000 (Nieuwesteeg 2015). The in vitro dual culture techniques were employed to analyze the effects of the antagonistic endophytic fungi isolated from the plants against different plant pathogens. Some of them are listed in Table 13.1. Direct methods of biological control involve production of lytic enzymes and antibiotics.

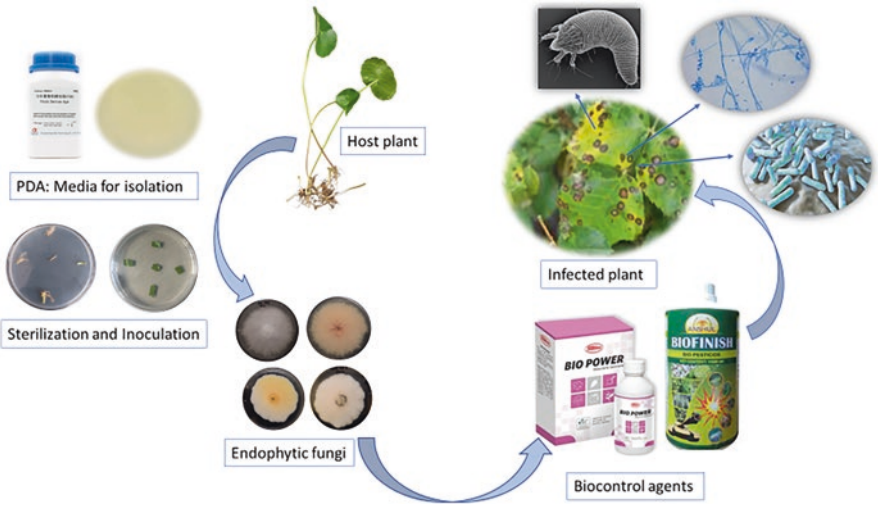

Fig. 13.1 Overview of fungal endophytes as biocontrol agents

Table 13.1 Antagonistic activity of fungal endophytes against phytopathogens

Sl no.	Fungal endophytes	Bioactive compounds/agents	Plants (hosts)	Plant pathogens	Disease	References
1.	*Clavicipitaceae* sp.	Alkaloids – ergovaline lolitrems, and lolines	*Poaceae*	*Rhizoctonia* sp., *Puccinia coronate*, *Alternaria triticina*	Oat crown rust, yellow dwarf disease, leaf blight	Clay and Schardl (2002)
2.	*Fusarium oxysporum*, *Pochonia chlamydosporia*	Coumarins – gibepyrone A, pyrrolo (1,2-a) pyrazine	*Dendrobium lindleyi*	*Fusarium* sp., *Sclerotium* sp., *Colletotrichum* sp., *Curvularia* sp., *and Phytophthora* sp.	Crown rot disease, leaf blight	Bungtongdee et al. (2019)
3.	*Alternaria* sp. *Samif01*	Alternariol 9-methyl ether	*Salvia miltiorrhiza Bunge*	*Agrobacterium tumefaciens*, *Bacillus subtilis*, *Ralstonia solanacearum*, *Staphylococcus haemolyticus*, *and Xanthomonas vesicatoria*	Wilt disease	Lou et al. (2016)
4.	*Epicoccum nigrum*	Beauvericin	*Entada abyssinica*	*Salmonella typhimurium and Bacillus cereus*	Gastroenteritis in humans and animals	Dzoyem et al. (2017)
5.	*Alternaria* sp.	3,6,6a,9,10-pentahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene	*Pinus ponderosa*	*Leishmania donovani*	Leishmaniasis in humans	Tantry et al. (2018)
6.	*Trichoderma citrinoviride*	Ginsenosides	*Panax ginseng*	*Botrytis cinerea*, *Alternaria panax*, *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* spp., *Botrytis cinerea*	Root rot disease	Park et al. (2019)

(continued)

Table 13.1 (continued)

Sl no.	Fungal endophytes	Bioactive compounds/agents	Plants (hosts)	Plant pathogens	Disease	References
7.	*Rhizopycnis vagum*	Allosecurinine	*Zingiber officinale* Rosc.	*Rhizoctonia solani*, *Corynespora cassiicola*, *Colletotrichum acutatum*, *Phytophthora infestans*, *Fusarium oxysporum*, *Sclerotium rolfsii*	Necrosis	Anisha et al. (2018)
9.	*Nigrospora sphaerica*	N/A	*Cornus florida*	*Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*	Root rot disease	Mmbaga et al. (2018)
10.	*Paenibacillus polymyxa*	Tenllone I (1)	*Morinda citrifolia* L.	*Aspergillus aculeatus*	Rot disease	Liu et al. (2019)
11.	*Alternaria* sp., *Diaporthe* sp., *Nigrospora oryzae*	Phenylethyl alcohol, 4-methylquinazoline, benzothiazole, benzyl alcohol, lilial, galaxolide	*Olea europaea* L.	*Colletotrichum acutatum*	Ripe rot and bitter rot disease	Landum et al. (2016)
12.	*Trichoderma viride*	N/A	*Spilanthes paniculata*	*Alternaria solani*, *Colletotrichum capsici*, *Fusarium solani*	Leaf spot and defoliation	Talapatra et al. (2017)
13.	*Penicillium commune*	3-methyl,1-butanol	*Monarda citriodora*	*Sclerotinia* sp.	White mold	Katoch and Pull (2017)
14.	*Lasiodiplodia pseudotheobromae*, *Fusarium* sp., *Xylariales* sp.	N/A	*Houttuynia cordata* (Thunb.)	*Fusarium oxysporum*, *Sclerotium rolfsii*, *Alternaria brassicicola*	Wilt and rot disease	Aramsirirujiwet et al. (2016)
15.	*Talaromyces funiculosus*, *Rhexocercosporidium* sp., *Fusarium solani*, *Fomitopsis* sp., *Purpureocillium lilacinum*	Flavonoids, alkaloids, polysaccharides, and saponins	*Sophora tonkinensis* Gapnep	*Fusarium solani*, *Colletotrichum gloeosporioides*	Bitter rot disease	Yao et al. (2017)

In contrast, competition between space and nutrition, enhancement of plant growth, and induction of systemic resistance are the indirect methods to antagonize the plant pathogens (Mmbaga et al. 2018). In biocontrol mechanisms, the organisms directly contribute to various mechanisms like parasitism, competition, and antibiosis against plant pathogens. Various types of biological control processes direct plant disease caused by pathogenic microorganisms. Endophytic organisms are directly involved in the biocontrol mechanisms such as competition and antibiosis against phytopathogens (Ownley et al. 2010). The production of elevated antipathogenic compounds has enhanced the host plant's defense mechanism against phytopathogens and pests. The endophytes also contribute to the different defense mechanisms of the host plants due to their secretion of various secondary metabolites (Katisky et al. 2018).

Clay and Schardl (2002) have investigated the potential of *Clavicipitaceae* sp. of fungus that they have isolated from the blades of the grass, against different plant pathogens like *Rhizoctonia* sp., *Puccinia coronata*, and *Alternaria triticina* which are known to cause oat crown rust disease, yellow dwarf disease, and leaf blight in the host plants. The alkaloids – ergovaline, lolitrems, and lolines – produced by the fungal endophytes showed antifungal activity against these phytopathogens. Coumarin derivatives such as gibepyrone A and pyrrolo(1,2-a)pyrazine which were produced by *Fusarium oxysporum* and *Pochonia chlamydosporia* also showed inhibition against crown rot and leaf blight disease causing *Sclerotium* sp. and *Colletotrichum* sp. in *Dendrobium lindleyi* plant (Bungtongdee et al. 2019). Wilt disease caused by *Staphylococcus haemolyticus*, *Ralstonia* sp., and *Xanthomonas* sp. was also inhibited by *Alternaria* sp. Samif01 – a fungal endophyte which was isolated from *Salvia miltiorrhiza* Bunge due to the composition of the compound alternariol 9-methyl ether (Lou et al. 2016). The extracts of endophytic fungi, *Epicoccum nigrum* and *Alternaria* sp., which consists of beauvericin and other metabolites also tested positive for their inhibition against human and animal pathogens- *Salmonella* sp., and *Leishmania donovani* which cause gastroenteritis and leishmaniasis (Dzoyem et al. 2017; Tantry et al. 2018). Park et al. (2019) conducted experiments on the stems of a ginseng plant where they isolated *Trichoderma citrinoviride*, an endophytic fungus, and tested it against different phytopathogens like *Botrytis cinerea*, *Alternaria panax*, *Cylindrocarpon* sp., and *Phytophthora cactorum* which causes root rot diseases and showed that the ginsenosides present in the endophyte cause the inhibition against these pathogens. The crude extract of the endophytic fungi – *Rhizopus vagum* – was isolated from *Zingiber officinale* Rosc. The scanning electron microscopy confirmed the inhibition against the soft rot pathogen *Pythium myriotylum* and various other plant pathogens (Anisha et al. 2018). Among 16 endophytes isolated from the stems of flowering dogwood or *Cornus florida* plant, Mmbaga et al. (2018) found that *Nigrospora sphaerica*, a fungal endophyte, showed antagonism toward the different root rot disease causing pathogens such as *Fusarium* sp. *and Macrophomina phaseolina.* Another rot disease causing pathogen – *Aspergillus aculeatus* – was also inhibited by a fungal endophyte, *Paenibacillus polymyxa* isolated from *Morinda citrifolia* L. which comprised of the compound, tenllone 1(1) (Liu et al. 2019). Fourteen endophytic fungi

isolated from the trees of *Olea europaea* L. were tested for their inhibitory activity toward ripe and bitter rot disease-causing pathogens and every isolate showed inhibition toward *Colletotrichum acutatum* (Landum et al. 2016). Talapatra et al. (2017) conducted studies on the fungal endophyte *Trichoderma viride* isolated from the leaves of *Spilanthes paniculata* and tested for their inhibitory activity against the leaf spot and defoliation causing pathogens *Alternaria solani*, *Fusarium solani*, and *Colletotrichum capsici* and resulted in positive antagonism. Among 82 endophytes isolated with different genera, 11% of the isolates showed activity against at least one pathogen, and *Penicillium commune* displayed complete inhibition toward the white mold-causing pathogen *Sclerotinia* sp., which can be attributed to the compound 3-methyl,1-butanol (Katoch and Pull 2017). *Lasiodiplodia pseudotheobromae*, *Fusarium* sp., and *Xyriales* sp., isolated from *Houttuynia cordata* (Thunb.), have showed positive inhibition toward wilt rot disease causing *Fusarium oxysporum*, *Sclerotium rolfsii*, and *Alternaria brassicicola* (Aramsirirujiwet et al. 2016). The fungal endophytes – *Talaromyces funiculosus*, *Rhexocercosporidium* sp., *Fusarium* sp., and *Purpureocillium lilacinum* – are also promising biocontrol agents against bitter rot disease-causing pathogens like *Fusarium solani* and *Colletotrichum gloeosporioides* due to their flavonoid, alkaloid, polysaccharide, and saponin content (Yao et al. 2017).

5 Fungal Endophytes as Biocontrol Agents

In 1914, C.F. von was the first to utilize biological weapons to combat plant pathogens. Agrochemicals can be broadly distinguished into two parts namely – pesticides and fertilizers. Pesticides can be further divided into fungicides, insecticides, and herbicides which can be used against weeds, pathogens, and insects (De Silva et al. 2019). Extensive usage of chemical pesticides and fertilizers may threaten the environment, causing soil and water contamination, endangering the nontarget organisms, animals, and plants. It may even lead to the induction of resistance of the weeds and pests against pesticides (Stangarlin et al. 2011). Misuse of pesticides also leads to the formation of toxic by-products. Induced resistance against pesticides and hazardous effects on the ozone layer along with overall contamination of the environment are various problems that arise due to the misuse of these chemical pesticides. Therefore, the use of these chemically derived compounds proves to be inefficient and hazardous to nature (Goudjal et al. 2014). The first two generations of pesticides comprise of organic substances rendering multisite inhibition with defense responses against various pests and pathogens. The third-generation pesticides are manufactured with lower toxicity compounds that are site specific and are used for managing plant diseases and pests (Silva et al. 2018). Since chemically derived pesticides have been proven to be the most effective toward eliminating pests and pathogens, without considering the detrimental effects on the environment, farmers worldwide have chosen chemically derived pesticides as agents for control. Inconsiderate and irresponsible usage of these chemicals has transformed

many plant pathogens to become resistant to antibiotics and other pathogens (Adnan et al. 2019).

The ecological microbiomes of endophytic fungi are complex and their bioactive compounds are still underexplored. So, these are promising biocontrol agents against plant pathogens and other human and animal pathogens as well (Zheng et al. 2017). Due to the EU regulations and the emergent pesticide-resistant plant pathogens leading to the failure in pathogen control, the use of many pesticides in agriculture is now withdrawn. So, this gives rise to the need of more sustainable and effective approaches toward the control of phytopathogens. Integration of physical, chemical, and biological control methods is now required to effectively and sustainably manage the control plant pathogens (Malandrakis et al. 2018). Due to its efficient reproducibility, plant growth promotion, nutrient utilization, and ability toward rhizosphere alteration and survivability in adverse conditions, it makes *Trichoderma* a competent agent against numerous plant pathogens (Adnan et al. 2019). By integration of various eradication and protection methods, pollution of environment, loss of crop yields, and control of plant pathogens can be maintained by incorporating biological control agents such as fungal and bacterial endophytes producing effective secondary metabolites such as alkaloids, sesquiterpenes, terpenoids, tannins, and other phenolic compounds (Arwiyanto 2014) as shown in Table 13.1.

6 Economic Impact of Plant Pathogens

Though pathogens have had detrimental effects on the healthcare sectors through infections on humans and animals, plant pathogens have specifically caused adverse effects on the food supply globally. According to the Food and Agriculture Organization of the United Nations (FAO), a loss of 20–40% of yield has occurred, estimated to be approximately 220 billion dollars per year. This is also attributed to climate change which have encouraged the spread of plant pathogens (Ristaino et al. 2021). India being the second-highest producer of wheat in the world has also endured severe losses in wheat production due to the wheat blast disease caused due to the spread of the plant pathogenic fungus *Magnaporthe oryzae* Triticum which was firstly identified in Brazil and reported in Bangladesh in 2016 and further spread to India and other neighboring countries as well. The sudden climate change to an atypically warm weather during the preharvest season is believed to be the cause of the outbreak of the wheat blast disease in 2016 besides anthropogenic activities that may have generated the transfer of the pathogens to other regions as well (Fones et al. 2020). Plant pathogens specifically parasitic nematodes have gained significance globally due to their impacts both in economy and global food supply. Food crops in particular have been estimated to have lost 10.7% of their yield due to pathogenic infections, and other crops of economic importance have suffered losses as high as 14%, averaging up to 12.33% (Chen 2020). Developing countries like India have also suffered losses at around 14.6%. According to the current prices, Rs. 400 million worth of crop produce was lost in Rajasthan in the 1960s due to the

Molya disease caused by the nematode *Heterodera avenae*, and *Anguina tritici* was also the cause of a loss of Rs. 450 million worth of wheat produce. Similar to these incidences, root lesions in coffee plants caused by *Pratylenchus* have damaged the coffee worth Rs. 200 million (Chaloner et al. 2021). *M. incognita* is a major cause of crop production losses all over the world. India has also been the main cause of food crop losses in vegetables like lady's finger, brinjals, tomatoes, and cucumbers. In Southeast Asia, the rice and wheat crops have endured major losses due to the plant pathogen – *Meloidogyne graminicola* (Fahad et al. 2021). Owing to the global climate change and emergence of more pesticide and antibiotic-resistant plant pathogens, there is an emergent need to address the current food and economic crisis and adopt more sustainable and eco-friendly ways for the management of plant pathogens to increase crop production all over the world (Durham and Mizik 2021).

7 Future Prospects

Fungal endophytes play vital roles toward the development of the host plants, in their physiology, and agricultural niches. Endophytic fungi as microbial biocontrol agents are promising sources with effective environmentally sustainable mechanisms to combat plant pathogens and their diseases. The extensive distribution of endophytes is prominent in close association with their host plants, providing various benefits such as production of secondary metabolites/bioactive compounds that promote plant growth, induce stress tolerance, and control plant pathogens. However, unstable environmental conditions challenge the steady growth and production of fungal endophytes as biocontrol agents in field conditions. Successful utilization of fungal endophytes as biocontrol agents requires extensive research. Initial studies should include the identification of fungal endophytes and understanding their morphology and optimal growth conditions in specific environments. The association between the fungal endophytes and their host plants and their interactions in different environmental conditions also require an in-depth study to reveal the extent of their benefits toward the protection of plants against plant pathogens and other stresses. Since the complex interaction between the endophytic fungi and the host plant may switch from mutualistic to opportunistic, the potent endophytes should be analyzed in planta under various environmental conditions with abiotic stresses.

Recent advancements in the field of molecular science have unveiled novel and distinctive information on endophytes to provide a better understanding of the diverse microbial interactions along with plant-microbe interactions. High-resolution microscopic instruments and enhanced fluorescent proteins have endowed to better understanding of plant-microbe interactions. The research on fungal endophytes as biocontrol agents provides inspiring and promising opportunities for the development of more sustainable agents to combat plant pests and pathogens, and management of plant diseases, simultaneously encouraging the enhancement of our knowledge in the fungal diversity, their complex mechanisms, and interactions with

different biological ecosystems and metabolites produced. Furthermore, better understanding of fungal microbiomes will lead to promising novel sources of plant biocontrol agents, greatly enhancing the agricultural sectors in plant disease control, thus increasing crop yield.

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References

Adnan M, Islam W, Shabbir A, Khan KA, Ghramh HA, Huang Z et al (2019) Plant defense against fungal pathogens by antagonistic fungi with Trichoderma in focus. Microb Pathog 129:7–18

Akello J, Sikora R (2012) Systemic acropedal influence of endophyte seed treatment on Acyrthosiphon pisum and Aphis fabae offspring development and reproductive fitness. Biol Control 61(3):215–221

Alvarez-Loayza P, White JF Jr, Torres MS, Balslev H, Kristiansen T, Svenning JC, Gil N (2011) Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, Iriartea deltoidea. PloS One 6(1):e16386

Anisha C, Jishma P, Bilzamol VS, Radhakrishnan EK (2018) Effect of ginger endophyte Rhizopycnis vagum on rhizome bud formation and protection from phytopathogens. Biocatal Agric Biotechnol 14:116–119

Aramsirirujiwet Y, Gumlangmak C, Kitpreechavanich V (2016) Studies on antagonistic effect against plant pathogenic fungi from endophytic fungi isolated from Hottuynia Cordata Thunb and screening for Siderophore and indole-3-acetic acid production. Asia-Pacific J Sci Technol 21(1):55–66

Arwiyanto T (2014) Biological control of plant disease caused by bacteria. Jurnal Perlindungan Tanaman Indonesia 18(1):1–12

Bai YB, Zhang AL, Tang JJ, Gao JM (2013) Synthesis and antifungal activity of 2-chloromethyl-1 H-benzimidazole derivatives against phytopathogenic fungi in vitro. J Agric Food Chem 61(11):2789–2795

Bruisson S, Zufferey M, L'Haridon F, Trutmann E, Anand A, Dutartre A, De Vrieze M, Weisskopf L (2019) Endophytes and Epiphytes From the Grapevine Leaf Microbiome as Potential Biocontrol Agents Against Phytopathogens. Front Microbiol 10:2726. https://doi.org/10.3389/fmicb.2019.02726

Bungtongdee N, Sopalun K, Laosripaiboon W, Iamtham S (2019) The chemical composition, antifungal, antioxidant and antimutagenicity properties of bioactive compounds from fungal endophytes associated with Thai orchids. J Phytopathol 167(1):56–64

Chaloner TM, Gurr SJ, Bebber DP (2021) Plant pathogen infection risk tracks global crop yields under climate change. Nat Clim Chang 11(8):710–715

Chaudhary P, Agri U, Chaudhary A, Kumar A, Kumar G (2022) Endophytes and their potential in biotic stress management and crop production. Front Microbiol 13:933017. https://doi.org/10.3389/fmicb.2022.933017

Chen X (2020) Pathogens which threaten food security: Puccinia striiformis, the wheat stripe rust pathogen. Food Security 12(2):239–251

Chouhan S, Agrawal L, Prakash A (2022) Amelioration in traditional farming system by exploring the different plant growth-promoting attributes of endophytes for sustainable agriculture. Arch Microbiol 204(2):151. https://doi.org/10.1007/s00203-021-02637-4

