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Bibhuti Bhusan Mishra *Editors*

Advances in Agricultural and Industrial Microbiology

Volume-2: Applications of Microbes for Sustainable Agriculture and in-silico Strategies

Springer

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ISBN 978-981-16-9681-7 ISBN 978-981-16-9682-4 (eBook)
https://doi.org/10.1007/978-981-16-9682-4

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

About the Editors

Suraja Kumar Nayak obtained Ph.D. from Odisha University of Agriculture and Technology in 2013 and is presently working as Assistant Professor, Department of Biotechnology, Odisha University of Technology and Research, Bhubaneswar, Odisha, India. Dr. Nayak has 9 years of teaching and research experience in the field of microbiology and biotechnology. His areas of teaching and research include general and environmental microbiology, soil and agricultural microbiology, industrial and food biotechnology, and microbial biotechnology. Dr. Nayak has 20 publications including book chapters in various journals and publishers of national and international repute (h-index: 7 and Citation: 300 in Google Scholar). He has also submitted six accession numbers to NCBI, USA, and has presented papers in various national and international conferences. He has edited three books in microbiology and biotechnology published by national and international publishers. Five M.Tech and more than 15 B.Tech students have successfully completed their dissertation under his guidance, and presently two more are actively working. Dr. Nayak has 20 publications including book chapters in various journals and publications of national and international repute.

Bighneswar Baliyarsingh is an academician, educator, and researcher, working as Assistant Professor, Department of Biotechnology, Odisha University of Technology and Research, Bhubaneswar, Odisha, India. He obtained one of his postgraduate degrees, M.Sc. in Biotechnology, from Ravenshaw University, Cuttack, India, followed by M.Tech in Biotechnology from West Bengal University of Technology, Kolkata, India, sponsored by the Dept. of Biotechnology (DBT), Govt. of India, in the year 2007. He has gained research and industrial work experience by serving in different institutes like ICAR-NRRI, Cuttack; Bose Institute, Kolkata; and AIIMS, New Delhi, with brief industrial exposure through BCIL-BITP, sponsored by DBT, Govt. of India. He qualified for CSIR-UGC National Eligible Test (NET) in the year 2007. He has successfully supervised a good number of M.Tech and B.Tech dissertation works, continuously. He has published more than 15 articles including research papers, reviews, and book chapters in various journals and publishers of national and international repute (h-index: 4 in Google Scholar).

Ashutosh Singh is presently working as Associate Professor in the School of Engineering at the University of Guelph, Canada. After completing B.Tech in Biotechnology from Vellore Institute of Technology, India, in 2007, he obtained M.Sc. and Ph.D. degrees in Bioresource Engineering from McGill University, Canada, in 2010 and 2014, respectively. Before joining the University of Guelph, he worked as a Postdoctoral Fellow at Dalhousie University, Canada, from 2014 to 2015 and later joined McGill University as a Research Associate. Dr. Singh's research work involves the development of novel food processing methods and the use of physical, chemical, engineering, bioinformatics, and biotechnological tools to improve the understanding of the nutritional component of food at the molecular level. In recent years, his research group has expanded the research areas to include the development of nondestructive food quality and safety testing techniques using ATR-FTIR and NIR. His research group also works in the area of design, fabrication, and application of microfluidic electrochemical, quartz-crystal microbalance (QCM), and colorimetric biosensors to identify food allergens and toxins. Till date, he and his research group members have published over 50 research articles (Citation: 1004, h-index: 18, and i10-index: 23 (Google Scholar)).

Ilaria Mannazzu, Ph.D. in Microbial Biotechnology, was promoted to Associate Professor in Microbiology at the University of Sassari, Sassari, Italy, in 2001 and Professor of Microbiology in 2014; she obtained the National Academic Qualification as full professor of microbiology. Her teaching activity mainly involves general microbiology, microbial biotechnology, and food microbiology and includes master and doctoral seminar courses. Her research interests cover the biodiversity, physiology, and genetics of microorganisms of biotechnological relevance, mainly *Saccharomyces cerevisiae* and nonconventional yeasts. Also, her research activity focuses on (i) microbial interactions for the development of natural antimicrobials, (ii) *Saccharomyces cerevisiae* and non-Saccharomyces yeasts in wine and beer, (iii) fermentative stress response in wine yeasts, (iv) biotechnological production of primary and secondary metabolites, and (v) heterologous expression of microbial proteins. She has coauthored 75 publications in international peer-reviewed journals with 4244 citations (h-index 27, www.scopus.com, as of today), 10 book chapters, and more than 100 communications, proceedings, and posters presented at national and international conferences and papers in technical journals. She has been invited as a speaker at many national and international conferences. She is also a reviewer of many international journals and member of the editorial board of Fermentation (MDPI), Microorganisms (MDPI), World Journal of Microbiology and Biotechnology (Springer), etc. Being a member of the teaching board of the School of Doctorate in Agricultural Sciences of the University of Sassari, she participates in the preparation of the doctoral degree study program in Biophysical Chemistry of Brno University of Technology, Czechia. She represents the University of Sassari in the General Assembly of MIRRI IT-JRU, and she is a member of Società di MicrobiologiaAgraria, Alimentare e Ambientale (SIM3A), and the Italian Group of Wine Microbiology.

Bibhuti Bhusan Mishra is presently working as President, Odisha Bigyan Academy under Science and Technology Department, Government of Odisha, India. He obtained M.Phil. and Ph.D. degrees in 1983 and 1987, respectively, from Berhampur University, Odisha. He served as ICAR-Emeritus Professor at the PG Department of Microbiology, College of Basic Science and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India, after superannuation in 2018 for 3 years and has been Emeritus Professor, OUAT, from 2021. He has more than 40 years of teaching and research experience. Thirteen students have been awarded doctoral degree under his supervision in the field of environmental, soil, and agricultural microbiology from various universities across India, and currently two are actively working. In addition, he has supervised more than 30 PG students. He has more than 75 publications including book chapters and research manuscripts in various journals of national and international repute (h-index: 22 and Citation: 1635 in Google Scholar). He is credited with 25 accession numbers submitted to NCBI, USA. He has contributed research articles and book chapters in many more edited books pertaining to environmental, soil, agricultural, and industrial microbiology. He has edited eight books in microbiology and biotechnology published by national and international publishers and one textbook on botany practical. He successfully completed one major project funded by the University Grants Commission (UGC), Govt. of India, and was the Chief Nodal Officer of a project funded by the Rashtriya Krishi Vikas Yojana (RKVY) on "Establishment of Biofertilizer Production Unit," Govt. of Odisha, amounting to Rs. 150 lakhs. He is a recipient of "Best Teacher Award" from the University in 2012, from the College of Basic Sciences and Humanities in 2015, and from Odisha Botanical Society in 2018. For significant contributions in microbiology, he was conferred with Prof. Harihar Patnaik memorial award by the Orissa Botanical Society in 2016.

Plant Growth-Promoting Rhizobacteria for Sustainable Agriculture

1

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are closely allied with roots and can improve plant growth and inhibit the invading pathogens. The PGPR stimulates plant growth by various means, viz., increased nutrient uptake and production of hormones (IAA, gibberellins, cytokinins, etc.) and bioactive substances (to antagonize phytopathogenic microbes) along with the synthesis of enzymes that regulates plant ethylene levels. Recently, PGPR has attracted many researchers' attention to the development of biofertilizers as an eco-friendly approach. However, potential PGPR selection is an important factor, as plants' responses to environmental conditions often vary based on plant genotype, experimental sites, and seasons. A PGPR isolated from the native crop plants or their ecological zone is considered productive and efficient with steady results if reused at the same site and crop. Extensive studies have suggested that PGPR could have emerged as a promising and substitute chemical fertilizer method for agriculture sustainability. With this background, the interactions involving PGPR

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_1

populations with plants are the current challenge to explore their use under various agroclimatic conditions. The diverse group of PGPR isolated from various plants' rhizosphere and their role in increasing soil fertility, stress management, bioremediation, etc. are reviewed and discussed in this chapter.

Keywords

Biocontrol · Biofertilizers · Plant hormones · Rhizosphere · Stress management

1.1 Introduction

The rhizosphere zone consists of numerous microorganisms and the zone itself influences plants the most due to numerous activities in the roots (Uren 2000). The term "rhizosphere" was defined first as "the soil compartment affected by the plant root" by Lorenz Hiltner, the German agronomist, in 1904. The plant's rhizosphere is a zone of exceptional microbial action and a few microorganisms are bounteously present in this zone, named rhizobacteria, and have shown their various capacities. The nutrients do not just profit a portion of these rhizobacteria (as supplements) secreted by the plant root yet gainfully impact plant growth through different phenomena (Gowtham et al. 2018; Hariprasad et al. 2021).

1.1.1 Plant Growth-Promoting Rhizobacteria (PGPR)

The bacteria that colonize the host plant's roots and enhance its growth are generally termed as plant growth-promoting rhizobacteria (PGPR) (Gowtham et al. 2018). They are utilized as biofertilizers, biopesticides, bio-herbicides, and biocontrol agents (Hariprasad et al. 2021). The study of PGPR's interactions with plants and other microorganisms is often complicated in their biotic environment. These bacteria are classified based on their beneficial traits as biofertilizers capable of nitrogen fixation. The phyto-stimulators with the aptitude to produce hormones may act as biocontrol agents to protect plants from phytopathogenic microbe infection. The use of PGPR as bio-inoculants on crops would be a cost-effective biological disease management technique. It reduces the usage of chemical fertilizers, which also pollutes the atmosphere and causes human health problems (Gowtham et al. 2020). Furthermore, PGPR use will assist in increasing crop production, thereby helping to feed the mounting population. For three decades, a variety of PGPR (such as *Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Azotobacter*, *Azospirillum*, *Serratia*) have been documented to suppress a variety of fungal diseases while also significantly improving seed germination, root growth, and plant water uptake (Akhtar and Siddiqui 2010).

1.1.2 Diversity of the PGPR

The rhizobacterial diversity has been studied to a greater extent in numerous crops and other organisms, with the release of plant growth promoters (auxin, cytokinin, gibberellin, jasmonic acid, salicylic acid, abscisic acid, and ethylene), antagonistic metabolites (siderophores, antibiotics, hydrogen cyanide), soil enzymes (urease, proteases, dehydrogenase, nitrogenase, phosphatase), and inducers of systemic disease resistance (ISR) being used to assess their functionality (Johri et al. 2003). Scientists have been researching the accessibility of modern tools to study the microbial communities allied for improved plant growth for over a century. Structural and functional diversity are two approaches to studying the bacterial population. To comprehend the systemic approach, we must first understand the classes of individuals, their organisms, and their abundance.

The functional diversity of rhizobacteria is also explored through the screening of beneficial traits in rhizobacteria. Since the culture-based methods cannot isolate unculturable bacteria, they may not be appropriate for studying soil bacterial diversity (Amann et al. 1995). Denaturing gradient gel electrophoresis (DGGE) is an imperative method for studying bacterial population diversity and dynamics (Muyzer and Ramsing 1995). Muyzer et al. (1993) introduced DGGE of polymerase chain reaction (PCR)-amplified rDNA (ribosomal DNA) fragmented into microbial ecology and used it to research the genetic diversity of microbes from a variety of environments to examine the rhizobacterial population using molecular techniques. The analysis used by Muyzer et al. (1995) provided information on the genetic diversity of microbial communities located around the hydrothermal vents. Different isolation and purification methods yielded distinct PCR-DGGE profiles in rhizosphere samples, which reflected different bacterial consortia (Niemi et al. 2001). Gelsomino et al. (1999) have also used PCR and DGGE analysis to establish the bacterial population structure in Flevo silt loam soil. By examining the amplification, they showed that the species of *Arthrobacter* and *Enterobacter* were dominant in soil. Griffiths et al. (2000) used DGGE microbial population analysis to discern the active portion (rRNA derived) from total bacterial diversity (rDNA derived) across horizons of an existing grassland soil. DGGE of PCR and reverse transcriptase (RT) PCR-amplified 16S rRNA was used to investigate the rhizosphere-resident bacterial communities of *Chrysanthemum* (*Dendranthema grandiflora* Tzvelev) that majorly consisted of previously mentioned soil bacteria (*Pseudomonas*, *Acetobacter*, *Bacillus*, and *Arthrobacter*) (Duineveld et al. 2001).

Fang et al. (2005) used PCR amplification and DGGE analyses to assess the bacterial diversity in transgenic and non-transgenic corn rhizospheres and confirmed that the diversity of bacteria did not vary among the evaluated samples. Costa et al. (2006) have used DGGE to investigate the rhizosphere-resident bacteria of *Brassica napus* L. and *Fragaria ananassa* and found that *Streptomyces* and *Rhizobium* species were dominant ribotypes in the *F. ananassa* rhizosphere. At the same time, *Arthrobacter* sp. was the dominant ribotype in the *B. napus*, according to DGGE bands found in the bacterial profiles. Brons and van Elsas (2008) used PCR-DGGE fingerprinting and cluster analysis to determine the soil bacterial

population's composition. Besides, Monteiro et al. (2009) investigated the bacterial communities of the rhizospheres of three different genotypes of Vetiver [*Chrysopogon zizanioides* (L.) Roberty] and found that the predominant rhizospheric bacterial community hardly differs depending on the Vetiver genotype, according to the DGGE profiles.

PCR-DGGE was used by Yuan et al. (2010) to investigate the divergence in rhizobacterial communities of *Fritillaria thunbergii* grown in different habitats. The bacterial diversity was determined using principal component analysis (PCA), which revealed significant differences between all the soil samples collected from various habitats. Also, the same technique was used to examine the diversity of bacteria from the rhizosphere of *Colobanthus quitensis* (Kunth) Bartl and *Deschampsia antarctica* É. Desv (Teixeira et al. 2010). The Pearson's correlation index revealed no specific cluster formation irrespective of sample sites with >90% similarity. The DGGE was used by Nimnoi et al. (2011) to investigate the effects of rhizobial inoculants of three plants which revealed distinct communities of rhizobacteria on the created dendrogram and Sorensen's index. The findings indicated that the host and its rhizosphere soil had a synergistic impact on rhizobacterial communities. They also discovered that the inoculants played a role in the rhizosphere group structure changes. According to the hierarchical cluster analysis, the population structure of *D. elliptica* was more different from that of the other plants evaluated. The culture-dependent and -independent methods were used to examine the diversity of bacteria associated with maize roots by Pereira et al. (2011). Firmicutes, predominantly of the *Bacillus* genus, were found in abundance combined with the roots using culturable methods, while the genera of *Achromobacter*, *Lysinibacillus*, and *Paenibacillus* were found infrequently.

For analyzing the actinobacterial diversity of Panxi and China, the researchers combined culture-dependent and -independent methods from seven medicinal plants' rhizosphere (Zhao et al. 2012). The amplification of V6–V8 regions of 16S rDNA sequence revealed that *Agrobacterium*, *Burkholderia*, *Enterobacter*, and *Pseudomonas* genera were abundant in the rhizosphere soil of canola (Farina et al. 2012). Several of these bacteria have been shown to produce IAA and siderophores, solubilize phosphate, fix nitrogen, and promote canola plant growth. The DGGE analysis on *Eucalyptus globulus* callus and stem base's superficial tissues revealed that the bacterial populations differed at different sampling times (Peralta et al. 2012).

The examination of pearl millet rhizosphere of Faridabad, India, revealed *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Streptomyces* as dominant bacterial isolates (Prashar et al. 2012). Simpson index (D), Shannon-Wiener index, and equitability were determined to be 0.81, 1.71, and 0.95, respectively. Under in vitro conditions, the isolates were found to produce HCN, IAA, and ammonia along with the ability to solubilize phosphate. The isolates from the genus *Pseudomonas* had the greatest potential for promoting plant growth, whereas those from the genera *Staphylococcus* and *Streptomyces* had the least. Likewise, Gaikwad and Sapre (2015) investigated the rhizobacterial diversity in plant roots cultivated in the Solapur district, Maharashtra, India. They found that the structural diversity reported was the highest in the coriander rhizosphere, which was supported by its higher Simpson index value. When bacterial isolates from coriander

and turmeric were compared to bacterial isolates from other plants, the functional diversity, assessed based on their PGPR traits and efficiency in controlling the growth of phytopathogen (*Sclerotium rolfsii*), revealed that the bacterial isolates produced IAA, siderophore, and HCN, and also possessed the ability to solubilize phosphate and chitin.

1.2 Mechanism of Actions of PGPR for Plant Growth Promotion and Disease Suppression

Use of biological agents, such as PGPR, is one of the most recent ways to counteract biotic and abiotic stresses' negative effects. PGPR are rhizosphere-competent bacteria that colonize and multiply on plant roots irrespective of their growth stage (Antoun and Kloepper 2001). Rhizobacteria serve as eco-friendly and sustainable alternatives to the unsafe chemicals used for growth promotion and control of plant diseases (Shankar et al. 2009). The PGPR strains used as fresh suspensions and powdered formulations have commercial potential in plant growth promotion and management of plant diseases as evident from several researchers (Chithrashree et al. 2011). The PGPR usage in agriculture will boost plants' growth under stress conditions (Dimkpa et al. 2009) and decrease chemical fertilizers' usage. The mechanisms underlying the PGPR-mediated growth promotion in many crop plants are still unclear but some mechanisms identified include solubilization of minerals, root colonization and competition, nitrogen fixation, ability to synthesize phytohormones, and antagonism against phytopathogens through the production of siderophores, antibiotics, cyanide, chitinases, and β-1,3-glucanase along with the ability to synthesize enzymes that regulates plant ethylene levels and hydrolytic enzymes (Fig. 1.1) (Gupta et al. 2015; Hariprasad et al. 2021).

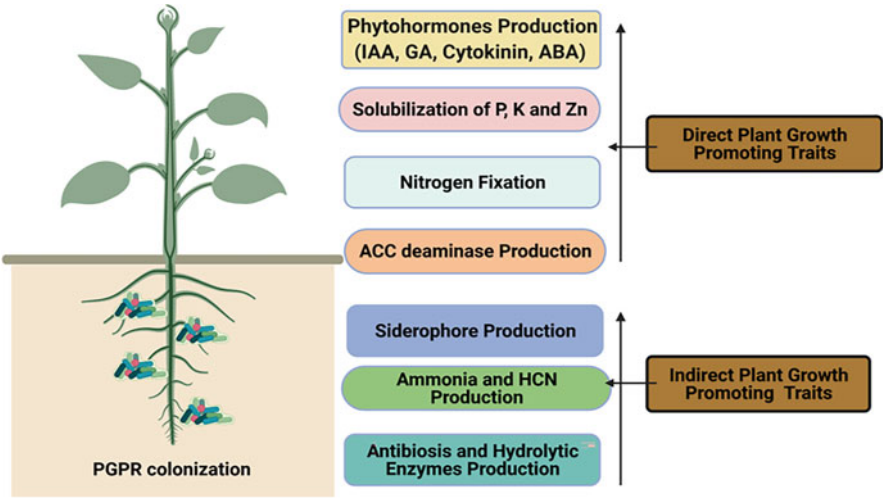

Fig. 1.1 Schematic representation of direct and indirect mechanisms of PGPR for plant growth

1.2.1 Root Colonization and Competition

Bacterial cells form a colony on the root's surface and further a biofilm made up of an extracellular polysaccharide matrix. The steps in root colonization include initial attachment, colony formation, and maturation of biofilm and it is necessary for its beneficial nature and to understand the mechanisms involved (Nayak et al. 2020). Microorganisms, including fungi, bacteria, protozoans, and nematodes, are all known to be inhibited or stimulated by the root's unidentified compounds. Further studies by Paterson et al. (1993) revealed that soil density, water-holding ability, and other factors influenced root colonization significantly. Similar experiments conducted by Beauchamp et al. (1993) in the rhizosphere soil of potato revealed the colonization of bacteria up to 8 cm length of roots at high temperatures. In addition to these factors, quorum sensing plays a significant part in finding out the root-colonizing bacterial density in the rhizosphere (Pierson et al. 1998). According to Gamalero et al. (2004), there was no major temporal difference in the density of total bacterial cells in any of the root zones examined. The microscopic analysis results revealed that all zones had a similar bacterial cell distribution pattern with lower density initially. But in later stages, zone A had the same pattern of colonization. Still, in contrast, zones B and C, which had root colonization to higher densities, thereby depicting the spatial pattern of colonization, were related to the differentiation in root zones.

To screen root-colonizing bacteria, Silva et al. (2003) established a simple root colonization bioassay. The bacteria that colonized roots in repeated experiments were considered positive for root colonization. The bacterized seeds were placed on 0.6 g of water agar and observed for the opaque zone around the growing roots. Mafia et al. (2009) used the same approach to screen root-colonizing bacteria in *Eucalyptus* seedlings. Apart from root colonization, PGPR must contend with native microbes for nutrients within the rhizosphere if pathogens can be successfully eliminated. Rhizobacteria that promote plant growth also battle with pathogens for nutrients in root exudates and eventually outnumbering them. PGPR populations on plant roots can serve as a sink for available nutrients, limiting the amount of nutrients available for invading pathogens (Bashan and de-Bashan 2005).

Biocontrol rhizosphere bacteria can multiply and spread throughout the rhizosphere system, colonizing possible infection sites on the root, thereby competing directly with the pathogens, including antibiotic production (Yasmin et al. 2009), siderophore (Singh et al. 2019), hydrolytic enzymes (Ramos-Solano et al. 2010), and fungal pathogen inhibition by hyphal colonization (Yang et al. 1994) and ISR (Fig. 1.2) (Gowtham et al. 2018). The colonization ability of PGPR to an acceptable density is required for successful biological control, but it is necessary to track its ability to colonize the root to screen an efficient root colonizer. Since tracking bacteria introduced into complex environments like soil necessitates the ability to distinguish them from native microflora, the markers used for this reason must meet certain criteria.

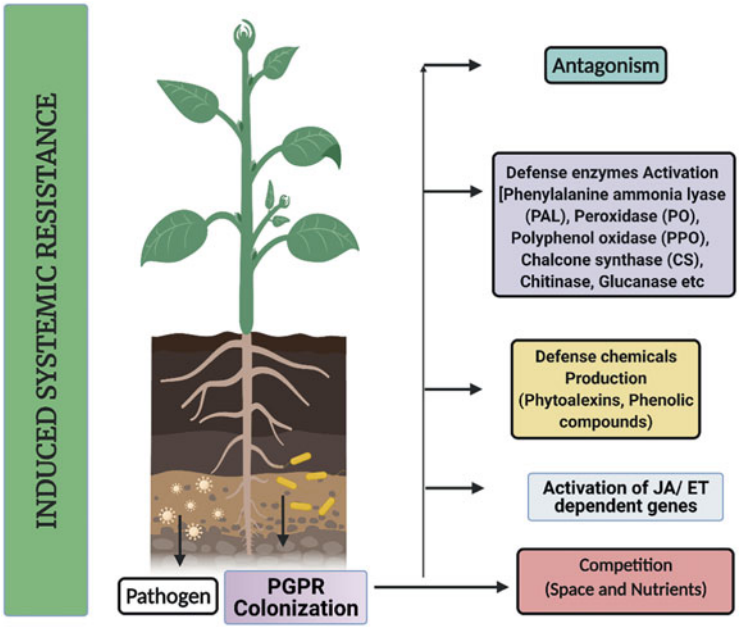

Fig. 1.2 Mode of induction of systemic resistance to various diseases

1.2.2 Nitrogen Fixation

For plant growth, nitrogen is the most limiting nutrient, and to fix this nitrogen for accessibility to plants, a specific microbe group is needed. Biological nitrogen fixers are microorganisms that fix nitrogen in the environment. They convert inert N_2 into a plant-friendly organic form (Reed et al. 2011). N_2 fixation accounts for up to 25% of total nitrogen in plants. Plant roots discharge substances that encourage colonization of bacteria and fix nitrogen, thereby effectively substituting the chemical fertilizers in various ways in dropping the environmental pollution. Even though many N_2-fixing bacteria are associated with legumes, members of the *Azotobacter* and *Azospirillum* genera have been extensively experienced in the field to increase legume and cereal yields (Nosheen et al. 2021).

The most common species present in the soil is *Azotobacter chroococcum*, but other species such as *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, and *A. vinelandii* can also be found (Kizilkaya 2009). The association of *A. chroococcum* in rhizospheres of plants was linked to increased seedling growth and germination (Sumbul et al. 2020). The presence of low levels of organic matter in soils is a significant limiting factor for *Azotobacter* proliferation; as a result, the rhizoplane is devoid of *Azotobacter* cells (Sammauria et al. 2020). *Azospirillum* mostly forms a symbiotic relationship with the plants to increase crop yield. It was shown that inoculating the plant with both *Azospirillum lipoferum* and *Bacillus megaterium*

provided balanced nitrogen nutrition and resulted in an enhanced crop yield than inoculating the wheat plant with only *Azospirillum* (El-Komy 2005).

1.2.3 Phosphate Solubilization

Phosphorus is the second important nutrient for plants. Even though total phosphorous levels in soils are typically high and most of them are insoluble, some emerge after applying chemical fertilizers (Penn and Camberato 2019). Microorganisms were believed to be involved in the solubilization of inorganic phosphates as early as 1903. Phosphate-solubilizing microbes are found everywhere, but their numbers differ from one soil to the next. The phosphate-solubilizing bacteria make up 50% of the soil's total population, while fungi make up 0.5–1%. Phosphate-solubilizing bacteria outnumber phosphate-solubilizing fungi by a factor of 2–150 (Khan et al. 2007). The phosphate-solubilizing microbes make up 40% of the culturable population which are largely isolated from rhizosphere soil (Sharma et al. 2013). The majority of phosphate-solubilizing bacteria have been isolated from the rhizospheric soil of different plants. They are metabolically more active than the bacteria that possess phosphate-solubilizing ability from different sources (Vazquez et al. 2000). Mineral phosphate solubilization is the mechanism of converting the insoluble form of phosphorus into soluble mono- and dibasic phosphate ions. As a result, phosphorus supply to plants increases (Gyaneshwar et al. 2002; Penn and Camberato 2019).

Similarly, Islam et al. (2007) revealed that some rhizobacteria isolated from the rice-grown soil were found to be phosphate solubilizers. Since they observed a decrease in pH of the culture and bacterial growth due to the accumulation of organic acids, phosphate solubilization was reported as supportive for organic acid production. Besides, these organisms boost the efficacy of nitrogen fixation and increase the availability of trace elements like Fe, Zn, and others (Nosheen et al. 2021). Khan and Khan (2001) demonstrated the management of wilt disease caused by *Fusarium* in tomato under field trials by applying phosphate-solubilizing microbes to the soil. Following soil application in the field, these phosphate solubilizers significantly increased vegetative and reproductive growth parameters. Certain PSM also reduced *Fusarium* incidence, which is linked to a lower *F. oxysporum* in the rhizosphere.

Dey et al. (2004) examined bacterial isolates from nine soil samples; eight produced siderophores and five produced IAA. Soilborne fungal pathogens like *Sclerotium rolfsii* were inhibited by ammonia and solubilized inorganic phosphate. The efficiency of these rhizobacterial isolates was tested in pot and field trials for 3 years. In both rainy and post-rain seasons, phosphate content in soil, shoots, and kernels increased significantly after bacterial inoculation. Similarly, Han et al. (2006) used phosphate- and potassium-solubilizing rhizobacteria to increase the nutrient availability and uptake capacity of pepper and cucumber in their experiment. Compared to other combinations, rock phosphate and rock potassium and co-inoculation improved the accessible P and K in potting medium significantly. The same combination increased pepper and cucumber plants' NPK content in shoots and roots and their dry weight and photosynthetic potential. Islam et al.

(2007) isolated phosphate-solubilizing bacteria from a rice rhizospheric soil sample and characterized them for PGPR traits, including ammonia (NH_3) synthesis, protease, chitinase, cellulase, and β-1,3-glucanase function. According to their findings, the isolate may have more than one trait that encouraged plant growth while also suppressing plant disease.

1.3 Phytohormone Synthesis

Plant hormones are generally referred to as endogenous (naturally occurring) growth substances in plants. Auxin (indole acetic acid), gibberellins (GAs), and cytokinin (zeatin) are examples of plant growth promoters, while abscisic acid, xanthoxin, and violaxanthin are examples of plant growth inhibitors. They are usually found in plants at <1 μM and above this concentration it is considered supraoptimal (Naqvi 2002). As sessile species, plants have evolved sophisticated adaptive mechanisms to respond to abiotic stress through phytohormones' mediation (Zhang et al. 2006). According to Davies and Zhang (1991), many physiological changes are linked to changes in these plant hormones' concentrations.

1.3.1 Indole Acetic Acid (IAA)

Indole acetic acid (IAA) is a natural and physiologically most active auxin found in plants that has a beneficial effect on root development (Miransari and Smith 2014). Up to 80% of rhizobacteria can synthesize IAA and colonize seed and/or root surfaces. They work in tandem with plants' IAA to promote cell proliferation and improve the host's absorption of micronutrients (Vessey 2003). It is involved in many processes, including cell division, differentiation and extension, germination, regulation of vegetative growth, initiation of adventitious and lateral root formation, mediation of light and gravity responses, photosynthesis, metabolite biosynthesis, pigment formation, as well as tolerance to stressful situations (Spaepen and Vanderleyden 2011). The PGPR, which possesses the ability to produce IAA, has increased the growth of many crop plants (Sachdev et al. 2009; Erturk et al. 2010; Gowtham et al. 2017; Singh et al. 2019; Hariprasad et al. 2021). Peyvandia et al. (2010) evaluated the effect of IAA-producing *P. fluorescens* on root formation and root architecture of olive micro shoots by measuring the number and length of adventitious and lateral roots. They found that the amount of IAA produced by rhizobacteria was dependent on the amount of tryptophan in the media and the addition of the same to media enhanced the total number and length of adventitious and lateral roots. Bacteria may take amino acid tryptophan, a physiological precursor molecule for IAA biosynthesis, from plant root exudates (Gupta et al. 2015). The ability of PGPR for increased grain production in *Brassica* sp. was positively correlated with tryptophan-dependent auxin production (Asghar et al. 2002). Ahmad et al. (2005) isolated IAA-producing *Pseudomonas* sp. and *Azotobacter* sp. from various rhizospheric soil samples and characterized them using cultural

and biochemical characteristics and its impact on IAA production. They discovered that as tryptophan concentrations increased from 0 to 5 mg/mL, IAA production increased in both rhizobacteria genera.

1.3.2 Cytokinins

Cytokinins affect plant physiological and developmental processes as they are directly involved in cell division and growth process (Srivastava 2002). Plant growth and development can be influenced by cytokinins released by nonpathogenic microorganisms living near the roots (Garcia de Salamone et al. 2001). Also, a wild-type strain *P. fluorescens* produced more of the cytokinins isopentenyl adenosine, zeatin riboside, and dihydroxyzeatin riboside than two mutants. It was also discovered that adding the precursor adenine to G20–18 cultures increased cytokinin activity. Garcia de Salamone et al. (2001) found that mutant strains were less capable of promoting radish plant growth than wild-type strain G20–18 in previous studies.

Bacillus cereus, *B. megaterium*, *B. subtilis*, *Escherichia coli*, *Halomonas desiderata*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Proteus vulgaris* all had phytohormones, including cytokinins, in their culture medium (Karadeniz et al. 2006). The cytokinin fractions isolated from the extract of bacteria were isolated by TLC and HPLC, according to Hussain and Hasnain (2009). In comparison to control, the bacterial extract increased cell division, cotyledon size, and fresh weight of cucumber cotyledons grown under light and dark conditions. Though the cytokinin-producing bacterial effect on plant cell division was studied primarily in the formation of root nodules (Markmann and Parniske 2009) it has been shown to promote cell division in inoculated wheat root tips (Molina-Favero et al. 2007). *Arabidopsis thaliana* mutant plants without receptors of cytokinin (*AHK2*, *AHK3*, and *CRE1*) and cytokinin signaling gene (*RPN12*) were treated with *Bacillus megaterium* to evaluate the function of cytokinin in plant growth upon treatment. The results of the study revealed that the knockout of triple-cytokinin receptors was insensitive to bacterial inoculation indicating their role in plant growth promotion (Ortiz-Castro et al. 2008). Accordingly, many PGPR have been proved to produce optimum levels of cytokinin than phytopathogens that function as inhibitors, thereby helping the plant in growth promotion (Kang et al. 2010).

1.3.3 Gibberellins (GAs)

Gibberellins (GAs) are tetracyclic diterpenoid acids that play various roles in plant development irrespective of their growth stage (Bottini et al. 2004). In the Egyptian Nile Delta, where rice has been rotated with *Trifolium alexandrinum* L. since antiquity, Yanni et al. (2001) found that indigenous *Rhizobium leguminosarum* pv. *trifolii* can colonize rice roots. *Rhizobium*-rice combination improves seedling vigor and grain yield by promoting root and shoot growth. They also discovered that *Rhizobium* formed GA, which they tentatively dubbed GA_7. In a bioassay, the dwarf

phenotype induced in alder by artificial treatment with paclobutrazol, an inhibitor of GA biosynthesis, was reversed when dwarf seedlings were treated with culture filtrate of PGPR (*Bacillus pumilus* and *B. licheniformis*) that were an inhabitant of alder rhizosphere (Gutierrez-Mannero et al. 2001). The presence of GA was discovered after GC-MS study of distilled fractions of culture filtrate. GA_1 had the highest concentration of the four types of GA detected, followed by GA_3. Probanza et al. (2002) also found that inoculating *Pinus pinea* plants with *B. licheniformis* and *B. pumilus* increased plant growth, probably through bacterial gibberellin development. *Azospirillum lipoferum* and *A. brasilense* fed with deutero GA_{20}-glycosides reversed the dwarf phenotype rice mutants, correlated with increased development (Cassan et al. 2001).

According to Joo et al. (2004), *B. cereus*, *B. macroides*, and *B. pumilus* produced GAs with the relative content of 3β-hydroxylated GAs (1, 3, 4 and 36) being higher than that of other GAs in the culture broth of the PGPR. Furthermore, Joo et al. (2005) found that using GA-producing rhizobacteria increased the fresh weight of pepper shoots and roots. It was also noted that among the three species of *Bacillus*, *B. cereus* was the most important as compared to the other two rhizobacteria as it increased the endogenous amount of GA in red pepper plants.

1.3.4 Abscisic Acid (ABA)

Abscisic acid (ABA) is one of the five "classical" plant hormones that control plant growth and development on a physiological and biochemical level (Kende and Zeevaart 1997). Abiotic stresses like salt, drought, cold, wounding, and others are directly linked to increased ABA levels (Gowtham et al. 2021). It has many effects during the plant life cycle, similar to other plant hormones. It plays a vital role in the effective alteration of plants to biotic and abiotic stresses by stomatal closure, thereby decreasing transpiration (Taiz and Zeiger 2010). The most common PGPR action mechanism to withstand stress is the induction of ABA synthesis in the plant by bacterial ABA (Cohen et al. 2001, 2009, 2015; Salomon et al. 2014). The bacterial ABA controls root elongation and architecture and water and nutrient levels and can also directly affect the concentration of hormones in the rhizosphere and leaf growth and gas exchange (Belimov et al. 2009; Dodd et al. 2010). No evidence on enhanced growth in plants is reported upon the ABA produced by the bacteria, but a few reports are available on the possible function of ABA-producing bacteria in suppressing abiotic stress in plants after bacterial inoculation. Cohen et al. (2001) showed that *Azospirillum lipoferum* inoculation partially reversed an inhibitor's effect (such as fluridone) in blocking ABA synthesis in maize seedlings and that the amount of ABA in seedlings increased and enhanced growth in comparison to fluridone treatment, thus maintaining a better water status. Cohen et al. (2008) measured the amount of ABA produced in *Arabidopsis thaliana* seedlings inoculated with the ABA-producing *Azospirillum brasilense* strain Sp245 and discovered that the ABA content was doubled when compared with uninoculated plants.

Furthermore, Cohen et al. (2009) investigated the impact of *A. lipoferum* in maize upon applying GA and ABA synthesis inhibitors, namely prohexadione-Ca and fluridone, to plants subjected to drought and adequate stress. They found that the bacterium application was as effective as that of inhibitors under both the stress conditions. Although drought-stressed plants were allowed to recover for a week, fluridone-treated and drought-stressed plants' relative water content was significantly lower, while *Azospirillum* completely nullified this impact. It was discovered to be related to ABA levels as measured by GC-EIMS. When plants were primed with only prohexadione-Ca or in combination with fluridone and subjected to drought, their growth was reduced and their ABA levels increased, implying that bacterial GAs are also essential in stress relief. The findings also indicated that both hormones released by *Azospirillum* might have helped plants cope with water stress. These findings bolstered the case for the use of beneficial bacteria with ABA-producing ability in plant stress alleviation under adverse environmental conditions. According to Salomon et al. (2014), ABA-producing *B. licheniformis* and *Pseudomonas fluorescens* increased ABA levels in 45-day-old in vitro-grown *Vitis vinifera* cv. Malbec plants by 76-fold and 40-fold, respectively, as a result of bacterization. Besides, as the amount of ABA increased, both bacteria reduced plant water loss. They hypothesized that both the bacteria serve as stress relievers by minimizing water loss and inducing ABA synthesis. Cohen et al. (2015) evaluated the morphological, physiological, and biochemical responses of *A. thaliana* Col-0 and aba2–1 mutant plants treated with ABA-producing *A. brasilense* Sp245 strain when watered and in drought stress and reported that the bacteria were effective in inducing stress tolerance.

1.3.5 Xanthoxin

Xanthoxin is an intermediate in ABA's biosynthesis and is classified as an endogenous plant growth inhibitor compared to the above five stimulatory plant hormones (Seo and Koshiba 2002). The fundamental structure and inhibitory function of xanthoxin are identical and similar to ABA (Burden et al. 1971; Taylor and Burden 1970); hence, it can be considered an ABA analog. The analog is also responsible for the stomatal closure and is found in various plant species (Raschke 1975). It is produced when violaxanthin is photooxidized and acts as an inhibitor of seed germination (Burden et al. 1971; Taylor and Burden 1972). Interestingly, Gowtham et al. (2021) confirmed the ability of *B. marisflavi* to produce ABA analog (xanthoxin-like compound) and its function in inducing drought stress tolerance in the host plant. According to their hypothesis, *B. marisflavi* catabolizes the carotenoid to produce ABA analog/xanthoxin in the rhizosphere under drought stress conditions. With the aid of xanthoxin oxidase and abscisic aldehyde oxidase, this low molecular compound (xanthoxin) can be taken up by plants, where it can either remain in its original form or be converted into ABA. Furthermore, they cause the plant to adapt physiologically to drought stress and first report ABA analog in conferring drought resistance in the host plant.

1.3.6 Ethylene

Plants can respond to any stress (both biotic and abiotic) by adjusting the level of hormones that trigger the expression of various stress-related proteins that defend plants from various negative effects of stressors (Singh et al. 2015; Murali et al. 2021a). Ethylene is a significant plant hormone responsible for the stress response and has an important role in plant response to growth and development (Abeles et al. 1992). Plants generate the necessary amount of ethylene under ideal conditions (plant-friendly), but this amount increases when plants are exposed to stressors (adversely affect the plants) (Glick 2014; Murali et al. 2021b). The first step in the synthesis of ethylene is converting methionine to S-adenosyl methionine, followed by 1-aminocyclopropane-1-carboxylic acid (ACC). Seedling emergence, root hair growth and elongation, tissue differentiation, lateral bud development, leaf and flower senescence, anthocyanin synthesis, fruit ripening, and processing of volatile compounds responsible for fruit fragrance are all processes in which ACC is involved (Singh et al. 2019; Gowtham et al. 2020; Hariprasad et al. 2021).

1.3.7 Production of 1-Aminocyclopropane-1-Carboxylate Deaminase

PGPR is known to support plant growth through various mechanisms, but ACC deaminase is more significant in today's environment because it protects plants from many stressors (Glick 2012). Certain plant-associated bacteria that produce ACC deaminase may minimize ethylene's stress in plants (Glick et al. 2007). ACC deaminase (EC 3.5.99.7) is a sulfhydryl multimeric enzyme with a monomeric subunit with a 35–42 kDa molecular mass. Honma and Shimomura discovered and published ACC deaminase for the first time in 1978. The enzyme ACC deaminase is located in the cytoplasm of soil bacteria and it catalyzes the conversion of ACC, an immediate precursor of ethylene, to α-ketobutyrate and ammonia, resulting in a decrease in ethylene levels in plants and the resumption of root/shoot development (Glick 2014). Induced systemic tolerance refers to the property of tolerance provided by certain bacteria to biotic or abiotic stressors by ACC deaminase activity to enhance plants' stress tolerance (Yang et al. 2009).

Among the enzymes, bacterial ACC deaminase is well known for its function in ethylene regulation that affects plants' growth and development. Rhizobacteria that produce ACC deaminase have been shown to help plants develop under abiotic stress conditions, including flooding, drought, salt, and heavy metals (Glick 2005). The increased root growth and/or enhanced development of lateral root hairs may increase tolerance to abiotic stress when the plant is inoculated with such bacteria. Rhizobacteria that develop ACC deaminase minimize ethylene's negative effects on plants caused by stress (Glick 2005). ACC deaminase producers have been identified in the bacteria *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacterium*, *Methylobacterium*, *Pseudomonas*, and *Rhizobium* (Penrose and Glick 2001; Pandey et al. 2005).

The decrease in ACC levels in plants caused by the ACC deaminase-synthesizing PGPR would also decrease ethylene levels, assisting the plant's growth and development (Glick 2014). According to Glick et al. (1998), PGPR with ACC deaminase activity are present at a lower level until stressors trigger it. Plant ethylene levels are dependent on the ratio of ACC oxidase to ACC deaminase, which should act before any ACC oxidase is induced since ACC oxidase has a higher affinity for ACC than ACC deaminase when PGPR with ACC deaminase is present (Glick et al. 1998). Mayak et al. (2004) found that PGPR with ACC deaminase activity endemic to rainy areas could protect plants from drought more effectively than bacteria isolated from water-rich areas. Many other researchers have confirmed the efficacy of rhizobacteria to produce ACC deaminase to protect plants against various abiotic stressors by equilibrating the amount of ethylene (Belimov et al. 2009; Gowtham et al. 2020), and the possible mechanism of action of ACC deaminase-producing PGPR is depicted in Fig. 1.3 as represented by Gowtham et al. (2020).

1.3.8 Siderophore

Iron is one of the essential micronutrients that are vital for the growth and development of plants and microbes. It has been observed that soil consists of a huge proportion of iron in its insoluble form, ferric hydroxide. The availability of iron in soil solutions is 10^{-18} M, which does not help in the sustenance of plants and can be overcome by applying microbes that can produce siderophores. Kloepper et al. (1988) were the first to discover that PGPR promotes plant growth by starving native microflora. Extracellular siderophores produced by PGPR effectively complex environmental iron, reducing its availability to certain native microflora. Many bacteria may produce multiple types of siderophores or have multiple iron-uptake systems to accommodate multiple siderophores. The species of *Bacillus*, *Serratia*, *Azotobacter*, *Pseudomonas*, *Enterobacter*, *Azospirillum*, and *Rhizobium* are only a few beneficial plant-associated bacterial genera that secrete different forms of siderophores (Ahemad and Kibret 2014). *Brucella abortus* strain 2308 is known to synthesize brucebactin (2,3-dihydroxybenzoate), a highly efficient catechol siderophore, according to Carrero et al. (2002), who used it as a siderophore for bacterial growth under iron-limited conditions. *Pseudomonas putida* DFC31 produced pyoverdine-type siderophores, and their analysis revealed the existence of hydroxymate and catecholate iron-chelating groups, according to Fu et al. (2007). The strain's IAA production and phosphate solubilization properties were also found to improve plant growth.

Helmy et al. (2008) isolated siderophores from *P. fluorescens* using affinity chromatography and identified them as 30 and 90 KDa, but they are polymers of many siderophores. *Erwinia carotovora*, the cause of bacterial soft rot in potatoes, was inhibited by a purified siderophore. The hydroxamate form of siderophores formed by *Rhizobium* isolated from *Sesbania sesban* was studied (Sridevi and Mallaiah 2008). Buyer et al. (1993) reported that PGPR produces siderophore in the rhizosphere under iron-limiting conditions using monoclonal antibodies. When

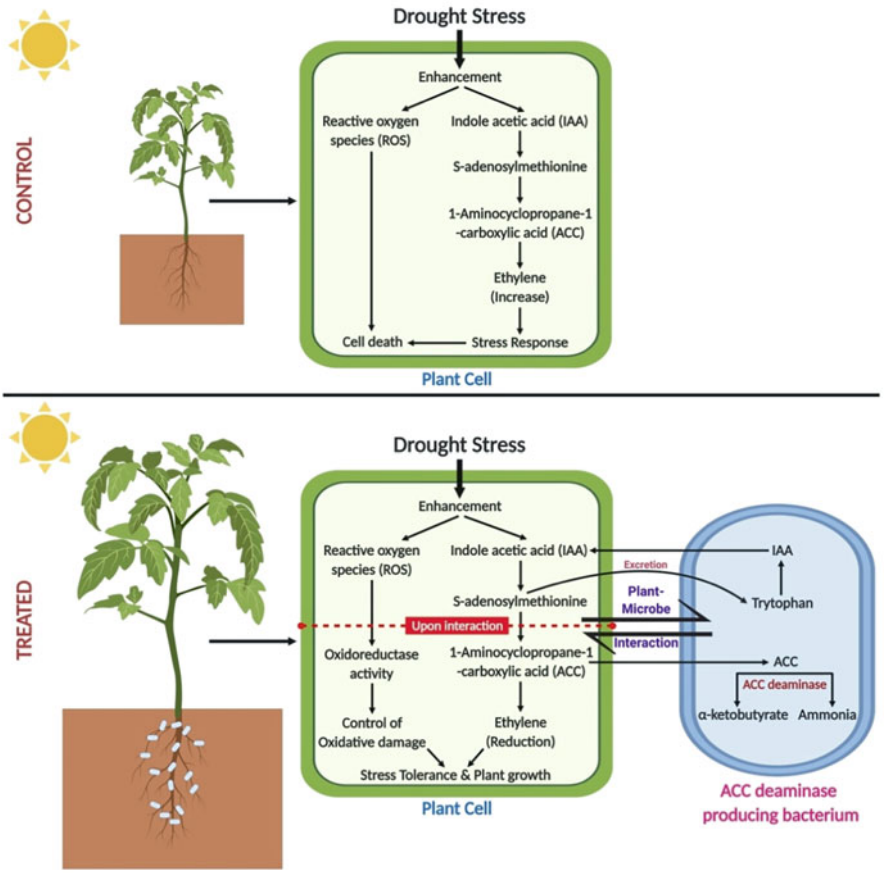

Fig. 1.3 Mechanism of action of ACC deaminase-producing PGPR for the induction of drought stress tolerance in plants (source: adopted from Gowtham et al. 2020)

grown in iron-limiting conditions, Terano et al. (2002) observed a new protein band of 75 kDa on the cell wall of *P. fluorescens* and increased development of protein of 54 kDa. This protein's expression may be involved in the siderophore-mediated iron-uptake process.

Siderophore is classified into three groups based on the iron-coordinating functional group. Hydroxamates (mycobactin and exochelin), catechols (enterobactin and vibriobactin), and thiazolines are examples of these compounds (pyochelin and yersiniabactin) (Essen et al. 2007). Iron solubilization, transport, and storage are the primary functions of siderophores (Stephan et al. 1993). There is a lot of evidence that various plant species can absorb bacterial Fe^{3+} siderophore complexes, and this process is important for plant iron absorption, particularly in calcareous soils (Masalha et al. 2000). A decrease often followed increased plant growth caused by *Pseudomonas* strains in root pathogen populations. There is strong evidence that

siderophore-mediated iron competition plays a direct role in these PGPR strains' biocontrol function (Loper and Buyer 1991).

For many plant diseases, the feasibility of using induced systemic resistance to protect plants has been demonstrated. Plants inoculated with the PGPR *P. putida* and *S. marcescens* biocontrols, for example, were covered against the cucumber pathogen *P. syringae* pv. *lachrymans* (Bashan and de-Bashan 2005). The role of siderophore concentration developed by *Pseudomonas* sp. in suppressing tomato bacterial wilt was investigated by Jagadeesh et al. (2001). Certain fluorescent *Pseudomonas* sp. strains synthesize siderophores that suppress soilborne plant diseases by opposing pathogen growth by sequestering iron from the atmosphere (Bashan and de-Bashan 2005). The pathogenic fungus *F. oxysporum* in tomato can be regulated more effectively by a mutant strain of *P. putida* that overproduces siderophores than the wild bacterium. The pyoverdine siderophore function produced by many *Pseudomonas* sp. in the control of *Pythium* and *Fusarium* species has been demonstrated in the rhizosphere microbial community structure (Yang and Crowley 2000). The role of iron and catechol siderophore concentrations in inducing systemic resistance in cucumber against *Colletotrichum orbiculare* infection was investigated by Press et al. (2001).

1.4 Secondary Metabolite Production

The research of rhizobacteria isolated from the rhizospheres of important medicinal plants is extremely important because they are well known for promoting plant growth and producing important metabolites (Solaiman and Anawar 2015). The inhibition or destruction of one organism by a metabolite created by another organism is known as antibiosis. Broad-spectrum antibiotics are agonists that develop strong growth inhibitory compounds effective against a wide range of microorganisms. Antibiotic production has been identified as a powerful mode of disease suppression in which the pathogen's development and/or activity is thought to be directly inhibited (Handelsman and Stabb 1996). Tomashow and Weller (1988) made the first convincing experiment on the bacterium-produced antibiotics that restrains plant disease in an ecosystem. The direct and indirect isolation techniques are used to isolate a wide variety of antifungal rhizobacteria from maize, barley, and chicory, including *P. fluorescens*, *P. cepacia*, *Serratia liquefaciens*, *S. plymuthica*, *Erwinia herbicola*, and *Bacillus* sp. (Lambert et al. 1987).

Many bacteria developed antimicrobial compounds in significant amounts (Solaiman and Anawar 2015). Pseudomonads inhibited soilborne fungal pathogens by producing antifungal compounds according to Dwivedi and Johri (2003). Using bioautography, the antifungal activity of *Pseudomonas cepacia* B37w was linked to the development of pyrrolnitrin, a particular antifungal compound (Burkhead et al. 1994). A novel antifungal compound, maltophilin, was developed by *Stenotrophomonas maltophilia* R3089 strain that was isolated from rape plants' rhizosphere (Jakobi et al. 1996). Compared to their wild type, nonmotile Tn5 transposon mutants of *Fusarium oxysporum* f.sp. *radicis-lycopersici* antagonistic

biocontrol strain *Pseudomonas chlororaphis* produce phenazine-1-carboxamide as the active metabolite which is at least 1000 times less successful in competitive tomato root-tip colonization (Chin-A-Woeng et al. 1998). From a sugar beet rhizobacterium, *Stenotrophomonas* sp. strain SB-K88, Nakayama et al. (1999) isolated three antifungal compounds known as xanthobaccins A, B, and C. They hypothesized that xanthobaccins produced by the bacterium played a crucial role in inhibiting damping-off disease in sugar beet. A fluorescent *Pseudomonas* sp. isolated from maize rhizosphere was found to be strongly antagonistic to maize foot, collar, and root rots along with wilting diseases caused by different species of *Fusarium* by producing different plant growth-promoting metabolites and fungal antibiotics (Pal et al. 2001). The three main antifungal compounds were found to be isomers of iturin A, a cyclic lipopeptide antibiotic produced by *Bacillus amyloliquefaciens* and used as a biocontrol agent against *Rhizoctonia solani* and other fungal plant pathogens, according to fast atom bombardment mass spectrometry/mass spectrometry collision-induced dissociation study (Yu et al. 2002).

Based on NMR and MS results, the antifungal metabolite produced by *Pseudomonas aeruginosa* PUPa3 has been classified as phenazine-1-carboxamide, which has broad-spectrum antifungal activity against a variety of phytopathogenic fungi (Kumar et al. 2005). Bacteria isolated from canola and soybean plants produced the antifungal organic volatile compounds (benzothiazole, cyclohexanol, n-decanal, etc.) that may play a key role in inhibiting sclerotial activity, limiting ascospore development, and lowering disease levels caused by *Sclerotinia sclerotiorum* (Fernando et al. 2005). *Pseudomonas fluorescens* produces antifungal metabolites such as pyrrolnitrin and pyoluteorin including 2,4-diacetylphloroglucinol and the evidence from the research suggests that these compounds are held in a balance that can be affected by certain plant and microbial phenolics (Baehler et al. 2005). A new nitrogen-containing heterocyclic antibiotic compound, "amino (5-(4-methoxyphenyl)-2-methyl-2-(thiophen-2-yl)-2,3-dihydrofuran-3-yl)methanol" (AMTM), was produced by *Delftia tsuruhatensis* WGR–UOM–BT1, a novel rhizobacterium from *Rauwolfia serpentina* with multiple PGPR properties for suppressing fungal phytopathogens (Prasannakumar et al. 2015).

1.4.1 Production of Hydrolytic Enzymes

Hydrolytic enzymes such as chitinases, β-1,3-glucanases, proteases, and lipases are among these substances. Any of these hydrolytic enzymes can be synthesized by a variety of *Pseudomonas* and *Bacillus* species. Extracellular chitinase and β-1,3-glucanase are produced by *Pseudomonas stutzeri*, which lyses the pathogen *Fusarium* sp. (Bashan and de-Bashan 2005). Fusaric acid (produced by *Fusarium*) can be hydrolyzed by *B. cepacia* and *Cladosporium werneckii*, causing severe plant damage.

Chitinases are glycol hydrolases that catalyze the hydrolytic degradation of chitin and non-soluble linear β-1,4-linked polymer of N-acetylglucosamine (GlcNAc) (Kurita 2001). Since these pathogenic fungi have a major cell wall component of

chitin, chitinase provided by chitinolytic rhizobacteria can degrade; rhizobacterial isolates' chitinolytic capacity had the potential to reduce soilborne root disease of many crop plants. Isolating possible chitinolytic rhizobacteria is thus a crucial step in the development of biopesticides. Three isolates of *Micromonospora carbonacea*, *Serratia marcescens*, and *Streptomyces viridodiasticus* produced high levels of chitinase that suppressed the growth of *Sclerotinia minor* (El-Tarabily et al. 2000). Aktuganov et al. (2003) investigated 70 *Bacillus* sp. strains that were antagonistic to phytopathogenic fungi and discovered that 19 of them had chitinolytic activity. Kamil et al. (2007) isolated 400 bacteria from the rhizospheres of maize, wheat, and rice plants and identified potent chitinolytic rhizobacteria in a minimal salt medium containing colloidal chitin as the sole carbon and energy source. In vitro, strains MS1 and MS3 inhibited the growth of all pathogenic fungi that were studied. Ajit et al. (2006) isolated fluorescent pseudomonads antagonistic to *F. oxysporum* f. sp. *dianthi*, the pathogen that causes carnation vascular wilt, and linked disease defense chitinase activity. Mycelial growth was also substantially inhibited by cell-free bacterial culture filtrate from chitin-containing media. According to Western blot analysis, chitinase is found in two isoforms with molecular masses of 43 kDa and 18.5 kDa.

Bacillus cereus CRS7-purified chitinase had a molecular weight of 47 kDa (Kishore and Pande 2007). Extracellular chitinase formed by the super-producing mutant strain *Serratia marcescens* M-1 was studied by Duzhak et al. (2009). They looked at four extracellular proteins with chitinase activity capable of binding chitin substrates, weighing 62, 54, 52, and 21 kDa. The proteins ChiA, ChiB, ChiC, and CBP21 were described as typical *S. marcescens* chitinases based on the data obtained. Furthermore, Kishore and Pande (2007) used chitinolytic *B. cereus* CRS7 and non-chitinolytic *Pseudomonas fluorescens* CRS31 to combat Botrytis gray mold, demonstrating the role of chitinase in plant disease management. Glucanases are another essential group of hydrolytic enzymes that degrade the phytopathogenic fungal cell wall. The rhizosphere proliferation of various phytopathogenic fungi was inhibited by β-1,3-glucanase-producing strain of *Pseudomonas cepacia* (Fridlender et al. 1993). The combined activity of the two hydrolytic enzymes chitinase and β-1,3-glucanase was more efficient than either enzyme alone in inhibiting fungal pathogens (Tanaka and Watanabe 1995). Inoculation of rice roots with endoglucanase-producing diazotrophs can boost root colonization and stimulate root and plant development. The ability to colonize plant roots will increase the plant's biological nitrogen-fixing activity (Asilah et al. 2009).