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160(S4):S99–S127
De Silva NI, Brooks S, Lumyong S, Hyde KD (2019) Use of endophytes as biocontrol agents. Fungal Biol Rev 33(2):133–148
Deepthi VC, Sumathi S, Faisal M, Elyas KK (2018) Isolation and identification of endophytic fungi with antimicrobial activities from the leaves of Elaeocarpus sphaericus (gaertn.) K. Schum and Myristica fragrans houtt. Int J Pharm Sci Res 9(7):2783–2791
Durham TC, Mizik T (2021) Comparative economics of conventional, organic, and alternative agricultural production systems. Economies 9(2):64
Dzoyem JP, Melong R, Tsamo AT, Maffo T, Kapche DG, Ngadjui BT et al (2017) Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus Epicoccum nigrum associated with Entada abyssinica. Rev Bras 27:251–253
Fahad S, Sonmez O, Saud S, Wang D, Wu C, Adnan M, Turan, V. (Eds.). (2021) Developing climate-resilient crops: improving global food security and safety. CRC Press
Fones HN, Bebber DP, Chaloner TM, Kay WT, Steinberg G, Gurr SJ (2020) Threats to global food security from emerging fungal and oomycete crop pathogens. Nat Food 1(6):332–342
Gao FK, Dai CC, Liu XZ (2010) Mechanisms of fungal endophytes in plant protection against pathogens. Afr J Microbiol Res 4(13):1346–1351
Gathage JW, Lagat ZO, Fiaboe KKM, Akutse KS, Ekesi S, Maniania NK (2016) Prospects of fungal endophytes in the control of Liriomyza leafminer flies in common bean Phaseolus vulgaris under field conditions. BioControl 61:741–753
Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538
Goudjal Y, Toumatia O, Yekkour A, Sabaou N, Mathieu F, Zitouni A (2014) Biocontrol of Rhizoctonia solani damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. Microbiol Res 169(1):59–65. https://doi.org/10.1016/j.micres.2013.06.014
Gurulingappa P, McGee PA, Sword G (2011) Endophytic Lecanicillium lecanii and Beauveria bassiana reduce the survival and fecundity of Aphis gossypii following contact with conidia and secondary metabolites. Crop Prot 30(3):349–353
Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320
Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342
Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 7:906
Katisky da Costa Stuart A, Stuart RM, Pimentel IC (2018) Effect of agrochemicals on endophytic fungi community associated with crops of organic and conventional soybean (Glycine max L. Merril). Agric Nat Resour 52(4):388–392
Katoch M, Pull S (2017) Endophytic fungi associated with Monarda citriodora, an aromatic and medicinal plant and their biocontrol potential. Pharm Biol 55(1):1528–1535
Khalil AMA, Hassan SED, Alsharif SM, Eid AM, Ewais EED, Azab E et al (2021) Isolation and characterization of fungal endophytes isolated from medicinal plant Ephedra pachyclada as plant growth-promoting. Biomolecules 11(2):140
Khan AL, Hamayun M, Khan SA, Kang SM, Shinwari ZK, Kamran M, Ur Rehman S, Kim JG, Lee IJ (2012) Pure culture of Metarhizium anisopliae LHL07 reprograms soybean to higher growth and mitigates salt stress. World J Microbiol Biotechnol 28(4):1483–1494. https://doi.org/10.1007/s11274-011-0950-9
Kim YM, Lee CH, Kim HG, Lee HS (2004) Anthraquinones isolated from Cassia tora (Leguminosae) seed show an antifungal property against phytopathogenic fungi. J Agric Food Chem 52(20):6096–6100

Knoch TR, Faeth SH, Arnott DL (1993) Endophytic fungi alter foraging and dispersal by desert seed-harvesting ants. Oecologia 95:470–473

Lacey LA, Neven LG (2006) The potential of the fungus, Muscodor albus, as a microbial control agent of potato tuber moth (Lepidoptera: Gelechiidae) in stored potatoes. J Invertebr Pathol 91(3):195–198

Landum MC, do Rosário Félix M, Alho J, Garcia R, Cabrita MJ, Rei F, Varanda CM (2016) Antagonistic activity of fungi of Olea europaea L. against Colletotrichum acutatum. Microbiol Res 183:100–108

Liu H, Chen Y, Li H, Li S, Tan H, Liu Z et al (2019) Four new metabolites from the endophytic fungus Diaporthe lithocarpus A740. Fitoterapia 137:104260

Lou J, Yu R, Wang X, Mao Z, Fu L, Liu Y, Zhou L (2016) Alternariol 9-methyl ether from the endophytic fungus Alternaria sp. Samif01 and its bioactivities. Braz J Microbiol 47:96–101

Malandrakis A, Daskalaki ER, Skiada V, Papadopoulou KK, Kavroulakis N (2018) A Fusarium solani endophyte vs fungicides: compatibility in a Fusarium oxysporum f.sp. radicis-lycopersici - tomato pathosystem. Fungal Biol 122(12):1215–1221. https://doi.org/10.1016/j.funbio.2018.10.003

Martinuz A, Schouten A, Sikora RA (2013) Post-infection development of Meloidogyne incognita on tomato treated with the endophytes Fusarium oxysporum strain Fo162 and Rhizobium etli strain G12. BioControl 58:95–104

Mishra VK, Passari AK, Singh BP (2016) In vitro antimycotic and biosynthetic potential of fungal endophytes associated with Schima Wallichii. In: Current trends in plant disease diagnostics and management practices. Springer, Cham, pp 367–381

Mmbaga MT, Gurung S, Maheshwari A (2018) Screening of plant endophytes as biological control agents against root rot pathogens of pepper (Capsicum annum L.). J Plant Pathol Microbiol 9(3):1000435

Moy M, Belanger F, Duncan R, Freehoff A, Leary C, Meyer W et al (2000) Identification of epiphyllous mycelial nets on leaves of grasses infected by clavicipitaceous endophytes. Symbiosis

Najam L, Alam T (2023) Occurrence, distribution, and fate of emerging persistent organic pollutants (POPs) in the environment. In: Emerging contaminants and plants: interactions, adaptations and remediation technologies. Springer International Publishing, Cham, pp 135–161

Nieuwesteeg B (2015) Biological control of fungal plant pathogens by tomato endosphere bacteria. Unpublished Master's Thesis

Notarte KIR, Devanadera MKP, Mayor ABR, Cada MCA, Pecundo MH, Macabeo APG (2019) Toxicity, antibacterial, and antioxidant activities of fungal endophytes Colletotrichum and Nigrospora spp. isolated from Uvaria grandiflora. Philipp J Sci 148(3):503–510

Ownley BH, Gwinn KD, Vega FE (2010) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl 55:113–128

Park YH, Mishra RC, Yoon S, Kim H, Park C, Seo ST, Bae H (2019) Endophytic Trichoderma citrinoviride isolated from mountain-cultivated ginseng (Panax ginseng) has great potential as a biocontrol agent against ginseng pathogens. J Ginseng Res 43(3):408–420

Pelo S, Mavumengwana V, Green E (2020) Diversity and antimicrobial activity of culturable fungal endophytes in Solanum mauritianum. Int J Environ Res Public Health 17(2):439

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York

Ristaino JB, Anderson PK, Bebber DP, Brauman KA, Cunniffe NJ, Fedoroff NV et al (2021) The persistent threat of emerging plant disease pandemics to global food security. Proc Natl Acad Sci 118(23):e2022239118

Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Segaran G, Sathiavelu M (2019) Fungal endophytes: a potent biocontrol agent and a bioactive metabolites reservoir. Biocatal Agric Biotechnol 21:101284

Sharma A, Kaushik N, Sharma A, Marzouk T, Djébali N (2022) Exploring the potential of endophytes and their metabolites for bio-control activity. 3 Biotech 12(10):277. https://doi.org/10.1007/s13205-022-03321-0

Shi D, An R, Zhang W, Zhang G, Yu Z (2017) Stilbene derivatives from Photorhabdus temperata SN259 and their antifungal activities against phytopathogenic fungi. J Agric Food Chem 65(1):60–65

Silva FDA, Liotti RG, Boleti APA, Reis ÉDM, Passos MBS, Dos Santos EL, Soares MA (2018) Diversity of cultivable fungal endophytes in Paullinia cupana (Mart.) Ducke and bioactivity of their secondary metabolites. PloS one 13(4):e0195874

Sikora RA, Schäfer K, Dababat AA (2007) Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australas Plant Pathol 36:124–134

Stangarlin JR, Kuhn OJ, Assi L, Schwan-Estrada KRF (2011) Control of plant diseases using extracts from medicinal plants and fungi. In: Science against microbial pathogens: communicating current research and technological advances, vol 2. Formatex, Badajoz, pp 1033–1042

Talapatra K, Das AR, Saha AK, Das P (2017) In vitro antagonistic activity of a root endophytic fungus towards plant pathogenic fungi. J Appl Biol Biotechnol 5(2):068–071

Talibi I, Boubaker H, Boudyach EH, Aoumar AB, A. (2014) Alternative methods for the control of postharvest citrus diseases. J Appl Microbiol 117(1):1–17

Tantry MA, Idris AS, Williamson JS, Shafi T, Dar JS, Malik TA et al (2018) Perylenequinones from an endophytic Alternaria sp. of Pinus ponderosa. Heliyon 4(12):e01046

Ting ASY, Mah SW, Tee CS (2010) Identification of volatile metabolites from fungal endophytes with biocontrol potential towards Fusarium oxysporum F. sp. cubense race 4. Am J Agric Biol Sci 5(2):177–182

Yamazaki H, Takahashi O, Kirikoshi R, Yagi A, Ogasawara T, Bunya Y et al (2020) Epipolythiodiketopiperazine and trichothecene derivatives from the NaI-containing fermentation of marine-derived Trichoderma cf. brevicompactum. J Antibiotics 73(8):559–567

Yao YQ, Lan F, Qiao YM, Wei JG, Huang RS, Li LB (2017) Endophytic fungi harbored in the root of Sophora tonkinensis Gapnep: diversity and biocontrol potential against phytopathogens. MicrobiologyOpen 6(3):e00437

Zheng YK, Miao CP, Chen HH, Huang FF, Xia YM, Chen YW, Zhao LX (2017) Endophytic fungi harbored in Panax notoginseng: diversity and potential as biological control agents against host plant pathogens of root-rot disease. J Ginseng Res 41(3):353–360. https://doi.org/10.1016/j.jgr.2016.07.005

Chapter 14
Endophytic Fungi and the Health Benefits from Their Potential Bioactive Secondary Metabolites

Mriganka Das, Sibashish Kityania, Rupshikha Nath, Rajat Nath, Deepa Nath, and Anupam Das Talukdar

Abstract Endophytic fungi colonize the inner parts of the plant tissues asymptomatically. Numerous research studies have demonstrated that endophytes directly generate bioactive compounds, improving their host plants' fitness. There has been a notable trend towards eco-friendly products in healthcare, medicine, and many other significant explorations of endophytic fungi, which have been discovered to generate diverse secondary metabolites with various biological properties, which has been a particular area of interest in this transformation. These fungi are an abundant source of secondary metabolites with functional properties, such as steroids, phenols, phenolic acids, quinines, phenolic, indole derivatives, amines, iso-coumarin derivatives, alkaloids, sesquiterpenes, flavonoids, diterpenes, lignans, terpenoids, peptides, chlorinated metabolites, and aliphatic compounds. New antibiotics, antimycotics, immunosuppressants, anticancer chemicals, and other bioactive secondary metabolites with diverse biological activity are now known to come from an endophytic fungus. Bioactive secondary metabolites produced by endophytic fungal organisms have been found to have unexpected medicinal promise. Additionally, it adds to the pharmaceutical and probiotic health products we regularly use to balance out diets that can improve health conditions. It also can enhance health conditions. The focus of the current study and emphasis is placed on endophytic fungi's significance in acquiring contemporary bioactive compounds that exhibit a variety of biological traits, including the potential to be antiparasitic, anti-pathogenic, antibacterial, antitumor, antioxidant, immunoregulatory, neuroprotective, and cytotoxic to cancer cells. Endophytes are an endless source of pharmacologically significant substances. Considerable attention is dedicated to studies that seek to elucidate the mechanism through which these metabolites exert health benefits.

M. Das · S. Kityania · R. Nath · R. Nath · A. D. Talukdar (✉)
Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India
e-mail: anupam@bioinfoaus.ac.in

D. Nath
Department of Botany, Guru Charan College, Silchar, Assam, India

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1 Introduction

Endophytic fungi, a diverse group of fungi, live inside the tissues of higher plants without causing any apparent symptoms or harm. This relationship between plants and fungi can be viewed as a mutualistic symbiosis in which both sides gain from the alliance. Most endophytes may be distinguished from plant components such as seeds, leaves, stems, roots, flowers, and meristems. The following categories can be used to classify endophytes:

1. Facultative: These endophytic fungi may colonize plant tissues but can live independently in their environment.
2. Obligate: These are endophytic fungi that must live within plant tissues to survive and grow. Endophytic fungi are a broad group of organisms, mainly belonging to the Ascomycotina and Deuteromycotina species, in terms of taxonomy and ecology (Rabiey et al. 2019). Endophytes, which may be characterized as a variety of distinct microorganisms living in plants with internal solid tissues and deriving from several evolutionary lineages, have been the subject of intense investigation over the years. These microorganisms have both covert and overt advantages for their host plants. They create complex biological interactions with their host plants and among themselves. Endophytes can create a wide range of secondary metabolites with various biological roles. According to studies and research, endophytic fungi play a crucial role in the health of their host plants by directly creating beneficial secondary metabolites. These substances are essential for shielding the host from herbivores and dangerous microorganisms. The mounting data underlines the expanding comprehension of endophytic fungi's ability to generate biologically active substances previously thought only to be produced by their host plants. This finding clarifies the medical importance of these phytochemicals (Ancheeva et al. 2020). Despite these incredible findings, only a tiny fraction of all known plant species—roughly 0.75% and 1.5%—have investigated their endophytes thus far. It means there is a significant probability that cryptic endophytic bacteria will produce novel bioactive chemicals. With an estimated 374,000–400,000 plant species and the range of ecological environments they occupy, the likelihood of discovering new and promising bioactive compounds is surprisingly great (Christenhusz and Byng 2016; Strobel 2018). Many bioactive chemicals are generated by endophytic fungi, some of which have pleiotropic and fascinating pharmacological effects such as antibacterial, antioxidant, antidiabetic, antimalarial, and anticancer properties. One of the most significant benefits of endophytic fungi as useful sources of new bioac-

tive chemicals is their capacity to generate these compounds in large quantities and under controlled circumstances, making them simpler to separate and purify for future study and development (Newman and Cragg 2020; Uzma et al. 2018). Furthermore, because endophytic fungi inhabit plant tissues, they have evolved defenses against plant infections and predators, making them an appealing source of bioactive compounds with potential uses in crop protection. The crucial significance of natural extracts in developing and discovering innovative drugs has received much attention in recent publications. These products offer a wealth of original and diverse pharmaceutical template options. It is becoming increasingly clear that many natural substances result from microbial production or interactions between microbes and the creatures that serve as their hosts (Singh et al. 2021). There are now exciting new prospects for study on endophytes to identify natural products, which can potentially change drug discovery and development. Recent writings have stressed the critical role that natural products play in discovering and developing novel medicines. These products offer a wealth of original and diverse pharmaceutical template options (Singh et al. 2021; Strobel 2018). This chapter will also look at the several challenges associated with employing endophytes as a different way to produce bioactive compounds derived from plants. We'll also discuss the potential use of these substances in creating new pharmaceuticals. It is predicted that the research discussed in this chapter will produce significant data that can be utilized to plan the optimal usage of endophytes as a consistent source of plant-derived metabolites.

2 Therapeutic Potential of Endophytic Fungi

Endophytic fungi are also significant because they have the potential to produce bioactive compounds that are structurally and functionally distinct from those produced by their host plants, making them a rich source of new natural products for drug discovery and development. Several studies have investigated the potential of endophytic fungi as a source of novel bioactive compounds. For example, a study isolated an endophytic fungus from a traditional Chinese medicinal plant, which produced a new compound (spirobisnaphthalene) with anti-inflammatory activity (Tan et al. 2020). Chemical epigenetic manipulation is a powerful tool used to manipulate the biosynthesis of secondary metabolites in various microorganisms, including endophytic fungi. In a recent study, to control the fungus' epigenetic regulation, the researchers in this work used a frequently used DNA methyltransferase inhibitor called 5-aza-2-deoxycytidine (5-Aza) (Guo et al. 2020). They noticed a striking result when they added 10 mg/L of 5-Aza to the *P. herquei* fermentation broth, forming three hitherto unidentified polyketides with a pyran-2-one scaffold. NMR, MS, and IR spectroscopy were among the many spectroscopic investigations used to identify these novel chemicals. Additionally, the researchers used quantum chemistry electronic circular dichroism (ECD) computations to ascertain the absolute configurations of these molecules. Penicillipyrones A–C were given to the three

newly discovered compounds identified from the fermentation broth of *P. herquei.* Penicillipyrones A and B were found to be diastereomers, while penicillipyrones B and C were found to be enantiomers. Notably, a panel of human tumor cell lines was inhibited by penicillipyrones A and C to a modest extent (Guo et al. 2020). To better understand how endophytic fungi produce secondary metabolites, this work demonstrates the usefulness of using chemical epigenetic modification. The researchers effectively induced the formation of hitherto undiscovered polyketides in *P. herquei* by using 5-aza-2-deoxycytidine (5-Aza) as an epigenetic modifier. The discovery of these novel compounds and their bioactivity highlight the importance of investigating endophytic fungi's metabolic potential in searching for new therapeutic medicines [5]. Furthermore, it has been demonstrated that endophytic fungus can produce substances with probiotic qualities. This work highlights the value of looking at endophytic fungus as a useful source for discovering and identifying chemical epigenetic manipulation. Low molecular weight polyphenols called lignans are generated by enzymes such as cinnamoyl-CoA reductase (CCR), cinnamyl-alcohol dehydrogenase (CAD), and PAL, as well as pinoresinol-lariciresinol reductase (PLR). A sugar moiety is joined to a non-sugar triterpene or steroid aglycone (sapogenin) in saponins which are glycosides (Bahabadi et al. 2012). Four classes of fungal secondary metabolites are connected to each other. The researchers have divided these various fungal secondary metabolites into several groups, including xanthones, quinones, coumarins, flavonoids, lignans, saponins, terpenes, coumarins, and other unspecified chemicals, to aid understanding and offer a thorough overview. For instance, coumarins are a kind of lactone defined by joining a pyrone ring and a benzene ring. Cinnamic acid serves as a precursor and is mostly used in their biosynthesis, which takes place via the shikimic acid pathway. As opposed to this, the enzymatic activity of phenylalanine ammonia lyase (PAL), flavanol reductase, chalcone synthase, and chalcone isomerase is required for the synthesis of flavonoids through the phenylpropanoid route. These substances, which are produced from the amino acid phenylalanine, have a variety of structural and functional properties (Mohanta, 2020). Utilizing enzymes such cytochromes P450 (P450s), UDP-glycosyltransferases (UGTs), and oxidosqualene cyclases (OSCs), they are produced from intermediates of the phytosterol pathway. Quinones can be made in a number of methods. For instance, the shikimate pathway synthesizes isoprenoid quinones using precursors obtained from chorismite. Terrequinone is produced from L-tryptophan by NRPS, dopaquinone from tyrosine by tyrosinase, and benzoquinone from catechol by catechol oxidase/PKS. The polyacetate/polymalonate process is used to create xanthones, which are oxygenated heterocyclic molecules. They are created when a single folded polyketide chain undergoes internal cyclization (Singh et al. 2021). A study is conducted to evaluate the probiotic potential of endophytic fungi isolated from medicinal plants (Gouda et al. 2016). The study found that the fungal extracts were able to modulate the gut microbiome in mice and also reduce inflammation, indicating their potential use as probiotics. The authors identified several bioactive compounds produced by the fungi, including alkaloids, flavonoids, terpenoids, and phenolics, which were responsible for their probiotic

activity. Similar examples of various potentials of extracts of endophytic fungi as pharmaceuticals and probiotics are discussed below:

2.1 Anticancer Drugs

Several fungal endophytes have been discovered to possess the ability to produce bioactive compounds with anticancer properties. Taxol is an illustration of this, a widely recognized anticancer medication synthesized by the endophytic fungus *Taxomyces andreanae* (Strobel et al. 1996). Beauvericin is a cyclic peptide generated from a particular kind of endophytic fungus that may be found in the South China Sea. It has been demonstrated to significantly slow the growth of cancer cells of the KB and KBv200 subtypes. Apoptosis, or planned cell death, is induced through the mitochondrial route as the mechanism behind this suppression. The effects of beauvericin on cells include a decrease in the generation of reactive oxygen species, disruption of the mitochondrial membrane potential, release of cytochrome c, activation of certain enzymes (Caspase-9 and -3), and cleavage of PARP, a protein involved in DNA repair. Notably, the beauvericin-induced apoptosis in these cells does not appear to be mediated by the regulation of Bcl-2 or Bax, which are proteins generally connected with apoptosis (Tao et al. 2015).

2.2 Antibiotics

Endophytic fungi are known to produce a variety of secondary metabolites with antibacterial and antifungal properties, which possess the potential for developing novel antibiotics. For example, the endophytic fungus *Fusarium oxysporum* produces the antifungal compound 2,4-diacetylphloroglucinol, which has been shown to have potential as a new antibiotic (Aini et al. 2022; Bora and Devi 2023; De Lamo and Takken 2020).

2.3 Immune System Modulators

The article reports on isolating and characterizing a polysaccharide from the endophytic fungus *Fusarium oxysporum* Dzf17, which was found to have immunomodulatory and antitumor activities in vitro and in vivo. The article suggests that the polysaccharide may have potential as an immunomodulator for use in cancer therapy (Manganyi and Ateba 2020).

2.4 Probiotics

Researchers demonstrated the probiotic potential of endophytic fungi in modulating the gut microbiome and reducing inflammation (Gouda et al. 2016). The potential of probiotics generated from therapeutic plantsherbal remedies and endophytic fungus *Azadirachta indica* and found that some fungal isolates might both boost the growth of good gut bacteria and prevent the growth of harmful bacteria (Björkman et al. 2015). The probiotic potential of endophytic fungus originating from therapeutic plants Herbal remedies *Gynura medica* and noted that probiotic and immunomodulatory polysaccharides may be produced by a few of the fungal isolates (Junya Wang et al. 2019).