1.5 Future Prospective and Conclusion

The availability of effective biocontrol agent formulations including survival during storage, rapid proliferation, and colonization ability after application plays a vital role in the success of biological control of plant diseases. One of the mechanisms for promoting growth by PGPR may be the activation of the host defense system and it warrants further study. While many biocontrol agents can control plant pathogens,

only a few commercial formulations have demonstrated consistently strong and stable efficacy in the field. The conflicting output of biocontrol agents under field study may be due to their ecological competence, soil, and microbiological factors. On the other hand, several studies showed that the field techniques performed consistently over time. Finally, safe biocontrol agent formulations are critical for subsistence gladiolus farming, where soilborne diseases are the key crisis and fungicide treatments are prohibitively expensive. When commercialized, the talc-based strain mixture formulation can become a favored input in integrated disease management systems. Further research on cost-effectiveness, performance evaluation using several pathogens, and/or evaluation in other agroclimatic regions will be needed to explore the formulation's commercialization.

Acknowledgements The authors would like to thank the Department of Studies in Biotechnology, Department of Studies in Microbiology and the Department of Studies in Botany, University of Mysore, for providing facilities.

References

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in plant biology, 2nd edn. Academic Press, San Diego

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of Azotobacter and fluorescent Pseudomonas in the presence and absence of tryptophan. Turk J Biol 29:29–34

Ajit NS, Verma R, Shanmugam V (2006) Extracellular chitinase of fluorescent pseudomonads antifungal to *Fusarium oxysporum* f.sp. *dianthi* causing carnation wilt. Curr Microbiol 52:310–316. https://doi.org/10.1007/s00284-005-4589-3

Akhtar MS, Siddiqui ZA (2010) Role of plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria, microbiology monographs 18. Springer-Verlag, Berlin Heidelberg, pp 157–195. https://doi.org/10.1007/978-3-642-13612-2_7

Aktuganov GE, Melentev AI, Kuzmina LY et al (2003) The chitinolytic activity of *Bacillus cohn* bacteria antagonistic to phytopathogenic fungi. Microbiol 72:313–317. https://doi.org/10.1023/A:1024200132596

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169. https://doi.org/10.1128/mr.59.1.143-169.1995

Antoun H, Kloepper JW (2001) Plant growth promoting rhizobacteria. Encyclopedia of genetics. Academic, New York, pp 1477–1480

Asghar HN, Zahir ZA, Arshad M et al (2002) Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea* L. Biol Fertil Soils35: 231–237. Doi:https://doi.org/10.1007/s00374-002-0462-8

Asilah AM, Radziah O, Radzali M (2009) Production of hydrolytic enzymes in rice (*Oryza sativa* L.) roots inoculated with N2-fixing bacteria. Malaysian J Soil Sci 13:43–57

Baehler E, Bottiglieri M, Pechy-Tarr M et al (2005) Use of green fluorescent protein-based reporters to monitor balanced production of antifungal compounds in the biocontrol agent *Pseudomonas fluorescens* CHA0. J Appl Microbiol 99(1):24–38. https://doi.org/10.1111/j.1365-2672.2005.02597.x

Bashan Y, de-Bashan LE (2005) Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination. Soil Biol Biochem 37:1795–1804. https://doi.org/10.1016/j.soilbio.2005.02.013

Beauchamp CJ, Kloepper JW, Antoun H (1993) Detection of genetically engineered bioluminescent pseudomonads in potato rhizosphere at different temperatures. Microb Releases 1:203–207

Belimov AA, Dodd IC, Hontzeas N et al (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 181(2):413–423. https://doi.org/10.1111/j.1469-8137.2008.02657.x

Bottini R, Cassan F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503. https://doi.org/10.1007/s00253-004-1696-1

Brons JK, van Elsas JD (2008) Analysis of bacterial communities in soil by use of denaturing gradient gel electrophoresis and clone libraries, as influenced by different reverse primers. Appl Environ Microbiol 74(9):2717–2727. https://doi.org/10.1128/AEM.02195-07

Burden RS, Firn RD, Hiron RWP et al (1971) Induction of plant growth inhibitor xanthoxin in seedlings by red light. Nature 234(46):95–96

Burkhead KD, Schisler DA, Slininger PJ (1994) Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. Appl Environ Microbiol 60(6):2031–2039. https://doi.org/10.1128/aem.60.6.2031-2039.1994

Buyer JS, Kartze MG, Sikora L (1993) A method for detection of pseudobactin, the siderophore produced by a plant-growth-promoting *Pseudomonas* strain, in the barley rhizosphere. Appl Environ Microbiol 59:677–681. https://doi.org/10.1128/aem.59.3.677-681.1993

Carrero MIB, Sangari FJ, Aguero J et al (2002) *Brucella abortus* strain 2308 produces brucebactin a highly efficient catecholic siderophore. Microbiol 148:353–360. https://doi.org/10.1099/00221287-148-2-353

Cassan F, Bottini R, Schneider G, Piccoli P (2001) *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in seedlings of rice dwarf mutants. Plant Physiol 125:2053–2058. https://doi.org/10.1104/pp.125.4.2053

Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ et al (1998) Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Mol Plant Microbe Interact 11:1069–1077. https://doi.org/10.1094/MPMI.2000.13.12.1340

Chithrashree, Udayashankar AC, Nayaka S et al (2011) Plant growth-promoting rhizobacteria mediated induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. Biol Control 59(2):114–122. https://doi.org/10.1016/j.biocontrol.2011.06.010

Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in Arabidopsis plants. Plant Growth Regul 54(2):97–103. https://doi.org/10.1007/s10725-007-9232-9

Cohen AC, Bottini R, Pontin M et al (2015) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153(1):79–90. https://doi.org/10.1111/ppl.12221

Cohen AC, Travaglia C, Reinoso H et al (2001) *Azospirillum* inoculation and inhibition of gibberellin and aba synthesis in maize seedling under drought. In: Proceedings-plant growth regulation society of America-annual meeting (Vol 28, p 88–93)

Cohen AC, Travaglia CN, Bottini R et al (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87(5):455–462. https://doi.org/10.1139/B09-023

Costa R, Götz M, Mrotzek N et al (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol Eco 56(2):236–249. https://doi.org/10.1111/j.1574-6941.2005.00026.x

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Ann Rev Plant Biol 42(1):55–76. https://doi.org/10.1146/annurev.pp.42.060191.000415

Dey R, Pal KK, Bhatt DM et al (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394. https://doi.org/10.1016/j.micres.2004.08.004

Dimkpa C, Weinand T, Asch F (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32(12):1682–1694. https://doi.org/10.1111/j.1365-3040.2009.02028.x

Dodd IC, Zinovkina NY, Safronova VI et al (2010) Rhizobacterial mediation of plant hormone status. Ann Appl Biol 157(3):361–379. https://doi.org/10.1111/j.1744-7348.2010.00439.x

Duineveld BM, Kowalchuk GA, Keijzer A et al (2001) Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Appl Environ Microbiol 67(1):172–178. https://doi.org/10.1128/AEM.67.1.172-178.2001

Duzhak AB, Panfilova ZI, Duzhak TG et al (2009) Extracellular chitinase of mutant super producing strain *Serratia marcescens* M-1. Biochemistry (Mosc) 74(2):209–214. https://doi.org/10.1134/s0006297909020126

Dwivedi D, Johri BN (2003) Antifungals from fluorescent pseudomonads: biosynthesis and regulation. Curr Sci 85(12):1693–1703

El-Komy HMA (2005) Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol Biotech 43:19–27

El-Tarabily KA, Soliman MH, Nassar AH et al (2000) Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Pl Pathol 49:573–583. https://doi.org/10.5423/PPJ.2006.22.2.107

Erturk Y, Ercisli S, Haznedar A et al (2010) Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biol Res 43:91–98

Essen SA, Johnsson A, Bylund D et al (2007) Siderophore production by Pseudomonas stutzeri under aerobic and anaerobic conditions. App Envi Microbiol 73(18):5857–5864. https://doi.org/10.1128/AEM.00072-07

Fang M, Kremer RJ, Motavalli PP et al (2005) Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. Appl Environ Microbiol 71(7):4132–4136. https://doi.org/10.1128/AEM.71.7.4132-4136.2005

Farina R, Beneduzi A, Ambrosini A et al (2012) Diversity of plant growth promoting rhizobacteria communities associated with the stages of canola growth. Appl Soil Eco:55, 44–52. https://doi.org/10.1016/j.apsoil.2011.12.011

Fernando WD, Ramarathnam R, Krishnamoorthy AS et al (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37(5):955–964. https://doi.org/10.1016/j.soilbio.2004.10.021

Fridlender M, Inbar J, Chet I (1993) Biological control of soil-borne plant pathogens by a ß-1,3-glucanase producing *Pseudomonas cepacia*. Soil Biol Biochem 25(9):1211–1221. https://doi.org/10.1016/0038-0717(93)90217-Y

Fu Z, Liu H, Yang Z, Ju H (2007) Recent developments in multianalyte immunoassay. Curr Trends Biotechnol Pharm 1:1–17

Gaikwad S, Sapre V (2015) Structural and functional diversity of rhizobacterial strains isolated from rhizospheric zone of different plants of Sholapur-Maharashtra region, India. Int J Curr Microbiol Appl Sci 4(9):263–273

Gamalero E, Lingua G, Capri GG et al (2004) Colonization pattern of primary tomato roots by *Pseudomonas fluorescence* A6RI characterized by dilution plating, flow cytometry, fluorescence, confocal and scanning electron microscopy. GEMS Microbiol Eco 48:79–87

Garcia de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47(5):404–411. https://doi.org/10.1139/w01-029

Gelsomino A, Keijzer-Wolters AC, Cacco G et al (1999) Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J Microbiol Met 38(1):1–15

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7. https://doi.org/10.1016/j.femsle.2005.07.030

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica (Cairo) 2012:963401

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39. https://doi.org/10.1016/j.micres.2013.09.009

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68. https://doi.org/10.1006/jtbi.1997.0532

Glick BR, Todorovic B, Czarny J et al (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242. https://doi.org/10.1080/07352680701572966

Gowtham HG, Brijesh Singh S, Murali M et al (2020) Induction of drought tolerance in tomato upon the application of ACC deaminase producing plant growth promoting rhizobacterium *Bacillus subtilis* Rhizo SF 48. Microbiol Res 234:126422. https://doi.org/10.1016/j.micres.2020.126422

Gowtham HG, Duraivadivel P, Ayusman S et al (2021) ABA analogue produced by *Bacillus marisflavi* modulates the physiological response of *Brassica juncea* L. under drought stress. Appl Soil Eco:159:103845. https://doi.org/10.1016/j.apsoil.2020.103845

Gowtham HG, Duraivadivel P, Hariprasad P et al (2017) A novel split-pot bioassay to screen indole acetic acid producing rhizobacteria for the improvement of plant growth in tomato [*Solanum lycopersicum* L.]. Sci Horticulturae 224:351–357. https://doi.org/10.1016/j.scienta.2017.06.017

Gowtham HG, Murali M, Brijesh Singh S et al (2018) Plant growth promoting rhizobacteria-*Bacillus amyloliquefaciens* improves plant growth and induces resistance in chilli against anthracnose disease. Biol Control 126:209–217. https://doi.org/10.1016/j.biocontrol.2018.05.022

Griffiths RI, Whiteley AS, O'Donnell AG et al (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66(12):5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000

Gupta G, Parihar SS, Ahirwar NK et al (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7(2):096–102

Gutierrez-Mannero FJ, Ramos-Solano B, Probanza A et al (2001) The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiologia Plant 111:206–211. https://doi.org/10.1034/j.1399-3054.2001.1110211.x

Gyaneshwar P, Kumar GN, Parekh LJ et al (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93

Han HS, Supanjani, Lee KD (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52:130–136

Handelsman J, Stabb EV (1996) Biocontrol of soil-borne plant pathogens. Plant Cell 8:1855–1869

Hariprasad P, Gowtham HG, Gourav C (2021) Beneficial plant-associated bacteria modulate host hormonal system enhancing plant resistance toward abiotic stress. In: Jogaiah S (ed) Biocontrol agents and secondary metabolites: applications and immunization for plant growth and protection. Woodhead Publishing, pp 113–151. https://doi.org/10.1016/B978-0-12-822919-4.00006-5

Helmy M, Baddar D, El'Masry HH (2008) Affinity purification of a siderophore that exhibits an antagonistic effect against soft rot bacterium. Biochemistry (Mosc) 73:776–782. https://doi.org/10.1134/s0006297908070055

Hussain A, Hasnain S (2009) Cytokinin production by some bacteria: its impact on cell division in cucumber cotyledons. Afri J Microbiol Res 3:704–712

Islam MT, Deora A, Hashidoko Y et al (2007) Isolation and identification of potential phosphate solubilizing bacteria from the rhizosphere of *Oryzae sativa* L. cv. BR29 of Bangladesh. Z Natureforsch C J Biosci 62:103–110

Jagadeesh KS, Kulkarni JH, Krishnaraj PU (2001) Evaluation of the role of fluorescent siderophore in the biological control of bacterial wilt in tomato using Tn5 mutants of fluorescent *Pseudomonas* sp. Curr Sci 81:882–888

Jakobi M, Winkelmann G, Kaiser D et al (1996) Maltophilin: a new antifungal compound produced by *Stenotrophomonas maltophilia* R3089. J Antibiot (Tokyo) 49(11):1101–1104. https://doi.org/10.7164/antibiotics.49.1101

Johri BN, Sharma A, Virdi JS (2003) Rhizobacterial diversity in India and its influence on soil and plant health. Adv Biochem Eng Biotech 84:49–89. https://doi.org/10.1007/3-540-36488-9_2

Joo GJ, Kim YM, Kim JT et al (2005) Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red pepper. The J Microbiol 43:510–515

Joo GJ, Kim YM, Lee IJ et al (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. Biotechnol Lett 26:487–491. https://doi.org/10.1023/B:BILE.0000019555.87121.34

Kamil Z, Rizk M, Saleh M et al (2007) Isolation and identification of rhizosphere bacteria and their potential in antifungal biocontrol. Glob J Mol Sci 2:57–66

Kang BG, Kim WT, Yun HS et al (2010) Use of plant growth promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep 4(3):179–183. https://doi.org/10.1007/s11816-010-0136-1

Karadeniz A, Topcuoglu SF, Inan S (2006) Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World J Microbiol Biotechnol 22:1061–1064. https://doi.org/10.1007/s11274-005-4561-1

Kende H, Zeevaart J (1997) The five "classical" plant hormones. Plant Cell 9(7):1197. https://doi.org/10.1105/tpc.9.7.1197

Khan MR, Khan SM (2001) Biomanagement of *Fusarium* wilt of tomato by the soil application of certain phosphate solubilizing micro-organisms. Int J Pest Management 47:223–227

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27:29–43. https://doi.org/10.1051/agro:2006011

Kishore GK, Pande S (2007) Chitin-supplemented foliar application of chitinolytic *Bacillus cereus* reduces severity of *Botrytis* gray mold disease in chickpea under controlled conditions. Lett Appl Microbiol 44:98–105. https://doi.org/10.1111/j.1472-765X.2006.02022.x

Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J Environ Biol 30:37–82

Kloepper JW, Hume DJ, Scher FM et al (1988) Plant growth-promoting rhizobacteria on canola (rape seed). Plant Dis 72:42–45

Kumar RS, Ayyadurai N, Pandiaraja P et al (2005) Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. J Appl Microbiol 98(1):145–154. https://doi.org/10.1111/j.1365-2672.2004.02435.x

Kurita A (2001) Controlled functionalization of the polysaccharide chitin. Prog Polym Sci 26:1921–1971

Lambert B, Leyns F, Van Rooyen L et al (1987) Rhizobacteria of maize and their antifungal activities. Appl Environ Microbiol 53:1866–1871

Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. Mol Pl Microbe Interaction 4:5–13

Mafia RG, Alfenas AC, Ferreira EM et al (2009) Root colonization and interaction among growth promoting rhizobacteria isolates and eucalypts species. Revista Arvore 33(1):1–9. https://doi.org/10.1590/S0100-67622009000100001

Markmann K, Parniske M (2009) Evolution of root endosymbiosis with bacteria: how novel are nodules? Trends Pl Sci 14:77–86

Masalha J, Kosegarten H, Elmaci O et al (2000) The central role of microbial activity for iron acquisition in maize and sunflower. Biol Fertil Soils 30:433–439. https://doi.org/10.1007/s003740050021

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166(2):525–530. https://doi.org/10.1016/j.plantsci.2003.10.025

Miransari M, Smith DL (2014) Plant hormones and seed germination. Environ Exp Bot 99:110–121

Molina-Favero C, Creus CM, Lanteri ML et al (2007) Nitric oxide and plant growth promoting rhizobacteria: common features influencing root growth and development. Adv Botanical Res 46:1–33. https://doi.org/10.1016/S0065-2296(07)46001-3

Monteiro JM, Vollu RE, MRR C et al (2009) Comparison of the bacterial community and characterization of plant growth-promoting rhizobacteria from different genotypes of *Chrysopogon zizanioides* (L.) Roberty (Vetiver) rhizospheres. J Microbiol 47(4):363–370. https://doi.org/10.1007/s12275-009-0048-3

Murali M, Gowtham HG, Singh SB et al (2021a) Bio-prospecting of ACC deaminase producing Rhizobacteria towards sustainable agriculture: A special emphasis on abiotic stress in plants. Appl Soil Ecol 168:104142. https://doi.org/10.1016/j.apsoil.2021.104142

Murali M, Singh SB, Gowtham HG et al (2021b) Induction of drought tolerance in Pennisetum glaucum by ACC deaminase producing PGPR-Bacillus amyloliquefaciens through Antioxidant defense system. Microbiol Res 253:126891. https://doi.org/10.1016/j.micres.2021.126891

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59(3):695–700. https://doi.org/10.1128/aem.59.3.695-700.1993

Muyzer G, Ramsing NB (1995) Molecular methods to study the organization of microbial communities. Water Sci Technol 32:1–9

Muyzer G, Teske A, Wirsen CO et al (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol 164(3):165–171. https://doi.org/10.1007/BF02529967

Nakayama T, Homma Y, Hashidoko Y et al (1999) Possible role of xanthobaccins produced by *Stenotrophomonas* sp. strain SBK88 in suppression of sugar beet damping-off disease. Appl Environ Microbiol 65(10):4334–4339. https://doi.org/10.1128/AEM.65.10.4334-4339.1999

Naqvi SSM (2002) Plant growth hormones: growth promoters and inhibitors. In: Pessarakli M (ed) Handbook of plant and crop physiology, 2nd edn. Marcel Dekker, Inc., New York, pp 501–525

Nayak SK, Nayak S, Patra JK (2020) Rhizobacteria and its biofilm for sustainable agriculture: a concise review. In: Yadav MK, Singh BP (eds) New and future developments in microbial biotechnology and bioengineering: microbial biofilms. Elsevier, Amsterdam, pp 165–175. https://doi.org/10.1016/B978-0-444-64279-0.00013-X

Niemi RM, Heiskanen I, Wallenius K et al (2001) Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. J Microbiol Methods 45(3):155–165

Nimnoi P, Lumyong S, Pongsilp N (2011) Impact of rhizobial inoculants on rhizosphere bacterial communities of three medicinal legumes assessed by denaturing gradient gel electrophoresis (DGGE). Ann Microbiol 61(2):237–245. https://doi.org/10.1007/s13213-010-0128-y

Nosheen S, Ajmal I, Song Y (2021) Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability 13(4):1868. https://doi.org/10.3390/su13041868

Ortiz-Castro R, Valencia-Cantero E, Lopez-Bucio J (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signal Behav 3:263–265. https://doi.org/10.4161/psb.3.4.5204

Pal KK, Tilak KVBR, Saxcna AK et al (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. Microbiol Res 156(3):209–223. https://doi.org/10.1078/0944-5013-00103

Pandey P, Kang SC, Maheshwari DK (2005) Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*. Curr Sci:177–180

Paterson E, Kemp JS, Gammack SM et al (1993) Leaching of genetically modified *Pseudomonas fluorescens* through intact soil microcosms: influence of soil type. Biol Fert Soils 15:308–314

Penn CJ, Camberato JJ (2019) A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture 9(6):120. https://doi.org/10.3390/agriculture9060120

Penrose DM, Glick BR (2001) Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. Can J Microbiol 47(4):368–372

Peralta KD, Araya T, Valenzuela S et al (2012) Production of phytohormones, siderophores and population fluctuation of two root-promoting rhizobacteria in *Eucalyptus globulus* cuttings. World J Microbiol Biotechnol 28(5):2003–2014. https://doi.org/10.1007/s11274-012-1003-8

Pereira P, Ibanez F, Rosenblueth M et al (2011) Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. ISRN Ecology 2011:1–10. https://doi.org/10.5402/2011/938546

Peyvandia M, Farahanib F, Hosseini Mazinanic M et al (2010) *Pseudomonas fluorescens* and its ability to promote root formation of olive micro shoots. Int J Plant Prod 4:63–66

Pierson EA, Wood DW, Cannon JA et al (1998) Interpopulation signaling via *N*-acyl homoserine lactones among bacteria in the wheat rhizosphere. Mol Plant Microbe Interaction 11:1078–1084

Prasannakumar SP, Gowtham HG, Hariprasad P et al (2015) *Delftia tsuruhatensis* WGR–UOM–BT1, a novel rhizobacterium with PGPR properties from *Rauwolfia serpentina* (L.) Benth. *ex* Kurz also suppresses fungal phytopathogens by producing a new antibiotic—AMTM. Lett Appl Microbiol 61(5):460–468. https://doi.org/10.1111/lam.12479

Prashar P, Kapoor N, Sachdeva S (2012) Structural and functional diversity of rhizobacteria of pearl millet in semi-arid agroclimatic Zone. Asian J Plant Sci Res 2(5):599–606

Press CM, Loper JE, Kloepper JW (2001) Role of iron in rhizobacteria mediated induced systemic resistance of cucumber. Phytopathology 91:593–598. https://doi.org/10.1094/PHYTO.2001.91.6.593

Probanza A, Garcia JAL, Palomino MR et al (2002) *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR Bacillus (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). Appl Soil Ecol 20(2):75–84. https://doi.org/10.1016/S0929-1393(02)00007-0

Ramos-Solano B, Garcia JAL, Garcia-Villaraco A et al (2010) Siderophore and chitinase producing isolates from the rhizosphere of *Nicotiana glauca* Graham enhance growth and induce systemic resistance in *Solanum lycopersicum* L. Plant Soil 334:189–197. https://doi.org/10.1007/s11104-010-0371-9

Raschke K (1975) Stomatal action. Ann Rev. Plant Physiol 26:309–340

Reed SC, Cleveland CC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. Ann Rev Ecol Evol Syst 42:489–512. https://doi.org/10.1146/annurev-ecolsys-102710-145034

Sachdev DP, Chaudhari HG, Kasture VM et al (2009) Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Indian J Exp Biol 47(12):993–1000

Salomon MV, Bottini R, de Souza Filho GA et al (2014) Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis

of defense-related terpenes in in vitro cultured grapevine. Physiol Plant 151(4):359–374. https://doi.org/10.1111/ppl.12117

Sammauria R, Kumawat S, Kumawat P et al (2020) Microbial inoculants: potential tool for sustainability of agricultural production systems. Arch Microbiol 202:677–693. https://doi.org/10.1007/s00203-019-01795-w

Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. Trends Plant Sci 7(1): 41–48

Shankar UAC, Nayaka CS, Niranjan-Raj S et al (2009) Rhizobacteria mediated resistance against *Bean common mosaic virus* strain blackeye cowpea mosaic in cowpea (*Vigna unguiculata*). Pest Manag Sci65(10):1059–1064. Doi:https://doi.org/10.1002/ps.1791

Sharma SB, Sayyed RZ, Trivedi MH et al (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587. https://doi.org/10.1186/2193-1801-2-587

Silva HSA, Romeiro R et al (2003) Development of a root colonization bioassay for rapid screening of rhizobacteria for potential biocontrol agents. J Phytopathol 151(1):42–46

Singh RP, Shelke GM, Kumar A et al (2015) Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. Front Microbiol 6:937. https://doi.org/10.3389/fmicb.2015.00937

Singh SB, Gowtham HG, Murali M et al (2019) Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). Biocatal Agric Biotechnol 18:101089. https://doi.org/10.1016/j.bcab.2019.101089

Solaiman ZM, Anawar HM (2015) Rhizosphere microbes interactions in medicinal plants. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants, soil biology, vol 42. Springer, Cham, pp 19–41. https://doi.org/10.1007/978-3-319-13401-7_2

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3(4):a001438. https://doi.org/10.1101/cshperspect.a001438

Sridevi M, Mallaiah KV (2008) Production of hydroxamate-type of siderophore by rhizobium strains from *Sesbania sesban* (L). Merr Int J Soil Sci 3:28–34. https://doi.org/10.3923/ijss.2008.28.34

Srivastava LM (2002) Plant growth and development: hormones and environment. Academic Press, San Diego

Stephan H, Freund S, Beck W et al (1993) Ornibactins—a new family of siderophores from Pseudomonas. Biometals 6:93–100

Sumbul A, Ansari RA, Rizvi R et al (2020) *Azotobacter*: a potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci 27(12):3634–3640. https://doi.org/10.1016/j.sjbs.2020.08.004

Taiz L, Zeiger E (2010) Plant physiology, 5thEdn. Sinauer Associates Inc., Sunderland, Massachusetts

Tanaka H, Watanabe T (1995) Glucanases and chitinases of *Bacillus circulans* WL-12. J Industrial Microbiol 14:478–483

Taylor HF, Burden RS (1970) Xanthoxin, a new naturally occurring plant growth inhibitor. Nature 227(5255):302–304

Taylor HF, Burden RS (1972) Xanthoxin, a recently discovered plant growth inhibitor. Proc R Soc Lond B Biol Sci 180(1060):317–346

Teixeira LC, Peixoto RS, Cury JC et al (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay. Maritime Antarctica ISME J 4(8):989–1001

Terano H, Nomota K, Takass S (2002) Siderophore production and induction of Iron-regulated proteins by a microorganism from rhizosphere of Barley. Biosci Biotechnol Biochem 66:2471–2473. https://doi.org/10.1271/bbb.66.2471

Tomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J Bact 170:3499–3508

Uren NC (2000) Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Willig S, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface. CRC Press, Boca Raton, pp 19–40

Vazquez MM, Cesar S, Azcon R et al (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and 1638 enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15:261–272. https://doi.org/10.1016/S0929-1393(00)00075-5

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2): 571–586. https://doi.org/10.1023/A:1026037216893

Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66:345–351. https://doi.org/10.1128/AEM.66.1.345-351.2000

Yang CH, Menge JA, Cooksey DA (1994) Mutations affecting hyphal colonization and pyoverdine production in pseudomonads antagonistic toward *Phytophthora parasitica*. Appl Environ Microbiol 60(2):473–481

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4

Yanni YG, Rizk RY, Abd El-Fattah FK et al (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* pv. *trifolii* with rice roots. Aust J Plant Physiol 28(9):845–870. https://doi.org/10.1071/PP01069

Yasmin F, Othman R, Sijam K et al (2009) Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. Afri J Microbiol Res 3:815–882

Yu GY, Sinclair JB, Hartman GL et al (2002) Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. Soil Biol Biochem 34(7):955–963. https://doi.org/10.1016/S0038-0717(02)00027-5

Yuan X, Xu J, Chai H et al (2010) Differences of rhizo-bacterial diversity and the content of peimine and peiminine of *Fritillaria thunbergii* among different habits. J Medicinal Plants Res 4(6):465–470. https://doi.org/10.5897/JMPR09.484

Zhang J, Jia W, Yang J et al (2006) Role of ABA in integrating plant responses to drought and salt stresses. Field Crops Res 97(1):111–119. https://doi.org/10.1016/j.fcr.2005.08.018

Zhao K, Penttinen P, Chen Q et al (2012) The rhizospheres of traditional medicinal plants in Panxi, China, host a diverse selection of actinobacteria with antimicrobial properties. Appl Microbiol Biotechnol 94(5):1321–1335. https://doi.org/10.1007/s00253-011-3862-6

Plant-Microbe Interactions and Its Effect on Crop Productivity

2

Sumer Singh Meena, Megha Mankoti, Prangya Ranjan Rout, and Anee Mohanty

Abstract

With the ever-increasing population combined with environmental degradation, the size of arable land is shrinking at an alarming rate. To meet the escalated human hunger, intensive agriculture is practised globally. Excessive usage of chemical fertilizers and pesticides combined with irrational irrigation practices is highly unsustainable. The challenge is to find ways to increase crop productivity without causing any negative impact on the environment and biodiversity. The microorganisms present in the soil could very well be the answer to move towards smart, eco-friendly ways of agriculture. The role of microorganisms in nitrogen fixation is well known and significant scientific efforts are unravelling the details of plant microbial interactions which could hold the key to sustainable agriculture. Advancements in molecular tools combined with high-throughput omics tools suggest that plant-microbe interactions are mediated by an array of mechanisms. Continued efforts in the direction of understanding the rhizosphere community dynamics will give the much-required impetus to organic and sustainable farming practices. This area of plant-microbe interaction would make a significant contribution towards the innovative biological strategies in agriculture and enable efficient utilization of this largely untapped resource. This chapter focuses on the beneficial communication between plants and microorganisms in terms of uptake of nutrients, biotic and abiotic stress tolerance and soil structure

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_2

improvement. Furthermore, it aims to provide in-depth understanding of the inter-kingdom interactions that are mostly driven by various metabolic pathways, phytohormones, siderophores and plant secondary metabolites.