3 Endophytic Fungi as a Reservoir of Bioactive Substances

It has been found that endophytic fungus produces a wide variety of beneficial secondary metabolites. Steroids, phenols, phenolic acids, quinines, indole derivatives, amines, alkaloids, sesquiterpenes, flavonoids, diterpenes, lignans, terpenoids, peptides, chlorinated metabolites, and aliphatic compounds are only a few of the many substances found in nature (Calixto 2019; Newman and Cragg 2016). These metabolites have been shown to possess displaying diverse biological properties encompassing a range of activities such as fighting against bacteria, fungi, viruses, and parasites, reducing inflammation, inhibiting tumor growth, providing antioxidant effects, suppressing the immune system, and protecting neurons and cytotoxic effects on cancer cells (Venieraki et al. 2017). The endophytic fungus has developed into a viable and vital reservoir of natural chemicals for investigating prospective medications and development due to the wide variety of bioactive molecules they generate (Table 14.1 and the overall bioactivity is shown in Fig. 14.1). The potential of these chemicals to cure a range of illnesses and ailments is currently being intensively investigated by researchers. Compared to manufactured pharmaceuticals, identifying new bioactive chemicals from endophytic fungus shows promise for improving the development of innovative medications with greater efficacy and fewer adverse effects (Newman and Cragg 2020; Shen 2015; Uzma et al. 2018). Figures 14.2, 14.3, and 14.4 illustrate the structure of a few essential phytochemicals.

3.1 Alkaloids

Alkaloids are an array of secondary metabolites found extensively in plant species, and their distribution spans widely among fungi. They exhibit a broad spectrum of biological effects, including antimicrobial, antitumor, and anti-inflammatory

Table 14.1 Some reported plant derived secondary metabolites from endophytic fungi and their bioactivity

Sl no.	Endophytic fungi source	Host plant	Plant-derived secondary metabolites	Bioactivity	References
1.	*Entrophospora infrequens*	*Nothapodytes foetida*	Camptothecin (flavonoid)	Antitumor	Satish Chander Puri et al. (2005)
	Xylaria sp.	*Camptotheca acuminata*			Amna et al. (2006)
	Fusarium solani	*Apodytes dimidiata*			Souvik Kusari et al. (2009c)
	Valsa mali	*Camptotheca acuminata*			Min and Wang (2009)
	Fusarium solani	*Apodytes dimidiata*			Souvik Kusari et al. (2009c)
	Galactomyces sp., *Fusarium* sp., *Diaporthe conorum*, *Fusarium verticillioides*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium sacchari*, *Fusarium subglutinans*, *Irpex lacteus*, *and Fusarium* sp.	*Nothapodytes nimmoniana*			Gurudatt et al. (2010)
	Alternaria alternata (black rot, black spot), *Fomitopsis* sp., *and Phomopsis* sp.	*Miquelia dentata* (syn: *Miquelia assamica* (Griff.) D.G. Lon)			Shweta et al. (2013)
	Aspergillus sp.	*Camptotheca acuminata (happy tree, cancer tree, or tree of life)*			Souvik Kusari et al. (2009c)
	Fusarium oxysporum	*Nothapodytes foetida*			Musavi et al. (2015)
	Botryosphaeria dothidea	*Camptotheca acuminata (happy tree, cancer tree, or tree of life)*			Ding et al. (2013)

(continued)

Table 14.1 (continued)

Sl no.	Endophytic fungi source	Host plant	Plant-derived secondary metabolites	Bioactivity	References
2.	*Unknown*	*Vinca minor*	Vincamine (flavonoid)	Antihypertensive, vasodilator	Yin and Sun (2011)
3.	*Paecilomyces* sp.	*Xanthium* spp.	Xanthatin(tarpene)	Antitumor	Nibret et al. (2011)
4.	*Fusarium* sp.	*Dysosma versipellis*	Podophyllotoxin (lignan)	Antitumor, antivirus	Tan et al. (2018)
	Phialocephala podophylli	*Podophyllum peltatum*			Arneaud and Porter (2015)
	Fusarium solani	*Podophyllum hexandrum*			Nadeem et al. (2012)
	Fusarium oxysporum	*Juniperus recurva*			Kour et al. (2008) and Satish Chandra Puri et al. (2006)
	Trametes hirsuta	*Podophyllum hexandrum*			Satish Chandra Puri et al. (2006)
	Alternaria sp.	*Juniperus vulgaris*			Satish Chandra Puri et al. (2006)
	Penicillium sp.	*Diphylleia sinensis*			Xianzhi et al. (2003)
	Alternaria sp., *Penicillium* spp	*Podophyllum hexandrum*			Xianzhi et al. (2003)
	Alternaria tenuissima	*Podophyllum emodi*			Liang et al. (2016)
	Mucor fragilis	*Podophyllum hexandrum*			Ebada et al. (2016)
	Aspergillus fumigatus	*Juniperus communis*			S Kusari et al. (2009a)
	Alternaria neesex	*Podophyllum hexandrum*			Li (2007)
	Phialocephala fortinii	*Podophyllum peltatum*			Eyberger et al. (2006)
	Penicillium implicatum	*Diphylleia sinensis*			Zeng et al. (2004)

5.	*Phomopsis phaseoli* (syn. *Diaporthe phaseolorum*), *Phomopsis* spp., *Phomopsis longicolla*, *Melanconium betulinum*	*Hippocrepis* sp., *Coronilla viminalis*, *lotus*, *Scorpiurus* sp., *and Securigera* sp.	(Beta-nitropropionic acid) 3-nitropropionic acid	Nematicidal, succinate dehydrogenase inhibitor, and antimycobacterial	Chomcheon et al. (2005) and Schwarz et al. (2004)
6.	*Anonymous*	*Digitalis lanata*	Digoxin	Cardiac, anticancer	Kaul et al. (2013)
7.	*Phoma glomerata*	*Salvia miltiorrhiza*	Salvianolic acid (polyphenol)	Antioxidant, cardiovascular, cerebrovascular diseases	Li et al. (2016)
8.	*Aspergillus fumigatus*	*Ribes* sp.	Tocopherol (phenol)	Anti-influenza, antioxidant	Xu et al. (2014)
9.	*Muscodor tigerii*	*Cinnamomum camphora*	Asarone (phenyl propane)	Antimicrobial	Saxena et al. (2015)
10.	*Talaromyces pinophilus*	*Withania* sp.	Withanolide	Treatment for Alzheimer's disease and cardiovascular disease and anticancer	Sathiyabama and Parthasarathy (2018)
11.	*Anonymous*	*Artemisia* spp.	Artemisinin (terpene)	Antimalarial	Huang et al. (2007)
12.	*Colletotrichum gloeosporioides*	*Centella asiatica*	Asiaticoside	Antidermatitic, anti-inflammatory, antioxidant, immunomodulatory	Gupta et al. (2018)
13.	*Rhizoctonia bataticola*	*Plectranthus barbatus*	Forskolin antiglaucoma	HIV and cancer prevention	Mir et al. (2015)

(continued)

Table 14.1 (continued)

Sl no.	Endophytic fungi source	Host plant	Plant-derived secondary metabolites	Bioactivity	References
14.	*Botryosphaeria* sp., *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Geotrichum* sp., *Alternaria* sp., *and Cephalosporium* sp.	*Vitis* spp.	(Stilbene polyphenol) resveratrol	Antioxidant, cancer preventing, and epigenetic manipulation	Shi et al. (2012)
15.	*Fusarium solani*	*Rheum palmatum*	Rhein	Anti-inflammatory, antimicrobial, antioxidant, anticancer, nephroprotective, hepatoprotective	You et al. (2013)
16.	*Epicoccum nigrum*	*Rubia tinctorum*	Quinizarin	Cytotoxicity, antibacterial	Dzoyem et al. (2017)
17.	*Gibberella moniliformis*	*Lawsonia inermis*	Lawsone	Cytotoxic	Sarang et al. (2017)
18.	*Dothideomycetes* sp.	*Syzygium aromaticum*	Eugenitin	Glucoamylase activation	Chomcheon et al. (2009)
19.	*Fusarium proliferatum*, *Fusarium solani*, *Fusarium oxysporum*, *Alternaria* sp.	*Cajanus cajan* (pigeon pea)	Cajaninstilbene acid	Antioxidant, anti-inflammatory, hypoglycemic, neuroprotective	JinTong Zhao et al. (2012)
20.	*Colletotrichum capsici*, *Alternaria alternata*, *Colletotrichum taiwanense*	*Passiflora incarnata*	Chrysin	Antiaging, anticonvulsant, antidiabetic, anti-inflammatory, antimicrobial, and hepatoprotective	Seetharaman et al. (2017)

21.	*Aspergillus awamori*, *Colletotrichum gloeosporioides*	*Rauwolfia serpentina*	Some saponins	Cardiovascular disease	Dhankhar et al. (2012)
	Coprinopsis cinerea, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus* sp., *Aspergillus tubingensis*, *Aspergillus terreus*, *Fusarium* sp.	*Eugenia jambolana*			Prabavathy (2013)
	Cladosporium sp., *Penicillium* sp., *Phomopsis* sp., *Trichoderma* sp., *Aspergillus flavus*, *Aspergillus niger*, *Alternaria alternata*	*Aegle marmelos*			Pragathi et al. (2013)
	Aspergillus neoniveus (syn. *Fennellia nivea*)	*Typhonium divaricatum*			Saraswaty et al. (2013)
	Phyllosticta sp., *Monochaetiakarstenii* (syn. *Pestalotiopsis maculans)*	*Shorea thumbuggaia*			Selvi and Balagengatharathilagam (2014)
	Cochliobolus lunatus (anamorph *Curvularia lunata*)	*Boswellia ovalifoliolata*			Yadav et al. (2014)
	Aspergillus sp.	*Justicia beddomei*			Nath et al. (2015)

(continued)

Table 14.1 (continued)

Sl no.	Endophytic fungi source	Host plant	Plant-derived secondary metabolites	Bioactivity	References
22.	*Cladosporium cladosporioides*	*Aconitum* spp.	Aconitine	Anticancer, anti-inflammatory, anti-neuralgic, cardiotoxic	Yang et al. (2013)
23.	*Colletotrichum gloeosporioides*	*Centella asiatica*	Asiaticoside	Antidermatitic, anti-inflammatory, antioxidant, immunomodulatory	Gupta et al. (2018)
24.	*Penicillium (Eupenicillium) parvum*	*Azadirachta indica*	Asiaticoside	Hepatoprotective, insecticidal	Souvik Kusari et al. (2012)
25.	*Ceriporia lacerata*	*Cleistocalyx operculatus*	Chalcone	Antibacterial, antifungal, antitumor, anti-inflammatory	Jie Wang et al. (2013)
26.	*Penicillium* sp., *Alternaria alternata*, *Alternaria* sp.	*Tabebuia argentea*	Lapachol	Antiparasitic, antimicrobial, antiviral, anti-inflammatory	Sadananda et al. (2011)
27.	*Penicillium* sp.	*Derris elliptica*	Rotenone	Insecticide, pesticide, piscicide	Sadananda et al. (2011)

28.	*Alternaria alternata*, *Colletotrichum capsici*, *Colletotrichum taiwanense*	*Passiflora incarnata*	Chrysin	Anti-inflammatory, antidiabetic, antiaging, anticonvulsant, antibacterial, calming, and hepatoprotective	Seetharaman et al. (2017)
29.	*Cladosporium delicatulum*	*Terminalia pallida*	Plumbagin	Anticancer	Venkateswarulu et al. (2018)
30.	*Paecilomyces* sp.	*Panax ginseng*	Panaxynol or falcarinol or carotatoxin (polyacetylene	Anticancer	Xu et al. (2009)

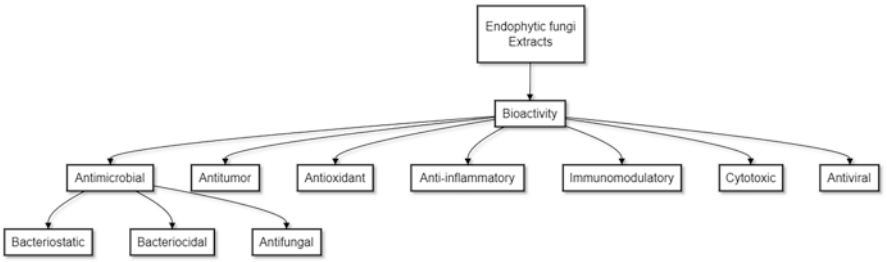

Fig. 14.1 Potential bioactivity of extracts from endophytic fungi

properties (Souvik Kusari et al. 2012). Endophytic fungi have been discovered to generate a diverse range of alkaloids, such as indole alkaloids, quinoline alkaloids, and pyrrolizidine alkaloids (Zhang et al. 2006). Some examples of alkaloids produced by endophytic fungi include ergot alkaloids produced by *Claviceps purpurea* and pyrrocidines, which *Beauveria bassiana* produces. Strobel and his team investigated indole alkaloid synthesis by endophytic fungi isolated from the medicinal plant *Uncaria tomentosa* (Strobel et al. 2004). The researchers found that the fungus residing within the plant tissues *Aspergillus* sp. produced a new indole alkaloid, 12-hydroxy-16-deoxo-11-oxo-strictosidine, which demonstrated significant cytotoxicity when tested against various human cancer cell lines. Another investigation was done on the generation of pyrrolizidine alkaloids by endophytic fungi derived from the medicinal plant *Symphytum officinale* (Souvik Kusari et al. 2012). The researchers discovered that the endophytic fungus *Fusarium* sp. produced several pyrrolizidine alkaloids, including lasiocarpine and echimidine, which exhibited significant antimicrobial activity against several bacterial and fungal pathogens.

3.2 Terpenoids

Terpenoids are a large and wide array of secondary metabolites with a broad distribution. They are recognized for their extensive spectrum of biological activities, such as antimicrobial, antitumor, and antioxidant properties. Endophytic fungi have been found to generate a multitude of terpenoids, such as sesquiterpenoids, diterpenoids, and triterpenoids. Some examples of terpenoids produced by endophytic fungi include taxol, which is produced by *Taxomyces andreanae*, and asperterpenes, which are produced by *Aspergillus terreus* (P. Kusari et al. 2013; Stierle et al. 1993). A study investigated taxol production by endophytic fungi isolated from the bark of *Taxus brevifolia*. The researchers found that the endophytic fungus *Taxomyces andreanae* produced taxol, demonstrating strong antitumor efficacy toward various lines of human cancer cells. Another study investigated the generation of triterpenoids by endophytic fungi obtained from the isolation of the medicinal plant *Euphorbia hirta* (Eze and Okoye 2017). The researchers found that the endophytic fungus *Penicillium* sp. produced several triterpenoids, including ursolic acid and betulinic acid, which exhibited significant antimicrobial and anti-inflammatory

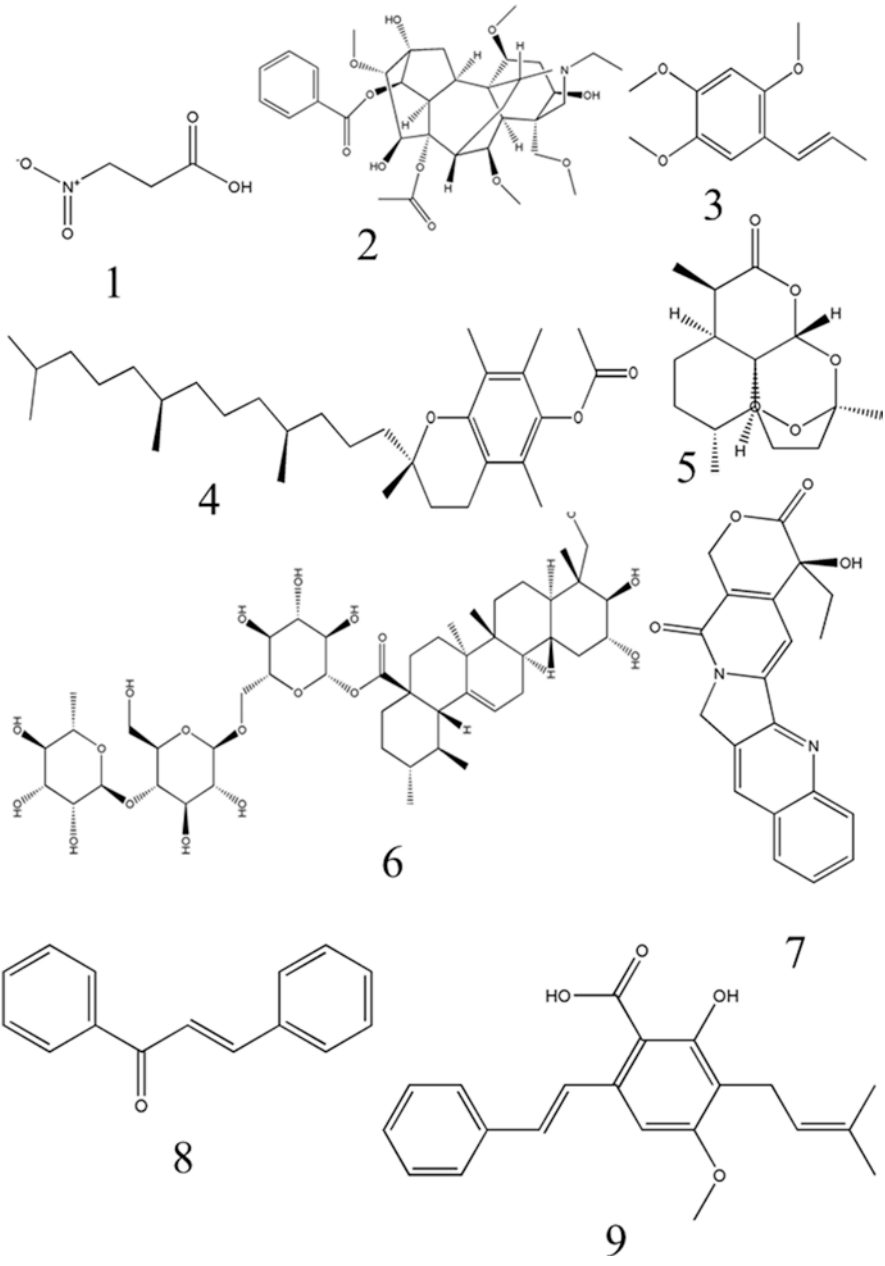

Fig. 14.2 (1) 3-nitropropionic acid, (2) aconitine, (3) asarone, (4) tocopherol, (5) artemisinin, (6) asiaticoside, (7) camptothecin, (8) chalcone, (9) cajaninstilbene acid

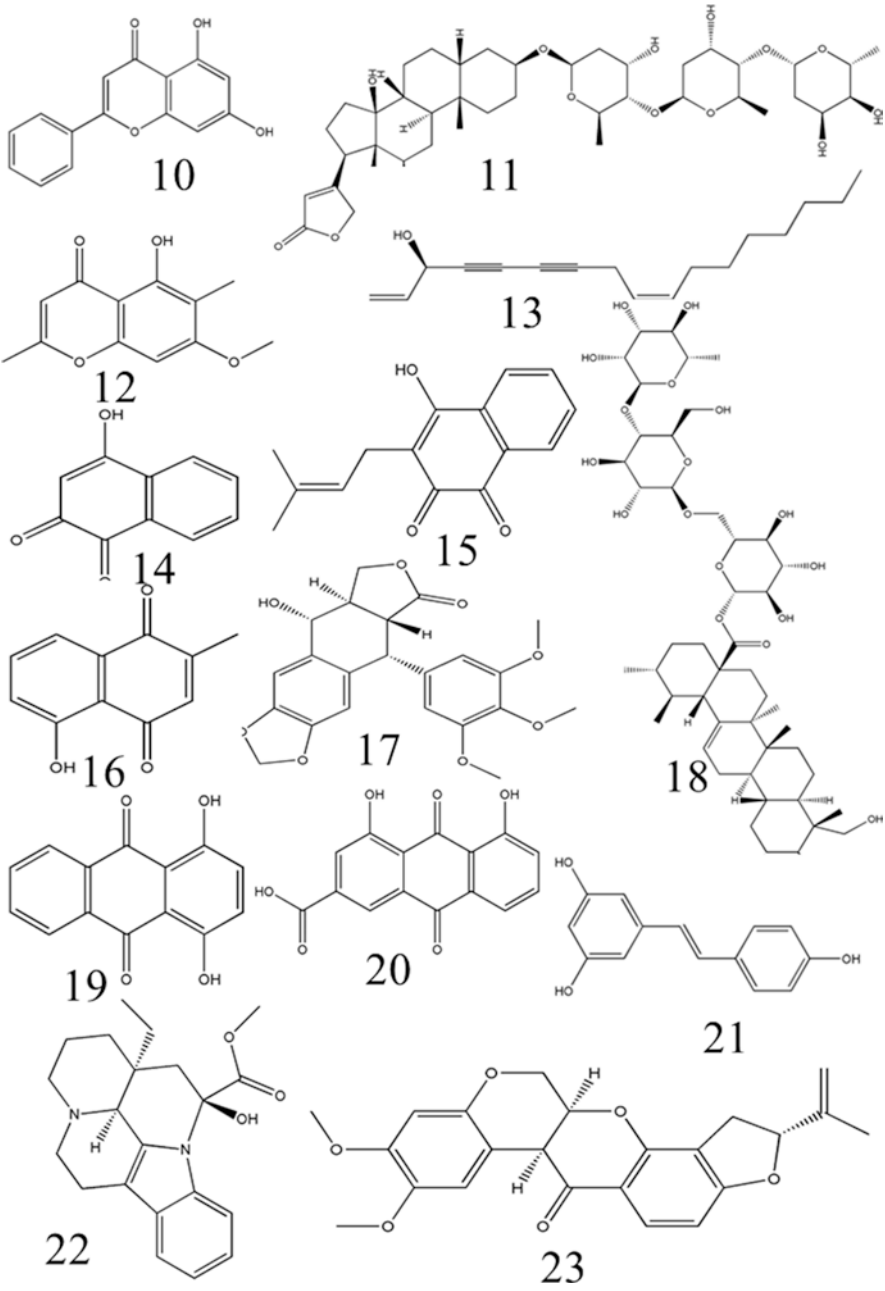

Fig. 14.3 (10) Chrysin, (11) digoxin, (12) eugenitin, (13) falcarinol, (14) lawsone, (15) lapachol, (16) plumbagin, (17) podophyllotoxin, (18) asiaticoside, (19) quinizarin, (20) rhein, (21) resveratrol, (22) vincamine, (23) rotenone

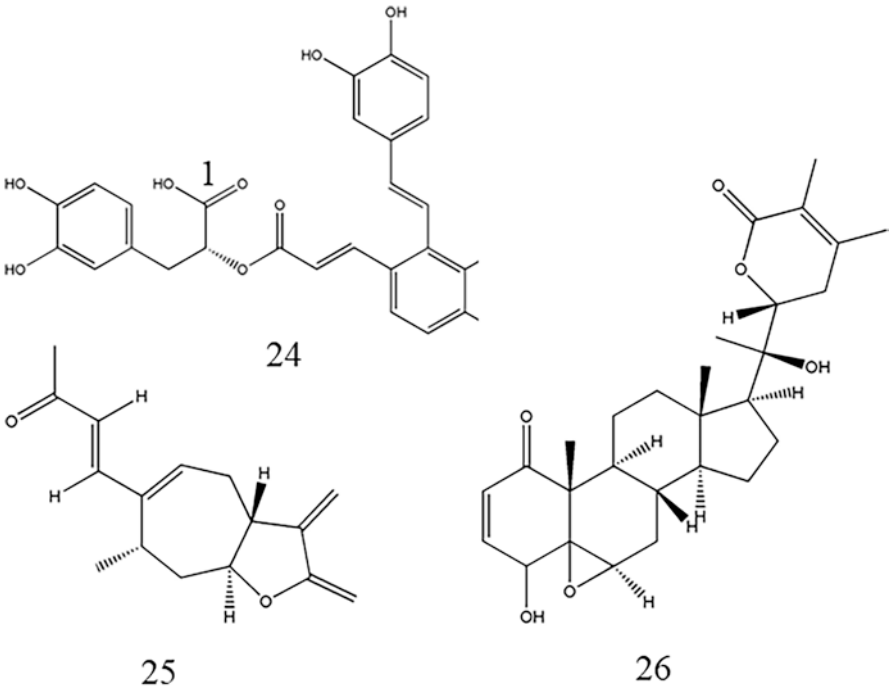

Fig. 14.4 (24) Salvianolic acid, (25) xanthatin, (26) withanolide

activity. The diterpenoid taxane family includes the complicated chemical molecule paclitaxel (PTX). It has a C-13 ester side chain and a four-membered oxetane ring. This substance is frequently used in chemotherapy to treat different forms of cancer. In 1971, it was initially taken from a *Taxus brevifolia* (Pacific yew tree) (Wani and Horwitz 2014). When paclitaxel binds to the mitotic spindle protein tubulin, it stabilizes microtubules. It stops the induction of mitotic arrest during the M phase and leads to the reversal of the cell cycle into the G0 phase, subsequently initiating apoptosis. In 1992, the FDA approved paclitaxel for treating ovarian cancer, and by 2004, the drug's market value had topped $3 billion. The endophytic fungus *T. andreanae* was the first organism found to produce paclitaxel from *Taxus* spp. Following this finding, 35 different host plant species included 83 different endophytic fungal species, including *Taxus* and non-*Taxus* species (Brito et al. 2008; Stierle et al. 1993; Wani and Horwitz 2014).