Keywords

Plant-microbe interaction · Rhizosphere · Biofertilizers · Biopesticide · Phytostimulant · Abiotic stress · Biotic stress

2.1 Introduction

Microbes have been found in both underground and aboveground regions and are classified into phyllospheric microbes that communicate on the surface of leaves, stems, flowers, fruits and rhizospheric microbes that live in soil or primary roots of plants. Similarly epiphytic microbes are located on the surface of plants, while endophytic microorganisms inhabit within the plant tissue. The most commonly found belowground microbes belong to Acidobacteria, Verrucomicrobia, Bacteroidetes, Proteobacteria, Planctomycetes and Actinobacteria groups. Most of the microbes are horizontally transferred while some of the bacteria are transferred through seeds which eventually transform into roots. The most dominant aboveground endophytic microbes belong to genera *Dyella*, *Staphylococcus*, *Bacillus*, *Propionibacterium*, *Pseudomonas*, *Ralstonia*, *Burkholderia* and *Mesorhizobium* and phyllospheric aboveground microbes such as *Pseudomonas*, *Sphingomonas*, *Frigoribacterium*, *Bacillus*, *Curtobacterium*, *Enterobacter* and *Acinetobacter* are the common representatives (Compant et al. 2019).

Plant-microbe interaction affects the growth of plants in both positive and negative ways. Positive interaction leads to nitrogen fixation, increased tolerance against biotic and abiotic stress, and production of biofilms or antibiotics that functions as biocontrol against pathogenic attack (Gupta et al. 2015; Backer et al. 2018) while negative interaction forms association with pathogenic bacteria, parasitic plants, fungi and invertebrate herbivores (Van der Putten et al. 2001).

2.2 Different Types of Plant-Microbe Interactions

Microbes also communicate with plants in other ways such as mutualism, commensalism, amensalism, symbiosis and pathogenesis. Some of the well-known examples of different plant-microbe interactions are listed in Table 2.1.

2.2.1 Mutualism

Mutualistic interaction between plants and microbes benefits each other. During this interaction the microbes colonize their host plants to gain access to nutrients. This

Table 2.1 Different types of plant-microbe interactions

Microorganism	Type of plant-microbe interaction	Reference
Azospirillum sp.	Symbiotic interaction with wheat plant and increases nitrogen content in plants	Arzanesh et al. (2011); Rosenblueth et al. (2018)
Erwinia amylovora	Pathogenic interaction with ornamental plants and fruit trees causing fire blight disease	Oh and Beer (2005)
Xanthomonas oryzae	Phyllospheric association with rice seedling	Buttimer et al. (2017)
Paraburkholderia unamae	Rhizospheric or endophytic association with maize, coffee, sorghum or sugarcane	Buttimer et al. (2017)
Pseudomonas stutzeri A1501	Symbiotic interaction with rice plant	Rosenblueth et al. (2018)
Pantoea sp., *Paraburkholderia* sp., *Pseudomonas* sp.	Fixation of atmospheric nitrogen, solubilization of phosphate, increased production of IAA, release of ACC deaminase in wheat and soyabean roots	Compant et al. (2019)
Xanthomonas sp.	Pathogenic interaction with potato and banana plants	Ocimati et al. (2018)
Pseudomonas strains	Production of ACC deaminase	Saravanakumar and Samiyappan (2007); Compant et al. (2019)
Pantoea stewartii, *Erwinia herbicola*	Phyllospheric association with corn	Buttimer et al. (2017)
Bacillus sp.	Production of ACC deaminase	Nayak et al. (2017)
Erwinia amylovora	Phyllospheric association with apple seedling and firethorn	Buttimer et al. (2017)
Colletotrichum sp.	Fungal endophyte in rice and tomato plant	Goh et al. (2013)
Fusarium sp., *Curvularia* sp.	Fungal endophyte in rice and tomato plant	Goh et al. (2013)
Ralstonia solanacearum, *Xylella fastidiosa*	Pathogenic interaction with potato and banana plants	Mansfield et al. (2012); Compant et al. (2019)
Bradyrhizobium	Symbiotic interaction with zornia, lupinus and galactia	Parker (2012)
Rhizobium etli, *Phaseolus vulgaris*	Mutualistic interaction under drought condition	Igiehon and Babalola (2018)
Pseudomonas putida	Mutualistic interaction with soybean	Compant et al. (2019)
Bacillus licheniformis K11	Mutualistic interaction with pepper plant under drought condition	Rubin et al. (2017)
Burkholderia sp., *Rhizopus* sp.	Symbiotic interaction	Lackner et al. (2009)
Glomus mosseae, *Arbuscular mycorrhiza*	Mutualistic interaction with soybean under drought condition	Igiehon and Babalola (2018)

(continued)

Table 2.1 (continued)

Microorganism	Type of plant-microbe interaction	Reference
Bacillus subtilis QST713	The bacteria produce antibiotic iturin A in tomato plants that helps to prevent disease	Fousia et al. (2016)
AMF consortium, *Bacillus thuringiensis*	Mutualistic interaction with *Trifolium repens* plant under drought condition	Igiehon and Babalola (2018)
Pseudomonas syringae	Pathogenic interaction with tomato, tobacco, olive and green beans	O'Brien et al. (2011)

interaction is commonly found in legume and rhizobia where bacteria infiltrate the plant root and form a specialized organ called nodule. Nodules formed by infiltration of bacteria into plants convert nitrogen to ammonia which is further used by plants and allows legumes to survive in nitrogen-depleted soil. In this way both legumes and bacteria benefit each other (Magnoli and Lau 2020). Such mutualistic interaction also takes place between plants and fungi where plants provide carbon to fungi in the form of sugar and organic molecules while the fungi capture water and nutrients from soil for the plants (Soto et al. 2009). Ectomycorrhizal and endomycorrhizal fungi (arbuscular mycorrhizal) are the two forms of fungi which interact with plants. Ectomycorrhizal fungi as the name suggests remain on the surface of plants and do not enter inside the plant tissues whereas endomycorrhizal fungi penetrate inside the plant tissue. Arbuscular mycorrhizal (AM) is the most common form which interacts with plants by the formation of hyphae accompanied by the development of appressorium leading to the formation of arbuscules and entry into the cortex of plant tissue (Scheublin and Van Der Heijden 2006; Badri et al. 2009). Metabolites spotted as strigolactones present in root exudates are found to attract AM fungi and there is hypothesis that strigolactones act as a signal for hyphal production in AM fungi (Badri et al. 2009).

2.2.2 Commensalism

Commensalism is a long-term interspecific interaction that is beneficial to one and has no effect on other. Chemolithotrophic ammonia-oxidizing bacteria and archaea convert ammonium to nitrite. *Nitrospira* and *Nitrobacter* then convert nitrite to nitrate. Nitrite is toxic to ammonia oxidizers; therefore, ammonium oxidizers are benefited. Both *Nitrospira* and *Nitrobacter* remain unaffected (Leung and Poulin 2008).

2.2.3 Amensalism

Chemicals released by one organism can harm or destroy another organism in the form of interaction. Amensalism also known as antibiosis occurs when one microorganism produces an antimicrobial agent that is harmful to other microorganisms.

For example, 2,4-DAPG (2,4-diacetylphloroglucinol) antibiotic produced by *Pseudomonas fluorescens* which provides resistance to protozoan *Acanthamoeba castellanii*, *Bacillus* sp., *Streptomyces* sp. and *Pseudomonas* sp. also produces bioactive lipopeptides that disrupt the cell membrane of pathogens such as oomycetes and *Naegleria americana* trophozoites and kill it (Soto et al. 2009; Igiehon and Babalola 2018).

2.2.4 Symbiosis

Parasymbiosis is the symbiosis in which new organism enters an existing symbiosis (Ponzio et al. 2016). Common symbiotic pathway (CSP) is initiated by binding of lipo-chitooligosaccharides (LCOs) to lysine motif (LysM) receptor present on the cell membrane of plants. Both legumes and non-legume plants have LysM receptors on their plasma membrane which binds to LCOs released by rhizobia and AM fungi (Leung and Poulin 2008).

2.2.5 Pathogenic Interaction

Pathogenic microbes can lead to progression of disease in plants and can stimulate plant defence system in them. Plant transmembrane receptors recognize general elicitors which are produced by pathogen and activate defence mechanisms known as microbial or pathogen-associated molecular patterns (MAMPs/PAMPs) and pattern recognition receptors (PRRs). PAMPs induce the first-line defence mechanism in plants. MAMPs/PAMPs further trigger two types of immunity: horizontal immunity which triggers MTI/PTI immunity and vertical immunity called as effector-triggered immunity (ETI)/R gene based. ETI is produced against avirulent proteins secreted by pathogens in plants. As a result the effector molecules released from MTI/PTI immunity or ETI induce hypersensitive response (HR) that restricts the entry of pathogens in plants. When pathogen releases elicitors, MAMPs/PAMPs and the PRRs present on plasma membrane prevent the colonization of pathogens and the infection. Defence factors produced are phenolic compounds, lignin, electrolytic leakage, phytoalexins and proteinase inhibitors. Nucleotide-binding leucine-rich repeat (NB-LRR) receptor has also been reported to play a significant role in defence against pathogens. NB-LRR blocks pattern recognition receptors (PRRs) via effector molecules and prevents infection (Imam et al. 2016; Soto et al. 2009).

2.3 Mechanisms of Plant-Microbe Interaction

2.3.1 Through Root Exudates

Roots secrete root exudates in the surrounding that have both positive and negative effects on the neighbouring plants and microbes (Bais et al. 2006, 2008). The region in which these compounds are secreted is known as rhizosphere. The rhizosphere contains three zones as the endorhizosphere that lies between cortical layer and endodermis, rhizoplane that lies between mucilage and epidermis, and ectorhizosphere which is nearby roots. Most of the microbes are commonly found in ectorhizosphere (Badri and Vivanco 2009). The exudates secreted in ectorhizosphere are amino acids, carbohydrates, phenolics, organic acid and other secondary metabolites such as volatile organic compounds (VOCs), jasmonic acid (JA) and salicylic acid (SA) which are of low molecular weight while mucilage (carbohydrate), proteins and lipopolysaccharides are of high molecular weight (Nayak and Mishra 2020; Narula et al. 2009; Vives-Peris et al. 2020). The plant growth-promoting rhizomicrobes (PGPR) are the microbes that are present in rhizosphere and are useful in defence priming and induced resistance in plant host (Mhlongo et al. 2018). Plant growth-promoting rhizobacteria (PGPR) benefit plants in direct and indirect ways. Nitrogen fixation, mineral solubilization, production of siderophore and phytohormone are the direct mechanisms whereas antibiotic production, lipopolysaccharides, hydrolytic enzymes and induced systemic resistance (ISR) are indirect mechanisms. Phenazine-1-carboxylic acid (PCA) is the antibiotic that affects *Sclerotium rolfsii* and *Gaeumannomyces graminis* which are involved in causing stem rot disease in groundnut and wheat plants (Olanrewaju et al. 2017; Backer et al. 2018).

Rhizospheric compounds are secreted by active and passive mechanisms (Vives-Peris et al. 2020). Active transport is mediated by ATP-binding cassette (ABC) and multidrug and toxic extrusion (MATE) membrane transporters. ABC transporters are primary active transporters that hydrolyse ATP for the transportation of various solutes while MATE are the secondary active transporters that enable the movement of different compounds across membrane using an electrochemical gradient. MATE also detoxify secondary metabolites such as phenol and metals (Moriyama et al. 2008; Baetz and Martinoia 2014). On the other hand, the passive transport involves diffusion, ionic channels and vesicle transport (More et al. 2019).

1. Diffusion: Gradient formation between the cytoplasm of root cells and rhizosphere facilitates the release of low-molecular-weight compounds such as sugars, carboxylic acid, amino acids and phenolics into rhizosphere.
2. Ionic channels: Several proteins are required for the activation of ionic channels which then facilitates the secretion of various carbohydrates such as malate and oxalate in large amount. There are two types of anionic channels: slow anion channel (SLAC) also known as S-type and fast anion channel (QUAC) also known as R-type (rapid type). Aluminium-activated malate transport (ALMT)

is the example of ionic channel where the Al^{3+} ions are used for the exudation of malate.

3. Vesicle transport: High-molecular-weight metabolites are secreted by vesicle transport. These molecules are enclosed in vesicles and are released into rhizosphere by exocytosis. Exuded metabolites are originated in the endoplasmic reticulum and Golgi apparatus from where they are transported to the cell membrane in enclosed vesicle active transport mechanism and released into the surrounding (Ryan et al. 2001; Neumann and Römheld 2007; More et al. 2019; Scavo et al. 2019).

2.3.1.1 Positive Plant-Microbe Interaction Mediated by Root Exudates

Positive interaction mediated by root exudates:

1. Legume nodulation by rhizobium: Rhizobia form symbiotic association with leguminous plants that results in fixation of atmospheric nitrogen via root nodules. This root nodule formation is stimulated by root exudates and expression of *nod* genes (Kiers et al. 2003; Abdel-Lateif et al. 2012).
2. Mycorrhizal fungi: As already stated earlier ectomycorrhizal and endomycorrhizal AM fungi are the two forms of fungi which interact with plants. Strigolactones in root exudates are found to draw the attention of AM fungi that further play a significant role in hyphal formation and development (Badri et al. 2009).
3. PGPR affects plants in direct and indirect ways. It enhances plant growth by supplying nutrients such as nitrogen, phosphorus, potassium and other essential minerals directly whereas it reduces or inhibits the effect of various pathogens as biocontrol agents indirectly. Table 2.2 summarizes the direct and indirect interactions mediated by PGPR between plants and microbes.
   (a) Direct plant-microbe interaction by PGPR
   PGPR is involved in nitrogen fixation symbiotically (with microbes) and asymbiotically (with free-living diazotrophs). It is also involved in phosphate solubilization and production of siderophores. Phosphate solubilization is done by phosphate-solubilizing microbes that convert insoluble phosphate to soluble phosphate which can be easily absorbed by plants. This process is initiated by the release of compounds such as protons, organic acid anions, hydroxyl ions, CO_2 and extracellular enzyme. *Enterobacter*, *Arthrobacter* and *Azotobacter* are the bacterial strains that solubilize phosphate by exopolysaccharide (EPS) (Zaidi et al. 2009). On the other hand, formation of siderophores occurs under iron-limiting condition. It stimulates the microbial uptake of iron from the soil and facilitates to plants. Hydroxamates, catecholates and carboxylates are the three major families of siderophores based on their functional group (Beneduzi et al. 2012; Gupta et al. 2015).
   (b) Indirect plant-microbe interaction by PGPR
4. Antibiosis: Antibiotics such as amphisin, 2,4-DAPG, phenazine and oomycin produced by microbes prevent pathogen proliferation in plants (Gupta et al. 2015; Sayyed et al. 2019).

Table 2.2 Type of plant-microbe interaction by PGPR

Type of plant-microbe interaction by PGPR	Classification	Description
Direct plant-microbe interaction by PGPR	Nitrogen fixation	PGPR can fix atmospheric nitrogen via symbiotic or asymbiotic interaction
	Phosphate solubilization	Phosphates are absorbed by plants in monobasic and dibasic forms
	Potassium solubilization	Potassium in soil results in lower crop yield. Solubilization of potassium by PGPR has improved the crop yield
	Siderophore production	Improves the iron uptake by plants
	Phytohormone production	PGPR enhance the release of IAA, cytokines and gibberellins in plants
Indirect plant-microbe interaction by PGPR	Antibiosis	Antibiotic activity by PGPR prevents phytopathogen attack
	Induced systemic resistance (ISR)	ISR is the defence system induced against biotic and abiotic stress or environmental stimuli
	Biofilm production	Production of EPS by certain bacteria results in the formation of biofilms

5. Induced systemic resistance (ISR): There are three pathways out of which two are involved in pathogenic attack and one in wounds and necrosis (Choudhary et al. 2007).
6. Biofilm production: Biofilm production requires two steps. First one is cell-cell adhesion and second is production of matrix of extracellular polymeric substance which is made up of polymer 1,6-linked N-acetylglucosamine (Costerton et al. 2005).

2.3.1.2 Negative Plant-Microbe Interaction Mediated by Root Exudates

Quorum-sensing inhibition is the negative plant-microbe interaction. Plants use symbiotic signals, volatiles and quorum sensing to colonize and communicate microbes. Small autoinducer (AI) molecules such as N-acyl-homoserine lactone (AHLs) are responsible for cell-cell interaction during the process of quorum sensing in plants (Fuqua et al. 2001; Czajkowski and Jafra 2009). Some species, such as the red algae *Delisea pulchra*, secrete halogenated furanones that mimic AHLs and can block quorum sensing (Bais et al. 2006). These molecules have the ability to disrupt QS-controlled processes including bioluminescence and swarming (Tan et al. 2020).

2.3.2 Siderophores

Iron is an essential element for plants and animals as it is involved in various functions such as nitrogen fixation, respiration, photosynthesis and detoxification. Plants absorb iron mostly in Fe^{2+} forms but sometimes in Fe^{3+} form. It is considered

as the fourth frequent element on earth (Huber 2005; Rout and Sahoo 2015). Regardless of this fact and due to low solubility of iron in alkaline soil, plants and microbes are not able to consume iron. To overcome this situation, microbes have developed specialized mechanism of "siderophores" to consume iron from the environment (Ahmed and Holmström 2014; Colombo et al. 2014).

Siderophore is a specialized structure of low molecular weight less than 10 KDa that has high affinity towards ferric ion (Sah and Singh 2015). It takes up Fe^{3+} ions from different habitats and facilitates to plants. It not only chelates iron but is also involved in weathering of soil minerals and enhancing of plant growth. Plant growth-promoting bacteria (PGPB) produce siderophores that bind to the iron and make it available to the host plant (Ansari et al. 2017). This mechanism is important as it provides iron to both plants and microbes and enhances their survival rate. PGPB are capable of converting Fe^{3+} to Fe^{2+}. Fe^{2+} is soluble in nature and can enter into bacterial cell easily whereas Fe^{3+} is insoluble and enters into the cell by siderophore (Scavino and Pedraza 2013; Ferreira et al. 2019). Siderophores produced by PGPB form a ferri-siderophore complex, which binds to a particular receptor present on the surface. Binding of complex to receptor is then followed by binding of transporter proteins to the complex (Pahari et al. 2017). These proteins then transport the complex to the cytoplasm. For bioremediation of iron, Gram-positive and Gram-negative bacteria use different transport mechanisms. Gram-negative bacteria contain lipopolysaccharide membrane on the outer side and TonB receptor located on the cytoplasmic membrane. Ferri-siderophore complex binds to lipopolysaccharide membrane and then binds to TonB receptor via crossing periplasmic space. Then from TonB receptor the complex binds to ATP complex which is also present on the cytoplasmic membrane and travels to cytoplasm where Fe^{3+} is converted to Fe^{2+}. Gram-positive bacteria have no lipopolysaccharide membrane. Therefore the complex directly binds to cytoplasmic membrane and travels to cytoplasm where Fe^{3+} is converted to Fe^{2+} (Page 2019; Yamano 2019).

Categorization of siderophores is due to their chemical structure and they are secreted by an array of PGPB groups. Such siderophores are hydroxamate, catecholate and carboxylate which are categorized on the basis of their chemical composition (Saha et al. 2016):

1. Hydroxamate siderophore: In bacteria hydrophilic siderophores are composed of alkylamines and acylated alkylamines whereas in fungi they are composed of hydroxylated and alkylated ornithine. N5-acyl-N5-hydroxyornithine and N6-acyl–hydroxylysine are the main components of siderophore in fungi.
2. Catecholate siderophore: It is composed of catecholate and hydroxyl group that binds to Fe^{3+} and converts them to Fe^{2+}. Catecholate siderophore is formed when two oxygen molecules, each from hydroxamate group and catecholate, join. It is only present in bacteria (Wittmann et al. 2004).
3. Carboxylate siderophore: Microbes such as *Sinorhizobium meliloti* and *Zygomycetes* produce carboxylate siderophores. These microbes for iron chelation use their functional groups such as hydroxyl and carboxyl groups. In addition, it also consists of one D-ornithine and two citric acid residues which

are linked by two amide bonds. Siderophores come in a variety of shapes, sizes and properties. Siderophores have conserved structures that bind to ferritin and lactoferrin molecules. Hydroxamate siderophores such as dihydroxybenzoylserine trimer derived from Enterobactin have complex structures and are more hydrophilic. Siderophores have hexadentate structures that fit the iron to six ferric coordination sites. Fe (III) affinity is higher in hexadentate siderophore than tetradentate siderophores (Carrano et al. 1996; Butler et al. 2021).

Siderophores also inhibit the growth of pathogens. *Pseudomonas putida* produces siderophores pseudobactins which inhibit the growth of the pathogen *Ralstonia solanacearum* in *Eucalyptus urophylla*, *Erwinia carotovora* in tobacco and *Botrytis cinerea* in tomato (Jain et al. 2016). Pseudobactins produced from *Pseudomonas fluorescens* protect the *Arabidopsis* plant from turnip crinkle virus and tobacco plant from tobacco necrosis virus (Voisard et al. 1994). *Rhizobium meliloti* RMP3 and RMP5 produce siderophores that protect the plant from the pathogen *Macrophomina phaseolina* (Arora et al. 2001).

2.4 Beneficial/Positive Interactions to Increase Plant Productivity

2.4.1 Nitrogen Fixation

Nitrogen fixation is an essential component of nitrogen cycle since it refills the total nitrogen content which is less vulnerable to leaching and volatilization. It is the process in which nitrogen is converted to ammonia by free-living bacteria or symbiotic bacteria and has the potential to sequester atmospheric CO_2 (Dixon and Kahn 2004). It is only limited to prokaryotes and absent in eukaryotes. Fermibacteria, cyanobacteria, proteobacteria, green sulphur bacteria, actinobacteria and archaea, mainly methanogens, are involved in nitrogen fixation. Nitrogen fixation microbes are aerobic (*Azotobacter*), facultative anaerobic (*Klebsiella*), anaerobic, heterotrophs (*Clostridium*), phototrophs (*Anabaena*), chemolithotrophs (*Leptospirillum ferrooxidans*), etc. (Rudnick et al. 2002).

Nitrogenases are the enzymes involved in nitrogen fixation. They are conserved metalloenzymes that reduce nitrogen (N_2) to ammonia. It contains two components: the smaller component is iron protein that transfers electrons to larger heterotetrameric component MoFe protein (Rubio and Ludden 2008; Luxem et al. 2020). MoFe is commonly found in diazotrophs and under MoFe-deficient condition some diazotrophs such as *Rhodobacter capsulatus* and *Azotobacter vinelandii* activate nitrogenase enzyme containing vanadium-Fe and Fe-Fe for nitrogen fixation. There are two metal centres present in MoFe protein: P cluster [8Fe-7S] and FeMo cofactor [MoFe7S9.homocitrate]. FeMo cofactor is involved in the reduction of Fe protein. Fe protein can undergo reduction by transfer of electrons from electron donor such as ferredoxin and flavodoxin to P cluster which is then transferred to

FeMo site. The process is Mg-ATP dependent. Inactivation of nitrogenase is mediated by DraT (dinitrogenase reductase ADP-ribosyltransferase) due to ADP-ribosylation of Fe protein whereas this process can be reversed by DraG (dinitrogenase reductase-activating glycohydrolase) resulting in the removal of ADP ribose from Fe protein, hence activating nitrogenase enzyme (Huergo et al. 2006; Moure et al. 2014). NifA genes in proteobacteria are activated by enhancer-binding protein along with RNA polymerase sigma factor. EBP region of Nif genes is conserved and contains amino regulatory domain and carboxy-terminal domain (Money et al. 1999; Martinez-Argudo et al. 2004). Nitrogenase is an oxygen-sensitive enzyme and gets inactivated in the presence of excess oxygen. The enzyme interacts with oxygen-responsive components FixL-FixJ-Fix-K which in turn inactivate the enzyme function (Oliveira et al. 2012). This is due to NifA and NifL genes. Under increased oxygen concentration NifL and NifA form inhibitory complex which results in the inhibition of NifA (Rudnick et al. 2002). This inhibition is regulated by GAF domain present in NifA. Another factor that controls the NifA genes under oxygen tension is oxidation and reduction of flavin molecules bound to N-terminal PAS domain of NifL. Inactivation of NifL genes results in the overexpression of NifA genes (Little et al. 2000). PII signalling is also an important factor for *nif* gene. For example, diazotrophs such as *Klebsiella pneumonia* GlnB proteins regulate NtrB-NtrC genes. The phosphorylation of NtrC is then followed by transcription of GlnK, NifA or NifL genes. Under nitrogen-limiting condition the uridylylated GlnB is followed by the phosphorylation of NtrC which activates sigma54. Sigma54 is dependent on nifLA and glnK promoter while under nitrogen-excess condition the non-uridylylated GlnB interacts with ghkL domain of NtrB that inhibits phosphorylation of NtrC and decreases the level of NifLA and glnK. This results in the expression of NifA. The interaction of GlnK with AmtB (ammonium transporter) is important for the PII signalling (Huergo et al. 2010). In high concentration of ammonium transporters, the GlnK becomes deuridylylated which further on decreases PII protein concentration in cytoplasm. Therefore the glnK-amtB is also an important factor for nitrogen fixation (Forchhammer 2008).

2.4.2 Phosphate Solubilization

Phosphate-solubilizing bacteria (PSB) can convert insoluble phosphorus to soluble phosphorus and act as biofertilizers. These biofertilizers are environmental friendly and enhance food productivity (Sharma et al. 2013). Furthermore, it induces production of phytohormones, enhances trace element availability and increases the efficiency of nitrogen fixation. *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kushneria*, *Paenibacillus*, *Rhodococcus*, *Serratia*, *Sinomona* and *Thiobacillus* genera have the most PSB (Zaidi et al. 2009; Gowami et al. 2019). Soil fungi can travel long distances faster than bacteria and can secrete acids such as lactic acid, acetic acid, oxalic acid, 2-ketogluconic acid and tartaric acid which are important for solubilization of phosphate. According to

reports 20% of actinobacteria such as *Actinomyces*, *Streptomyces* and *Micromonospora* are involved in phosphate solubilization (Barnard et al. 2013).

Inorganic phosphate solubilization by PSM: The organic acids with their carboxyl and hydroxyl ions are capable of forming complex with cations. The excretion of these organic acids results in pH drop that causes acidification in the surrounding which is followed by substitution of hydrogen ions with cations, leading to release of phosphorus ions in the surrounding (Sharma et al. 2013). Other compounds involved in the release of phosphorus are siderophores, CO_2, protons and hydroxyl ions. The secretion of organic acids in soil causes the acidification of microbial cells and surrounding (Rashid et al. 2004). Alternatively, there is another mechanism for solubilization of phosphorus with the H^+ translocation ATPase. In the process the cation is exchanged with H^+ surface. The gluconic acid is considered as the most important organic acid responsible for phosphate solubilization. Gram-negative bacteria solubilize phosphate by converting glucose to gluconic acid through direct oxidation (Sashidhar and Podile 2010).

Organic phosphorus mineralization by PSM: Phosphate mineralization is the solubilization of only organic phosphorus of the molecule and degrades the remaining portion. Sources reported that 30–50% of organic phosphate is present in soil and mostly present in the form of inositol phosphate. Phosphomonoesters, phosphodiesters, phospholipids, nucleic acids and phosphotriesters are some of the other organic phosphorus compounds (Kalayu 2019). Organic P is also present in large amounts as xenobiotic phosphonates such as pesticides, detergent additives, antibiotics and flame retardants (Oehl et al. 2004). Organic compounds have high molecular weight and are resistant to chemical hydrolysis; therefore it is necessary to convert them into soluble ionic phosphate like HPO_4^{2}, H_2PO_4 or low-molecular-weight organic phosphate before being assimilated by the cell (Alori et al. 2017). Phosphate mineralization is accomplished by the use of various enzyme classes. The enzymes that dephosphorylate thephosphor-ester or phosphor-anhydride bond of organic compounds are known as non-specific acid phosphatases (NSAPs). NSAP enzymes are phosphomonoesterases and secreted by PSM (Sharma et al. 2020). Phytase is another enzyme released by PSM which is involved in phosphate solubilization. This enzyme releases phosphorus from phytate obtained from plant seed and pollen in the soil (Rodríguez et al. 2007).

2.4.3 Biofertilizers

The widespread use of fertilizers on crops has collapsed the productivity of agricultural system and increased the cost of cultivation at a rapid rate which has resulted in stagnant farmer income and has given rise to the concern about food security (Mazid and Khan 2015). Nitrogen- and phosphate-based chemical fertilizers are commonly used to enhance crop productivity but also have the potential to pollute the atmosphere and groundwater as well as cause eutrophication and soil acidification. Plants grown in high level of nitrogen lead to the formation of carcinogenic compounds such as nitrosamines which are found in foods such as lettuce and spinach leaves.

Nitrate is the basic component of nitrogen fertilizer and is involved in water contamination (Vessey 2003; Vassilev et al. 2015). The utilization of nitrogenous fertilizers by plants is only 50%, 2–20% of fertilizer is evaporated, 15–25% reacts with organic compounds in clay soil and the remaining 2–20% interferes with surface and groundwater (Savci 2012). Another disadvantage of nitrogenous fertilizers is that they are not completely absorbed by plants and destroy underground and aboveground habitat. It affects not only plants but also humans by causing health problems such as methemoglobinemia or blue baby syndrome. The main cause of this disease is the accumulation of nitrate in blood which leads to conversion of nitrate to nitrite and inhibition of oxygen transport to cells and the source is nitrate-contaminated water (Elsayed et al. 2020). Nitrate can be removed by electrocoagulation method by adsorption on $Al(OH)_3$ and reduction of nitrate to nitrite or ammonium (Amarine et al. 2020). Biofertilizers on the other hand are the alternative to chemical fertilizers which are now needed to reduce our reliance on nitrogen fertilizers. Biofertilizers provide a use of renewable inputs by combining biological with beneficial microorganisms to increase crop productivity and to impart nutrients to farm (Barsha et al. 2021). Microbes in biofertilizers colonize the rhizosphere and encourage the nutrient status of the host plant when applied to plant surface, seed or soil (Mishra et al. 2021).

2.4.3.1 Production of Biofertilizer

The production of biofertilizer includes isolation, identification and functional characterization of desired and non-toxic microbes that promote plant growth. These microbes are isolated from soil or rhizosphere. Microbes are then grown in culture media and qualitative testing is performed to characterize the microbial strain functional properties. Then the pure strain based on the desired requirement of biofertilizers such as nutrient solubilization, nutrient mobilization, nitrogen fixation and phytohormone production is chosen. The selected strain is then determined by growing them on selective media and quantitative testing. Then the formulation material such as granular powder or slurry is chosen. The formulation material can be liquid or solid. The carrier is used to keep microbes alive. Next step is the cultivation of selected strain in optimal conditions in the laboratory which will increase the number of selected strains. Next step is prototyping in which the best product is chosen and the last step involves the testing of product in field and in different conditions to determine its efficiency and limitations (Mącik et al. 2020; Renjith et al. 2020; Raimi et al. 2021; Roy 2021).

2.4.3.2 Different Types of Biofertilizers

Types of biofertilizers are as follows:

1. Phosphate-solubilizing biofertilizer: Phosphate-solubilizing bacteria (PSB) have the potential to convert insoluble inorganic phosphorus to soluble forms by acid production, chelation and ion-exchange method. PSB fix phosphorus in the form of HPO_4^{2-} and H_2PO_4. *Pseudomonas*, *Burkholderia*, *Micrococcus*, *Erwinia*, *Agrobacterium*, *Achromobacter* and *Flavobacterium* are the bacterial population

that solubilizes inorganic phosphate compounds such as rock phosphate, tricalcium phosphate, dicalcium phosphate and hydroxyapatite (Mącik et al. 2020; Tian et al. 2021).

2. Phosphate-mobilizing biofertilizers: Phosphate-mobilizing microbes (PMM) scavenge phosphate from plants and mobilize it. Mycorrhizae are the important phosphate mobilizers in the symbiotic relationship with roots of the plants where fungus gets carbon from plants and plants get benefitted by micro- and macronutrients from fungus (Abhijith et al. 2020; Mącik et al. 2020).
3. Zinc-solubilizing biofertilizers: Zinc is the much-needed micronutrient of a plant. Zinc sulphate is expensive to use and therefore zinc-solubilizing bacteria such as *Bacillus* sp. are now being preferred. Zinc-solubilizing microbes combined with cheaper zinc compounds such as zinc oxide, zinc carbonate and zinc sulphide are also being used (Khoshru et al. 2020; Pradhan et al. 2021).
4. Nitrogen-fixing biofertilizers: Nitrogen fixation is the second most crucial mechanism in plants after photosynthesis in which nitrogen gas is converted to ammonium. Nitrogen fixation can generate 300–400 kg/N/ha/year. Nitrogen-fixing bacteria are *Rhizobium*, *Azospirillum*, *Cyanobacteria*, *Azolla* and *Azotobacter*. Rhizobium has symbiotic relationship with leguminous and non-leguminous plants such as parasponia which can fix 50–100 kg nitrogen. *Azospirillum* sp. belongs to Spirillaceae family and is heterotrophic in nature. It can fix 20–40 kg of nitrogen. These species are Gram negative and are found in rice fields. Cyanobacteria and Azolla are found in rice fields and have the potential to fix 20–30 kg of nitrogen. *Azotobacter* is a Gram-negative free-living bacterium which is present in rhizospheric soil of various plant species. It can only fix 10 mg of nitrogen and 1 g of carbon (Kour et al. 2020; Yadav 2020).
5. Sulphur-oxidizing biofertilizers: Combination of *Thiobacilli* with sulphur makes alkali soil suitable for crop cultivation. *Thiobacillus* produces sulphuric acid in soil which enhances nutrient mobilization and attracts other nutrients such as phosphate, potassium, calcium, manganese, aluminium and magnesium to increase their levels in soil (da Silva et al. 2020).
6. Silicate-solubilizing biofertilizers: Silicate-solubilizing bacteria not only provide H^+ ions to the soil but also maintain acidic condition. It also adds organic compounds such as citric acids, oxalic acid and hydroxyl carbolic acid to soil. It can also remove organic acid from soil by forming complex with cations (Mącik et al. 2020).

2.4.3.3 Advantages of Biofertilizers

Biofertilizers are cost effective and easy to produce in large quantities. Following are the most important benefits of biofertilizers (Akram et al. 2020; Yadav 2020; Sansinenea 2021):

(a) Biofertilizers enrich the soil with essential nutrients and microorganisms.
(b) Biofertilizers are involved in nitrogen fixation.
(c) Biofertilizers make plants more resistant against stress by facilitating the hormones and antimetabolites.

(d) It is also involved in bioremediation of heavy plants in soil.
(e) It enhances crop productivity by 25%.
(f) It is involved in removing toxins from the ecosystem.

2.4.4 Biopesticides

Pesticides have been used to prevent crops and livestock from pests for many years but despite their beneficial effects they have also given rise to environmental and food safety concerns. Furthermore they have reduced crop productivity and caused soil degradation, groundwater contamination and nutrition deficiency in plants (Copping and Menn 2000; Popp et al. 2013). Biopesticides are advantageous over pesticides as they are less detrimental to people's health and improve plant nutritional status (Nayak et al. 2018). Biopesticides contain living pathogenic microbes that target specific pests. Biofungicides, bioherbicides and bioinsecticides are among them (Gupta and Dikshit 2010; Leng et al. 2011).

2.4.4.1 Classification of Biopesticides

1. Biopesticides contain active ingredients such as bacteria, fungi, virus, protozoa or algae. These active ingredients target unique pests and should be monitored by humans to ensure that they do not affect essential species. For example, *Bacillus thuringiensis* or Bt secretes a protein that is poisonous to many insect pests (Senthil-Nathan 2015).
2. Plant-incorporated protectants (PIPs): Plants secrete pesticidal substances after the insertion of pesticidal gene into the plant. The pesticidal gene transferred to plants results in the transcription of this gene and produces a specific protein that kills the pest. Insertion of the Bt pesticidal gene into plants produces the protein that kills the pest, which is an example of plant biopesticide (Matten et al. 2012; Nelson and Alves 2014).
3. Biochemical pesticides: Biochemical pesticides produce scented plant extracts to trap insects or may interfere in the mating process of insects in order to control pest. Special committee made by the Environmental Protection Agency (EPA) makes decisions regarding the same (Sarwar 2015).
   (a) *Bacillus thuringiensis*: This is widely used as a biopesticide and mainly functions against lepidopterous pests such as bollworm and stem borers. Once entered into the midget of pests, Bt secretes toxins in midgut and kills the pest. The strains such as *Kurstaki*, *Galleria* and *Dendrolimus* are subspecies of *Bacillus thuringiensis* used as biopesticide.
   (b) *Baculovirus* is involved in killing various harmful plant pests such as Lepidoptera that affect cotton, rice and vegetables but is restricted to small areas. It is manufactured by integrated pest management and agricultural departments on a small scale (Szewczyk et al. 2006).
   (c) *Trichoderma* is a biopesticide that kills pests on major pulses. *Trichoderma* species such as *T. harzianum*, *T. asperellum*, *T. longibrachiatum* and

T. reesei are isolated from various regions and have been found to act as biopesticides in pulses (Harman et al. 2012).

(d) Antifungal activity of bacterial endophytes such as *Pseudomonas*, *Bacillus* and *Paenibacillus* controls pathogens like *Rhizoctonia bataticola*, *Fusarium udam*, *F. oxysporum* and *S. rolfsii* in pulses (Kumar et al. 2013).

(e) *Verticillium* and *Paecilomyces* are used as biopesticides in legume plants while *Paecilomyces*, *Pochonia chlamydosporia*, *Aspergillus nidulans* and *T. harzianum* are used as biopesticides in soybean (Mishra et al. 2018).

2.4.5 Phytostimulants

Phytostimulant is any substance or microbe that enhances nutritional status, crop quality, crop productivity and abiotic stress tolerance when added exogenously to plant or soil. According to the European Biostimulant Industry Control (EBIC), biostimulant added to crops or rhizosphere improves nutrient uptake, crop quality and abiotic stress tolerance (Calvo et al. 2014; Du Jardin 2015). Phytostimulants can be living or non-living. Living-based phytostimulants are rhizospheric microbes such as PGPR whereas non-living phytostimulants are humid acid, amino acid, nitrobenzene, gibberellic acid and seaweed (Du Jardin 2015; Yakhin et al. 2017). Humic acid is formed from decomposition of microbes such as bacteria and fungi and chemical degradation of plants and animals. Humic acid combined with fulvic acid forms organic compounds that increase the water penetration in soil and reduce the toxins and harmful substances absorbed by plants (Peña-Méndez et al. 2005). Amino acids are important for phytohormones, seed germination, cell division, seed size, yield, pollination, fruit production and metabolic processes in plants. Therefore, the exogenous supply of amino acid to plants enhances plant growth in many ways (Kocira 2019). Nutritive elements such as iron, seaweed, nitrogen, manganese and potassium also act as phytostimulants. Seaweed such as brown, red and green algae are used. Brown seaweed including *Ascophyllum nodosum*, *Sargassum* sp., *Laminaria* sp. and *Turbinaria* sp. are focused as phytostimulants that provide enhanced nutrient adsorption, faster seed germination, fruit quality, flowering, etc. (Khan et al. 2009; Battacharyya et al. 2015).

Phytostimulant formulation is a very important process for the development and efficiency of plant growth. In this process natural raw materials are converted to effective, safe and economical product that can be stored, transported and applied. Natural raw materials used are seeds, leaves and roots. Their biological activity is assessed by using organic solvents (Hazra 2018; Rouphael and Colla 2018; Hazra and Purkait 2019).

1. Inputs given for the formulation design:
   - Physical, chemical and biological properties of compounds
   - Marketing inputs such as reliability, economy, user friendliness and protection
   - Application inputs such as plant, climate and equipment
   - Manufacturing process input such as quality control and production equipment

2. Developmental steps:
   - Research such as laboratory preparation, physical and chemical test
   - Investigation of formulation involving bio-efficacy, phytotoxicity, shelf life, analytical method and small-scale field trials
   - Commercial level involving packaging design
3. Other formulation design requirements:
   - It should not have negative effect when applied.
   - Product should be of greatest biological impact and less expensive.
   - Affordable large-scale manufacturing.
   - Packaging and storage should be clean.
   - Long shelf life and easy to transport.
   - It should be registered properly.

2.4.5.1 Advantages of Phytostimulants

Advantages of phytostimulants (Posmyk and Szafrańska 2016; Povero et al. 2016) are as follows:

1. Improves the efficiency of crop quality and metabolism by improving plant health and vigour
2. Improves crop tolerance to abiotic stress and recovery
3. Improves nutrient absorption, translocation and utilization
4. Improves the quality of products such as sugar content, colour and fruit seedling
5. Improves water efficiency and nutrient uptake

2.5 Plant-Microbe Interactions in Protecting Against Biotic Stresses

2.5.1 Plant Defence Mechanism Against Herbivore

Herbivores utilize a variety of feeding strategies to acquire nutrients from plants. Some of the herbivorous arthropods feed on small or single plants and are known as monophagous while some have association with a diverse range of host plants (Santamaria et al. 2013). Herbivores are potential feeders and can consume all plant organs. Some of the herbivores such as spiders or thrips contain tube-like structure and some such as aphids and whiteflies contain specialized stylets that suck nutrients from parenchymatic cells or vascular tissues of the plant (Skaljac et al. 2019). Leaf eaters are found to be resistant against plant defence system. They eat epidermal cell layers in leaf tissues and have adapted the digestive strategies and digestive physiology such as detoxification system, protease inhibitors, pH and endo- or exo-peritrophic compartments (Pallini et al. 1997; Alba et al. 2012).

Plants react against pathogens by activating defence mechanisms that inhibit, destroy or alter pathogen metabolism. Plant defence system involves physical barriers such as surface waxes, trichomes, thorns, spines and cell wall modification, and toxins to reduce herbivore growth and digestion (Belete 2018; Tiku 2021).

Trichomes are hairlike epidermal structures that are present on the leaves and stems and provide primary protection against herbivores. They also prevent the attachment of insect/mite eggs to plant tissues after oviposition (Wu et al. 2021). Trichomes also secrete toxic metabolites, protease inhibitors and harmful compounds against pests and herbivores. For the entrapment of herbivores other materials such as latex, oils, resins and other sticky materials are also released by plants. These substances are mostly released from wounded plants. Latex is composed of abundant proteins such as lectins, proteases and secondary metabolites such as terpenoids, alkaloids, furanocoumarins and phenolics (Noman et al. 2021). Other pathogen-released molecules are herbivore-associated elicitors (HAEs) and herbivore-associated molecular patterns (HAMPs) which are detected by plant defence mechanism. Detection of these elicitors activates a cascade of events involving Ca^{2+} resulting in the development of reactive oxygen species (ROS), phosphorylation cascades and transcriptional activation events, which triggers plant defence responses. Salivary enzymes such as glucose oxidases, oxidoreductases, beta-glucosidases and fatty acid amino conjugates (FACs) of herbivorous insects are examples of HAMPs and HAE (Turlings and Ton 2006; Kaplan 2012).

Plant defence system is also elicited by cryptic peptides, inceptins and volicitin. Inceptins are the proteolytic product of ATP synthase c subunit (cATPC) which was discovered in cowpea (Schmelz et al. 2006). Volicitin is the FAC molecule present in *Spodoptera exigua* which was the first FAC to be studied. Inceptin and volicitin bind to plant cell membrane in a reversible manner via specific receptors and trigger the release of volatile terpenes which is considered as indirect defence (Aljbory and Chen 2018).

2.6 Plant-Microbe Interactions in Protecting Against Abiotic Stress

2.6.1 Water

Osmotic stress caused by salinity, cold or drought increases the risk of dehydration in plants. To reduce the dehydration stress, osmoprotectants are released that lowers the water potential and prevents water loss (Singh et al. 2015). Some of the microbes such as *Arabidopsis* release VOCs in order to prevent water loss. Such VOCs are choline, glycine and betaine. *Arabidopsis* plant, when treated or inoculated with GBo3 in the presence of VOCs, showed improved tolerance to dehydration stress (Zhang et al. 2008). VOC treatments to plants also increase the level of phosphoethanolamine N-methyltransferase (PEAMT) transcripts for inducing dehydration tolerance. PEAMT performs three methylation steps and converts phosphoethanolamine to phosphocholine. PEAMT is considered to be an important enzyme in the biosynthesis pathway of choline, glycine, betaine and VOC that triggers plant tolerance to dehydration (Liu and Zhang 2015). Another compound 2,3-butanediol, found in GBo3, also promotes water stress tolerance and disease resistance (Cho et al. 2013). 2,3-Butanediol in VOCs of several other PGPR stains such as *Pseudomonas chlororaphis* strain O6 is capable of inducing ISR in plants

(Mercado-Blanco and Bakker 2007; Farag et al. 2013). 2,3-Butanediol in Arabidopsis also showed improved drought tolerance in *P. chlororaphis* O6 after inoculation of *Arabidopsis* with *P. chlororaphis* O6. 2,3-Butanediol is found to affect JA, SA and ethylene (Han et al. 2006; Park et al. 2018). It has been reported that 2,3-butanediol is also involved in increased production of nitric oxide (NO) and hydrogen peroxide in plants. It suggested that NO signalling significant in drought tolerance is induced by 2,3-butanediol. Abscisic acid (ABA) is also known to regulate response under dehydration condition. However, when treated with GBo3 VOC3, the osmoprotectants were found to be unrelated to ABA. Therefore the relation between ABA, NO, SA and hydrogen peroxide signalling pathways plays an indirect role in water stress (Vishwakarma et al. 2020). PGPR was also found to play a role in drought tolerance. Wheat inoculated with *Bacillus safensis* strain W10 has been found to increase antioxidant responses at the enzymatic and metabolite levels (Vurukonda et al. 2016; Sarkar et al. 2018). PGPR-treated potato increased proline aggregation and gene expression of ROS scavenging enzymes which increased tolerance to abiotic stresses (Batool et al. 2020). EPS produced by some PGPR strains such as *Pseudomonas aeruginosa* strain Pa2 has been found to retain soil moisture content and enhance drought tolerance in plants.

2.6.2 Temperature

Temperature controls the physiology and metabolism of microbes by various mechanisms. Microbes adapt to low and high temperature due to their intrinsic properties to survive in such adverse conditions (Theocharis et al. 2012). These microorganisms have developed mechanisms such as expression of heat- and cold-tolerant enzymes in order to protect their proteins, membranes and nucleic acids. The expression of heat-shock proteins such as HSP20, HSP60, HSP70, HSP90, HSP100 and ROS scavenging enzyme such as catalase and ascorbate peroxidase is essential for heat and cold tolerance (Hasanuzzaman et al. 2013). Microbes tolerating heat and cold stress are classified into several groups based on their growth: psychrophilic (true psychrophilic and psychrotrophs), mesophiles and thermophiles. True psychrophilic microbes grow best at or less than 15 °C while psychrotrophs grow best between 20 and 30 °C. Mesophiles grow best at 20–40 °C and thermophiles grow best at 50 °C or higher. Heat stress triggers the expression of a gene involved in microbe survival. During heat stress, the Dnak gene in *Alicyclobacillus acidoterrestris* increases its expression to code HSP, which protects it from heat. Heat-shock protein induction is an essential factor for surviving under extreme heat stress. Most of the microbes survive heat stress by obtaining efficient nutrients and water uptake, as well as increasing photosynthesis (Jiao et al. 2015; Zhao et al. 2021). Trehalose produced by microbes such as *Saccharomyces cerevisiae* is another important compound that resists heat stress and prevents microorganisms from heat, cold and oxidative stress by inducing denaturation of heat stress-induced protein (Eleutherio et al. 1993).

Microbes such as *Arthrobacter nicotianae*, *Pseudomonas carina* and *Brevundimonas terrae* survive at cold temperature because of PGPR. Psychrophilic bacteria isolated from Antarctica contain heat-shock proteins and other compounds such as trehalose that protect it from such low temperature. High temperature for some microbes such as *Agrobacterium* has been reported to inhibit pilus formation and virulence while some bacteria such as soft-rot bacterium *Pectobacterium atrosepticum* showed increased virulence, development of plant cell wall-degrading enzyme and quorum sensing (Kumar and Verma 2018; Kushwaha et al. 2020). Some rhizosphere bacteria and endophytes can help plants cope with the negative effects of temperature stress. The symbiotic relationship between tropical panic grass *Dichanthelium lanuginosum* and the fungus *Curvularia protuberata* helps both species to thrive at high soil temperature. *Burkholderia phytofirmans* strain has antifungal properties and has been found to increase heat resistance in tomatoes, cold tolerance in grapevine, drought in wheat, and salt and freezing in Arabidopsis (Govindasamy et al. 2018).

Another mechanism for temperature tolerance is the circadian clock that becomes activated with change in environmental conditions (Seo and Mas 2015). Circadian clock-associated 1 (CCA1) and late elongated hypocotyl are two transcription factors (LHY) which are involved in this process. Glycine-rich RNA-binding protein 7 is an RNA-binding protein that binds directly to the PRR transcripts. Genes flagellin-sensitive 2 (FLS2) and Ef-tu receptor (EFR) proteins act downstream of CCA1 and LHY genes (Dong et al. 2011; Cheng et al. 2019). PRR is responsible for the control of stomatal closure and prevents pathogen invasion. The clock also influences the biosynthesis and signalling of the defensive hormones SA and jasmonate in addition to PTI and ETI (Robert-Seilaniantz et al. 2011).

2.6.3 Heavy Metal

Heavy metal accumulation in soil is mainly caused by industrialization, farming methods and anthropogenic activities. Heavy metals are with greater density than 4 g/cm^3 and have a negative effect on plants as well as human health (Mishra et al. 2019). Therefore, it is really important to remove heavy metals from the environment. It is a cost-effective and long-term method (Nagajyoti et al. 2010). Plant-associated microbes such as mycorrhiza, rhizobacteria and firmicutes have the potential to promote plant growth. Biosorption, bioaccumulation, immobilization, bioventing and direct-indirect enzymatic reduction are the methods involved in bioremediation of heavy metals (Mishra 2017; Padhan et al. 2021).

1. Biosorption: metals are absorbed on negatively charged microbial surface.
2. Bioaccumulation: metals enter into the cells through proteins and get accumulated in the cells.
3. Immobilization: metals get fixed to Fe oxides and organic colloids.

4. Direct and indirect enzymatic reduction: in direct reduction method the metals are reduced by organic oxidation and in indirect reduction method the metals are reduced by Fe or S oxidation process.
5. Bioventing: this supplies O_2 to soil microbes and favours their growth.

Nitrogen fixation, nutrient mobilization, siderophores and phosphate solubilization are other methods used for removal of heavy metals (Rajkumar et al. 2009). AM fungi reduce cadmium stress in plants by decreasing malonaldehyde and hydrogen peroxide. *Enterobacter* sp. and *Klebsiella* sp. are effective in removing Cd, Pb and Zn from polluted soil (Rajkumar et al. 2009; Mitra et al. 2018). Arsenic-resistant bacteria (ARB) from *Pteris vittata* enhanced phosphate solubilization. The siderophore production from *Pteris vittata* improves plant growth and nutrition uptake (Das et al. 2017). Two bacterial strains *Bradyrhizobium japonicum* E109 and *Azospirillum brasilense* Az39 accumulate arsenic from the soil. Extracellular and intracellular aggregation, sequestration and biotransformation are the main mechanisms adopted by microbes to cope (Verma et al. 2019; Vezza et al. 2020). Heavy metals can be totally degraded by certain microbes. *Pseudomonas* sp. MBR, for example, shows the biotransformation and elimination of single Fe^{3+}, zinc and Cd citrate complex.

Endophytic microbes are capable of synthesizing nitrogenase under metal stress and low-nitrogen conditions. Endophytic microbes such as *Burkholderia* sp., *Rahnella* sp., *Sphingomonas* sp. and *Acinetobacter* sp. synthesize nitrogenase and fix atmospheric nitrogen in *Populus trichocarpa* and *Salix sitchensis* (Ma et al. 2016; Chandran et al. 2020). In addition, PGPM produces a number of low-molecular-weight organic acids that are useful in phytoremediation. Gluconic, oxalic and citric acids are the most powerful in mobilizing heavy metals and making them available to plants. Furthermore oxidation or reduction influences heavy metals such as arsenic, selenium, chromium and mercury (Mandal et al. 2016; Swamy et al. 2019). Another method for heavy metal mobilization is biomethylation. Biomethylation is the transfer of a methyl group by microbes. Bacteria are involved in the methylation of Pb, Hg, Se, As, Tn and Sn. Phytochelatins (PCs) are metal-binding cysteine-rich peptides that are synthesized from glutathione in certain fungi and plants against stress (Ullah et al. 2015).

Indirect mechanism of phytoremediation improves the growth of plants and inhibits infection caused by pathogen and increased heavy metal accumulation (Ma et al. 2016). High concentration of heavy metals in rhizosphere disrupts the uptake of nutrients and inhibits plant growth. PGPB are capable of fixing atmospheric nitrogen and protecting plants from metal stress through symbiotic association. Phosphorus is present in higher amount in soil but is inaccessible to plants in its complex form (Pii et al. 2015). Microbes in response to metal stress produce organic acids and supply to plants. Endophytic bacteria help plants thrive in metal-stressed environments by controlling pathogens or inducing systemic resistance (Parray et al. 2016).

2.7 Conclusion

The population of humankind is explosively increasing and has reached 7.8 billion at the end of 2020 which is further expected to reach 9.9 billion by 2050. Combined with population explosion, diversification of land usage will result in less arable land available for food production. It will be crucial to achieve a balance between different components associated with crop productivity for food sustainability. The nutrient composition of soil is an integral factor in soil fertility and stability. Therefore, soil structure needs to be intact for continuous crop production. The role of microbes associated with different parts of the plant is paramount in nutrient uptake and protection against biotic and abiotic stresses. Understanding these interactions will go a long way in exploiting these beneficial microorganisms as biofertilizers, phytostimulants and biopesticides which will enable sustainable agricultural practices in the future.