3.3 Polyketide-Derived Lactones and Polyketides

Polyketide-derived lactones are a class of secondary metabolites produced by a wide range of microorganisms, including endophytic fungi. They are known to have a range of biological functions, including antitumor and antifungal properties, and

insecticidal properties. Some examples of polyketide-derived lactones generated by endophytic fungi include beauveriolides, produced by *Beauveria bassiana* (Patocka, 2016), and xylarilides that *Xylaria* produces. Researchers found nine polyketides, encompassing two recently discovered benzophenone derivatives, generated by *Penicillium* sp. ZJ-SY2, an endophytic fungus, is extracted from *Sonneratia apetala* leaves. These substances exhibited powerful influence on the immune system, with 5.9–9.3 g/mL as the IC50 value. The structures were established using NMR in 1D and 2D spectroscopy. Substances 1, 3, 5, and 7 demonstrated that these compounds demonstrated moderate immunosuppressive effects on the growth of splenic lymphocytes in mice induced by Con A (T cell) and LPS (B cell). The study provides insight into the potential use of endophytic fungi in developing immunosuppressive drugs (Liu et al. 2016). Some polyketides, such as amphotericin B, lovastatin, rapamycin, and erythromycin B, have already been developed into commercial drugs for antifungal, anticholesterol, immunosuppressive, and antibiotic purposes. Recently, two new polyketide compounds, A and C, have been discovered from an endophytic fungus. Due to their promising biological activity, these compounds have shown potential for development as drugs in the medical industry. Further research is needed to fully explore the potential of these compounds as drugs (Debbab et al. 2011).

3.4 Peptides

It is well established that endophytic fungi can synthesize a broad range of biologically active substances, including peptides, with various health benefits. These peptides have gained increasing attention due to their potential therapeutic effects, such as antibacterial, antioxidant, and anticancer properties and activities. For example, the endophytic fungus *Aspergillus flavus* produces the peptide flavoglaucin, which has been found to exhibit strong antimicrobial activity against various pathogenic bacteria and fungi. Similarly, the peptide cyclo-(L-Pro-L-Tyr) produced by the endophytic fungus *Penicillium* sp. has been found to possess potent antioxidant and antitumor activities. Other endophytic fungal peptides, such as emericellipsin A and B, have also shown promising health benefits, including antifungal and anticancer properties. Overall, the discovery and characterization of peptides produced by endophytic fungi provide a valuable source of potential therapeutics for a range of health conditions. An antibiotic called cyclosporin A (CyA) has antifungal and immunosuppressive properties. Cyclosporin A has been manufactured in vast quantities using a fermentation approach and a range of fungus, including *Tolypocladium*, *Trichoderma*, *Fusarium*, *Penicillium*, *and Aspergillus* spp. (Srivastava 2017). In another study, researchers found endomorphin-2: An endophytic fungus, *Penicillium* sp., derived from the leaves of the medicinal plant *Tabernaemontana divaricata*, synthesizes an opioid peptide that exhibits analgesic effects and interacts with the mu-opioid receptor, influencing gut motility (Dekan et al. 2019).

3.5 Quinones

Endophytic fungi create a large number of quinones, such as naphthoquinones and anthraquinones. Two examples of quinones generated by endophytic fungi include emodin, which is formed by *Alternaria* sp., and rubrofusarin, which is made by *Fusarium* sp. (Strobel and Daisy, 2003). In a separate study, An et al. (2020) looked at the production of rubrofusarin by endophytic fungi isolated from the medicinal plant *Uncaria rhynchophylla*. Numerous rubrofusarin chemicals were found to be produced by the endophytic fungus *Fusarium* sp. When used against a variety of plant pathogenic fungus, these compounds demonstrated strong antifungal effectiveness. A substance produced from *Hypericum perforatum* called hypericin is well known for having antidepressant, antineoplastic, anticancer, antiviral, and photosensitizer effects. It hinders the reabsorption of serotonin, norepinephrine, and dopamine, enhances the activity of IL-6, and activates sigma receptors, acting as an antidepressant (Pizzorno et al. 2016). Hypericin has been shown to stabilize the HIV virus's capsid and limit reverse transcriptase release, preventing the virus from becoming uncoated. Additionally, endophytic fungi that were isolated from *H. perforatum*, such as *C. globosum*, *Thielaviasub thermophila*, and *Epicoccum nigrum*, have been found to generate hypericin (Souvik Kusari et al. 2008, 2009b; Vigneshwari et al. 2019). *Salvia* spp. roots contain a group of diterpenoid quinine metabolites known as tanshinones, which have been demonstrated to have promise as anticancer, antiatherosclerotic, antihypertensive, and neuroprotective medicines. They consist of cryptotanshinone, isotanshinone I, tanshinone IIA, and tanshinone IIB. Their anticancer action includes altering the PTEN-mediated regulation of the PI3K/AKT pathway, which in turn inhibits DNA replication, arrests cell cycle progression, controls oxidative stress, decreases mitochondrial membrane potential, and starts apoptosis. By phosphorylating STAT3 at Tyr705 and STAT3, tanshinone I specifically lowers the levels of HIF-1 in cancer cells and prevents tumor angiogenesis. Tanshinones' anti-cardiovascular effects include the prevention of myocardial cell death, cardiac tissue scarring, artery plaque formation, uptake of oxidized low-density lipoprotein, activation of blood clotting factor thrombin, and blood clot formation. Tanshinones also significantly reduce phosphorylated tau expression, protect neurons from the neurotoxicity of A, and protect them from numerous neurodegenerative disorders by specifically diminishing the expression of inflammatory genes in activated microglia (Jiang et al. 2019).

3.6 Saponins

Dioscorea zingiberensis is the main source of diosgenin, a natural substance with anti-inflammatory and anticancer activities. The MAPK/AKT/NF-B signaling pathway is inhibited, ROS generation is decreased, and inflammatory mediator levels such NO and IL-1 and IL-6 are decreased. Cell cycle arrest, immune system

modification, regulation and inhibition of the activity of the caspase-3 enzyme, and the induction of the STAT3 signaling pathway are all associated with diosgenin's anticancer properties. The native populations of *Dioscorea zingiberensis* are dwindling because it takes so long to reach maturity. Endophytes may therefore be a potential substitute source of diosgenin (C.-H. Ding et al. 2014; Jesus et al. 2016; Ligang et al. 2004). In Northeast China, 96 fungus isolates from 12 distinct species were recovered from *A. elata*. The most prevalent genera, accounting for 25% and 12.5% of the isolates, respectively, were *Diaporthe* and *Alternaria*. Many of the isolated fungi were capable of producing saponins, according to an analysis of the saponins they generated. G22 (*Penicillium* sp., 2.049 mg/mL) had the greatest concentration. Ginsenosides Rb2 and Re were also found in G22, suggesting that it has the ability to produce ginsenosides and may have uses in this field (Wu et al. 2012).

3.7 Flavonoids

An isoflavone molecule called cajanol, which is present in the *Cajanus cajan* root extract, has antitumor, antibacterial, and antimalarial effects. It can trigger apoptosis through the pathway involving reactive oxygen species (ROS) and mitochondria-mediated processes and stop the cell cycle at the G2/M phase check point. Additionally, it has been revealed that the cajanol produced by endophytic strains of *Hypocrea lixii*, which live inside the roots of *C. cajan*, has anticancer properties (Zhao et al. 2013). By controlling various inflammatory mediators, inhibition of various inflammatory markers such as COX-2, lipoxygenase, TNF-alpha, IL-1, IL-2, IL-6, and IL-8, as well as Janus kinases, curcumin (*Chaetomium globosum*), and an unnamed strain are two examples of the fungal endophytes from which curcumin has been isolated (Wang et al. 2012; Yan et al. 2014). A powerful chemical found in *Curcuma* spp. demonstrates extraordinary anti-inflammatory and antioxidant actions. By inhibiting cyclin D1 and CDK4, stopping the cell cycle; upregulating the expression of Fas, FasL, and DR5; activating caspase via p-53; and inhibiting TNF-induced NF-B activation, it also has promising anticancer effects (Fadus et al. 2017). According to a new study, curcumin may be able to influence epigenetic processes. It can control the regulation of histone acetyltransferases (HATs) and histone deacetylases (HDACs), as well as limit the activity of DNA methyltransferases (DNMTs). Along with interacting with DNA and transcription factors, curcumin can control the expression of microRNAs (miRNA) (Hassan et al. 2019).

Fruits and vegetables contain flavonoid, kaempferol, a substance with anti-inflammatory, anticancer, cardioprotective, neuroprotective, hepatoprotective, and antidiabetic characteristics. It functions by lowering iNOS, COX-2 protein, and inflammatory cytokine expression. Additionally, kaempferol slows the proliferation of cancer cells by stopping the cell cycle and concentrating on vital

signaling pathways, including MAPK/ERK and PI3K/AKT. It also modifies the expression of EMT-related markers and inhibits the activation of NF-B, AP-1, and AKT and the phosphorylation of these three transcription factors. In treating cancer, kaempferol is a powerful chemopreventive drug (Chen and Chen 2013; Imran et al. 2019).

3.8 Fungal Endophyte-Derived Coumarins or Benzopyrones

Since ancient times, coumarins have been used as herbal medicine's natural cures. They were initially found and isolated from the seeds of the *Coumarouna odorata* species of *Dipteryx* (Matos et al. 2015). Celery (*Apium graveolens*) and Siberian ginseng (*Acanthopanax senticosus*) both contain the coumarin chemical isofraxidin, which has a number of health advantages. It regulates lipid metabolism by lowering triglyceride buildup and preventing the production of fatty acid synthesis-related enzymes. Isofraxidin's anti-inflammatory capabilities decrease the number of inflammatory cytokines and cells in the liver. It is a powerful hyperpigmentation agent because of its capacity to increase melanin formation. It does this by raising the activity and expression of tyrosinase and the transcription factor that controls melanogenesis, microphthalmia-associated transcription factor (MITF), in melanocytes (Li et al. 2017). Umbelliferone (7-hydroxycoumarin) is a chemical that may be found in the Rutaceae and Apiaceae families of plants. It is used as a sunscreen and is well recognized for its fluorescence capabilities. It has been discovered to provide a number of health advantages, including anti-inflammatory, antihyperglycemic, anticancer, and antioxidant properties. It has been demonstrated that umbelliferone causes apoptosis and cell cycle arrest, making it a viable cancer therapy (J. Li et al. 2017). A member of the coumarin family, scopoletin (6-methoxy-7-hydroxycoumarin), has antifungal, anti-acetylcholinesterase (AChE), and anticancer activities. By causing apoptosis, decreasing the protein levels, and inhibiting the activity of acid phosphatase (ACP), it has been discovered to prevent the growth of cancer cells (Hornick et al. 2011). *Citrus bergamia* and *Balanites aegyptiaca* contain the furocoumarin chemical bergapten, commonly referred to as 5-methoxypsoralen. It has been found as a potential photosensitizer for the oral photochemotherapy treatment of psoriasis. DNA damage can result from bergapten's ability to attach to pyrimidine nucleotides in DNA. Additionally, it activates PTEN, which causes the activation of autophagy, which may have anticancer characteristics (Seida et al. 1981). Meranzin is well recognized for controlling the 2-adrenoceptor in a manner that has an antidepressant effect. Along with bergapten, it may be found in grapefruit peels. Endophytic fungi including *Alternaria brassicae*, *Botryodiplodia theobromae*, and *Penicillium* sp. generate these substances (Xie et al. 2013).

3.9 Lignans

Lignans are a group of secondary metabolites that serve various biological purposes, making them a study topic of interest in many fields. Endophytic bacteria generate seven of the various plant-derived lignans with therapeutic value**.** Significant anticancer and antiviral activities are provided by podophyllotoxin, an aryl tetralin lactone lignan found in medicinal plants including *Podophyllum*, *Diphylleia*, *Dysosma*, *and Juniperus*. Etoposide, teniposide, and etopophos (etoposide phosphate) are only a few examples of helpful anticancer medications that may be chemically synthesized using it. Podophyllotoxin prevents mitosis in the late S/early G2 phase by destabilizing microtubules as an anti-tubulin drug and blocking the enzyme topoisomerase II, which is essential for unwinding the DNA double helix (Canel et al. 2000)**.** Researchers have discovered podophyllotoxin in 17 fungi living within ten different host plant types**.**

4 Investigating the Potential and Addressing Issues with the Use of Endophytes as Supplemental Sources of Natural Plant Extracts

Natural substrates are successful in medical and drug development because of their wide range of structural complexity, pharmacological activity, safety, and innate ability to bind to other biomolecules. Recent developments in the techniques for fermentation, extraction, purification, characterization, and bioassay, along with studies on the biosynthesis of compounds from plants by endophytic fungi, have made it possible to characterize new and original natural sources quickly and gain access to endophytes and its resources that were previously off-limits (Potterat and Hamburger 2013). Fungal fermentation methods offer significant flexibility in manipulating the synthesis of bioactive chemicals by utilizing feeding precursors, elicitors, specialized enzymes, and modifiers. These methods are typically characterized by their brevity, simplicity, and economic viability (Ebada et al. 2016; Kumar et al. 2013). Endophytes have a unique ability to biotransform original bioactive chemicals produced from plants into more effective derivatives, resulting in structural and functional diversity. In some instances, endophytes can activate the expression of genes within the plant host and facilitate the host's synthesis of particular biomolecules. As a result, every study on the biosynthesis of nature-derived products obtained from plants utilizing fungal endophytes offers a promising strategy for the precise and effective synthesis of important bioactive natural products employing endophytes as stable and capable "biolaboratories."

However, there are certain difficulties with this strategy. First, a search for endophytic fungi that exhibit high productivity and produce desirable plant-based substances continues. Next, epigenetic alterations, mutations, and genetic

engineering are used to improve the fungal strains' adaptability for industrial uses. Additionally, we must unravel the entire biosynthetic pathway with all the necessary enzymes and associated genes involved to control and manage the biosynthesis process for increased productivity. This may be done through the "omics" fields of genomics, transcriptomics, proteomics, and metabolomics. Alternatively, the identified bioactive compounds' biosynthesis pathway may be put together and imitated in practical systems, providing a method to generate the target molecules easily. Secondly, to overcome the problems of attenuation and low yield, which are the primary barriers to this innovative approach's commercial success (Amirkia and Heinrich 2015; Hautbergue et al. 2018; Hillman et al. 2017; Swift et al. 2019). Our comprehension of the relationships between host plants and endophytes, the requirements for plant environments, and identifying specific signals or elicitors must be improved. Reduced product yields in axenic monocultures have been linked to the downregulation of genes, which prevents observable signals or molecules from emerging interactions between the host-endophyte and endophyte-endophyte. The precise nature of the presumed activator signals and chemicals must yet be characterized, though. Thirdly, for the effective industrial-scale manufacturing of pharmaceutically useful compounds or leads, this sector needs cooperation between researchers in this field and the pharmacological business. The drug-related or medical sector must focus on the endophyte-dependent production of natural plant chemicals (Amirkia and Heinrich 2015).

5 Endophytic Fungal Extracts and Plant Derivatives: Prospects for the Future

It is encouraging for the future of endophytic fungal extract because the endophytic fungus has established itself as a significant source of bioactive secondary metabolites with a range of functional capabilities. Peptides, chlorinated metabolites, aliphatic compounds, alkaloids, sesquiterpenes, flavonoids, diterpenes, lignans, phenols, phenolic acids, quinines, phenolic, indole derivatives, amines, and derivatives of iso-coumarin are some of the metabolites that make up this list. Many of these bioactive compounds have been demonstrated to have therapeutic properties such as cytotoxicity, antibacterial, antiparasitic, anti-pathogenic, antitumor, antioxidant, and immunoregulatory activity. Certain endophytic fungal extracts, for example, have been identified to produce antibiotics that can be used to treat diseases caused by bacteria resistant to standard treatments. In contrast, others have shown promise as anticancer drugs. Endophytic fungal extracts are increasingly being utilized in healthcare and medicine due to the growing demand for natural and environmentally friendly products. These extracts may be used to develop new drugs, probiotics, and other health products that help improve well-being and treat various illnesses. They may also provide an environmentally

friendly and sustainable alternative to traditional medicines. Overall, the future is quite promising for endophytic fungal extract. With further research and development, these extracts may become a significant source of cutting-edge drugs and health products that are beneficial for both the health of persons and the environment.

6 Conclusion

In conclusion, endophytic fungi are a significant source of biologically active substances created as by-products and have a wide range of functional properties. These metabolites have shown great promise in healthcare and medicine, with the potential to be used to develop innovative drugs, probiotics, and other health products. Because they provide a workable and responsible alternative to traditional treatments, endophytic fungal extracts are becoming more and more popular with consumers who choose natural and environmentally friendly products. Due to their diverse spectrum of biological actions, which include their potential as antiparasitic, anti-pathogenic, antibacterial, antitumor, antioxidant, immunoregulatory, neuroprotective, and cytotoxic agents, these compounds represent a never-ending supply of pharmacologically significant molecules. Future studies should focus on determining the mechanisms of action of these metabolites' health benefits and their effectiveness and safety. Overall, there is a lot of promise for endophytic fungal extracts to improve the environment and human health.