References

Abdel-Lateif K, Bogusz D, Hocher V (2012) The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria. Plant Signal Behav 7(6):636–641

Abhijith R, Vennila A, Purushothaman CS et al (2020) Phosphorus mobilizers from mangrove ecosystem and their role in desalination of agricultural lands. In: Naresh RK (ed) Research trends in agriculture sciences, vol 23. AkiNik Publications, New Delhi p, pp 61–81

Ahmed E, Holmström SJM (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7(3):196–208

Akram MS, Cheema MA, Waqas M et al (2020) How the biofertilizers play a role in the regulation and sustainability of agriculture. Preprints 2020:2020030262

Alba JM, Bleeker PM, Glas JJ et al (2012) The impact of induced plant volatiles on plant-arthropod interactions. In: Smagghe G, Diaz I (eds) Arthropod-plant interactions. Progress in biological control, vol 14. Springer, Dordrecht, pp 15–73. https://doi.org/10.1007/978-94-007-3873-7_2

Aljbory Z, Chen MS (2018) Indirect plant defense against insect herbivores: a review. Insect Sci 25(1):2–23

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.00971

Amarine M, Lekhlif B, Mliji E et al (2020) Nitrate removal from groundwater in Casablanca region (Morocco) by electrocoagulation. Groundw Sustain Dev 11:100452

Ansari RA, Mahmood I, Rizvi R et al (2017) Siderophores: augmentation of soil health and crop productivity. In: Kumar V, Kumar M, Sharma S, Prasad R (eds) Probiotics in agroecosystem. Springer, Singapore, pp 291–312. https://doi.org/10.1007/978-981-10-4059-7_15

Arora N, Sun Chul S, Maheshwari DK (2001) Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81(6):673–677

Arzanesh MH, Alikhani HA, Khavazi K et al (2011) Wheat (*Triticum aestivum L.*) growth enhancement by *Azospirillum* sp. under drought stress. World J Microbiol Biotechnol 27(2): 197–205

Backer R, Rokem JS, Ilangumaran G et al (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32(6): 666–681. https://doi.org/10.1111/j.1365-3040.2008.01926.x

Badri DV, Weir TL, der Lelie D et al (2009) Rhizosphere chemical dialogues: plant–microbe interactions. Curr Opin Biotechnol 20(6):642–650

Baetz U, Martinoia E (2014) Root exudates: the hidden part of plant defense. Trends Plant Sci 19(2):90–98

Bais HP, Broeckling CD, Vivanco JM (2008) Root exudates modulate plant—microbe interactions in the rhizosphere. In: Karlovsky P (ed) Secondary metabolites in soil ecology. Soil biology, vol 14. Springer, Berlin, Heidelberg, pp 241–252. https://doi.org/10.1007/978-3-540-74543-3_11

Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7(11):2229–2241. https://doi.org/10.1038/ismej.2013.104

Barsha KC, Pandey B, Chand H et al (2021) Promotion of organic and sustainable agriculture through the use of bio-fertilizers. J Wastes Biomass Manag 3(1):01–08. https://doi.org/10.26480/jwbm.01.2021.01.08

Batool T, Ali S, Seleiman MF et al (2020) Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. Sci Rep 10(1):1–19

Battacharyya D, Babgohari MZ, Rathor P et al (2015) Seaweed extracts as biostimulants in horticulture. Sci Hortic-Amsterdam 196:39–48

Belete T (2018) Defense mechanisms of plants to insect pests: from morphological to biochemical approach. Trends Tech Sci Res 2(2):30–38

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35(4):1044–1051. https://doi.org/10.1590/s1415-47572012000600020

Butler A, Harder T, Ostrowski AD et al (2021) Photoactive siderophores: structure, function and biology. J Inorg Biochem 221:111457. https://doi.org/10.1016/j.jinorgbio.2021.111457

Buttimer C, McAuliffe O, Ross RP et al (2017) Bacteriophages and bacterial plant diseases. Front Microbiol 8:34. https://doi.org/10.3389/fmicb.2017.00034

Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383(1): 3–41. https://doi.org/10.1007/s11104-014-2131-8

Carrano CJ, Drechsel H, Kaiser D et al (1996) Coordination chemistry of the carboxylate type siderophore rhizoferrin: the iron (III) complex and its metal analogs. Inorg Chem 35(22): 6429–6436

Chandran V, Shaji H, Mathew L (2020) Endophytic microbial influence on plant stress responses. In: Kumar A, Radhakrishnan EK (eds) Microbial endophytes: functional biology and applications. Woodhead Publishing, Sawston, pp 161–193. https://doi.org/10.1016/C2019-0-00366-4

Cheng YT, Zhang L, He SY (2019) Plant-microbe interactions facing environmental challenge. Cell Host Microbe 26(2):183–192

Cho S-M, Kim YH, Anderson AJ et al (2013) Nitric oxide and hydrogen peroxide production are involved in systemic drought tolerance induced by 2R, 3R-butanediol in *Arabidopsis thaliana*. Plant Pathol J 29(4):427–434. https://doi.org/10.5423/PPJ.OA.07.2013.0069

Choudhary DK, Prakash A, Johri BN (2007) Induced systemic resistance (ISR) in plants: mechanism of action. Indian J Microbiol 47(4):289–297

Colombo C, Palumbo G, He JZ et al (2014) Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. J Soils Sediments 14(3):538–548. https://doi.org/10.1007/s11368-013-0814-z

Compant S, Samad A, Hanna Faist H et al (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J Adv Res 19:29–37

Copping LG, Menn JJ (2000) Biopesticides: a review of their action, applications and efficacy. Pest Manag Sci 56(8):651–676

Costerton J, Montanaro L, Arciola CR (2005) Biofilm in implant infections: its production and regulation. Int J Artif Organs 28(11):1062–1068

Czajkowski R, Jafra S (2009) Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules. Acta Biochim Pol 56(1):1–16

da Silva EVN, Stamford NP, Oliveira WS et al (2020) Nutrient availability in phosphate and potassic rocks induced by *Acidithiobacillus* oxidizing bacteria to produce biofertilizers. Aust J Crop Sci 14(2):250–258

Das S, Chou M-L, Jean J-S et al (2017) Arsenic-enrichment enhanced root exudates and altered rhizosphere microbial communities and activities in hyperaccumulator Pteris vittata. J Hazard Mater 325:279–287. https://doi.org/10.1016/j.jhazmat.2016.12.006

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2(8): 621–631. https://doi.org/10.1038/nrmicro954

Dong MA, Farré EM, Thomashow MF (2011) Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in Arabidopsis. Proc Natl Acad Sci 108(17):7241–7246. https://doi.org/10.1073/pnas.1103741108

Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic-Amsterdam 196:3–14

Eleutherio EC, Araujo PS, Panek AD (1993) Protective role of trehalose during heat stress in *Saccharomyces cerevisiae*. Cryobiology 30(6):591–596. https://doi.org/10.1006/cryo.1993.1061

Elsayed SIM, Glala AA, Abdalla AM et al (2020) Effect of biofertilizer and organic fertilization on growth, nutrient contents and fresh yield of dill (*Anethum graveolens*). Bull Natl Res Cent 44(1): 1–10. https://doi.org/10.1186/s42269-020-00375-z

Farag MA, Zhang H, Ryu C-M (2013) Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles. J Chem Ecol 39(7): 1007–1018. https://doi.org/10.1007/s10886-013-0317-9

Ferreira MJ, Silva H, Cunha A (2019) Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: a review. Pedosphere 29(4): 409–420

Forchhammer K (2008) PII signal transducers: novel functional and structural insights. Trends Microbiol 16(2):65–72. https://doi.org/10.1016/j.tim.2007.11.004

Fousia S, Paplomatas EJ, Tjamos SE (2016) *Bacillus subtilis* QST 713 confers protection to tomato plants against *Pseudomonas syringae* pv tomato and Induces Plant Defence-related Genes. J Phytopathol 164(4):264–270. https://doi.org/10.1111/jph.12455

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35(1):439–468. https://doi.org/10.1146/annurev.genet.35.102401.090913

Goh CH, Vallejos DFV, Nicotra AB et al (2013) The impact of beneficial plant-associated microbes on plant phenotypic plasticity. J Chem Ecol 39(7):826–839. https://doi.org/10.1007/s10886-013-0326-8

Govindasamy V, George P, Raina SK et al (2018) Plant-associated microbial interactions in the soil environment: role of endophytes in imparting abiotic stress tolerance to crops. In: Bal S, Mukherjee J, Choudhury B, Dhawan A (eds) Advances in crop environment interaction. Springer, Singapore, pp 245–284. https://doi.org/10.1007/978-981-13-1861-0_10

Gowami SP, Maurya BR, Dubey AN et al (2019) Role of phosphorus solubilizing microorganisms and dissolution of insoluble phosphorus in soil. Int J Chem Stud 7(3):3905–3913

Gupta G, Parihar SS, Ahirwar NK et al (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7(2):096–102

Gupta S, Dikshit A (2010) Biopesticides: an ecofriendly approach for pest control. J Biopest 3:186–188

Han SH, Lee SJ, Moon JH et al (2006) GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. Mol Plant-Microbe Interact 19(8):924–930. https://doi.org/10.1094/MPMI-19-0924

Harman GE, Herrera-Estrella AH, Horwitz BA et al (2012) Trichoderma–from basic biology to biotechnology. Microbiology 158(1):1–2. https://doi.org/10.1099/mic.0.056424-0

Hasanuzzaman M, Nahar K, Alam MM et al (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci 14(5):9643–9684. https://doi.org/10.3390/ijms14059643

Hazra DK (2018) Conventional and futuristic smart herbicide formulations for environment friendly weed management: a review. J Crop Weed 14(2):130–136s

Hazra DK, Purkait A (2019) Role of pesticide formulations for sustainable crop protection and environment management: a review. J Pharmacogn Phytochem 8:686–693

Huber DL (2005) Synthesis, properties, and applications of iron nanoparticles. Small 1(5):482–501. https://doi.org/10.1002/smll.200500006

Huergo LF, Chubatsu LS, Souza EM et al (2006) Interactions between PII proteins and the nitrogenase regulatory enzymes DraT and DraG in *Azospirillum brasilense*. FEBS Lett 580(22):5232–5236. https://doi.org/10.1016/j.febslet.2006.08.054

Huergo LF, Noindorf L, Gimenes C et al (2010) Proteomic analysis of *Herbaspirillum seropedicae* reveals ammonium-induced AmtB-dependent membrane sequestration of PII proteins. FEMS Microbiol Lett 308(1):40–47. https://doi.org/10.1111/j.1574-6968.2010.01986.x

Igiehon NO, Babalola OO (2018) Below-ground-above-ground plant-microbial interactions: focusing on soybean, rhizobacteria and mycorrhizal fungi. Open Microbiol J 12:261. https://doi.org/10.2174/1874285801812010261

Imam J, Singh PK, Shukla P (2016) Plant microbe interactions in post genomic era: perspectives and applications. Front Microbiol 7:1488. https://doi.org/10.3389/fmicb.2016.01488

Jain S, Varma A, Tuteja N et al (2016) Bacteria-mediated elicitation of induced resistance in plants upon fungal phytopathogen. In: Choudhary D, Varma A, Tuteja N (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore, pp 249–269. https://doi.org/10.1007/978-981-10-2854-0_12

Jiao L, Junjian R, Xixi X et al (2015) Heat, acid and cold stresses enhance the expression of DnaK gene in *Alicyclobacillus acidoterrestris*. Food Res Int 67:183–192. https://doi.org/10.1016/j.foodres.2014.11.023

Kalayu G (2019) Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int J Agron 2019:4917256. https://doi.org/10.1155/2019/4917256

Kaplan I (2012) Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? Biol Control 60(2):77–89

Khan W, Rayirath UP, Subramanian S et al (2009) Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul 28:386–399. https://doi.org/10.1007/s00344-009-9103-x

Khoshru B, Mitra D, Mahakur B et al (2020) Role of soil rhizobacteria in utilization of an indispensable micronutrient zinc for plant growth promotion. J Crit Rev 7(12):4644–4654

Kiers ET, Rousseau RA, West SA et al (2003) Host sanctions and the legume–rhizobium mutualism. Nature 425(6953):78–81. https://doi.org/10.1038/nature01931

Kocira S (2019) Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. Chil J Agric Res 79(1):17–25

Kour D, Rana KL, Yadav AN et al (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487

Kumar A, Verma JP (2018) Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52. https://doi.org/10.1016/j.micres.2017.11.004

Kumar RS, Moorthy K, Vinodhini R et al (2013) Antimicrobial efficacy and phytochemical analysis of *Indigofera trita* Linn. Afr J Tradit Complement Altern Med 10(3):518–525

Kushwaha P, Kashyap PL, Kuppusamy P (2020) Microbes for cold stress resistance in plants: mechanism, opportunities, and challenges. In: Goel R, Soni R, Suyal D (eds) Microbiological advancements for higher altitude agro-ecosystems & sustainability. Rhizosphere biology. Springer, Singapore, pp 269–292. https://doi.org/10.1007/978-981-15-1902-4_14

Lackner G, Möbius N, Scherlach K et al (2009) Global distribution and evolution of a toxinogenic Burkholderia-Rhizopus symbiosis. Appl Environ Microbiol 75(9):2982–2986. https://doi.org/10.1128/AEM.01765-08

Leng P, Zhang Z, Pan G et al (2011) Applications and development trends in biopesticides. Afr J Biotechnol 10(86):19864–19873

Leung T, Poulin R (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie et Milieu 58(2):107

Little R, Reyes-Ramirez F, Zhang Y et al (2000) Signal transduction to the *Azotobacter vinelandii* NIFL–NIFA regulatory system is influenced directly by interaction with 2-oxoglutarate and the PII regulatory protein. EMBO J 19(22):6041–6050. https://doi.org/10.1093/emboj/19.22.6041

Liu X-M, Zhang H (2015) The effects of bacterial volatile emissions on plant abiotic stress tolerance. Front Plant Sci 6:774

Luxem KE, Kraepiel AML, Zhang L et al (2020) Carbon substrate re-orders relative growth of a bacterium using Mo-, V-, or Fe-nitrogenase for nitrogen fixation. Environ Microbiol 22(4):1397–1408. https://doi.org/10.1111/1462-2920.14955

Ma Y, Rajkumar M, Zhang C et al (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25

Mącik M, Gryta A, Frąc M (2020) Biofertilizers in agriculture: an overview on concepts, strategies and effects on soil microorganisms. Adv Agron 162:31–87. https://doi.org/10.1016/bs.agron.2020.02.001

Magnoli SM, Lau JA (2020) Novel plant–microbe interactions: rapid evolution of a legume–rhizobium mutualism in restored prairies. J Ecol 108(4):1241–1249. https://doi.org/10.1111/1365-2745.13366

Mandal A, Thakur JK, Sahu A et al (2016) Plant-microbe interaction for the removal of heavy metal from contaminated site. In: Choudhary D, Varma A, Tuteja N (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore, pp 227–247. https://doi.org/10.1007/978-981-10-2854-0_11

Mansfield J, Genin S, Magori S et al (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13(6):614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Martinez-Argudo I, Little R, Shearer N et al (2004) The NifL-NifA system: a multidomain transcriptional regulatory complex that integrates environmental signals. J Bacteriol 186(3):601–610. https://doi.org/10.1128/JB.186.3.601-610.2004

Matten SR, Frederick RJ, Reynolds AH (2012) United States Environmental Protection Agency insect resistance management programs for plant-incorporated protectants and use of simulation modeling. In: Wozniak C, McHughen A (eds) Regulation of agricultural biotechnology: the United States and Canada. Springer, Dordrecht, pp 175–267. https://doi.org/10.1007/978-94-007-2156-2_11

Mazid M, Khan TA (2015) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3(3):10–23

Mercado-Blanco J, Bakker PA (2007) Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. Antonie Van Leeuwenhoek 92(4):367–389. https://doi.org/10.1007/s10482-007-9167-1

Mhlongo MI, Piater LA, Madala NE et al (2018) The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112. https://doi.org/10.3389/fpls.2018.00112

Mishra BB, Nayak SK, Pahari A (eds) (2021) Agriculturally important microorganisms: mechanisms and applications for sustainable agriculture. New India Publishing Agency, New Delhi

Mishra GK (2017) Microbes in heavy metal remediation: a review on current trends and patents. Recent Pat Biotechnol 11(3):188–196. https://doi.org/10.2174/1872208311666170120121025

Mishra RK, Bohra A, Kamaal N et al (2018) Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects. Egypt J Biol Pest Co 28(1): 1–11

Mishra S, Bharagava RN, More N et al (2019) Heavy metal contamination: an alarming threat to environment and human health. In: Sobti R, Arora N, Kothari R (eds) Environmental biotechnology: for sustainable future. Springer, Singapore, pp 103–125. https://doi.org/10.1007/978-981-10-7284-0_5

Mitra S, Pramanik K, Sarkar A et al (2018) Bioaccumulation of cadmium by *Enterobacter* sp. and enhancement of rice seedling growth under cadmium stress. Ecotoxicol Environ Saf 156:183–196. https://doi.org/10.1016/j.ecoenv.2018.03.001

Money T, Jones T, Dixon R et al (1999) Isolation and properties of the complex between the enhancer binding protein NIFA and the sensor NIFL. J Bacteriol 181(15):4461–4468. https://doi.org/10.1128/JB.181.15.4461-4468.1999

More S, Shinde SE, Kasture MC (2019) Root exudates a key factor for soil and plant: an overview. Pharm Innov J 8(6):449–459

Moriyama Y, Hiasa M, Matsumoto T et al (2008) Multidrug and toxic compound extrusion (MATE)-type proteins as anchor transporters for the excretion of metabolic waste products and xenobiotics. Xenobiotica 38(7–8):1107–1118. https://doi.org/10.1080/00498250701883753

Moure VR, Costa FF, Cruz LM et al (2014) Regulation of nitrogenase by reversible mono-ADP-ribosylation. Curr Top Microbiol Immunol 384:89–106. https://doi.org/10.1007/82_2014_380

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8(3):199–216. https://doi.org/10.1007/s10311-010-0297-8

Narula N, Kothe E, Behl RK (2009) Role of root exudates in plant-microbe interactions. J Appl Bot Food Quality 82(2):122–130

Nayak SK, Mishra BB (eds) (2020) Frontiers in soil and environmental microbiology. CRC Press, Boca Raton

Nayak SK, Dash B, Baliyarsingh B (2018) Microbial remediation of persistent agro-chemicals by soil bacteria: an overview. In: Patra J, Das G, Shin HS (eds) Microbial biotechnology. Springer, Singapore, pp 275–301. https://doi.org/10.1007/978-981-10-7140-9_13

Nayak SK, Nayak S, Mishra BB (2017) Antimycotic role of soil *Bacillus* sp. against rice pathogens: a biocontrol prospective. In: Patra J, Vishnuprasad C, Das G (eds) Microbial biotechnology. Springer, Singapore, pp 29–60. https://doi.org/10.1007/978-981-10-6847-8_2

Nelson ME, Alves AP (2014) Plant incorporated protectants and insect resistance. In: Onstad DW (ed) Insect resistance management: biology, economics, and prediction, 2nd edn. Academic Press, London, pp 99–147

Neumann G, Römheld V (2007) The release of root exudates as affected by the plant physiological status. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface, 2nd edn. CRC Press, Boca Raton, pp 23–72. https://doi.org/10.1201/9781420005585_2

Noman A, Aqeel M, Islam W et al (2021) Insects–plants-pathogens: toxicity, dependence and defense dynamics. Toxicon 197:87–98

O'Brien HE, Thakur S, Guttman DS (2011) Evolution of plant pathogenesis in *Pseudomonas syringae*: a genomics perspective. Annu Rev Phytopathol 49:269–289. https://doi.org/10.1146/annurev-phyto-072910-095242

Ocimati W, Were E, Groot JCJ et al (2018) Risks posed by intercrops and weeds as alternative hosts to *Xanthomonas campestris* pv. *musacearum* in banana fields. Front Plant Sci 9:1471. https://doi.org/10.3389/fpls.2018.01471

Oehl F, Frossard E, Fliessbach A et al (2004) Basal organic phosphorus mineralization in soils under different farming systems. Soil Biol Biochem 36(4):667–675. https://doi.org/10.1016/j.soilbio.2003.12.010

Oh C-S, Beer SV (2005) Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. FEMS Microbiol Lett 253(2):185–192. https://doi.org/10.1016/j.femsle.2005.09.051

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33(11):1–16. https://doi.org/10.1007/s11274-017-2364-9

Oliveira MAS, Aquinoa B, Bonatto AC et al (2012) Interaction of GlnK with the GAF domain of *Herbaspirillum seropedicae* NifA mediates NH4+-regulation. Biochimie 94(4):1041–1047. https://doi.org/10.1016/j.biochi.2012.01.007

Padhan D, Rout PP, Kundu R et al (2021) Bioremediation of heavy metals and other toxic substances by microorganisms. In: Parray JA, Mahmoud AHAE, Sayyed R (eds) Soil bioremediation: an approach towards sustainable technology. Wiley, UK, pp 285–329. https://doi.org/10.1002/9781119547976.ch12

Page MG (2019) The role of iron and siderophores in infection, and the development of siderophore antibiotics. Clin Infect Dis 69(7):S529–S537

Pahari A, Pradhan A, Nayak SK et al (2017) Bacterial siderophore as a plant growth promoter. In: Patra JK, Vishnuprasad C, Das G (eds) Microbial biotechnology. Springer, Singapore, pp 163–180. https://doi.org/10.1007/978-981-10-6847-8_7

Pallini A, Janssen A, Sabelis MW (1997) Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. Oecologia 110(2):179–185. https://doi.org/10.1007/s004420050147

Park KY, Seo SY, Oh BR et al (2018) 2, 3-butanediol induces systemic acquired resistance in the plant immune response. J Plant Biol 61(6):424–434. https://doi.org/10.1007/s12374-018-0421-z

Parker MA (2012) Legumes select symbiosis island sequence variants in Bradyrhizobium. Mol Ecol 21:1769–1778

Parray JA, Jan S, Kamili AN et al (2016) Current perspectives on plant growth-promoting rhizobacteria. J Plant Growth Regul 35(3):877–902. https://doi.org/10.1007/s00344-016-9583-4

Peña-Méndez EM, Havel J, Patocka J (2005) Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. J Appl Biomed 3(1):13–24

Pii Y, Mimmo T, Tomasi N et al (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review Biol Fertil Soils 51(4):403–415. https://doi.org/10.1007/s00374-015-0996-1

Ponzio C, Weldegergis BT, Dicke M et al (2016) Compatible and incompatible pathogen–plant interactions differentially affect plant volatile emissions and the attraction of parasitoid wasps. Funct Ecol 30(11):1779–1789

Popp J, Pető K, Nagy J (2013) Pesticide productivity and food security. A review Agron Sustain Dev 33(1):243–255. https://doi.org/10.1007/s13593-012-0105-x

Posmyk MM, Szafrańska K (2016) Biostimulators: a new trend towards solving an old problem. Front Plant Sci 7:748

Povero G, Mejia JF, Di Tommaso D et al (2016) A systematic approach to discover and characterize natural plant biostimulants. Front Plant Sci 7:435. https://doi.org/10.3389/fpls.2016.00435

Pradhan S, Meena RP, Ram H et al (2021) Zn-solubilizing microorganism: a novel perspective for sustainable agriculture. In: Rakshit A, Meena VS, Manoj P, Singh HB, Singh AK (eds) Biofertilizers. Woodhead Publishing, Sawston, pp 69–81. https://doi.org/10.1016/B978-0-12-821667-5.00025-7

Raimi A, Roopnarain A, Adeleke R (2021) Biofertilizer production in Africa: current status, factors impeding adoption and strategies for success. Scientific African 11:e00694

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77(2):153–160. https://doi.org/10.1016/j.chemosphere.2009.06.047

Rashid M, Khalil S, Ayub N et al (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak J Biol Sci 7(2): 187–196. https://doi.org/10.3923/pjbs.2004.187.196

Renjith PS, Sheetal KR, Kumar S et al (2020) Microbial and biotechnological approaches in the production of biofertilizer. In: Singh A, Srivastava S, Rathore D, Pant D (eds) Environmental microbiology and biotechnology. Springer, Singapore, pp 201–219. https://doi.org/10.1007/978-981-15-6021-7_10

Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49:317–343. https://doi.org/10.1146/annurev-phyto-073009-114447

Rodríguez H, Fraga R, Gonzalez T et al (2007) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Developments in plant and soil sciences, vol 102. Springer, Dordrecht, pp 15–21. https://doi.org/10.1007/978-1-4020-5765-6_2

Rosenblueth M, Ormeño-Orrillo E, López-López A et al (2018) Nitrogen fixation in cereals. Front Microbiol 9:1794. https://doi.org/10.3389/fmicb.2018.01794

Rouphael Y, Colla G (2018) Synergistic biostimulatory action: designing the next generation of plant biostimulants for sustainable agriculture. Front Plant Sci 9:1655. https://doi.org/10.3389/fpls.2018.01655

Rout GR, Sahoo S (2015) Role of iron in plant growth and metabolism. Rev Agric Sci 3:1–24. https://doi.org/10.7831/ras.3.1

Roy A (2021) Biofertilizers for agricultural sustainability: current status and future challenges. In: Yadav AN, Singh J, Singh C, Yadav N (eds) Current trends in microbial biotechnology for sustainable agriculture. Environmental and microbial biotechnology. Springer, Singapore, pp 525–553. https://doi.org/10.1007/978-981-15-6949-4_21

Rubin RL, van Groenigen KJ, Hungate BA (2017) Plant growth promoting rhizobacteria are more effective under drought: a meta-analysis. Plant Soil 416(1):309–323. https://doi.org/10.1007/s11104-017-3199-8

Rubio LM, Ludden PW (2008) Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annu Rev Microbiol 62:93–111. https://doi.org/10.1146/annurev.micro.62.081307.162737

Rudnick P, Kunz C, Gunatilaka MK et al (2002) Role of GlnK in NifL-mediated regulation of NifA activity in *Azotobacter vinelandii*. J Bacteriol 184(3):812–820. https://doi.org/10.1128/JB.184.3.812-820.2002

Ryan P, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52(1):527–560. https://doi.org/10.1146/annurev.arplant.52.1.527

Sah S, Singh R (2015) Siderophore: structural and functional characterisation—a comprehensive review. Agriculture 61(3):97

Saha M, Sarkar S, Sarkar B et al (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23(5):3984–3999. https://doi.org/10.1007/s11356-015-4294-0

Sansinenea E (2021) Application of biofertilizers: current worldwide status. In: Rakshit A, Meena VS, Manoj P, Singh HB, Singh AK (eds) Biofertilizers. Woodhead Publishing, Sawston, pp 183–190. https://doi.org/10.1016/B978-0-12-821667-5.00004-X

Santamaria ME, Martínez M, Cambra I et al (2013) Understanding plant defence responses against herbivore attacks: an essential first step towards the development of sustainable resistance against pests. Transgenic Res 22:697–708. https://doi.org/10.1007/s11248-013-9725-4

Saravanakumar D, Samiyappan R (2007) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102(5):1283–1292

Sarkar J, Chakraborty U, Chakraborty BN (2018) Induced defense response in wheat plants against *Bipolaris sorokiniana* following application of *Bacillus safensis* and *Ochrobactrum pseudogrignonense*. Ind Phytopathol 71(1):49–58. https://doi.org/10.1007/s42360-018-0006-2

Sarwar M (2015) Information on activities regarding biochemical pesticides: an ecological friendly plant protection against insects. Int J Eng Adv Res Technol 1(2):27–31

Sashidhar B, Podile AR (2010) Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. J Appl Microbiol 109(1):1–12

Savci S (2012) Investigation of effect of chemical fertilizers on environment. Apcbee Procedia 1: 287–292

Sayyed RZ, Ilyas N, Tabassum B et al (2019) Plausible role of plant growth-promoting rhizobacteria in future climatic scenario. In: Sobti R, Arora N, Kothari R (eds) Environmental biotechnology: for sustainable future. Springer, Singapore, pp 175–197. https://doi.org/10.1007/978-981-10-7284-0_7

Scavino AF, Pedraza RO (2013) The role of siderophores in plant growth-promoting bacteria. In: Maheshwari D, Saraf M, Aeron A (eds) Bacteria in agrobiology: crop productivity. Springer, Berlin, Heidelberg, pp 265–285. https://doi.org/10.1007/978-3-642-37241-4_11

Scavo A, Abbate C, Mauromicale G (2019) Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. Plant Soil 442(1):23–48. https://doi.org/10.1007/s11104-019-04190-y

Scheublin TR, Van Der Heijden MG (2006) Arbuscular mycorrhizal fungi colonize nonfixing root nodules of several legume species. New Phytol 172(4):732–738. https://doi.org/10.1111/j.1469-8137.2006.01858.x

Schmelz EA, Carroll MJ, LeClere S et al (2006) Fragments of ATP synthase mediate plant perception of insect attack. Proc Natl Acad Sci 103(23):8894–8899. https://doi.org/10.1007/s11248-013-9725-4

Senthil-Nathan S (2015) A review of biopesticides and their mode of action against insect pests. In: Thangavel P, Sridevi G (eds) Environmental sustainability. Springer, New Delhi, pp 49–63. https://doi.org/10.1007/978-81-322-2056-5_3

Seo PJ, Mas P (2015) STRESSing the role of the plant circadian clock. Trends Plant Sci 20(4): 230–237. https://doi.org/10.1016/j.tplants.2015.01.001

Sharma D, Gahtyari NC, Chhabra R et al (2020) Role of microbes in improving plant growth and soil health for sustainable agriculture. In: Yadav A, Rastegari A, Yadav N, Kour D (eds) Advances in plant microbiome and sustainable agriculture. microorganisms for sustainability, vol 19. Springer, Singapore, pp 207–256. https://doi.org/10.1007/978-981-15-3208-5_9

Sharma SB, Sayyed RZ, Trivedi MH et al (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2(1):1–14

Singh M, Kumar J, Singh S et al (2015) Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. Rev Environ Sci Biotechnol 14(3):407–426. https://doi.org/10.1007/s11157-015-9372-8

Skaljac M, Vogel H, Wielsch N et al (2019) Transmission of a protease-secreting bacterial symbiont among pea aphids via host plants. Front Physiol 10:438. https://doi.org/10.3389/fphys.2019.00438

Soto MJ, Domínguez-Ferreras A, Pérez-Mendoza D et al (2009) Mutualism versus pathogenesis: the give-and-take in plant–bacteria interactions. Cell Microbiol 11(3):381–388. https://doi.org/10.1111/j.1462-5822.2009.01282.x

Swamy MK, Nalina N, Nalina D et al (2019) Heavy metal stress and tolerance in plants mediated by rhizospheric microbes. In: Akhtar MS (ed) Salt stress, microbes, and plant interactions: causes and solution. Springer, Singapore, pp 181–198. https://doi.org/10.1007/978-981-13-8801-9_8

Szewczyk B, Hoyos-Carvajal L, Paluszek M et al (2006) Baculoviruses—re-emerging biopesticides. Biotechnol Adv 24(2):143–160

Tan W-S, Law JW-F, Law LN-S et al (2020) Insights into quorum sensing (QS): QS-regulated biofilm and inhibitors. Prog Microbes Mol Biol 3(1):a0000141. https://doi.org/10.36877/pmmb.a0000141

Theocharis A, Clément C, Barka EA (2012) Physiological and molecular changes in plants grown at low temperatures. Planta 235(6):1091–1105. https://doi.org/10.1007/s00425-012-1641-y

Tian J, Ge F, Zhang D et al (2021) Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. Biology 10(2):158. https://doi.org/10.3390/biology10020158

Tiku AR (2021) Direct and indirect defence against insects. In: Singh IK, Singh A (eds) Plant-pest interactions: from molecular mechanisms to chemical ecology. Springer, Singapore, pp 157–192. https://doi.org/10.1007/978-981-15-2467-7_8

Turlings TCJ, Ton J (2006) Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. Curr Opin Plant Biol 9(4): 421–427

Ullah A, Heng S, Munis MFH et al (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40. https://doi.org/10.1016/j.envexpbot.2015.05.001

Van der Putten WH, Vet LEM, Harvey JA et al (2001) Linking above-and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol Evol 16(10):547–554. https://doi.org/10.1016/S0169-5347(01)02265-0

Vassilev N, Vassileva M, Lopez A et al (2015) Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl Microbiol Biotechnol 99(12): 4983–4996. https://doi.org/10.1007/s00253-015-6656-4

Verma M, Mishra J, Arora NK (2019) Plant growth-promoting rhizobacteria: diversity and applications. In: Sobti R, Arora N, Kothari R (eds) Environmental biotechnology: for sustainable future. Springer, Singapore, pp 129–173. https://doi.org/10.1007/978-981-10-7284-0_6

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2):571–586

Vezza ME, Nicotra MFO, Agostini E et al (2020) Biochemical and molecular characterization of arsenic response from *Azospirillum brasilense* Cd, a bacterial strain used as plant inoculant. Environ Sci Pollut Res Int 27(2):2287–2300. https://doi.org/10.1007/s11356-019-06959-1

Vishwakarma K, Kumar N, Shandilya C et al (2020) Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture: a review. Front Microbiol 11:560406. https://doi.org/10.3389/fmicb.2020.560406

Vives-Peris V, de Ollas C, Gómez-Cadenas A et al (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39(1):3–17. https://doi.org/10.1007/s00299-019-02447-5

Voisard C, Bull CT, Keel C et al (1994) Biocontrol of root diseases by *Pseudomonas fluorescens* CHA0: current concepts and experimental approaches. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular ecology of rhizosphere microorganisms: biotechnology and the release of GMOs. Wiley, Germany, pp 67–89. https://doi.org/10.1002/9783527615810.ch6

Vurukonda SSKP, Vardharajula S, Shrivastava M et al (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24. https://doi.org/10.1016/j.micres.2015.12.003

Wittmann S, Heinisch L, Scherlitz-Hofmann I et al (2004) Catecholates and mixed catecholate hydroxamates as artificial siderophores for mycobacteria. Biometals 17(1):53–64. https://doi.org/10.1023/A:1024409517626

Wu R, Lev-Yadun S, Sun L et al (2021) Higher elevations tend to have higher proportion of plant species with glandular trichomes. Front Plant Sci 12:632464. https://doi.org/10.3389/fpls.2021.632464

Yadav AN (2020) Plant microbiomes for sustainable agriculture: current research and future challenges. In: Yadav A, Singh J, Rastegari A, Yadav N (eds) Plant microbiomes for sustainable agriculture. sustainable development and biodiversity, vol 25. Springer, Cham, pp 475–482. https://doi.org/10.1007/978-3-030-38453-1_16

Yakhin OI, Lubyanov AA, Yakhin IA et al (2017) Biostimulants in plant science: a global perspective. Front Plant Sci 7:2049. https://doi.org/10.3389/fpls.2016.02049

Yamano Y (2019) In vitro activity of cefiderocol against a broad range of clinically important gram-negative bacteria. Clin Infect Dis 69(7):S544–S551

Zaidi A, Khan MS, Ahemad M et al (2009) Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: Khan M, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, Heidelberg, pp 23–50. https://doi.org/10.1007/978-3-642-01979-1_2

Zhang H, Xie X, Kim M-S et al (2008) Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56(2):264–273. https://doi.org/10.1111/j.1365-313X.2008.03593.x

Zhao N, Zhang J, Qi Y et al (2021) New insights into thermo-acidophilic properties of *Alicyclobacillus acidoterrestris* after acid adaptation. Food Microbiol 94:103657. https://doi.org/10.1016/j.fm.2020.103657

Rhizobacterial Biostimulants: Efficacy in Enhanced Productivity and Sustainable Agriculture

3

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Abstract

Increasing global population coupled with climate change necessitates higher crop production with lesser chemical inputs. From both human and environmental health perspectives, it is better to find safe alternatives for the otherwise harmful synthetic pesticides and fertilizers. Plant growth-promoting rhizobacteria (PGPRs) are extremely valuable biostimulants promoting plant growth and health, without any harmful side effects. PGPRs are widely used as biofertilizers in a wide variety of plants for enhanced productivity. PGPR bio-inoculants ensure enhanced crop productivity through increased nutrient assimilation and uptake, increased phytohormone production, and phosphate solubilization. Further, PGPRs are also used as biological control agents/inducers of host resistance to a wide range of phytopathogens in different crops. Integrated use of PGPR along with certain synthetic chemicals is fast gaining acceptance as a cost-effective means for growth enhancement with plant disease management. Biotechnological advances in rhizosphere engineering and genetically modified rhizospheric microorganisms have made significant improvements in the efficiency of PGPR in terms of both growth promotion and disease management. It also extended in

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_3

the perseverance, fitness, and effectiveness of the introduced biocontrol communities in the new environment of soil. Manipulating the rhizosphere to exploit or enhance this innate genetic potential through manipulation of root/soil interactions will definitely play an important role in the future development of sustainable farming processes. This chapter provides a comprehensive overview of the advancements and applications of PGPR-mediated plant growth and health promotion, updates on PGPR mechanisms, and deliberates the potential of using rhizosphere engineering for sustainable agriculture.

Keywords

Plant growth-promoting rhizobacteria · Biostimulants · Induction systemic resistance · Rhizosphere engineering

3.1 Introduction

To increase yield and control plant diseases, agricultural practices are becoming increasingly reliant on the application of enormous quantities of synthetic fertilizers, pesticides, and growth regulators. Chemical use has concerns such as environmental pollution and health risks. Furthermore, overreliance on chemical pesticides disrupts natural nutrient cycling in the environment and kills biological ecosystems that would otherwise sustain crop production. The use of bioresources is a sustainable substitute to synthetic fertilizers, pesticides, and growth regulators. Plant immunization is the most widely studied and a rapidly evolving phenomenon (Heil and Bostock 2002; Durrant and Dong 2004).

Induced resistance denotes a variety of plant responses in which a prior treatment with an inducing agent, which may be biotic or abiotic in nature, increases the plant's resistance to a later pathogen challenge. Plants have a number of challenge-inducible resistance mechanisms that can be separated into two groups: local and systemic defenses. Local defenses normally result in the death of the cell that is responding to the threat, either by dehydration and abscission or through the hypersensitive reaction. As a result, complete induced resistance can only be expressed by systemic, uninfected parts of the plant, which necessitates the presence of systemic signals from necrotic areas to other parts of the plant (Joshi et al. 2016).

In response to pathogen attack or chemical treatment, systemic acquired resistance (SAR) will be expressed throughout plants. Use of nonpathogenic rhizospheric bacteria is projected as a potential biological substitute to chemical synthetic pesticides (Walsh et al. 2001; Zahir et al. 2004). PGPRs are a form of beneficial rhizosphere bacteria, and resistance mediated by them is recognized as induced systemic resistance (ISR), which is expressed systemically. PGPRs are free-living bacteria that help plants grow by improving emergence, colonizing roots, and stimulating development (Bhattacharyya et al. 2016).

In current years, plant growth promotion by PGPR and disease control is receiving global significance and acceptance (Van Loon et al. 1998). PGPRs are also

known to have beneficial effects in biological disease control as well as with their inducing systemic defense mechanisms (Kloepper et al. 2004). Besides using the plant's existing protection mechanisms, PGPR may be a useful component in environmentally sound pest control programs and a way to reduce our dependence on pesticides (Van Loon et al. 1998; Wang et al. 2005).

In greenhouse and field studies, applications with certain of these PGPRs have decreased the occurrence of plant infections. ISR has been shown to be effective not only against different bacterial, fungal, and viral diseases, but also against several nematodes and insects. Such efficacies of PGPR in inducing ISR have been successfully proved in various crop diseases in field conditions (Zehnder et al. 2001). Rhizobacteria serve as eco-friendly and sustainable alternatives to the unsafe chemicals used for growth promotion and control of plant diseases (Shankar et al. 2009). The PGPR strains used as fresh suspensions and powdered formulations have commercial potential in plant growth promotion and management of plant diseases as evident from several researchers (Chithrashree et al. 2011).

3.2 Rhizosphere

The volume of soil immediately surrounding the roots of plant in which bacterial improvement is stimulated is termed as "rhizosphere" which is the territory where numerous significant interactions and processes take place. The region of rhizosphere is where most microbial activity happens, resulting in a restrained nutrient pool from where important micro- and macronutrients are obtained. Because of the presence of root exudates, which serve as a source of nutrients for microbial growth, the population of rhizosphere microbes is distinct from that of its surroundings (Burdman et al. 2000). The volume of bacteria covering the plant roots is usually 10–100 times greater than that in the bulk soil, indicating that the narrow rhizosphere zone is nutrient rich compared to the bulk soil (Vejan et al. 2016).

The rhizosphere, comprising the root, root surface, and region close to the surfaces, the "rhizoplane," is more intensively colonized by microbes that are attracted by nutrients in plant root exudates. Indeed, this "rhizosphere effect" was first defined by Hiltner in 1904, who detected increased activity and number of microbes in the locality of roots of the plant. Fungi, actinobacteria, bacteria, algae, and protozoa are the microorganisms that widely colonize the rhizosphere and bacteria are the common among them (Kaymak 2010; Mishra et al. 2021a).

3.3 Plant Growth-Promoting Rhizobacteria (PGPR)

In the rhizosphere, bacteria abundantly exist, most often structured in microcolonies, and are referred to as rhizobacteria. They are a subgroup of total rhizosphere bacteria that have the capacity to colonize the emerging root structure in the presence of challenging soil microflora when reintroduced to seeds or vegetative plant parts. Rhizobacteria from the rhizosphere which have beneficial impact on the

development and health of plants are termed as PGPR (Ghosh and Gangopadhyay 2019). These rhizospheric bacteria not only gain from the secretion of nutrients from roots of the plant, but also have a useful influence on the plant, stimulating its development in an indirect or direct mode. Commonly found PGPR genera comprise *Azotobacter*, *Alcaligenes*, *Azospirillum*, *Arthrobacter*, *Acetobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Paenibacillus*, *Flavobacterium*, *Pseudomonads*, *Serratia*, and few Enterobacteriaceae members.

Extracellular PGPR (ePGPR) and intracellular PGPR (iPGPR) are two major types of PGPR reported. The ePGPRs are located on the rhizoplane or in between the cortex cells of root, while iPGPRs are established predominantly inside root cell's specific nodular structure (Viveros et al. 2010). The ePGPRs are represented by *Serratia*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Caulobacter*, *Flavobacterium*, *Micrococcus*, *Erwinia*, *Arthrobacter*, *Agrobacterium*, and *Pseudomonas*. Similarly the iPGPRs are *Bradyrhizobium*, *Frankia*, *Allorhizobium*, *Rhizobium*, and *Mesorhizobium* (Bhattacharyya and Jha 2012; Gouda et al. 2018).

PGPRs, besides their growth-stimulating abilities, also play a significant part in the control and management of many phytopathogenic microorganisms (Son et al. 2014; Ahemad and Kibret 2014) and serve as a major active constituent in biofertilizer preparation. Based on their relation with hosts, PGPRs have been categorized into two groups: rhizobacteria which live within plants plus share metabolites directly are referred to as symbiotic bacteria; furthermore bacteria which live in plant cells' outer surface and interchange chemical components with them are often referred to as free-living rhizospheric bacteria. The mechanism like indirect and direct method of functioning of PGPR is also the basis of categorization. Biofertilization, root stimulation, rhizoremediation, and plant stress management are the direct mechanisms. However, rhizobacteria indirectly encourage plant development by cutting down the rate of plant infections by systemic acquired resistance, antibiosis, and competition for nutrient and niche (Egamberdieva and Lugtenberg 2014; Vejan et al. 2016).

Owing to their dual advantages of encouraging plant growth as well as controlling plant pests and diseases, PGPRs have gained significance, and are extensively used as microbial inoculants for improving productivity in farming (Barea et al. 2005). The usage of PGPR mixtures with other approaches such as host resistance and chemicals has proved to be effective against a number of plant diseases. The practical application of PGPR-based products has improved, and many PGPR formulations are commercialized, with more in production. In addition, novel formulation technologies have been developed (Kilian et al. 2000; Kloepper et al. 2004).

3.4 PGPR Growth-Promoting Mechanisms

The main mechanism of PGPRs includes alteration in the equilibrium of rhizosphere's microbial population along with changes in host's physiology (Glick et al. 1999). However, different mechanisms are attributed for the occurrence of plant growth promotion when agricultural crops are treated with PGPR. Increase in nitrogen fixation, auxin, gibberellin, cytokinin, and ethylene production (Byrne et al. 2019); phosphorus solubilization, sulfur oxidation, and enhanced nitrate availability (Glick et al. 1998); increased extracellular antibiotics, lytic enzymes, and hydrocyanic acid production (Jadhav et al. 2017); and enhanced root permeability, root site competition and several nutrients, inhibition of harmful bacteria, and enhanced uptake of vital nutrients (Goswami et al. 2016; Kumar et al. 2019; Mekonnen and Kibret 2021) are among them.

3.4.1 Phytohormone Production

Phytohormones are organic compounds and growth regulators, which encourage, inhibit, or alter plant development and growth at small concentrations (below 1 mM) (Damam et al. 2016). They are messenger chemicals that play an important role in improving the plant development and growth (Jiang et al. 2017). Cytokinins, gibberellins, auxins, ethylene, and abscisic acid are all produced by rhizobacteria. Auxin production by PGPR promotes the emergence of adjacent roots, improves the length of root hair and compactness, and speeds up the lateral root elongation, all of which increase root surface area. Plant cell proliferation or elongation is induced by indole acetic acid, resulting in the increase of ACC by the plant (Dobbelaere et al. 2001). Plant responses to several developmental and environmental signals are mediated by ethylene. It has been demonstrated that when excreted around the roots, ethylene aids in plant development. Plant hormones, for example cytokinins, ethylene, abscisic acid, gibberellins, brassinosteroids, and auxins, cause proliferation of root cells by enhanced water and nutrient uptake as a result of overproduction of root hairs and adjacent roots (Sureshbabu et al. 2016).

Phytohormones are separated into groups on the basis of where they act upon: root-invigorating hormones and shoot-invigorating hormones. During root invigoration, cross talk occurs between some hormone-mediated pathways and the pathways that distinguish and react to exterior environmental changes (Jung et al. 2013). Cytokinins, gibberellins, and auxins also function in controlling the main features of plant development and plant growth. Rather than root growth, higher cytokinin concentrations positively regulate shoot development. Shoot-invigorating hormones are produced by *Bacillus subtilis*, *Azotobacter* sp., *Pseudomonas fluorescens*, *Rhodospirillum rubrum*, *Paenibacillus polymyxa*, *Pseudomonas* sp., *Rhizobium leguminosarum*, and *Pantoea agglomerans* (Prathap and Ranjitha 2015; Gouda et al. 2018).

One of *Bacilli* rhizobacteria's direct PGPR mechanisms is the synthesis of gibberellic acid (GA) and indole acetic acid (IAA) (García-Fraile et al. 2015; Aloo

et al. 2019). IAA influences cell separation and elongation, which aids in shoot and root growth (Pin-Ng et al. 2015). About 80% of PGPR produces IAA, which promotes cell elongation, separation, and differentiation (Ahmed et al. 2017). *Acinetobacter*, *Azospirillum*, *Bacillus*, *Klebsiella*, *Pseudomonas*, and *Rhizobium* are the most common rhizosphere bacteria which biosynthesize IAA in various crops (Choudhary et al. 2018). The tomato seedlings inoculated with *Bacillus subtilis* improve root and shoot development, leaf area, and seedling vigor, as well as greater amounts of GA and IAA in inoculated plants compared to uninoculated plants (Chowdappa et al. 2013). *Peucedanum japonicum* Thunb. shoot growth and dry weight were enhanced following *Bacillus* (SH1RP8) inoculation (Hong and Lee 2014).

Increased plant growth has been linked to the presence of cytokinin including IAA and GA in plant rhizospheres (Patel and Minocheherhomji 2018). Among other things, cytokinin enhances germination and enlargement of leaf, root, and shoot production, thereby promoting plant growth (Jha and Saraf 2015). Some of the cytokinins producing bacterial genera include *Arthrobacter*, *Achromobacter*, *Enterobacter*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Flavobacterium*, *Bacillus*, *Paenibacillus*, *Klebsiella*, and *Pseudomonas*.

3.4.2 Utilization of 1-Aminocyclopropane-1-Carboxylate

1-Aminocyclopropane-1-carboxylate (ACC) acts as a molecule for the biosynthesis of ethylene, which is used by some rhizobacteria like *Actinobacteria* (Nascimento et al. 2018). Nevertheless, ethylene has a number of biological roles, including controlling the ripening process and seed germination, because of its role in ripening, senescence, and abscission, and is often referred to as "ageing hormone" (Schaller 2012). The ACC deaminase enzyme degrades the ACC to NH_3 and also α-ketobutyrate. *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Streptomyces* produce ACC deaminase which improves plant health (Glick 2014). Tomato plants treated with *Streptomyces filipinensis* along with *S. atrovirens* enhanced plant growth by increasing ACC deaminase production (Buzón-Durán et al. 2020; Fasusi and Babalola 2021).

3.4.3 Exopolysaccharide (EPS) Production

A variety of bacteria, algae, and plants produce high-molecular-weight, biodegradable polymers called exopolysaccharides (EPSs) made up of monosugar residues and products (Sanlibaba and Cakmak 2016). EPSs promote plant growth and development by retaining water potential, confirming obligate interaction between roots of plant and rhizobacteria, soil particle aggregation, pathogenesis, and sustaining the plant in stress (Pawar et al. 2016). Exopolysaccharides generating PGPR include *Agrobacterium* sp., *Azotobacter vinelandii*, *Enterobacter cloacae*, *Bacillus*

drentensis, *Rhizobium* sp., *Rhizobium leguminosarum*, and *Xanthomonas* sp. (Mahmood et al. 2016).

3.4.4 Phosphate Solubilization

Phosphorus is a vital component for plants because it aids in their development and is the second most significant nutrient essential in sufficient amounts for optimal plant development. All major metabolic processes like photosynthesis, macromolecular biosynthesis, energy transfer, signaling, and respiration require phosphorus (Anand et al. 2016). However, plants cannot absorb it, since most of it exists in insoluble and precipitated form. Phosphate is absorbed as monobasic ($H_2PO_4^-$) as well as dibasic (HPO_4^{-2}) ions. An important PGPR trait of phosphate-solubilizing bacteria to promote growth is phosphorus solubilization and mineralization into forms (Alori et al. 2017) which release phosphates from organic molecules to solubilize inorganic phosphate (Gouda et al. 2018).

Plant stimulatory properties of PGPR may also be attributed to phosphate-solubilizing and diazotrophic bacteria increasing the accessibility of scarce plant nutrients including phosphorus, amino acids, nitrogen, and B vitamins from rhizosphere (Nautiyal et al. 2000; Rozycki et al. 1999). Inorganic phosphorus is solubilized by small-molecular-weight organic acids formed from bacteria (Sharma et al. 2013). Best examples of phosphate-solubilizing rhizobacteria are *Arthrobacter*, *Flavobacterium*, *Enterobacter*, *Mesorhizobium ciceri*, *Beijerinckia*, *Microbacterium*, *Burkholderia*, *Bacillus*, *Serratia*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Mesorhizobium mediterraneum*, and *Rhodococcus* (Parmar and Sindhu 2013; Oteino et al. 2015; Gouda et al. 2018). Actinobacteria such as *Streptomyces aureofaciens*, *S. alboniger*, *S. venezuelae*, and *S. lienomycini* have also been shown to produce phytases, which are phosphomonoesterases that start the stepwise degradation of phytate (Barman et al. 2019).

3.4.5 Potassium Solubilization

Pseudomonas strains isolated from the rhizosphere of black peppers derived inorganic phosphate from tricalcium phosphate. The higher uptake of nutrients by the treated plants confirmed the role of *P. fluorescens* in nutrient variability in the soil microcosm in PGPR-mediated plant growth stimulation. The treated black pepper showed substantial nitrogen and potassium uptake. Since these strains generate siderophores, the release of available phosphate could be achieved by metal ion chelation related with complex types of P, in addition to the other mechanisms of phosphatase enzyme production and organic acid release. In bacterized plants, potassium uptake was also found to be higher. Thus, PGPR treatment resulted in increased nutrient utilization from the black pepper rhizosphere, resulting in increased plant vigor (Diby et al. 2003; Anandaraj and Sarma 2003).

3.4.6 Production of Enzymes

Studies show that under phosphate-limiting conditions, the activity of phytase by *Bacillus* species is critical for plant growth promotion. *Bacillus amyloliquefaciens* FZB45's extracellular phytase activity contributed to plant growth promotion. In the presence of phytate, diluted culture filtrates of *B. amyloliquefaciens* FZB45 stimulated maize seedling development under phosphate limitation. The sequence of amino acids of the phytase *phyA* gene cloned from FZB45 was found to be very similar to that of known *Bacillus* phytases (Idriss et al. 2002).

Recent research has shown that the activity of ACC deaminase minimizes inhibition of plant root elongation by ethylene (Glick et al. 1998; Ma et al. 2002). The cleavage of ACC to ammonia and α-ketobutyrate is catalyzed by this enzyme. The strains of bacteria isolated from seven different soils (one strain from each soil sample) collected from different locations were capable to utilize ACC as a nitrogen source and stimulated root growth in canola seedlings (Glick et al. 1998). ACC deaminase genes present in *Pseudomonas* and *Azospirillum* strains facilitated the root elongation of canola seedling (Shah et al. 1998; Holguin and Glick 2001).

3.5 PGPR for Disease Management

Several PGPRs have been found to decrease the effects of host stresses by reducing the damage done by phytopathogens. This can be achieved by biocontrol or stimulation of systemic resistance to phytopathogens in the plant.

3.5.1 Biocontrol

Some of the mechanisms used by bacteria for biocontrol include the production of antibiotics, the physical shift of phytopathogens, the production of siderophores to inhibit phytopathogens in the infected area from multiplying, the production of a variety of small compounds that can prevent plant pathogen development, and the synthesis of enzymes that can prevent plant pathogen growth (Van Loon and Glick 2004). A range of enzymes, antibiotics, and siderophores are among the substances that PGPR can generate to help reduce pathogen damage. Pathogens may be able to compete with these microorganisms for nutrients and establishment.

Despite this, biological control has not so far been commonly used for a number of reasons. For example, many environmental factors such as temperature, water content, pH, and relations with other microbes are likely to influence the efficacy of a biocontrol strain in the field. In addition, several biocontrol agents that showed promise in earlier tests failed to colonize the rhizosphere under more complex biological conditions. Competition and antagonistic effects mediated by antimicrobial metabolites and extracellular lytic enzymes could be involved in direct biocontrol interactions with pathogens. Several new biocontrol mechanisms have recently been identified. Some of them deal with pathogenicity factors, plant susceptibility

factors, pathogen cell-cell communication, and structures being degraded. Other novel mechanisms involve inhibiting pathogenic factor synthesis and enhancing the pathogen's vulnerability to stress (Defago 2003).

3.5.2 Mechanisms of Biological Control

3.5.2.1 Antibiosis

Beneficial rhizobacteria have been studied for the development of secondary metabolites, referred to as antimicrobial compounds, which are metabolic by-products that are not required for their growth (Gislason et al. 2020). Antibiotics developed by soil-dwelling bacteria function as fungistatics, inhibiting phytopathogens by directing either important compounds or signaling pathways in the rhizosphere, and their growth is affected by a diversity of influences such as aeration, temperature, and occurrence of challenging microbes (Shaikh and Sayyed 2015; Omran and Kadhem 2016).

The *B. subtilis* 168 and *B. amyloliquefaciens* FZB42 are known to release an array of antibiotics like bacilysin, difficidin, mycobacillin, rhizocticin, and subtilin (Chang et al. 2007; Leclere et al. 2005). Lantibiotics are synthesized by *Bacillus subtilis* with antibacterial characteristics against Gram-positive bacteria (Stein 2005), though their role in the biological control of phytopathogens still remains unclear (Ramadan et al. 2016).

3.5.2.2 Competition

Most microbes compete with each other for nutrients, space, oxygen, light, and water. It happens when two or more species need similar nutrients, and usage by one decreases the amount available to the other (Egamberdieva and Lugtenberg 2014). In order to compete for limiting factors, the aim is to exploit the growing environment so that non-pathogens are preferred over other pathogens. *Bacillus* sp. on the other hand, while they are not aggressive root colonizers, they outcompete other microbes in the area by secreting secondary metabolites, lethal to other microbes. One example is the antagonism for nutrients and appropriate niches on the root exterior.

3.5.2.3 Siderophore Production

Siderophores have been proposed as a mode of pathogen suppression by limiting pathogen survival by chelating iron, thus restricting iron nutrition (Chaiharn et al. 2009). As a result, rhizobacteria which produce siderophores play a significant role in the biocontrol of plant diseases (Sayyed et al. 2005). The *Bacillus* species derived from the rhizosphere of maize produced large quantities of siderophores (Bjelić et al. 2018). Siderophores produced by *Bacillus* sp. antagonized *Rhizoctonia solani* (Kumar et al. 2013). Production of siderophores by *Bacillus antiquum* prevented *Macrophomina phaseolina*-causing sorghum charcoal rot disease (Gopalakrishnan et al. 2011).

Alcaligenes sp., *Bacillus* sp., *Bradyrhizobium* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Rhizobium* sp. all produce siderophore (Shaikh and Sayyed 2015).

PGPR has a competitive edge in root colonization by removing additional microbes from ecological niche due to the development of siderophores. Plants and phytopathogens fight for iron because microbial siderophores prefer iron chelation, which makes iron unavailable to plants.

3.5.2.4 Production of Hydrogen Cyanide

The development of hydrogen cyanide (HCN) is a key feature of certain beneficial rhizobacteria. HCN's action mechanism is thought to inhibit the respiratory chain's terminal cytochrome-c oxidase by binding to metalloenzymes, which is the reason for its phytopathogen-suppressing ability (Rosier et al. 2018). *Aeromonas*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Streptomyces* species produce HCN. Hydrogen cyanide also aids in mineral mobilization and phosphate release, increasing the availability of nutrient for both host plants and *Actinobacteria* (Rijavec and Lapanje 2016). Due to HCN's ability to contain plant pathogens and increase nutrient availability, HCN-producing rhizobacteria are efficient biocontrol agents and biofertilizers for promoting plant growth and health (Fasusi and Babalola 2021).

Plant growth-promoting strains' capacity to stimulate plant development is reliant on HCN production. In the agricultural production systems, HCN is generally used as a biocontrol agent due to its plant pathogen toxicity, metal ion chelation, and increasing phosphate availability (Rijavec and Lapanje 2016). The usage as biofertilizer and disease-preventing efficiency of several HCN-producing PGPRs have been described in tomato plants (Ahmed et al. 2017).