References

Aini K, Wildjajanti H, Setiawan A, Kurniawati AR (2022) Antibacterial activity of endophytic fungi isolated from the stem bark of jambu mawar (Syzygium jambos). Biodiversitas J Biol Divers 23(1)

Amirkia V, Heinrich M (2015) Natural products and drug discovery: a survey of stakeholders in industry and academia. Front Pharmacol 6:237

Amna T, Puri SC, Verma V, Sharma JP, Khajuria RK, Musarrat J et al (2006) Bioreactor studies on the endophytic fungus Entrophospora infrequens for the production of an anticancer alkaloid camptothecin. Can J Microbiol 52(3):189–196

An C, Ma S, Shi X, Xue W, Liu C, Ding H (2020) Diversity and antimicrobial activity of endophytic fungi isolated from Chloranthus japonicus Sieb in Qinling Mountains, China. Int J Mol Sci 21(17):5958

Ancheeva E, Daletos G, Proksch P (2020) Bioactive secondary metabolites from endophytic fungi. Curr Med Chem 27(11):1836–1854

Arneaud SL, Porter JR (2015) Investigation and expression of the secoisolariciresinol dehydrogenase gene involved in podophyllotoxin biosynthesis. Mol Biotechnol 57:961–973

Bahabadi SE, Sharifi M, Behmanesh M, Safaie N, Murata J, Araki R et al (2012) Time-course changes in fungal elicitor-induced lignan synthesis and expression of the relevant genes in cell cultures of Linum album. J Plant Physiol 169(5):487–491

Björkman KM, Church MJ, Doggett JK, Karl DM (2015) Differential assimilation of inorganic carbon and leucine by Prochlorococcus in the oligotrophic North Pacific Subtropical Gyre. Front Microbiol 6:1401

Bora P, Devi NN (2023) Exploration of the chemical constituents and its antioxidant, antibacterial activities of endophytic fungi isolated from the medicinal plant Dillenia indica. Arch Microbiol 205(2):67

Brito DA, Yang Z, Rieder CL (2008) Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. J Cell Biol 182(4):623–629

Calixto JB (2019) The role of natural products in modern drug discovery. An Acad Bras Cienc 91

Canel C, Moraes RM, Dayan FE, Ferreira D (2000) Podophyllotoxin. Phytochemistry 54(2):115–120

Chen AY, Chen YC (2013) A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food Chem 138(4):2099–2107

Chomcheon P, Wiyakrutta S, Sriubolmas N, Ngamrojanavanich N, Isarangkul D, Kittakoop P (2005) 3-Nitropropionic acid (3-NPA), a potent antimycobacterial agent from endophytic fungi: is 3-NPA in some plants produced by endophytes? J Nat Prod 68(7):1103–1105

Chomcheon P, Wiyakrutta S, Sriubolmas N, Ngamrojanavanich N, Mahidol C, Ruchirawat S, Kittakoop P (2009) Metabolites from the endophytic mitosporic Dothideomycete sp. LRUB20. Phytochemistry 70(1):121–127

Christenhusz MJ, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261(3):201–217-201–217

De Lamo FJ, Takken FL (2020) Biocontrol by Fusarium oxysporum using endophyte-mediated resistance. Front Plant Sci 11:37

Debbab A, Aly AH, Proksch P (2011) Bioactive secondary metabolites from endophytes and associated marine derived fungi. Fungal Divers 49(1):1

Dekan Z, Sianati S, Yousuf A, Sutcliffe KJ, Gillis A, Mallet C et al (2019) A tetrapeptide class of biased analgesics from an Australian fungus targets the μ-opioid receptor. Proc Natl Acad Sci 116(44):22353–22358

Dhankhar S, Kumar S, Dhankhar S, Yadav J (2012) Antioxidant activity of fungal endophytes isolated from Salvadora oleoides Decne. Int J Pharm Pharm Sci 4(2):380–385

Ding X, Liu K, Deng B, Chen W, Li W, Liu F (2013) Isolation and characterization of endophytic fungi from Camptotheca acuminata. World J Microbiol Biotechnol 29:1831–1838

Ding C-H, Du X-W, Xu Y, Xu X-M, Mou J-C, Yu D et al (2014) Screening for differentially expressed genes in endophytic fungus strain 39 during co-culture with herbal extract of its host Dioscorea nipponica Makino. Curr Microbiol 69:517–524

Dzoyem JP, Melong R, Tsamo AT, Maffo T, Kapche DG, Ngadjui BT et al (2017) Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus Epicoccum nigrum associated with Entada abyssinica. Rev Bras 27:251–253

Ebada SS, Eze P, Okoye FB, Esimone CO, Proksch P (2016) The fungal endophyte Nigrospora oryzae produces quercetin monoglycosides previously known only from plants. ChemistrySelect 1(11):2767–2771

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from Podophyllum peltatum produce podophyllotoxin. J Nat Prod 69(8):1121–1124

Eze PM, Okoye FB (2017) Metabolites of endophytic fungi isolated from Euphorbia hirta growing in Southern Nigeria. Chem Sci Rev Lett 6(21):12–19

Fadus MC, Lau C, Bikhchandani J, Lynch HT (2017) Curcumin: an age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med 7(3):339–346

Gouda S, Das G, Sen SK, Shin H-S, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538

Guo D-L, Qiu L, Feng D, He X, Li X-H, Cao Z-X et al (2020) Three new α-pyrone derivatives induced by chemical epigenetic manipulation of Penicillium herquei, an endophytic fungus isolated from Cordyceps sinensis. Nat Prod Res 34(7):958–964

Gupta S, Bhatt P, Chaturvedi P (2018) Determination and quantification of asiaticoside in endophytic fungus from Centella asiatica (L.) Urban. World J Microbiol Biotechnol 34:1–10
Gurudatt P, Priti V, Shweta S, Ramesha B, Ravikanth G, Vasudeva R et al (2010) Attenuation of camptothecin production and negative relation between hyphal biomass and camptothecin content in endophytic fungal strains isolated from Nothapodytes nimmoniana Grahm (Icacinaceae). Curr Sci 98(8):1006–1010
Hassan F-u, Rehman M S-u, Khan MS, Ali MA, Javed A, Nawaz A, Yang C (2019) Curcumin as an alternative epigenetic modulator: mechanism of action and potential effects. Front Genet 10:514
Hautbergue T, Jamin EL, Debrauwer L, Puel O, Oswald IP (2018) From genomics to metabolomics, moving toward an integrated strategy for the discovery of fungal secondary metabolites. Nat Prod Rep 35(2):147–173
Hillman ET, Readnour LR, Solomon KV (2017) Exploiting the natural product potential of fungi with integrated-omics and synthetic biology approaches. Curr Opin Syst Biol 5:50–56
Hornick A, Lieb A, Vo N, Rollinger J, Stuppner H, Prast H (2011) The coumarin scopoletin potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and ameliorates anticholinergic-and age-impaired memory. Neuroscience 197:280–292
Huang W-Y, Cai Y-Z, Xing J, Corke H, Sun M (2007) A potential antioxidant resource: endophytic fungi from medicinal plants. Econ Bot 61(1):14–30
Imran M, Salehi B, Sharifi-Rad J, Aslam Gondal T, Saeed F, Imran A et al (2019) Kaempferol: a key emphasis to its anticancer potential. Molecules 24(12):2277
Jesus M, Martins AP, Gallardo E, Silvestre S (2016) Diosgenin: recent highlights on pharmacology and analytical methodology. J Anal Methods Chem 2016:1
Jiang Z, Gao W, Huang L (2019) Tanshinones, critical pharmacological components in Salvia miltiorrhiza. Front Pharmacol 202
Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK (2013) Prospecting endophytic fungal assemblage of Digitalis lanata Ehrh(foxglove) as a novel source of digoxin: a cardiac glycoside. 3 Biotech 3:335–340
Kour A, Shawl AS, Rehman S, Sultan P, Qazi PH, Suden P et al (2008) Isolation and identification of an endophytic strain of Fusarium oxysporum producing podophyllotoxin from Juniperus recurva. World J Microbiol Biotechnol 24:1115–1121
Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus Fusarium oxysporum isolated from Catharanthus roseus. PLoS One 8(9):e71805
Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod 71(2):159–162
Kusari S, Lamshöft M, Spiteller M (2009a) Aspergillus fumigatus Fresenius, an endophytic fungus from Juniperus communis L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J Appl Microbiol 107(3):1019–1030
Kusari S, Zühlke S, Kosuth J, Cellarova E, Spiteller M (2009b) Light-independent metabolomics of endophytic Thielavia subthermophila provides insight into microbial hypericin biosynthesis. J Nat Prod 72(10):1825–1835
Kusari S, Zühlke S, Spiteller M (2009c) An endophytic fungus from Camptotheca acuminata that produces camptothecin and analogues. J Nat Prod 72(1):2–7
Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012) An endophytic fungus from Azadirachta indica A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28(3):1287–1294
Kusari P, Kusari S, Spiteller M, Kayser O (2013) Endophytic fungi harbored in Cannabis sativa L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. Fungal Divers 60:137–151
Li C (2007) Fermentation conditions of Sinopodophyllum hexandrum endophytic fungus on production of podophyllotoxin. Food Ferment Ind 33(9):28

Li X, Zhai X, Shu Z, Dong R, Ming Q, Qin L, Zheng C (2016) Phoma glomerata D14: An endophytic fungus from Salvia miltiorrhiza that produces salvianolic acid C. Curr Microbiol 73:31–37

Li J, Li X, Li Z, Zhang L, Liu Y, Ding H, Yin S (2017) Isofraxidin, a coumarin component improves high-fat diet induced hepatic lipid homeostasis disorder and macrophage inflammation in mice. Food Funct 8(8):2886–2896

Liang Z, Zhang J, Zhang X, Li J, Zhang X, Zhao C (2016) Endophytic fungus from Sinopodophyllum emodi (Wall.) Ying that produces Podophyllotoxin. J Chromatogr Sci 54(2):175–178

Ligang Z, Xiaodong C, Chengzong Y, Xuehong W, Liqun Z (2004) Endophytic fungi of {\sl Paris polyphylla} var.{\sl Yunnanensis} and steroid analysis in the fungi. Nat Prod Res Dev 16(3):198–200

Liu H, Chen S, Liu W, Liu Y, Huang X, She Z (2016) Polyketides with immunosuppressive activities from mangrove endophytic fungus Penicillium sp. ZJ-SY2. Mar Drugs 14(12):217. Retrieved from https://www.mdpi.com/1660-3397/14/12/217

Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms 8(12):1934

Matos MJ, Santana L, Uriarte E, Abreu OA, Molina E, Yordi EG (2015) Coumarins—an important class of phytochemicals. In: Phytochemicals-isolation, characterisation and role in human health, vol 25, pp 533–538

Min C, Wang X (2009) Isolation and identification of the 10-hydroxycamptothecin-producing endophytic fungi from Camptotheca acuminata Decne. Acta Botan Boreali-Occiden Sin 29(3):614–617

Mir R, Kaushik S, Chowdery R, Anuradha M (2015) Elicitation of forskolin in cultures of Rhizactonia bataticola-a phytochemical synthesizing endophytic fungi. Int J Pharm Pharm Sci 7:185–189

Mohanta TK (2020) Fungi contain genes associated with flavonoid biosynthesis pathway. J Funct Foods 68:103910

Musavi SF, Dhavale A, Balakrishnan RM (2015) Optimization and kinetic modeling of cell-associated camptothecin production from an endophytic Fusarium oxysporum NFX06. Prep Biochem Biotechnol 45(2):158–172

Nadeem M, Ram M, Alam P, Ahmad MM, Mohammad A, Al-Qurainy F et al (2012) Fusarium solani, P1, a new endophytic podophyllotoxin-producing fungus from roots of Podophyllum hexandrum. Afr J Microbiol Res 6(10):2493–2499

Nath A, Chattopadhyay A, Joshi S (2015) Biological activity of endophytic fungi of Rauwolfia serpentina Benth: an ethnomedicinal plant used in folk medicines in Northeast India. Proc Natl Acad Sci India Sect B Biol Sci 85:233–240

Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79(3):629–661

Newman DJ, Cragg GM (2020) Plant endophytes and epiphytes: burgeoning sources of known and "unknown" cytotoxic and antibiotic agents? Planta Med 86(13/14):891–905

Nibret E, Youns M, Krauth-Siegel RL, Wink M (2011) Biological activities of xanthatin from Xanthium strumarium leaves. Phytother Res 25(12):1883–1890

Patocka J (2016) Bioactive metabolites of entomopathogenic fungi Beauveria bassiana. Mil Med Sci Lett 85(2):80

Pizzorno JE, Murray MT, Joiner-Bey H (2016) The Clinician's handbook of natural medicine E-book: Elsevier Health Sciences

Potterat O, Hamburger M (2013) Concepts and technologies for tracking bioactive compounds in natural product extracts: generation of libraries, and hyphenation of analytical processes with bioassays. Nat Prod Rep 30(4):546–564

Prabavathy D (2013) Cytotoxic potential and phytochemical analysis of Justicia beddomei and its endophytic Aspergillus sp. Asian J Pharm Clin Res:159–161

Pragathi D, Vijaya T, Mouli K, Anitha D (2013) Diversity of fungal endophytes and their bioactive metabolites from endemic plants of Tirumala hills-Seshachalam biosphere reserve. Afr J Biotechnol 12(27):4317

Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from Nothapodytes f oetida that produces Camptothecin. J Nat Prod 68(12):1717–1719

Puri SC, Nazir A, Chawla R, Arora R, Riyaz-ul-Hasan S, Amna T et al (2006) The endophytic fungus Trametes hirsuta as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol 122(4):494–510

Rabiey M, Hailey LE, Roy SR, Grenz K, Al-Zadjali MA, Barrett GA, Jackson RW (2019) Endophytes vs tree pathogens and pests: can they be used as biological control agents to improve tree health? Eur J Plant Pathol 155:711–729

Sadananda T, Nirupama R, Chaithra K, Govindappa M, Chandrappa C, Vinay Raghavendra B (2011) Antimicrobial and antioxidant activities of endophytes from Tabebuia argentea and identification of anticancer agent (lapachol). J Med Plants Res 5(16):3643–3652

Sarang H, Rajani P, Vasanthakumari MM, Kumara PM, Siva R, Ravikanth G, Uma Shaanker R (2017) An endophytic fungus, Gibberella moniliformis from Lawsonia inermis L. produces lawsone, an orange-red pigment. Antonie Van Leeuwenhoek 110:853–862

Saraswaty V, Srikandace Y, Simbiyani N, Setiyanto H, Udin Z (2013) Antioxidant activity and total phenolic content of endophytic fungus Fennellia nivea NRRL 5504. Pak J Biol Sci: PJBS 16(22):1574–1578

Sathiyabama M, Parthasarathy R (2018) Withanolide production by fungal endophyte isolated from Withania somnifera. Nat Prod Res 32(13):1573–1577

Saxena S, Meshram V, Kapoor N (2015) Muscodor tigerii sp. nov.-volatile antibiotic producing endophytic fungus from the Northeastern Himalayas. Ann Microbiol 65(1):47–57

Schwarz M, Köpcke B, Weber RW, Sterner O, Anke H (2004) 3-Hydroxypropionic acid as a nematicidal principle in endophytic fungi. Phytochemistry 65(15):2239–2245

Seetharaman P, Gnanasekar S, Chandrasekaran R, Chandrakasan G, Kadarkarai M, Sivaperumal S (2017) Isolation and characterization of anticancer flavone chrysin (5, 7-dihydroxy flavone)-producing endophytic fungi from Passiflora incarnata L. leaves. Ann Microbiol 67:321–331

Seida AA, Kinghorn AD, Cordell GA, Farnsworth NR (1981) Isolation of bergapten and marmesin from Balanites aegyptiaca. Planta Med 43(09):92–93

Selvi BK, Balagengatharathilagam P (2014) Isolation and screening of endophytic fungi from medicinal plants of Virudhunagar district for antimicrobial activity. Int J Sci Nat 5(1):147–155

Shen B (2015) A new golden age of natural products drug discovery. Cell 163(6):1297–1300

Shi J, Zeng Q, Liu Y, Pan Z (2012) Alternaria sp. MG1, a resveratrol-producing fungus: isolation, identification, and optimal cultivation conditions for resveratrol production. Appl Microbiol Biotechnol 95:369–379

Shweta S, Gurumurthy BR, Ravikanth G, Ramanan US, Shivanna MB (2013) Endophytic fungi from Miquelia dentata Bedd., produce the anti-cancer alkaloid, camptothecine. Phytomedicine 20(3–4):337–342

Singh A, Singh DK, Kharwar RN, White JF, Gond SK (2021) Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: insights, avenues, and challenges. Microorganisms 9(1):197

Srivastava RK (2017) A study on cyclosporine a production by fungal cells

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Strobel G (2018) The emergence of endophytic microbes and their biological promise. J Fungi 4(2):57

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel G, Yang X, Sears J, Kramer R, Sidhu RS, Hess W (1996) Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology 142(2):435–440

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67(2):257–268

Swift CL, Podolsky IA, Lankiewicz TS, Seppälä S, O'Malley MA (2019) Linking 'omics' to function unlocks the biotech potential of non-model fungi. Curr Opin Syst Biol 14:9–17

Tan X-m, Zhou Y-q, Zhou X-L, Xia X-H, Wei Y, He L-l et al (2018) Diversity and bioactive potential of culturable fungal endophytes of Dysosma versipellis; a rare medicinal plant endemic to China. Sci Rep 8(1):5929

Tan Y, Guo Z, Zhu M, Shi J, Li W, Jiao R et al (2020) Anti-inflammatory spirobisnaphthalene natural products from a plant-derived endophytic fungus Edenia gomezpompae. Chin Chem Lett 31(6):1406–1409

Tao Y-w, Lin Y-c, She Z-g, Lin M-t, Chen P-x, Zhang J-y (2015) Anticancer activity and mechanism investigation of beauvericin isolated from secondary metabolites of the mangrove endophytic fungi. Anticancer Agents Med Chem (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 15(2):258–266

Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV et al (2018) Endophytic fungi—alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:309

Venieraki A, Dimou M, Katinakis P (2017) Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hellenic Plant Protect J 10(2):51–66

Venkateswarulu N, Shameer S, Bramhachari P, Basha ST, Nagaraju C, Vijaya T (2018) Isolation and characterization of plumbagin (5-hydroxyl-2-methylnaptalene-1, 4-dione) producing endophytic fungi Cladosporium delicatulum from endemic medicinal plants. Biotechnol Rep 20:e00282

Vigneshwari A, Rakk D, Németh A, Kocsubé S, Kiss N, Csupor D et al (2019) Host metabolite producing endophytic fungi isolated from Hypericum perforatum. PLoS One 14(5):e0217060

Wang Y, Xu L, Ren W, Zhao D, Zhu Y, Wu X (2012) Bioactive metabolites from Chaetomium globosum L18, an endophytic fungus in the medicinal plant Curcuma wenyujin. Phytomedicine 19(3–4):364–368

Wang J, Yao L-y, Lu Y-h (2013) Ceriporia lacerata DMC1106, a new endophytic fungus: isolation, identification, and optimal medium for 2′, 4′-dihydroxy-6′-methoxy-3′, 5′-dimethylchalcone production. Biotechnol Bioprocess Eng 18:669–678

Wang J, Wang H, Zhang H, Liu Z, Ma C, Kang W (2019) Immunomodulation of ADPs-1a and ADPs-3a on RAW264. 7 cells through NF-κB/MAPK signaling pathway. Int J Biol Macromol 132:1024–1030

Wani MC, Horwitz SB (2014) Nature as a remarkable chemist: a personal story of the discovery and development of Taxol®. Anti-Cancer Drugs 25(5):482

Wu H, Yang H, You X, Li Y (2012) Isolation and characterization of saponin-producing fungal endophytes from Aralia elata in Northeast China. Int J Mol Sci 13(12):16255–16266. Retrieved from https://www.mdpi.com/1422-0067/13/12/16255

Xianzhi Y, Shiping G, Lingqi Z, Hua S (2003) Select of producing podophyllotoxin endophytic fungi from podophyllin plant. Nat Prod Res Dev 15(5):419–422

Xie Y, Huang X, Hu S-y, Qiu X-j, Zhang Y-j, Ren P et al (2013) Meranzin hydrate exhibits antidepressive and prokinetic-like effects through regulation of the shared alpha 2-adrenoceptor in the brain–gut axis of rats in the forced swimming test. Neuropharmacology 67:318–325

Xu L-L, Han T, Wu J-Z, Zhang Q-Y, Zhang H, Huang B-K et al (2009) Comparative research of chemical constituents, antifungal and antitumor properties of ether extracts of Panax ginseng and its endophytic fungus. Phytomedicine 16(6–7):609–616

Xu J, Luo X, Zhong W, Zhang J, Tan R (2014) Characterization of volatile constituents from an endophytic Aspergillus fumigatus strain. J Chem Pharm Res 6(4):893–897

Yadav M, Yadav A, Yadav JP (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. Asian Pac J Trop Med 7:S256–S261

Yan J, Qi N, Wang S, Gadhave K, Yang S (2014) Characterization of secondary metabolites of an endophytic fungus from Curcuma wenyujin. Curr Microbiol 69:740–744

Yang K, Liang J, Li Q, Kong X, Chen R, Jin Y (2013) Cladosporium cladosporioides XJ-AC03, an aconitine-producing endophytic fungus isolated from Aconitum leucostomum. World J Microbiol Biotechnol 29:933–938

Yin H, Sun Y-H (2011) Vincamine-producing endophytic fungus isolated from Vinca minor. Phytomedicine 18(8–9):802–805

You X, Feng S, Luo S, Cong D, Yu Z, Yang Z, Zhang J (2013) Studies on a rhein-producing endophytic fungus isolated from Rheum palmatum L. Fitoterapia 85:161–168

Zeng S, Shao H, Zhang L (2004) An endophytic fungus producing a substance analogous to podophyllotoxin isolated from Diphylleia sinensis. J Microbiol 24:1–2

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771

Zhao J, Fu Y, Luo M, Zu Y, Wang W, Zhao C, Gu C (2012) Endophytic fungi from pigeon pea [Cajanus cajan (L.) Millsp.] produce antioxidant cajaninstilbene acid. J Agric Food Chem 60(17):4314–4319

Zhao J, Li C, Wang W, Zhao C, Luo M, Mu F et al (2013) Hypocrea lixii, novel endophytic fungi producing anticancer agent cajanol, isolated from pigeon pea (Cajanus cajan [L.] Millsp.). J Appl Microbiol 115(1):102–113

Chapter 15
Endophytic Fungi for Microbial Phytoremediation: Prospects for Agricultural and Environmental Sustainability

Ratna Prabha, Dhananjaya P. Singh, Amrender Kumar, M. Kumar, Ritika, Shwetank Singh, Vishva Deepak Chaturvedi, and Rajan Singh

Abstract Owing to their ability to colonize plant tissues and metabolize a wide range of organic and inorganic toxic pollutants, endophytic fungi are a promising tool for environmental bioremediation. They reside within the living tissues of plants and have emerged as a promising group of organisms for mycoremediation, which involves using fungi to degrade and/or detoxify environmental pollutants, including organic and inorganic compounds and heavy metals. In the current years, the identification of endophytic fungi with mycoremediation potential has emerged as an area of focus. Researchers are exploring various plant species to isolate and identify endophytic fungi with the potential to degrade environmental pollutants. The use of molecular techniques and high-throughput screening methods has accelerated the identification of such fungi. Understanding the mechanisms by which endophytic fungi detoxify or degrade environmental pollutants is crucial for devel-

R. Prabha (✉) · A. Kumar · Ritika
Agricultural Knowledge Management Unit (AKMU), ICAR-Indian Agricultural Research Institute,
New Delhi, India
e-mail: ratna.prabha@icar.gov.in

D. P. Singh · R. Singh
ICAR-Indian Institute of Vegetable Research, Varanasi, India

M. Kumar
ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, MauNath Bhanjan, Uttar Pradesh, India

S. Singh
Department of Plant Pathology, Acharya Narendra Dev University of Agriculture and Technology,
Ayodhya, Uttar Pradesh, India

V. D. Chaturvedi
Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Kangra, Himachal Pradesh, India

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oping effective mycoremediation strategies. Overall, the field of endophytic fungi and their potential as mycoremediators is rapidly evolving, and there is significant potential for these organisms to play a critical role in developing sustainable environmental remediation strategies. In this chapter, attempts are being taken to explore the genetic and biochemical pathways involved in phytoremediation processes besides the potentials of endophytic fungi.

Keywords Endophytic fungi · Microbial phytoremediation · Environmental sustainability · Agriculture sustainability

1 Introduction

Mismanagement of agricultural and household wastes and industrial effluents poses threatening environmental pollution concerns. It further deteriorates the vulnerable soil and water ecosystem for plant growth and crop production. Therefore, effective strategies are necessary to combat soil and water pollution caused by various chemical contaminants and heavy metals. Existing physical and chemical methods for decontamination, degradation, remediation, and removal of pollutants from the ecosystem are cumbersome and costly, produce toxic by-products, and struggle with low-concentration yet highly toxic chemicals. Therefore, it is crucial to develop strategies that overcome these limitations and enable in situ remediation of pollutants in a more sustainable and eco-friendly way.

Excessive reliance on synthetic insecticides, weedicides, herbicides, fungicides, and antibiotics during the green revolution has led to adverse environmental effects like pesticide resistance, insect resurgence, toxicity to nontarget organisms, and expanded soil and water pollution (Meena et al. 2020; Shukla et al. 2020; Singh et al. 2020; Jacquet et al. 2022). A sustainable plant protection and soil health approach based on eco-friendly microbial inoculants and free from chemical contaminants and heavy metals is advised (Singh et al. 2016; Renu et al. 2022). Microbial inoculants as a wide range of microorganisms with beneficial functional traits like biocontrol, biopesticides, biofertilizers, and biostimulants could offer a reliable and eco-friendly alternative to chemical pesticides and fertilizers (Singh and Prabha 2019; Adeleke et al. 2022; Verma et al. 2022).

Fungi are heterotrophic eukaryotic organisms that grow as elongated, polarized cells known as hyphae or budding yeasts. Their reproductive processes involve meiosis and/or mitosis (Naranjo-Ortiz and Gabaldón 2019; dos Reis et al. 2022). Fungi have independently evolved a unique lifestyle within the tree of life, contributing to their remarkable biodiversity of species among eukaryotes (Hawksworth and Lucking 2018; Willis 2018; dos Reis et al. 2022). Endophytic fungi, also known as fungal endophytes, are a diverse group of organisms with polyphyletic origins (Chi et al. 2019). These fungi can colonize plant tissues, such as stems, flowers, leaves,

fruits, and roots intra- or extracellularly (Li et al. 2016a, b). Importantly, they do so without causing noticeable disease symptoms in the colonized tissues (Bacon and White 2000; dos Reis et al. 2022). In contrast to mycorrhizal fungi that colonize plant roots and extend into the rhizosphere, endophytes exclusively inhabit plant tissues, including roots, stems, and leaves, and may emerge to produce spores during plant or host tissue senescence (Sherwood and Carroll 1974; Carroll 1988; Stone et al. 2004; Rodriguez et al. 2009). Endophytic fungi (EF) are intriguing fungal communities that establish beneficial relationships with host tissues, residing intercellularly or intracellularly, providing advantages to both. Endophytic fungi (EF) have garnered significant attention due to their potential as sources of novel cytotoxic compounds, including anticarcinogenic molecules and antibacterial substances (Radic and Strukelj 2012; Uzma et al. 2018; Alam et al. 2021). Endophytic fungi can be crucial in mitigating climate change effects on crops (Yan et al. 2019; Grabka et al. 2022). These fungi colonize plant tissues and positively impact plant productivity by enhancing photosynthesis, promoting growth, improving nutrient uptake efficiency, and conferring tolerance to abiotic and biotic stresses (Harman and Uphoff 2019; Grabka et al. 2022; Liu-Xu et al. 2022). Endophytic fungi contribute to the plant's growth by producing secondary metabolites (Wen et al. 2022). These metabolites enhance the plant's ability to withstand both biotic and abiotic stresses. Broad-spectrum endophytic fungi benefit plants through direct and indirect mechanisms. Direct mechanisms include phytohormone production, nitrogen fixation, phosphate solubilization, siderophore production, and antimicrobial metabolite production (El Enshasy et al. 2020). Indirect means involve abiotic and biotic resistance, biocontrol, bioremediation, and phytoremediation (Dubey et al. 2020; Singh et al. 2021; Verma et al. 2022). Furthermore, endophytic fungi can biosynthesize medically significant "phytochemicals," previously believed to be exclusive to the host plant (Wen et al. 2022).

Fungal endophytes are categorized using various criteria. Firstly, based on their ecological characteristics, they are classified as clavicipitaceous and non-clavicipitaceous endophytes; clavicipitaceous endophytes (C-endophytes) infect certain grasses. In contrast, nonclavicipitaceous endophytes (NC-endophytes) can be isolated from symptomless tissues of nonvascular plants, ferns, conifers, and angiosperms (Rodriguez et al. 2009). Secondly, their mode of reproduction distinguishes them as sexual or asexual endophytes. Thirdly, according to their transmission patterns, they can be vertically or horizontally transmitted. Fourthly, their source of nutrition determines whether they are biotrophic or necrotrophic endophytes (Adeleke et al. 2022).

Additionally, endophytes can be classified as symptomatic or asymptomatic based on their expression of infection. Lastly, they can be categorized as foliar or root endophytes depending on the plant body part they colonize (Bamisile et al. 2018; Adeleke et al. 2022). Certain fungal endophytes can colonize a broad range of plant species, while others exhibit a more specific association and are restricted to a limited number of plants. Additionally, specificity can also exist regarding the colonized plant part (Aly et al. 2011; Bamisile et al. 2018). Vertically transmitted fungi appear to establish more mutualistic relationships with plants, whereas horizontally

transmitted fungi are more likely to have antagonistic interactions (Aly et al. 2011; Baron and Rigobelo 2021).

Endophytic fungi play a crucial role in enhancing the self-defense mechanisms of plants by activating induced systemic resistance (ISR) pathways, which can overlap with acquired systemic resistance (ASR) pathways, leading to improved plant growth (Berg 2009; Busby et al. 2016) and protection against pests and pathogens (Chadha et al. 2015). Apart from inducing the production of defense molecules by the host plant, endophytic fungi serve as a vast source of bioactive compounds that benefit their plant hosts. They are proficient in producing a wide array of compounds with antimicrobial and insecticidal activities, including alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, chlorinated compounds, and volatile organic compounds (VOCs) (Card et al. 2016; Lugtenberg et al. 2016; Latz et al. 2018; Kaddes et al. 2019). Studies have also reported the production of compounds with antiviral, antibacterial, antifungal, and insecticidal properties by endophytic fungi (Card et al. 2016; Latz et al. 2018). Iron is an essential micronutrient for all living cells (Rana et al. 2020; Turbat et al. 2020). Siderophores are small molecules produced by certain microorganisms, including endophytic fungi, which possess iron-chelating properties. They bind to ferric ions in the rhizosphere, thereby aiding plants' acquisition and utilization of iron (Chowdappa et al. 2020).

There has been extensive work on the role of endophytic fungi as per their functional traits on plant growth promotion, soil quality improvement, stress alleviation, secondary metabolites, and bio−/phytoremediation. Bibliographic records of the past two decades suggest the importance of endophytic fungi in research and development perspectives (endophytic fungi – Search Results – PubMed (nih.gov); seen on 29.6.2023). As per the PubMed records, there are 10,145 research work entries with the keyword "endophytic fungi." With the keyword "endophytic fungi phytoremediation," there has been 346 records, while the keywords "endophytic fungi bioremediation" and "endophytic fungi secondary metabolites" have fetched 333 and 1171 records, respectively, in the last two decades. Because of the potential applicability of these organisms in environmental remediation and plant and soil health, the interest in research on these fungi is exponentially growing. Table 15.1 provides information about different terms associated with remediation and also about endophytic fungi.

2 Potential of Endophytic Fungi as Mycoremediators

Fungal endophytes play various roles such as phytostimulation, phytoimmobilization, phytostabilization, phytotransformation, phytoremediation, and biocontrol (Sahoo et al. 2017; Radziemska et al. 2021; Adeleke et al. 2022). They produce secondary metabolites like antimicrobial siderophores that defend against pathogens (Srinivas et al. 2020). Some fungal endophytes solubilize and mobilize phosphorus, potassium, and zinc salts, enhancing plant metabolic activity and promoting

Table 15.1 Different terminologies associated with remediation and endophytic fungi

S. no.	Term	Definition
1.	Endophyte	Any microorganism, such as fungus, bacteria, or virus, which resides within the tissues of a plant without causing any visible harm to the host
2.	Endophytic fungi	Fungi that colonize the internal tissues of plants, including the leaves, stems, and roots, forming a symbiotic relationship with the host plant
3.	Plant-fungal interaction	Relationship between plants and fungi, including endophytic fungi; interaction can be mutualistic or symbiotic
4.	Fungal colonization	Establishment and growth of endophytic fungi within the tissues of plants
5.	Biodegradation	Process by which living organisms break down complex organic compounds into simpler forms, reducing the concentration and toxicity of contaminants
6.	Phytodegradation	Breakdown or degradation of pollutants by plants and associated microorganisms
7.	Rhizodegradation	Degradation or transformation of contaminants in the rhizosphere facilitated by the activities of plants and associated microorganisms
8.	Phytoremediation	Process that utilizes plants and associated microorganisms to remove, degrade, or immobilize contaminants from soil, water, or air
9.	Mycoremediation	Use of fungi, including endophytic fungi, for environmental remediation purposes
10.	Bioaugmentation	Involves the introduction of specific microorganisms or microbial communities to the contaminated site to enhance the remediation process
11.	Phytoaugmentation	Combination of phytoremediation with the addition of amendments or beneficial organisms to improve the remediation process
12.	Phytostimulation	A phenomenon where plants release certain compounds or signals that stimulate the growth and activity of endophytic fungi, enhancing their remediation capabilities
13.	Mycofiltration	Technique that utilizes endophytic fungi to filter and remove contaminants from water or other liquid environments
14.	Biomineralization	Ability to transform metal contaminants into less toxic or less mobile forms, can precipitate metals as insoluble minerals, reducing their bioavailability and potential for harm
15.	Biofilm formation	Some endophytic fungi have the ability to form biofilms on plant surfaces or in the rhizosphere. These biofilms can act as hot spots for microbial activity, facilitating the degradation or immobilization of contaminants through microbial interactions and metabolic processes
16.	Remediation effectiveness	Degree of success in achieving the desired remediation outcomes

plant growth for higher crop production (Mehta et al. 2019; Haro and Benito 2019; Yung et al. 2021; Verma et al. 2022). Endophytic fungi possess the ability to enhance the uptake of essential macronutrients, including phosphorus, nitrogen, potassium, and magnesium, as well as micronutrients like zinc, iron, and copper, from the soil and organic matter, thereby increasing nutrient availability to their plant hosts (Rana et al. 2020; Baron and Rigobelo 2021). Endophytic fungi exert a broad range of effects on plant growth through direct and indirect mechanisms (Adeleke and Babalola 2022). In the direct mechanism, these fungi regulate plant hormones such as cytokinins, ethylene, and auxins and enhance soil nutrient availability through processes like phosphorus and iron solubilization, siderophore production, and nitrogen fixation. In the indirect mechanism, endophytes protect plants by releasing enzymes, antibiotics, hydrogen cyanide, and volatile compounds that inhibit pathogen activities and induce systemic resistance (Segaran and Sathiavelu 2019). Furthermore, endophytic fungi can produce important plant growth regulators such as auxins, gibberellins (GAs), and cytokinins. However, the potential for phytohormone production by endophytic fungi, particularly gibberellins, remains unexplored mainly despite their crucial role as chemical messengers and signaling molecules in various environmental conditions affecting plant growth (Khan et al. 2015). Among the phytohormones produced, indole-3-acetic acid (IAA) is the primary auxin synthesized by fungi. Auxins serve as key regulators of plant growth, exhibiting positive effects on shoot and root development, including tropism responses, cell division and elongation, vascular tissue differentiation, and initiation of root formation (Jaroszuk-Ściseł et al. 2014; Baron and Rigobelo 2021).

Mycoremediation offers an economical, environmentally friendly, and effective approach to address the escalating issue of soil and water pollution. Fungi exhibit robust growth, extensive hyphal networks, and the ability to produce versatile extracellular ligninolytic enzymes (Akhtar and Mannan 2020). They possess a high surface area to volume ratio, resistance to heavy metals, and adaptability to varying pH and temperature and contain metal-binding proteins, making them ideal candidates for remediating diverse pollutants. Mycoremediation can be applied for in situ remediation of pollutants like synthetic dyes, herbicides, insecticides, organometallic compounds, conjugates, and pharmaceutical drugs released by industries (Akhtar and Mannan 2020). Many beneficial fungal endophytes have been identified and can be used as biofertilizers and biocontrol agents in agriculture (Busby et al. 2016; Bastami et al. 2021; Grabka et al. 2022; Verma et al. 2022). Moreover, endophytic fungi have shown potential in environmental remediation, including agrochemical and metal pollutants' solubilization, assimilation, and mineralization (Gavrilaş et al. 2022). They can also serve as nanosensors for detecting contaminants, contributing to environmental conservation efforts (Sahoo et al. 2017; Khanam et al. 2020; Verma et al. 2022).

Microorganisms have the potential to accumulate heavy metals and other pollutants, enhance plant growth, and facilitate pollutant uptake from the soil through mobilization/immobilization processes (Ma et al. 2011). Endophytes have emerged as crucial components in ecological communities, aiming to mitigate land and water spoilage caused by excessive toxic insecticides, environmental degradation,

industrial wastewater, harmful gases, and biodiversity loss. As a new and efficient method, biological control utilizing endophytes has gained widespread recognition for ecological remediation and pest/pathogen control (Guo et al. 2008). Moreover, endophytes have found a novel application in phytoremediation, which involves the removal of xenobiotics and heavy metals from soil with the assistance of plants (Ma et al. 2011). Endophytes can exert both direct and indirect roles in the phytoremediation process and the degradation of environmental toxins. Indirectly, they enhance plant growth, accelerating the phytoremediation process due to the plants' inherent phytoremediation abilities. Directly, endophytes can degrade and/or accumulate pollutants themselves (Sudha et al. 2016). Endophytes in the ecosystem have a cryptic existence and primarily function as decomposers, among the primary colonizers of deceased plant tissues (Kumaresan and Suryanarayanan 2002; Oses et al. 2008). Endophytic fungi have demonstrated resistance to heavy metals and also exhibit the capacity to degrade organic contaminants. Furthermore, they not only mitigate the toxic effects of metals on plants and reduce the evapotranspiration of volatile contaminants but also enhance plant growth and facilitate phytoremediation through improved phytoextraction capabilities. Their adaptability to environments with high metal content allows them to modulate the translocation and accumulation of metals in plants (Mishra and Sarma 2017). Fungal endophytes play a significant role in the degradation of plastics, including polyester and polyurethane, and have been extensively studied for their ability to break down plastic and rubber materials. These fungi produce diverse enzymes such as lipases, proteases, tyrosinases, and xylanases, among others, making them promising candidates for enzyme production in waste treatment applications (Mishra and Sarma 2017).

The extensive array of metabolic pathways employed by endophytes renders them invaluable tools for various bioremediation applications. These include the assimilation of methane; nitrogen fixation; remediation of diverse pollutants such as pesticides, herbicides, insecticides, petrochemicals, polychlorobiphenyls, and phenols/chlorophenols; and the biotransformation of organic compounds like propylene into epoxypropane, as well as the production of chiral alcohols (Gai et al. 2009; Kim et al. 2012; Stępniewska and Kuźniar 2013). Additionally, endophytic microorganisms possess the capability to synthesize secondary metabolites that can exert antifungal and antibacterial properties; serve as precursors for plant hormones and growth factors, including vitamins B12 (Ivanova et al. 2006) and B1 (Mercado-Blanco and Bakker 2007; Simons et al. 1997); and act as bioprotectants (Trotsenko and Khmelenina 2002). Using endophytic fungi, phytotoxicity to plants and evapotranspiration of volatile soil contaminants can be minimized by limiting metal translocation and accumulation in plant tissues. Since these fungi are usually resistant to heavy metals and are capable of degrading pesticides, weedicides, organometallic compounds, antibiotics, xenobiotics, and many other kinds of organic pollutants/contaminants, the in situ phytoremediation for decontaminating soils based on endophytic fungi is becoming a promising technology (Fig. 15.1).

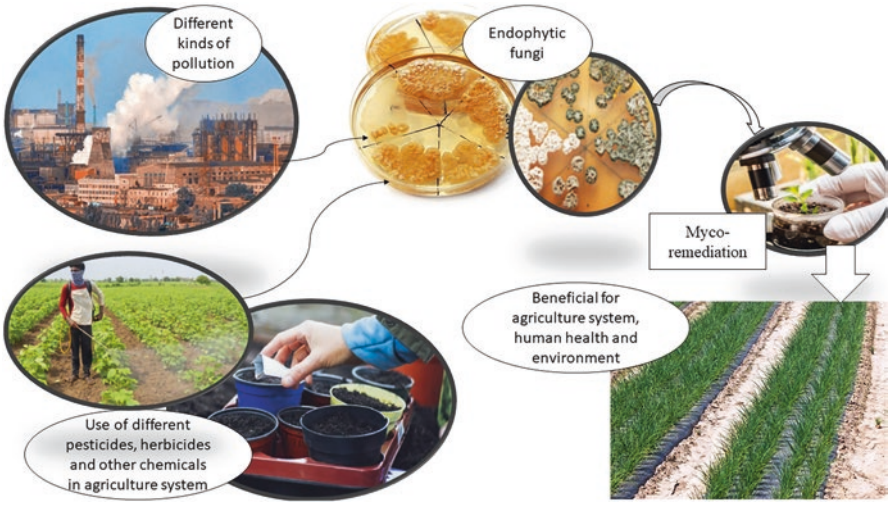

Fig. 15.1 Removal of different kinds of pollutants by endophytic fungi

3 Different Pollutants and Mycoremediation

There are two primary categories of environmental pollutants: (i) elemental pollutants and (ii) organic pollutants. The first category encompasses elements such as uranium (U), cerium (Ce), and tellurium (Te), as well as heavy metals and metalloids including mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), cesium (Cs), strontium (Sr), arsenic (As), zinc (Zn), manganese (Mn), and others (Nandy et al. 2020). On the other hand, organic pollutants include polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, polychlorinated biphenyls (PCBs), and similar compounds. Elemental pollutants are mainly removed through absorption, phytoextraction, transformation, hyperaccumulation, and translocation processes. Organic pollutants, on the other hand, are primarily sequestered through degradation, mineralization, and detoxification mechanisms (Meagher 2000; Nandy et al. 2020).

The indiscriminate use of pesticides and herbicides is prevalent in agrarian practices. These chemicals, such as glyphosate, endosulfan, paraquat, fipronil, and aldrin, have been reported to possess carcinogenic properties, act as endocrine disruptors, exhibit neurotoxic effects, and cause severe damage to the reproductive system as well as vital organs like the liver and kidneys (Akhtar and Mannan 2020). Furthermore, chemicals like DDT, aldrin, and dieldrin can persist in the environment due to their chemical stability (Purnomo et al. 2017; Akhtar and Mannan 2020). Numerous health hazards and chronic diseases are aggravated by toxic pollutants present in our ecosystem. Prolonged exposure to air pollutants such as volatile organic compounds (VOCs), polychlorinated biphenyls (PCBs), benzene, particulate matter, and tobacco smoke particles has been linked to diseases

including atopic dermatitis, lung cancer, and cardiopulmonary disorders such as asthma, hypertension, and chronic obstructive pulmonary disorder (Landrigan et al. 2002; Everett et al. 2008; Ahn 2014; Nandy et al. 2020). Lead (Pb), arsenic (As), and mercury (Hg) poisoning, resulting from soil and water pollution, are particularly dangerous as they can trigger cardiovascular diseases, renal failure, arthritis, emphysema, skin cancer, and various other ailments. Specific conditions like itai-itai, arsenicosis, and Minamata disease are associated with the toxicity of Pb, As, and Hg, respectively (Arnemo et al. 2016; Matta and Gjyli 2016; Nandy et al. 2020).

Mycoremediation offers a promising, environmentally friendly, cost-effective, and in situ approach for treating soil and water contaminated with herbicides and pesticides. Ligninolytic enzymes released by various fungi offer promising strategies for removing polycyclic aromatic hydrocarbons (PAHs). This approach is more cost-effective and environmentally friendly than conventional methods used to remediate PAH-contaminated sites (Rodarte-Morales et al. 2012; Akhtar and Mannan 2020). The release of antibiotics into the environment can significantly impact the economy, environment, and public health (Kraemer et al. 2019; Gothwal and Shashidhar 2015; Akhtar and Mannan 2020). Several fungi have demonstrated the ability to eliminate different antibiotics, such as bifonazole, clotrimazole, sulfonamides, oxacillin, oxytetracycline, and fluoroquinolone (Gothwal and Shashidhar 2015; Copete-Pertuz et al. 2018; Migliore et al. 2012; Kryczyk-Poprawa et al. 2019; Akhtar and Mannan 2020). Aquatic and white-rot fungi had represented their capability as viable candidates for treating pharmaceutical wastes (Akhtar and Mannan 2020). Anthropogenic activities contribute to eutrophication, leading to cyanobacteria blooms' proliferation (Zanchett and Oliveira-Filho 2013). Aquatic fungi, such as *Mucor hiemalis*, exhibit the ability to accumulate and degrade cyanotoxins, suggesting that mycoremediation could be a potential strategy for the degradation of cyanotoxins and the inhibition of algal bloom formation (Akhtar and Mannan 2020). Mycoremediation also represents an effective strategy for phthalate's efficient and rapid degradation (Akhtar and Mannan 2020). Different fungi, such as *Penicillium verrucosum*, *Cladosporium cladosporioides*, and *Geotrichum candidum*, have demonstrated the ability to degrade different commercially available detergents (Jakovljevic and Vrvic 2017, 2018; Akhtar and Mannan 2020).

Heavy metals, which originate from industrial sources, wastewater, sewage systems, and other human activities, significantly impact human health and environmental equilibrium. However, removing metals from contaminated land is costly and labor-intensive (Acikel 2011; Pan and Wang 2012; Hua et al. 2012; Nandy et al. 2020). Fungi demonstrate higher tolerance to metal toxicity compared to endophytic bacteria, as they can prevent metal-induced cell damage in the plant root system, enhance total biomass and mycelial coverage to facilitate water uptake and metal accumulation, and prevent interplant metal transport more effectively (Kidd et al. 2009; Wenzel 2009). It has been observed that endophytes associated with heavy metal hyperaccumulating plants (Robinson et al. 2003; Rosa et al. 2004) develop metal resistance due to long-term exposure and adaptation to high metal concentrations in their host plants (Idris et al. 2004; Nandy et al. 2020). Phytotoxicity poses a significant challenge in phytoremediation, potentially disrupting

plant-endophyte metabolic signaling. However, endophytes exhibit key characteristics for phytotoxicity reversal, including the production of organic acids, iron chelators, siderophores, and degrading enzymes (Soleimani et al. 2010a; Yanni and Dazzo 2010; Nandy et al. 2020).

The inherent degradation system, coupled with the metabolic symbiosis process between plants and fungal endophytes, triggers metal sequestration or metal-chelating reactions to ensure the host's survival in contaminated soils (Aly et al. 2011; Nandy et al. 2020). The tolerance mechanisms that enable certain fungi to survive under high metal concentrations include metal sequestration or accumulation, precipitation, intracellular compartmentalization of metals within fungal cell walls, mineral weathering, bioabsorption, and volatilization (Fomina et al. 2005; Finlay et al. 2009). Some fungi can alleviate aluminum (Al) toxicity through oxalate exudation (van Scholl et al. 2008).

Examples of metal-resistant endophytic fungi have been reported from the genera *Aspergillus*, *Mucor*, *Phoma*, *Microsphaeropsis*, *Alternaria*, *Peyronellaea*, and *Steganosporium* (Nandy et al. 2020). When collected from other plant or tissue systems, the same fungal isolates have shown different sensitivities to metals, indicating the niche utilization aspect and variability of metal toxicity adaptation (Li et al. 2012).

Significant diversity of endophytes has been observed in many wastelands contaminated with hazardous elements and heavy metals. Fungi such as *Phoma* sp., *Alternaria* sp., and *Peyronellaea* sp. have been isolated from lead (Pb)- and zinc (Zn)-contaminated soil (Nandy et al. 2020). However, plant roots collected from wastelands exhibited a high occurrence of arbuscular mycorrhizal fungi (AMF), predominantly *Glomus* sp. and *Acaulospora* sp. AMF can enhance arsenic (As) uptake in the hyperaccumulating fern *Pteris vittata* L. (Trotta et al. 2006) by overexpressing the As-translocation factor (Nandy et al. 2020). Due to the chemical similarity between arsenate and phosphate, *P. vittata* L. can absorb it via the phosphate uptake system (Wang et al. 2002), even under low As concentrations, as mycorrhiza demonstrates more efficient absorption than the plant root system.

Mucor sp. and endophytic yeasts (*Cryptococcus* sp. CBSB78 and *Rhodotorula* sp. CBSB79) have been isolated from *Brassica chinensis* L. grown in metal-rich soil, which can enhance the bioaccumulation of Pb, Zn, copper (Cu), and cadmium (Cd) (Deng et al. 2011, 2012; Wang et al. 2013; Nandy et al. 2020). *Penicillium* sp. and *Trichoderma* sp. have been widely recognized as metal-accumulating fungi. *Trichoderma atroviride* F6 has been found to alleviate cellular toxicity caused by Cd2+ and nickel (Ni2+) in *B. juncea* (L.) coss. Var. foliosa (Cao et al. 2008; Nandy et al. 2020). *Exophiala pisciphila* has been found to absorb Pb and Cd up to 20% and 5% of its dry weight, respectively (Zhang et al. 2008; Nandy et al. 2020). *Microsphaeropsis* sp. LSE10 from *Solanum nigrum* L. absorbs up to 247.5 mg/g Cd (Xiao et al. 2010). Additionally, certain arbuscular mycorrhizal fungi (AMF) can accumulate radioactive pollutants such as uranium oxides (Fomina et al. 2007; Nandy et al. 2020).

The endophyte *Penicillium janthinellum* LK5 has been reported to alleviate Cd-induced membrane injury and oxidative stress damage by reducing electrolyte

and lipid peroxidation levels and upregulating glutathione and catalase activities (Khan et al. 2014). *Festuca arundinacea* Schreb. and *Festuca pratensis* Huds., belonging to the Poaceae family, are examples of plants that exhibit both higher biomass accumulation and improved total petroleum hydrocarbon degradation when infected with endophytic fungi (Soleimani et al. 2010b; Nandy et al. 2020).

The highly effective endophytic fungus *Pestalotiopsis palmarum* BM-04 has demonstrated remarkable tolerance to extreme salinity and crude oil pollution (Naranjo-Briceno et al. 2013; Nandy et al. 2020). *P. microspora* has been found to degrade plastic polyester polyurethane (PUR) in *Dendrobium* sp., while *Phomopsis liquidambari* efficiently degrades polycyclic aromatic hydrocarbon (PAH) in *Bischofia polycarpa* (H. Lév.) Airy Shaw (Chen et al. 2013; Dai et al. 2010). *Ceratobasidium stevensii* can degrade phenolic acids in *Citrullus lanatus* (Thunb.) Mansf. (Xiao et al. 2014; Nandy et al. 2020).

4 Identification and Isolation of Endophytic Fungi with Mycoremediation Potential

The rhizosphere harbors a diverse community of endophytic microorganisms, which are known to play a crucial role in plant growth and development by modulating the physical and chemical characteristics of the soil, influencing mineral content, soil debris deposition, and water uptake capacity (Caballero-Mellado et al. 2004; Schulz et al. 2015). Presence of contaminants can disrupt the microbe-rhizosphere interface, altering the structure and density of endophytic communities and significantly impacting plant-microbe interactions. Consequently, the presence of xenobiotic- or metal-resistant endophytic fungal strains and subsequent phytoremediation processes positively influence various aspects of plant physiology, such as growth, survival, stress tolerance, salinity and acidity tolerance, and metabolic activities (Rajkumar et al. 2010; Nandy et al. 2020). With approximately 300,000 plant species on Earth, each individual plant can harbor one or more types of endophytes (Petrini 1991; Strobel and Daisy 2003; Stępniewska and Kuźniar 2013). These endophytes have been isolated from various parts of plants, including roots, stems, leaves, and inflorescences, found in weeds, fruit plants, and important vegetables (Bulgari et al. 2012; Bhore et al. 2010; Munif et al. 2012).

The hyphal structure of endophytic fungi provides a larger surface area for the absorption of toxic pollutants and facilitates an explorative growth strategy, making them ideal candidates for phytoremediation (Gadd 2007). Harnessing the phytoextraction, phytoaccumulation, or biotransformation abilities of both plants and their endophytic fungi is essential for sustainable detoxification of contaminated soil and water (Stepniewska and Kuzniar 2013; Nandy et al. 2020). Mycorrhizal fungi, responsible for nutrient absorption and translocation, play a vital role in phytoremediation by assisting in the accumulation of potentially harmful elements, maintaining soil pH and water pressure, and facilitating interplant nutrient transfer (Fomina

et al. 2005). Plant-microbe interactions mediate environmentally triggered stress conditions and employ various modes of bioremediation (Zhao et al. 2013).

AMF, primarily zygomycetous fungi, are commonly associated with herbaceous plants, while ectomycorrhizal fungi (EMF), predominantly Basidiomycetes, are found in association with woody plants (Mandyam and Jumpponen 2005). Dark septate endophytic fungi (DSEF), on the other hand, are commonly found in plants belonging to the Brassicaceae family (Usuki and Narisawa 2007). The microclimatic conditions in the rhizosphere strongly influence the host specificity, as well as the organ and tissue specificity, of endophytic fungi. They also govern stress tolerance, organic and inorganic element cycling, mycogenic mineral formation, and metal augmentation, which may be triggered by specific fungal metabolite production (Gadd 2007; Aly et al. 2011; Suryanarayanan et al. 2012; Suryanarayanan 2013; Nandy et al. 2020).

The fungal endophyte *Penicillium funiculosum* exhibits anti-copper stress activity and promotes plant growth, making it a potential candidate for bioremediation in cultivated areas using stress-mediating endophytes (Khan and Lee 2013; Sudha et al. 2016). In the case of *Festuca arundinacea* (Schreb.) and *F. pratensis* (Huds.) plants infected with endophytic fungi *Neotyphodium coenophialum* and *N. uncinatum*, it was observed that the infected plants exhibited greater shoot and root biomass compared to uninfected plants when grown in petroleum-contaminated soil (Soleimani et al. 2010a; Sudha et al. 2016). *Neotyphodium* endophytes have been shown to enhance cadmium tolerance in *Festuca arundinacea* and *F. pratensis* plants compared to noninfected plants (Soleimani et al. 2010b; Sudha et al. 2016). This highlights the significant role of endophytes in phytoremediation processes.

Several enzyme classes, including cytochrome P450 monooxygenases, glutathione, glycosyl transferases, reductases, dehalogenases, and glutathione-S-transferases, play vital roles in the phytoremediation process. Fungi such as *Trametes versicolor* and *Coriolopsis polyzona* secrete laccase, which is capable of degrading aromatic compounds, while peroxidase produced by *Phanerochaete chrysosporium* and *Phanerochaete laevis* is known for aliphatic hydrocarbon dehalogenation. *Aspergillus niger*-derived nitrilase, on the other hand, cleaves cyanide groups from aromatic and aliphatic nitriles. Although these fungi are not endophytic, enzymes isolated from fungal sources, including these examples, have contributed to various forms of pollutant sequestration.

5 Advantages of Using Endophytic Fungi for Mycoremediation

Reports suggest that endophytic fungi promote rhizodeposition in plants and influence microbial mineralization in the soil without altering the microbial community's diversity (Van Hecke et al. 2005; Nandy et al. 2020). The remedial effectiveness of endophytic fungi has paved the way for a wide range of interdisciplinary research,

focusing on the simultaneous interactions among three key elements: plants, microbes, and contaminants. The selection of the appropriate microbe and its underlying rationale constitutes the first crucial factor. While endophytic bacteria have predominantly been investigated in the context of endophyte-mediated phytoremediation, the distinctive characteristics of endophytic fungi also suggest their potential as bioremediation agents. Comparative monitoring of metal resistance, organic pollutant degradation capabilities, and phytotoxicity reversal is essential. The microenvironment of the symbiotic interaction and its impact on bioaccumulation, transformation, or extraction processes must be thoroughly understood, as not all endophytic microbes can enhance phytoremediation efficiency in plants (Nandy et al. 2020). Selection and genetic engineering of potent endophytic microbes, establishment of transgenic plants, niche division, host specificity, fungal metabolite-mediated reactions, increased pollutant uptake and tolerance, enhanced root and shoot biomass, intact root architecture, maintenance of pH, salinity, and pollutant-induced toxicity in the rhizosphere, management of the microbial population, and balanced symbiotic interaction are critical aspects of successful and environmentally friendly phytoremediation using endophytes (Kidd et al. 2009; Nandy et al. 2020).

6 Factors Influencing the Activity of Endophytic Fungi in Mycoremediation

Microbe-mediated processes such as ion and ligand exchange, secretion of metabolite-rich exudates, release of microbial siderophores, pH-regulated mobilization and immobilization of chemicals, high tolerance levels, and biotransformation capacity facilitated by endogenous enzymatic systems strongly influence total metal uptake, enhance absorption, transport, and accumulation of pollutants. Sequestration processes such as phytostabilization (immobilization within the rhizosphere) or bioaccumulation (translocation, transformation, and storage in aboveground tissue) are common for heavy metals, while detoxification or phyto-decontamination processes are preferred for organic substances. Detoxification encompasses mineralization, transformation, enzymatic degradation, conjugation, and compartmentalization.

Using endophyte-plant interactions to counter xenobiotic toxicity offers several advantages but presents challenges. Positive traits of endophytic microbes include the absence of tissue-specific interspecies competition, long-term sustenance, utilization of host metabolite reservoirs, and ease of genetic modification (Mercado-Blanco 2015; Nandy et al. 2020).

The final stage of the plant-microbe interaction in phytoremediation involves the removal of contaminants from the environment, which occurs after multiple biochemical and physiological reactions (Redfern and Gunsch 2016; Nandy et al. 2020). The exploration of the complex network of microbial communities dispersed throughout various parts of the plant, and their pluralistic interactions

(plant-microbiome-soil-pollutant) can only be determined through the application of next-generation sequencing and high-end technologies, such as real-time high-throughput whole plant functional phenotyping (Deng and Cao 2017).

In conjunction with phytoremediation, in situ bioremediation represents an alternative, more environmentally sustainable treatment approach than conventional remediation technologies (van Cauwenberghe and Roote 1998). Bioaugmentation, a commonly employed bioremediation strategy, involves the introduction of exogenous microorganisms, such as endophytes, to facilitate the remediation of contaminated soils and sediments. The combined utilization of endophytic augmentation and phytoremediation, referred to as endophytic phytoaugmentation, presents a promising and effective approach for in situ treatment of runoff and waste systems (Redfern and Gunsch 2016). Endophytic phytoaugmentation utilizing indigenous xenobiotic-degrading endophytes offers the inherent advantage of decreased competition within the internal plant tissue, eliminating the necessity for repeated inoculation (Redfern and Gunsch 2016).

7 Conclusion

The application of endophyte-assisted phytoremediation technique represents a futuristic and in situ approach to remediate biohazardous contaminants, metals, metalloid pollutants, carcinogenic agents, industrial organic waste materials, inorganic pesticides and herbicides, hydrocarbon-based elements, and chlorinated products from the environment. This technique operates as a sustainable and multifactorial pollution control strategy, exhibiting low environmental impact and operational expenses. This chapter aims to provide a concise overview of the underlying mechanisms involved in endophytic fungi-mediated phytoremediation techniques. Furthermore, the chapter extensively discusses various strategies, their limitations, and the application of transgenic technology in this emerging field of research.

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References

Acıkel YS (2011) Use of biosurfactants in the removal of heavy metal ions from soils. In: Khan MS, Zaidi A, Goel R, Musarrat J (eds) Biomanagement of metal-contaminated soils. Springer, Dordrecht, pp 183–223

Adeleke BS, Babalola OO (2022) Meta-omics of endophytic microbes in agricultural biotechnology. Biocatal Agric Biotechnol 42:102332. https://doi.org/10.1016/j.bcab.2022.102332

Adeleke BS, Ayilara MS, Akinola SA et al (2022) Biocontrol mechanisms of endophytic fungi. Egypt J Biol Pest Control 32:46. https://doi.org/10.1186/s41938-022-00547-1

Ahn K (2014) The role of air pollutants in atopic dermatitis. J Allergy Clin Immunol 134:993–999
Akhtar N, Mannan MA (2020) Mycoremediation: expunging environmental pollutants. Biotechnol Rep (Amst) 26:e00452. https://doi.org/10.1016/j.btre.2020.e00452
Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S, Yuán Y, Gǒng W (2021) Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa? Front Plant Sci 12:791033. https://doi.org/10.3389/fpls.2021.791033
Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90:1829–1845
Arnemo JM, Andersen O, Stokke S, Thomas VG, Krone O, Pain DJ, Mateo R (2016) Health and environmental risks from lead-based ammunition: science versus socio-politics. EcoHealth 13:618–622
Bacon CW, White J (2000) Microbial endophytes. CRC, New York
Bamisile BS, Dash CK, Akutse KS, Keppanan R, Wang L (2018) Fungal endophytes: beyond herbivore management. Front Microbiol 9:544
Baron NC, Rigobelo EC (2021) Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. Mycology 13:39–55. https://doi.org/10.1080/21501203.2021.1945699
Bastami A, Amirnia R, Sayyed RZ, Enshasy HAE (2021) The effect of mycorrhizal fungi and organic fertilizers on quantitative and qualitative traits of two important Satureja species. Agronomy 11:1285. https://doi.org/10.3390/agronomy11071285
Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84(1):11–18. https://doi.org/10.1007/s00253-009-2092-7
Bhore SJ, Nithya R, Loh CY (2010) Screening of endophytic bacteria isolated from leaves of Sambung Nyawa [*Gynura procumbens* (Lour.) Merr.] for cytokinin-like compounds. Bioinformation 5:191–197
Bulgari D, Bozkurt AI, Casati P (2012) Endophytic bacterial community living in roots of healthy and 'Candidatus Phytoplasma Mali'-infected apple (*Malus domestica*, Borkh.) trees. A Van Leeuw 102:677–687
Busby PE, Ridout M, Newcombe G (2016) Fungal endophytes: modifiers of plant disease. Plant Mol Biol 90:645–655
Caballero-Mellado J, Martínez-Aguilar L, Paredes-Valdez G, Estrada-De Los Santos P (2004) *Burkholderiaunamae* sp. nov, an N2-fixing rhizospheric, endophytic species. Int J Syst Evol Microbiol 54:1165–1172
Cao L, Jiang M, Zeng Z, Du A, Tan H, Liu Y (2008) *Trichoderma atroviride* F6 improves phytoextraction efficiency of mustard (*Brassica juncea* (L.) coss. var. foliosa Bailey) in Cd, Ni contaminated soils. Chemosphere 71:1769–1773
Card S, Johnson L, Teasdale S, Caradus J (2016) Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. FEMS Microbiol Ecol 92(8):19. https://doi.org/10.1093/femsec/fiw114
Carroll G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69:2–9
Chadha N, Mishra M, Rajpal K, Bajaj R, Choudhary DK, Varma A (2015) An ecological role of fungal endophytes to ameliorate plants under biotic stress. Arch Microbiol 197(7):869–881. https://doi.org/10.1007/s00203-015-1130-3
Chen Y, Wang HW, Li L, Dai CC (2013) The potential application of the endophyte Phomopsis liquidambari to the ecological remediation of long-term cropping soil. Appl Soil Ecol 67:20–26
Chi WC, Chen W, He CC, Guo SY, Cha HJ, Tsang LM, Ho TW, Pang KL (2019) A highly diverse fungal community associated with leaves of the mangrove plant acanthus ilicifolius var xiamenensis revealed by isolation and metabarcoding analyses. PeerJ. https://doi.org/10.7717/peerj.7293
Chowdappa S, Jagannath S, Konappa N, Udayashankar AC, Jogaiah S (2020) Detection and characterization of antibacterial siderophores secreted by endophytic fungi from cymbidium aloifolium. Biomol Ther 10:18. https://doi.org/10.3390/biom10101412

Copete-Pertuz LS, Plácido J, Serna-Galvis EA, Torres-Palma RA, Mora A (2018) Elimination of Isoxazolyl–Penicillins antibiotics in waters by the ligninolytic native Colombian strain Leptosphaerulina sp. considerations on biodegradation process and antimicrobial activity removal. Sci Total Environ 630:1195–1204. https://doi.org/10.1016/j.scitotenv.2018.02.244

Dai CC, Tian LS, Zhao YT, Chen Y, Xie H (2010) Degradation of phenanthrene by the endophytic fungus Ceratobasidum stevensii found in Bischofia polycarpa. Biodegradation 21:245–255

Deng Z, Cao L (2017) Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere 168:1100–1106

Deng Z, Cao L, Huang H, Jiang X, Wang W, Shi Y, Zhang R (2011) Characterization of Cd-and Pb-resistant fungal endophyte *Mucor* sp. CBRF59 isolated from rapes (*Brassica chinensis*) in a metal-contaminated soil. J Hazard Mater 185:717–724

Deng Z, Wang W, Tan H, Cao L (2012) Characterization of heavy metal-resistant endophytic yeast Cryptococcus sp. CBSB78 from rapes (*Brassica chinensis*) and its potential in promoting the growth of Brassica spp. in metal-contaminated soils. Water Air Soil Pollut 223:5321–5329

dos Reis JBA, Lorenzi AS, do Vale HMM (2022) Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. Arch Microbiol 204:675. https://doi.org/10.1007/s00203-022-03283-0

Dubey A, Malla MA, Kumar A, Dayanandan S, Khan ML (2020) Plants endophytes: unveiling hidden agenda for bioprospecting toward sustainable agriculture. Crit Rev Biotechnol 40:1210–1231. https://doi.org/10.1080/07388551.2020.1808584

El Enshasy H, Ambehabati K, El Baz A, Ramchuran SO, Sayyed R et al (2020) Trichoderma: biocontrol agents for promoting plant growth and soil health. In: Yadav A, Mishra S, Kour D, Yadav N, Kumar A (eds) Agriculturally important fungi for sustainable agriculture. Functional annotation for crop protection, vol 2. Springer, Cham. https://doi.org/10.1007/978-3-030-48474-3_8

Everett CJ, Mainous AG III, Frithsen IL, Player MS, Matheson EM (2008) Association of polychlorinated biphenyls with hypertension in the 1999–2002 national health and nutrition examination survey. Environ Res 108:94–97

Finlay R, Wallander H, Smits M, Holmstrom S, Van Hees P, Lian B, Rosling A (2009) The role of fungi in biogenic weathering in boreal forest soils. Fungal Biol Rev 23:101–106

Fomina MA, Alexander IJ, Colpaert JV, Gadd GM (2005) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biol Biochem 37:851–866

Fomina M, Charnock JM, Hillier S, Alvarez R, Gadd GM (2007) Fungal transformations of uranium oxides. Environ Microbiol 9:1696–1710

Gadd GM (2007) Geomycology:biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 11:3–49

Gai CS, Lacava PT, Quecine MC, Auriac MC, Lopes JRS, Araújo WL, Miller TA, Azevedo JL (2009) Transmission of Methylobacterium mesophilicum by Bucephalogonia xanthophis for paratransgenic control strategy of citrus variegated chlorosis. J Microbiol 47:448–454. https://doi.org/10.1007/s12275-008-0303-z

Gavrilaş S, Ursachi CŞ, Perţa-Crişan S, Munteanu F-D (2022) Recent trends in biosensors for environmental quality monitoring. Sensors 22:1513. https://doi.org/10.3390/s22041513

Gothwal R, Shashidhar T (2015) Antibiotic pollution in the environment:a review. Clean Soil Air Water 43:479–489. https://doi.org/10.1002/clen.201300989

Grabka R, d'Entremont TW, Adams SJ, Walker AK, Tanney JB, Abbasi PA et al (2022) Fungal endophytes and their role in agricultural plant protection against pests and pathogens. Plan Theory 11:384. https://doi.org/10.3390/plants11030384

Guo B, Wang Y, Sun X, Tang K (2008) Bioactive natural products from endophytes: a review. Appl Biochem Microbiol 44:136–142

Harman GE, Uphoff N (2019) Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. Scientifica 25:1–25. https://doi.org/10.1155/2019/9106395

Haro R, Benito B (2019) The role of soil fungi in K+ plant nutrition. Int J Mol Sci 20:3169. https://doi.org/10.3390/ijms20133169

Hawksworth DL, Lücking R (2018) Fungal diversity revisited:2.2 to 3.8 million species. In: Heitman J, Howlett BJ, Crous PW, Stukenbrock EH, James TY, Gow NAR (eds) The fungal kingdom. ASM Press, Washington, pp DC79–DC95

Hua M, Zhang S, Pan B, Zhang W, Lv L, Zhang Q (2012) Heavy metal removal from water/wastewater by nanosized metal oxides: a review. J Hazard Mater 211:317–331

Ibiang SR, Sakamoto K, Kuwahara N (2020) Performance of tomato and lettuce to arbuscular mycorrhizal fungi and *penicillium pinophilum* EU0013 inoculation varies with soil, culture media of inoculum, and fungal consortium composition. Rhizosphere 16:10

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyper accumulator Thlaspi goesingense. Appl Environ Microbiol 70:2667–2677

Ivanova EG, Fedorov DN, Doronina NV, Trotsenko YA (2006) Production of vitamin B12 in aerobic methylotrophic bacteria. Microbiology 75:494–496. https://doi.org/10.1134/S0026261706040217

Jacquet F, Jeuffroy MH, Jouan J et al (2022) Pesticide-free agriculture as a new paradigm for research. Agron Sustain Dev 42:8. https://doi.org/10.1007/s13593-021-00742-8

Jakovljevic VD, Vrvic MM (2017) *Penicillium verrucosum* as promising candidate for bioremediation of environment contaminated with synthetic detergent at high concentration. Appl Biochem Microbiol 53:368–373. https://doi.org/10.1134/S0003683817030164

Jakovljevic VD, Vrvic MM (2018) Potential of pure and mixed cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* for application in bioremediation and detergent industry. Saudi J Biol Sci 25:529–536. https://doi.org/10.1016/j.sjbs.2016.01.020

Jaroszuk-Ściseł J, Kurek E, Trytek M (2014) Efficiency of indoleacetic acid, gibberellic acid and ethylene synthesized in vitro by *fusarium culmorum* strains with different effects on cereal growth. Biologia 69(3):281–292. https://doi.org/10.2478/s11756-013-0328-6

Kaddes A, Fauconnier ML, Sassi K, Nasraoui B, Jijakli MH (2019) Endophytic fungal volatile compounds as solution for sustainable agriculture. Molecules 24(6):16

Khan AL, Lee IJ (2013) Endophytic *Penicillium funiculosum* LHL06 secretes gibberellin that reprograms *Glycine max* L. growth during copper stress. BMC Plant Biol 13:86

Khan AL, Waqas M, Hussain J, Al-Harrasi A, Lee IJ (2014) Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe). Biol Fertil Soils 50:75–85

Khan AR, Ullah I, Waqas M, Shahzad R, Hong SJ, Park GS, Jung BK, Lee IJ, Shin JH (2015) Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. World J Microbiol Biotechnol 31:1461–1466

Khanam Z, Gupta S, Verma A (2020) Endophytic fungi-based biosensors for environmental contaminants- a perspective. S Afr J Bot 132:401–406. https://doi.org/10.1016/j.sajb.2020.08.007

Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root–soil interface:implications in phytoremediation. Environ Exp Bot 67:243–259

Kim T-U, Cho S-H, Han J-H, Shin Y-M, Lee HB, Kim SB (2012) Diversity and physiological properties of root endophytic Actinobacteria in native herbaceous plants of Korea. J Microbiol 50:50–57. https://doi.org/10.1007/s12275-012-1417-x

Kraemer SA, Ramachandran A, Perron GG (2019) Antibiotic pollution in the environment: from microbial ecology to public policy. Microorganisms 7:180

Kryczyk-Poprawa A, Zmudzki P, Maslanka A, Piotrowska J, Opoka W, Muszynska B (2019) Mycoremediation of azole antifungal agents using in vitro cultures of Lentinula edodes. 3 Biotech 9:1. https://doi.org/10.1007/s13205-019-1733-5

Kumaresan V, Suryanarayanan TS (2002) Endophytic assemblages in young, mature and senescent leaves of Rhizophora apiculata:evidence for the role of endophytes in mangrove litter degradation. Fungal Divers 9:81–91

Landrigan PJ, Schechter CB, Lipton JM, Fahs MC, Schwartz J (2002) Environmental pollutants and disease in American children:estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities. Environ Health Perspect 110:721–728

Latz MA, Jensen B, Collinge DB, Jørgensen HJ (2018) Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. Plant Ecol Divers 11:555–567. https://doi.org/10.1080/17550874.2018.1534146

Li H-Y, Wei D-Q, Shen M, Zhou Z-P (2012) Endophytes and their role in phytoremediation. Fungal Divers 54:11–18. https://doi.org/10.1007/s13225-012-0165-x

Li JL, Sun X, Chen L, Guo LD (2016a) Community structure of endophytic fungi of four mangrove species in southern China. Mycology 7(4):180–190

Li P, Wu Z, Liu T, Wang Y (2016b) Biodiversity, phylogeny, and antifungal functions of endophytic fungi associated with zanthoxylum bungeanum. Int J Mol Sci 17(9):1541

Liu-Xu L, Vicedo B, García-Agustín P, Llorens E (2022) Advances in endophytic fungi research:a data analysis of 25 years of achievements and challenges. J Plant Interact 17:244–266. https://doi.org/10.1080/17429145.2022.2032429

Lugtenberg BJJ, Caradus JR, Johnson LJ (2016) Fungal endophytes for sustainable crop production. FEMS Microbiol Ecol 92(12):17. https://doi.org/10.1093/femsec/fiw194

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud Mycol 53:173–189

Matta G, Gjyli L (2016) Mercury, lead and arsenic: impact on environment and human health. J Chem Pharm Sci 9:718–725

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162

Meena RS, Kumar S, Datta R, Lal R, Vijayakumar V et al (2020) Impact of agrochemicals on soil microbiota and management: A review. Land 9:34. https://doi.org/10.3390/land9020034

Mehta P, Sharma R, Putatunda C, Walia A (2019) Endophytic fungi:role in phosphate solubilization. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 183–209

Mercado-Blanco J (2015) Life of microbes inside the plant. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer International Publishing, Switzerland, Heidelberg, pp 25–32

Mercado-Blanco J, Bakker PAHM (2007) Interaction between plants and beneficial Pseudomonas spp.:exploiting bacterial traits for crop protection. Antonie Leeuwenhoek 92:367–389. https://doi.org/10.1007/s10482-007-9167-1

Migliore L, Fiori M, Spadoni A, Galli E (2012) Biodegradation of oxytetracycline by Pleurotus ostreatus mycelium:a mycoremediation technique. J Hazard Mater 215–216:227–232. https://doi.org/10.1016/j.jhazmat.2012.02.056

Mishra R, Sarma V (2017) Mycoremediation of heavy metal and hydrocarbon pollutants by endophytic fungi. In: Prasad R (ed) Mycoremediation and environmental sustainability. Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-319-68957-9_8

Munif A, Hallmann J, Sikora RA (2012) Isolation of endophytic bacteria from tomato and their biocontrol activities against fungal disease. Microbiol Indones 6:148–156. https://doi.org/10.5454/mi.6.4.2

Nandy S, Das T, Tudu CK, Pandey DK, Dey A, Ray P (2020) Fungal endophytes: futuristic tool in recent research area of phytoremediation. S Afr J Bot 134:285–295

Naranjo-Briceño L, Pernía B, Guerra M, Demey JR, De Sisto Á, Inojosa Y, González M, Fusella E, Freites M, Yegres F (2013) Potential role of oxidative exoenzymes of the extremophilic fungus Pestalotiopsis palmarum BM-04 in biotransformation of extra-heavy crude oil. Microb Biotechnol 6:720–730

Naranjo-Ortiz MA, Gabaldón T (2019) Fungal evolution:major ecological adaptations and evolutionary transitions. Biol Rev Cambridge Philos Soc 94(4):1443–1476

Oses R, Valenzuela S, Freer J, Sanfuentes E, Rodriguez J (2008) Fungal endophytes in xylem of healthy chilean trees and their possible role in early wood decay. Fungal Divers 33:77–86

Pan K, Wang WX (2012) Trace metal contamination in estuarine and coastal environments in China. Sci Total Environ 421:3–16

Petrini O (1991) Fungal endophytes of tree leaves. In: Fokkema NJ, van den Heuvel I (eds) Microbial ecology of the leaves. Cambridge University Press, Cambridge, pp 185–187

Purnomo AS, Nawfa R, Martak F, Shimizu K, Kamei I (2017) Biodegradation of Aldrin and Dieldrin by the white-rot fungus Pleurotus ostreatus. Curr Microbiol 74:320–324. https://doi.org/10.1007/s00284-016-1184-8

Radic N, Strukelj B (2012) Endophytic fungi:the treasure chest of antibacterial substances. Phytomedicine 19:1270–1284. https://doi.org/10.1016/j.phymed.2012.09.007

Radziemska M, Gusiatin A, Cydzik-Kwiatkowska A, Cerda V, Pecina A (2021) Insight into metal immobilization and microbial community structure in soil from a steel disposal dump phytostabilized with composted, pyrolyzed or gasified wastes. Chemosphere 272:129576. https://doi.org/10.1016/j.chemosphere.2021.129576

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Rana KL, Kour D, Kaur T, Devi R, Yadav AN, Yadav N, Dhaliwal HS, Saxena AK (2020) Endophytic microbes:biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Anton Leeuw Int J Gen Mol Microbiol 113(8):1075–1107. https://doi.org/10.1007/s10482-020-01429-y

Redfern LK, Gunsch CK (2016) Endophytic phytoaugmentation: treating wastewater and runoff through augmented phytoremediation. Ind Biotechnol (New Rochelle NY) 12(2):83–90. https://doi.org/10.1089/ind.2015.0016

Renu S, Sarim KM, Singh DP, Sahu U, Bhoyar MS, Sahu A, Kaur B, Gupta A, Mandal A, Thakur JK, Manna MC, Saxena AK (2022) Deciphering cadmium (Cd) tolerance in newly isolated bacterial strain, Ochrobactrum intermedium BB12, and its role in alleviation of Cd stress in spinach plant (*Spinacia oleracea* L.). Front Microbiol 12:758144

Robinson BH, Lombi E, Zhao FJ, McGrath SP (2003) Uptake and distribution of nickel and other metals in the hyperaccumulator Berkheya coddii. New Phytol 158:279–285

Rodarte-Morales AI, Feijoo G, Moreira MT, Lema JM (2012) Biotransformation of three pharmaceutical active compounds by the fungus *Phanerochaete chrysosporium* in a fed batch stirred reactor under air and oxygen supply. Biodegradation 23:145–156

Rodriguez R, White J Jr, Arnold A, Redman AR (2009) Fungal endophytes:diversity and functional roles. New Phytol 182:314–330

Rosa G, Peralta-Videa JR, Montes M, Parsons JG, Cano-Aguilera I, Gardea-Torresdey JL (2004) Cadmium uptake and translocation in tumbleweed (Salsola kali), a potential Cd-hyperaccumulator desert plant species:ICP/OES and XAS studies. Chemosphere 55:1159–1168

Sahoo S, Sarangi S, Kerry RG (2017) Bioprospecting of endophytes for agricultural and environmental sustainability (Chapter-19). In: Patra JK (ed) Microbial biotechnology, pp 429–458. https://doi.org/10.1007/978-981-10-6847-8_19

Schulz B, Haas S, Junker C, Andrée N, Schobert M (2015) Fungal endophytes are involved in multiple balanced antagonisms. Current Science, pp 39–45

Segaran G, Sathiavelu M (2019) Fungal endophytes:a potent biocontrol agent and a bioactive metabolites reservoir. Biocatal Agric Biotechnol 21:101284

Sherwood M, Carroll G (1974) Fungal succession on needles and young twigs of old-growth Douglas fir. Mycologia 66:499–506

Shukla R, Sarim KM, Singh DP (2020) Microbe-mediated management of arsenic contamination:current status and future prospects. Environmental Sustainability 3:83–90. https://doi.org/10.1007/s42398-019-00090-0

Simons M, Permentier HP, de Weger LA, Wijffelman CA, Lugtenberg BJJ (1997) Amino acid synthesis is necessary for tomato root colonization by Pseudomonas fluorescens strain WCS365. Mol Plant Microb Interact 10:102–106. https://doi.org/10.1094/MPMI.1997.10.1.102

Singh DP, Prabha R (Eds.) (2019) Microbial interventions in agriculture and environment. Vol. 3: Soil and crop health management. Springer, 491 p, https://doi.org/10.1007/978-981-32-9084-6
Singh DP, Singh HB, Prabha R (Eds.) (2016) Microbial inoculants in sustainable agricultural productivity. Vol. 2: Functional applications. 1st ed. Springer, X, 440 p. ISBN:978-81-322-2644-4
Singh S, Kumar V, Singla S, Sharma M, Singh DP, Prasad R, Thakur VK, Singh J (2020) Kinetic study of the biodegradation of acephate by indigenous soil bacterial isolates in the presence of humic acid and metal ions. Biomol Ther 10(3):433. https://doi.org/10.3390/biom10030433
Singh N, Singh A, Dahiya P (2021) Plant growth-promoting endophytic fungi from different habitats and their potential applications in agriculture. In: Yadav AN (ed) Recent trends in mycological research. Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-030-60659-6_3
Soleimani M, Afyuni M, Hajabbasi MA, Nourbakhsh F, Sabzalian MR, Christensen JH (2010a) Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non-infected grasses. Chemosphere 81:1084–1090
Soleimani M, Hajabbasi MA, Afyuni M, Mirlohi A, Borggaard OK, Holm PE (2010b) Effect ofendophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. Int J Phytoremediation 12:535–549
Srinivas C, Shubha S, Narasimhamurthy K, Arakere U, Sudisha J (2020) Detection and characterization of antibacterial siderophores secreted by endophytic fungi from cymbidium aloifolium. Biomol Ther 10:1412. https://doi.org/10.3390/biom10101412
Stępniewska Z, Kuźniar A (2013) Endophytic microorganisms—promising applications in bioremediation of greenhouse gases. Appl Microbiol Biotechnol 97:9589–9596
Stone JK, Polishook JD, White JRJ (2004) Endophytic fungi. In: Mueller G, Bills GF, Foster MS (eds) Biodiversity of fungi:inventory and monitoring methods. Elsevier, Burlington, pp 241–270
Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502
Sudha V, Govindaraj R, Baskar K, Abdullah Al-Dhabi N, Duraipandiyan V (2016) Biological properties of endophytic fungi. Braz Arch Biol Technol 59:e16150436
Suryanarayanan TS (2013) Endophyte research:going beyond isolation and metabolite documentation. Fungal Divers 6:561–568
Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Gopalan V (2012) Fungal endophytes: an untapped source of biocatalysts. Fungal Ecol 54:19–30
Trotsenko YA, Khmelenina VN (2002) Biology of extremophilic and extremotolerant methanotrophs. Arch Microbiol 177:123–131. https://doi.org/10.1007/s00203-001-0368-0
Trotta A, Falaschi P, Cornara L, Minganti V, Fusconi A, Drava G, Berta G (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern Pteris vittata L. Chemosphere 65:74–81
Turbat A, Rakk D, Vigneshwari A, Kocsube S, Thu H, Szepesi A, Bakacsy L, Skrbic BD, Jigjiddorj EA, Vagvolgyi C (2020) Characterization of the plant growth-promoting activities of endophytic fungi isolated from sophora flavescens. Microorganisms 8(5):15
Usuki F, Narisawa K (2007) A mutualistic symbiosis between a dark septate endophytic fungus, Heteroconium chaetospira, and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99:175–184
Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV et al (2018) Endophytic fungi-alternative sources of cytotoxic compounds: a review. Front Pharmacol 9:309. https://doi.org/10.3389/fphar.2018.00309
van Cauwenberghe L, Roote DS (1998) In situ bioremediation. Available at: https://clu-in.org/download/toolkit/insbio_o.pdf
Van Hecke MM, Treonis AM, Kaufman JR (2005) How does the fungal *endophyte Neotyphodium coenophialum* affect tall fescue (*Festuca arundinacea*) rhizodeposition and soil microorganisms? Plant Soil 275:101–109
Van Schöll L, Kuyper TW, Smits MM, Landeweert R, Hoffland E, Van Breemen N (2008) Rock-eating mycorrhizas:their role in plant nutrition and biogeochemical cycles. Plant Soil 303:35–47

Verma A, Shameem N, Jatav HS, Sathyanarayana E, Parray JA, Poczai P, Sayyed RZ (2022) Fungal endophytes to combat biotic and abiotic stresses for climate-smart and sustainable agriculture. Front Plant Sci 13:953836. https://doi.org/10.3389/fpls.2022.953836

Wang J, Zhao FJ, Meharg AA, Raab A, Feldmann J, McGrath SP (2002) Mechanisms of arsenic hyperaccumulation in Pteris vittata. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol 130:1552–1561

Wang W, Deng Z, Tan H, Cao L (2013) Effects of Cd, Pb, Zn, Cu-resistant endophytic Enterobacter sp. CBSB1 and Rhodotorula sp. CBSB79 on the growth and phytoextraction of Brassica plants in multimetal contaminated soils. Int J Phytoremediation 15:488–497

Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, Hu Y, (2022) Endophytic Fungi: An effective alternative source of plant-derived bioactive compounds for pharmacological studies. J Fungi 8:205. https://doi.org/10.3390/jof8020205

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

Willis KJ (ed) (2018) State of the world fungi report. Royal Botanic Gardens, Kew

Xiao X, Luo S, Zeng G, Wei W, Wan Y, Chen L, Guo H, Cao Z, Yang L, Chen J, Xi Q (2010) Biosorption of cadmium by endophytic fungus (EF) Microsphaeropsis sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. Bioresour Technol 6:1668–1674

Xiao X, Dai CC, Wang X, Liu FY, Wang HW, Li XG (2014) Effect of the endophyte Ceratobasidium stevensii on 4-HBA degradation and watermelon seed germination. Afr J Microbiol Res 8:1535–1543

Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao D (2019) Beneficial effects of endophytic fungi colonization on plants. Appl Microbiol Biotechnol 103:3327–3340. https://doi.org/10.1007/s00253-019-09713-2

Yanni YG, Dazzo FB (2010) Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. trifolii in extensive field inoculation trials within the Egypt Nile delta. Plant Soil 336:129–142

Yung L, Sirguey C, Azou-Barré A, Blaudez D (2021) Natural fungal endophytes from Noccaea caerulescens mediate neutral to positive effects on plant biomass, mineral nutrition and Zn Phytoextraction. Front Microbiol 12:689367. https://doi.org/10.3389/fmicb.2021.689367

Zanchett G, Oliveira-Filho EC (2013) Cyanobacteria and cyanotoxins:from impacts on aquatic ecosystems and human health to anticarcinogenic effects. Toxins (Basel) 5:1896–1917. https://doi.org/10.3390/toxins5101896

Zhang Y, Zhang Y, Liu M, Shi X, Zhao Z (2008) Dark septate endophyte (DSE) fungi isolated from metal polluted soils:their taxonomic position, tolerance, and accumulation of heavy metals in vitro. J Microbiol 46:624–632

Zhao J, Li C, Wang W, Zhao C, Luo M, Mu F et al (2013) *Hypocrea lixii*, novel endophytic fungi producing anticancer agent cajanol, isolated from pigeon pea (*Cajanus cajan* [L.] M illsp.). J Appl Microbiol 115:102–113

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