3.6 Rhizobacterium-Mediated Induced Systemic Resistance

Induced systemic resistance (ISR) is a term that describes how PGPR triggers the defense mechanism in plants and thus increases pathogen resistance. PGPR has been established to trigger host defense by preventing phytopathogens in a number of studies. *Bacillus amyloliquefaciens*, *Lactobacillus paracasei*, *Propionibacterium fluorescens*, and *Propionibacterium putida*, for example, elicit ISR against tomato phytopathogens. Plants have been bred to develop a system of tolerance. Thickenings of cell wall or quick collapse of infected cells has been identified to elicit defense during ISR to prevent pathogen spread (Lugtenberg et al. 2002; Mhlongo et al. 2018).

Although majority of rhizobacteria elicit host plant resistance and their use could transform agronomy, basic investigation on PGPR and the application of advanced technology to help plant's transition from the lab to the field is still not completely done (Gouda et al. 2018). PGPRs systemically trigger the host innate resistance mechanism and therefore enhance host resistance to phytopathogens termed as ISR. There are several studies on PGPR-induced host resistance by preventing phytopathogens. *B. amyloliquefaciens*, *P. putida*, *P. fluorescens*, and *Lactobacillus paracasei* are all identified to induce defense towards different pathogens in tomato

plant (Singh et al. 2016; Narasimhamurthy et al. 2020; Boukerma et al. 2017; Wu et al. 2018).

3.6.1 Production of Enzymes

The lysis of the fungal cell wall is assisted by hydrolytic enzymes. These enzymes deform components of fungal pathogens' cell walls. It is one of the most critical mechanisms for managing soil-inhabiting pathogens in an eco-friendly manner (Tariq et al. 2017). Many microbial strains, such as *B. cereus*, *Serratia marcescens*, *B. thuringiensis*, and others, have the ability to generate degrading enzymes for the control of phytopathogens such as *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium oxysporum*, and through hyphal swelling, hyphal curling, or hyphal bursting (Jadhav and Sayyed 2016). It is widely assumed that bacteria with chitin and glucans in their cell walls can be used to biologically protect crops from pathogens (Rai and Nabti 2017).

The two devastating plant pathogens such as *R. solani* and *Phytophthora capsici* are also prevented by PGPR (Islam et al. 2016). Since many plant pathogenic fungi have chitin-based cell walls, chitinase-producing rhizobacteria are of particular importance for the biocontrol of pathogens, reducing the dependence on chemicals. In hosts like wheat and soybean, the capacity of *B. subtilis* to mobilize and solubilize zinc has been demonstrated (Ramesh et al. 2014), as well as in mung bean and soybean plants (Sharma et al. 2013). In studies involving *B. aryabhattai*, zinc solubilization has also been documented (Mumtaz et al. 2017).

Beneficial rhizobacteria as well as their consortia produce lytic enzymes like protease, acylases, and lactonases that hydrolyze pathogenic bacteria, fungi, and protozoa cell walls, containing pathogen infection in hosts (Nayak et al. 2020). *Streptomyces* sp. secretes chitinases, and *Streptomyces* RC1071 from cerrado soils was found to have antagonistic activity against fungi (Shafi and Khattab 2020). Proteases, lipases, chitinases, and cellulases have all been documented to be produced by actinobacteria (El-Sherbiny et al. 2017). Actinobacteria that generate enzymes have biocontrol ability and can help to boost plant growth features (Wani and Gopalakrishnan 2019). Numerous bacterial species have proven to be effective antipathogens in the fight against plant pathogens; these include *Azospirillum*, *Bacillus*, *Pythium*, *Coniothyrium*, *Pseudomonas*, and *Serratia* (Murthy et al. 2014; Heidarzadeh and Baghaee-Ravari 2015). Amylase, cellulase, chitinase, esterase, lipase, protease, and urease are important players in N, H, and C bacteria's biological transformation processes (Rai and Nabti 2017; Xun et al. 2015).

3.6.2 Production of Volatile Organic Compounds (VOCs)

Bacterial volatile organic compounds (VOCs) are revealed to be determinants of plant ISR elicitation (Sharifi and Ryu 2016). Improved defense, tolerance to abiotic stress, and biomass of plant are all mediated, either directly or indirectly, by VOCs

from PGPR strains (Hassen et al. 2016). A wide number of soil microorganisms emit VOCs and include benzene, methyl, decane, cyclohexane, benzene (1-methylnonadecyl), 2-(benzyloxy)ethanamine, dotriacontane, 11-decyldocosane, 1-(N-phenylcarbamyl)-2-morpholinocyclohexene, dodecane, 1-chlorooctadecane, and 2,6,10-trimethyl tetradecane, despite the fact that the amount and form of VOCs produced differ by PGPR (Kanchiswamy et al. 2015; Gouda et al. 2018).

Biocontrol strains synthesize VOCs that promote plant development, inhibit plant pathogens, and mediate ISR against different plant pathogens (Raza et al. 2016a, b). Some species such as *Bacillus*, *Serratia*, *Arthrobacter*, *Stenotrophomonas*, and *Pseudomonas* are just a few of the bacteria that produce VOCs; these affect host development. The acetoin and 2,3-butanediol, both created by *Bacillus* species, are the greatest powerful VOCs for preventing fungal progress and improving plant development (Santoro et al. 2016; Shafi et al. 2017).

3.7 Commercialization of Rhizobacteria

Because of environment and health concerns about long-term pesticide use, there is a lot of interest in exploring alternate control methods for use in combined crop management approaches (Reuveni 1995). Pesticides will almost definitely be used less in the future, with a focus on biological alternatives, such as the usage of beneficial microbes as biological control agents (Mishra et al. 2021b). In contrast to synthetic pesticides, microbes as biocontrol agents characteristically have a limited range of action, and their performance in real-world agriculture is often inconsistent, resulting in their limited commercialization (Baker 1991).

During the 1996 growing season, more than five million ha of yields were applied with these produces mainly to control *R. solani* and *Fusarium* sp., which caused root diseases in addition to promoting mass of root and seedling vigor. Growing and registering biopesticides is half the price of conventional pesticides. They are eco-friendly, ensure a high cost-benefit ratio, and do not put the pathogen at risk of developing resistance. They are easy to use and work well with biofertilizers. The use of five marketable chitosan-based preparations of PGPR grown at Auburn University in the United States has demonstrated growth promotion of cucumber, pepper, and tomato plants. Amendment with each of the formulations in rice field soil in a 1:40 (formulation:soil) ratio showed a substantial twofold rise in weights of shoot and root, as well as grain yield. The findings indicate that commercial bacterial formulations can be applied as microbial inoculums to increase rice plant development (Vasudevan et al. 2002).

3.8 Future Trends and Prospects

Rhizobacteria that promote plant growth are the most important players in the rhizosphere, and their composition and biomass have a direct effect on the plant's response to the environment. Rhizobacterium-mediated plant growth promotion is

attributed to increased uptake of water and nutrients and enhanced phytohormone synthesis. The activity of rhizosphere is greatly influenced by various aspects like water content, microbial population, type of soil, and their communication with roots and also physiology of plants. Rhizo-atmosphere depends on root exudates which aid in enhanced nutrient acquisition, mitigate mineral deprivation, and promote microbial proliferation. Understanding of the genes involved in such exudation processes has enabled us to transfer them into plants since many of the genes controlling these exudates have now been identified; it is possible to manipulate conditions in the rhizosphere by genetic engineering. The activity of rhizosphere bacteria is largely dependent on various factors like colonization ability and completion with resident microflora. These crucial determinants are genetically altered in improved strains. Introduced chiA genes in rhizosphere bacteria efficiently suppressed several soil phytopathogens. Genetically modified rhizosphere bacteria with genes encoding for higher expression of plant growth hormones positively affected root growth, nutrient uptake, and stress tolerance. Another application of rhizosphere engineering is the rhizoremediation for restoration of contaminated soil and groundwater. Efficient rhizoremediation is dependent on the synergy between plant (exudates), soil (chemistry), and microbial communities. Interactions between certain bacterial populations of the grass rhizospheres promoted the development of definite hydrocarbon-degrading bacteria, which eventually enhanced removal of hydrocarbons (Mezzari et al. 2011).

Rhizosphere engineering mainly aims at plant modification to enhance nutrient uptake, tolerate stresses, and support beneficial microflora. Microbial profiling is essential to determine their influence on plant progress and health. Type of soil and plant exudation mainly determine the bacterial profile in the soil. Rhizosphere engineering deals with extending the persistence, fitness, and effectiveness of the introduced bacterial communities in the new environment soil. This bias is achieved by microbe-utilizable substrates either added to the soil or released by the plants (e.g., opines). Rhizosphere engineering using microbes is generally done by managing beneficial microbial populations by exploiting their natural processes, modifying their metabolic activities, or manipulating the soil or host plant inoculations. Transfer of soil suppressiveness attributed to a range of beneficial microbes from disease-free soils to disease-conducive soils is known to manage several soilborne phytopathogens (Haas and Défago 2005). Disease-protective ability of suppressive soils is largely due to the microbial consortia rather than individual taxon and such consortia can be exploited for rhizosphere engineering (Mendes et al. 2011).

Finally, rhizosphere engineering aims to reduce our dependence on agrochemicals by substituting or enhancing the behavior of beneficial microbes. In spite of the advent of latest technologies like next-generation sequencing technologies, majority of the rhizosphere still remain unexplored and uncultured. Phylogenetic analysis and developmental stage-dependent microbial activities have to be emphasized for their optimal exploitation. Greater understanding dynamics of soil root and bacterial interface in the rhizosphere in the light of new technological developments through effective scientific collaborations will ease the challenges of pesticides and heavy metal remediation.

References

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahmed B, Zaidi A, Khan MS et al (2017) Perspectives of plant growth promoting rhizobacteria in growth enhancement and sustainable production of tomato. In: Zaidi A, Khan M (eds) Microbial strategies for vegetable production. Springer, Cham, pp 125–149. https://doi.org/10.1007/978-3-319-54401-4_6

Aloo BN, Makumba BA, Mbega ER (2019) The potential of Bacilli rhizobacteria for sustainable crop production and environmental sustainability. Microbiol Res 219:26–39. https://doi.org/10.1016/j.micres.2018.10.011

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.0097

Anand K, Kumari B, Mallick MA (2016) Phosphate solubilizing microbes: an effective and alternative approach as bio-fertilizers. Int J Pharm Sci 8(2):37–40

Anandaraj M, Sarma YR (2003) The potential of PGPRs in disease management of spie crops. Abstracts and Short Papers. 6th International PGPR Workshop, 5–10 October 2003, Indian Institute of Spices Research, Calicut, India, p 27–39

Baker R (1991) Diversity in biological control. Crop Prot 10(2):85–94. https://doi.org/10.1016/0261-2194(91)90054-U

Barea J-M, Pozo MJ, Azcón R et al (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Barman S, Das S, Bhattacharya SS (2019) The prospects of bio-fertilizer technology for productive and sustainable agricultural growth. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, Chennai, pp 233–253. https://doi.org/10.1016/B978-0-444-64191-5.00017-1

Bhattacharyya PN, Goswami MP, Bhattacharyya LH (2016) Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. J Phytology 8:26–41. https://doi.org/10.19071/jp.2016.v8.3022

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbial Biotechnol 28:1327–1350. https://doi.org/10.1007/s11274-011-0979-9

Bjelić D, Marinković J, Tintor B et al (2018) Antifungal and plant growth promoting activities of indigenous rhizobacteria isolated from maize (*Zea mays* L.) rhizosphere. Commun Soil Sci Plant Anal 49:88–98. https://doi.org/10.1080/00103624.2017.1421650

Boukerma L, Benchabane M, Charif A et al (2017) Activity of plant growth promoting rhizobacteria (PGPRs) in the biocontrol of tomato Fusarium wilt. Plant Prot Sci 53(2):78–84. https://doi.org/10.17221/178/2015-PPS

Burdman S, Jurkevitch E, Okon Y (2000) Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. In: Subba Rao NS, Dommergues YR (eds) Microbial interactions in agriculture and forestry. Science Publishers, Enfield, NH, pp 229–250

Buzón-Durán L, Pérez-Lebeña E, Martín-Gil J et al (2020) Applications of *Streptomyces* spp. enhanced compost in sustainable agriculture. In: Biology of composts. Springer, Cham, pp 257–291. https://doi.org/10.1007/978-3-030-39173-7_13

Byrne MP, Tobin JT, Forrestal P et al (2019) The nitrogen cycle a mini review. Biosys Eng Res Rev Dublin, University College Dublin

Chaiharn M, Chunhaleuchanon S, Lumyong S (2009) Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. World J Microbiol Biotechnol 25:1919–1928. https://doi.org/10.1007/s11274-009-0090-7

Chang WT, Chen YC, Jao CL (2007) Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shell fish chitin wastes. Bioresour Technol 98(6):1224–1230. https://doi.org/10.1016/j.biortech.2006.05.005

Choudhary M, Ghasal PC, Yadav RP et al (2018) Towards plant-beneficiary rhizobacteria and agricultural sustainability. In: Meena V (ed) Role of rhizospheric microbes in soil. Springer, Singapore, pp 1–46. https://doi.org/10.1007/978-981-13-0044-8_1

Chowdappa P, Kumar SM, Lakshmi MJ et al (2013) Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol Control 65:109–117. https://doi.org/10.1016/j.biocontrol.2012.11.009

Chithrashree, Udayashankar AC, Nayaka S et al (2011) Plant growth-promoting rhizobacteria mediated induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. Biol Control 59(2):114–122. https://doi.org/10.1016/j.biocontrol.2011.06.010

Damam M, Kaloori K, Gaddam B et al (2016) Plant growth promoting substances (phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal plants. Int J Pharm Sci Rev 37(1):130–136

Defago G (2003) Mechanisms of biological control. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (eds) Abstracts and Short Papers. 6th International PGPR Workshop, 5–-10 October 2003, Indian Institute of Spices Research, Calicut, India, p 106

Diby P, Srinivasan V, Anandaraj M et al (2003) Abstracts and short papers. In 6th International PGPR Workshop, 5–10 October 2003, Indian Institute of Spices Research, Calicut, India, p 27–39

Dobbelaere S, Croonenborghs A, Thys A et al (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Australian J Plant Physiol 28:871–879

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209. https://doi.org/10.1146/annurev.phyto.42.040803.140421

Egamberdieva D, Lugtenberg B (2014) Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses. Springer, New York, NY, pp 73–96. https://doi.org/10.1007/978-1-4614-9466-9_4

El-Sherbiny GM, Darwesh OM, El-Hawary AS (2017) Taxonomic characterization of the chitinolytic actinomycete *Cellulomonas chitinilytica* strain HwAC11. Int J Adv Res Biol Sci 4:292–299

Fasusi OA, Babalola OO (2021) The multifaceted plant-beneficial rhizobacteria toward agricultural sustainability. Plant Prot Sci 57(2):95–111. https://doi.org/10.17221/130/2020-PPS

García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2(3):108–205. https://doi.org/10.3934/bioeng.2015.3.183

Ghosh A, Gangopadhyay D (2019) Impact of plant growth promoting rhizobacteria in sustainable agriculture: an important natural resource for crop improvement. Int J Plant Environ 5(3):210–214. https://doi.org/10.18811/ijpen.v5i03.10

Gislason AS, Fernando WD, de Kievit TR (2020) Biosynthesized secondary metabolites for plant growth promotion. In: Keswani C (ed) Bioeconomy for sustainable development. Springer, Singapore, pp 217–250. https://doi.org/10.1007/978-981-13-9431-7_12

Glick B (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39. https://doi.org/10.1016/j.micres.2013.09.009

Glick BR, Patten CL, Holguin G et al (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68. https://doi.org/10.1006/jtbi.1997.0532

Gopalakrishnan S, Humayun P, Kiran BK et al (2011) Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. World J Microbiol Biotechnol 27:1313–1321. https://doi.org/10.1007/s11274-010-0579-0

Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2:1–19. https://doi.org/10.1080/23311932.2015.1127500

Gouda S, Kerry RG, Das G et al (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res 206:131–140. https://doi.org/10.1016/j.micres.2017.08.016

Haas D, Défago G (2005) Biological control of soilborne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hassen AI, Bopape FL, Sanger LK (2016) Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. In: Singh D, Singh H, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 23–36. https://doi.org/10.1007/978-81-322-2647-5_2

Heidarzadeh N, Baghaee-Ravari S (2015) Application of Bacillus pumilus as a potential biocontrol agent of Fusarium wilt of tomato. Archives of Phytopathology and Plant Protection 48:841-849. https://doi.org/10.1080/03235408.2016.1140611

Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot 89(5):503–512. https://doi.org/10.1093/aob/mcf076

Holguin G, Glick BR (2001) Expression of the ACC deaminase gene from *Enterobacter cloacae* UW4 in *Azospirillum brasilense*. Microbial Ecol 41(3):281–288. https://doi.org/10.1007/s002480000040

Hong SH, Lee EY (2014) Vegetation restoration and prevention of coastal sand dunes erosion using ion exchange resins and the plant growth-promoting rhizobacteria *Bacillus* sp. SH1RP8 isolated from indigenous plants. Int Biodeterior Biodegrad 95:262–269. https://doi.org/10.1016/j.ibiod.2014.05.026

Idriss EE, Makarewicz M, Farouk A et al (2002) Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiol 148:2097–2109

Islam S, Akanda AM, Prova A et al (2016) Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol 6:1360. https://doi.org/10.3389/fmicb.2015.01360

Jadhav H, Sayyed R (2016) Hydrolytic enzymes of rhizospheric microbes in crop protection. MOJ Cell Sci Rep 3(5):135–136. https://doi.org/10.15406/mojcsr.2016.03.00070

Jadhav HP, Shaikh SS, Sayyed RZ (2017) Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: an overview. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation microorganisms for sustainability, vol 2. Springer, Singapore, pp 183–203. https://doi.org/10.1007/978-981-10-4862-3_9

Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. E3 J Agric Res Dev 5:108–119

Jiang CJ, Liu XL, Liu XQ et al (2017) Stunted growth caused by blast disease in rice seedlings is associated with changes in phytohormone signaling pathways. Front Plant Sci 8:1558. https://doi.org/10.3389/fpls.2017.01558

Joshi R, Wani SH, Singh B et al (2016) Transcription factors and plants response to drought stress: current understanding and future directions. Front Plant Sci 7:1029. https://doi.org/10.3389/fpls.2016.01029

Jung H, Janelle K, McCouch S (2013) Getting to the roots of it: genetic and hormonal control of root architecture. Front Plant Sci 4(186):1–32. https://doi.org/10.3389/fpls.2013.00186

Kanchiswamy CN, Malnoy M, Maffei ME (2015) Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front Plant Sci 6:151. https://doi.org/10.3389/fpls.2015.00151

Kaymak DC (2010) Potential of PGPR in agricultural innovations. In: Maheshwari D (ed) Plant growth and health promoting bacteria. Microbiology monographs, vol 18. Springer, Berlin, Heidelberg, pp 45–79. https://doi.org/10.1007/978-3-642-13612-2_3

Kilian M, Steiner U, Krebs B et al (2000) FZB24 *Bacillus subtilis*—mode of action of a microbial agent enhancing plant vitality. Pflanzenschutz-Nachrichten, Bayer 1:72–93

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94(11):1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Kumar A, Meena VS, Roy P et al (2019) Role of Rhizobia for sustainable agriculture: lab to land. In: Kumar A, Meena V (eds) Plant growth promoting rhizobacteria for agricultural sustainability. Springer, Singapore, pp 129–149. https://doi.org/10.1007/978-981-13-7553-8_7

Kumar SS, Ram KR, Kumar DR et al (2013) Biocontrol by plant growth promoting rhizobacteria against black scurf and stem canker disease of potato caused by *R. solani*. Arch Phytopathol Plant Prot 46:487–502. https://doi.org/10.1080/03235408.2012.745054

Leclere V, Bechet M, Adam A et al (2005) Mycosubtilin overproduction by *Bacillus subtilis* BBG100 enhances the organism's antagonistic and biocontrol activities. Appl Environ Microbiol 71:4577–4584

Lugtenberg BJ, Chin-A-Woeng TFC, Bloemberg GV (2002) Microbe-plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81(1–4):373–383. https://doi.org/10.1023/a:1020596903142

Ma W, Penrose DM, Glick BR (2002) Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Can J Microbiol 48:947–954. https://doi.org/10.1139/w02-100

Mahmood S, Daur I, Al-Solaimani SG et al (2016) Plant growth promoting rhizobacteria and silicon synergistically enhance salinity tolerance of mung bean. Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.00876

Mekonnen H, Kibret M (2021) The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. Chem Biol Technol Agric 8:15. https://doi.org/10.1186/s40538-021-00213-y

Mendes R, Kruijt M, de Bruijn I et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100. https://doi.org/10.1126/science.1203980

Mezzari MP, Zimermann DMH, Corseuil HX et al (2011) Potential of grasses and rhizosphere bacteria for bioremediation of diesel-contaminated soils. Rev Bras Ciênc Solo 35:2227–2236. https://doi.org/10.1590/S0100-06832011000600038

Mhlongo MI, Piater LA, Madala NE et al (2018) The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112. https://doi.org/10.3389/fpls.2018.00112

Mishra BB, Nayak SK, Pahari A (eds) (2021a) Agriculturally important microorganisms: mechanisms and applications for sustainable agriculture. New India Publishing Agency, New Delhi

Mishra BB, Nayak SK, Mohapatra S et al (eds) (2021b) Environmental and agricultural microbiology: applications for sustainability. Wiley-Scrivener Publisher, Beverly

Mumtaz MZ, Ahmad M, Jamil M et al (2017) Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. Microbiol Res 202:51–60. https://doi.org/10.1016/j.micres.2017.06.001

Murthy KN, Uzma F, Chitrashree Srinivas C (2014) Induction of systemic resistance in tomato against *Ralstonia solanacearum* by *Pseudomonas fluorescens*. Am J Plant Sci 5(12):1799–1811. https://doi.org/10.4236/ajps.2014.512193

Narasimhamurthy K, Soumya K, Udayashankar AC et al (2020) Efficacy of indigenous plant growth promoting rhizobacteria and Trichoderma strains in eliciting resistance against bacterial wilt in tomato. Egypt J Biol Pest Control 30:106. https://doi.org/10.1186/s41938-020-00303-3

Nascimento FX, Rossi MJ, Glick BR (2018) Ethylene and 1-Aminocyclopropane-1-carboxylate (ACC) in plant-bacterial interactions. Front Plant Sci 9:114. https://doi.org/10.3389/fpls.2018.00114

Nautiyal CS, Bhadauria S, Kumar P et al (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol Lett 182:291–296. https://doi.org/10.1111/j.1574-6968.2000.tb08910.x

Nayak SK, Nayak S, Patra JK (2020) Rhizobacteria and its biofilm for sustainable agriculture: a concise review. In: Yadav MK, Singh BP (eds) New and future developments in microbial

biotechnology and bioengineering: microbial biofilms current research and future trends in microbial biofilms. Elsevier, Amsterdam, pp 165–175. https://doi.org/10.1016/B978-0-444-64279-0.00013-X

Omran R, Kadhem MF (2016) Production, purification, and characterization of bioactive metabolites produced from rare actinobacteria *Pseudonocardia alni*. Asian J Pharma and Clinical Res 9:1–9

Oteino N, Lally RD, Kiwanuka S et al (2015) Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Front Microbiol 6:745. https://doi.org/10.3389/fmicb.2015.00745

Parmar P, Sindhu SS (2013) Potassium solubilisation by Rhizosphere Bacteria: influence of nutritional and environmental conditions. J Microbial Res 3:25–31. https://doi.org/10.5923/j.microbiology.20130301.04

Patel ST, Minocheherhomji FP (2018) Review: plant growth promoting rhizobacteria: blessing to agriculture. Int J Pure Appl Biosci 6:481–492

Pawar ST, Bhosale AA, Gawade TB et al (2016) Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. J Microbiol Biotechnol Res 3(3): 24–31

Pin-Ng JL, Perrine-Walker F, Wasson AP et al (2015) The control of auxin transport in parasitic and symbiotic root-microbe interactions. Plan Theory 4:606–643. https://doi.org/10.3390/plants4030606

Prathap M, Ranjitha KBD (2015) A critical review on plant growth promoting rhizobacteria. J Plant Pathol Microbiol 6(4):1–4. https://doi.org/10.4172/2157-7471.1000266

Rai A, Nabti E (2017) Plant growth-promoting bacteria: importance in vegetable production. In: Zaidi A, Khan M (eds) Microbial strategies for vegetable production. Springer, Cham, pp 23–48. https://doi.org/10.1007/978-3-319-54401-4_2

Ramadan EM, AbdelHafez AA, Hassan EA et al (2016) Plant growth promoting rhizobacteria and their potential for biocontrol of phytopathogens. Afr J Microbiol Res 10:486–504

Ramesh A, Sharma SK, Sharma MP et al (2014) Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in vertisols of central India. Appl Soil Ecol 73:87–96. https://doi.org/10.1016/j.apsoil.2013.08.009

Raza W, Ling N, Yang L et al (2016a) Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. Sci Rep 6:24856. https://doi.org/10.1038/srep24856

Raza W, Yousaf S, Rajer FU (2016b) Plant growth promoting activity of volatile organic compounds produced by bio-control strains. Sci Lett 4(1):40–43

Reuveni R (1995) Novel approaches for integrated pest management. Lewis Publishers, Boca Raton, FL

Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785. https://doi.org/10.3389/fmicb.2016.01785

Rosier A, Medeiros FH, Bais HP (2018) Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant Soil 428:35–55. https://doi.org/10.1007/s11104-018-3679-5

Rozycki H, Dahm H, Strzelczyk E et al (1999) Diazotrophic bacteria in root-free soil and in the root zone of pine (*Pinus sylvestris* L.) and oak (*Quercus robur* L.). Appl Soil Ecol 5:29–56

Sanlibaba P, Cakmak GA (2016) Exo-polysaccharides production by lactic acid bacteria. Appl Microbiol 2(2):1–5

Santoro MV, Bogino PC, Nocelli N et al (2016) Analysis of plant growth promoting effects of Fluorescent pseudomonas strains isolated from *Mentha piperita* rhizosphere and effects of their volatile organic compounds on essential oil composition. Front Microbiol 7(1085):1–17

Sayyed RZ, Naphade BS, Chincholkar SB (2005) Ecologically competent rhizobacteria for plant growth promotion and disease management. In: Rai MK, Chikhale NJ, Thakare PV,

Wadegaonkar PA, Ramteke PA (eds) Recent trends in biotechnology. Scientific Publishers, Jodhpur, pp 1–16
Schaller GE (2012) Ethylene and the regulation of plant development. BMC Biol 10:9. https://doi.org/10.1186/1741-7007-10-9
Shafi J, Tian H, Ji M (2017) *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnol Biotechnol Equip 31:446–459. https://doi.org/10.1080/13102818.2017.1286950
Shafi ME, Khattab AE-NA (2020) Improvement of chitinase produced from chitin of desert locust by *Streptomyces halstedii* strain isolated from Jeddah, KSA. Adv Env Biol 14:7–19. https://doi.org/10.22587/aeb.2020.14.1.2
Shah S, Li J, Moffatt BA et al (1998) Isolation and characterization of ACC deaminase genes from two different plant growth promoting rhizobacteria. Can J Microbiol 44:833–843
Shaikh S, Sayyed RZ (2015) Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, India, pp 337–351. https://doi.org/10.1007/978-81-322-2068-8_18
Shankar UAC, Nayaka CS, Niranjan-Raj S et al (2009) Rhizobacteria mediated resistance against *Bean common mosaic virus* strain blackeye cowpea mosaic in cowpea (*Vigna unguiculata*). Pest Manag Sci 65:1059–1064. https://doi.org/10.1002/ps.1791
Sharifi R, Ryu CM (2016) Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, Alarm signals to Arabidopsis seedlings for eliciting induced resistance, or both? Front Microbiol 7(196):1–10. https://doi.org/10.3389/fmicb.2016.00196
Sharma SB, Sayyed RZ, Trivedi MH et al (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2:587
Singh D, Yadav DK, Chaudhary G et al (2016) Potential of *Bacillus amyloliquefaciens* for biocontrol of bacterial wilt of tomato incited by *Ralstonia solanacearum*. J Plant Pathol Microbiol 7(327):1–6. https://doi.org/10.4172/2157-7471.1000327
Son JS, Sumayo M, Hwang YJ et al (2014) Screening of plant growth promoting rhizobacteria as elicitor of systemic resistance against grey leaf spot dieses in pepper. Appl Soil Ecol 73:1–8
Stein T (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 56(4):845–857. https://doi.org/10.1111/j.1365-2958.2005.04587.x
Sureshbabu K, Amaresan N, Kumar K (2016) Amazing multiple function properties of plant growth promoting rhizobacteria in the rhizosphere soil. Int J Curr Microbiol Appl Sci 5(2):661–683
Tariq M, Noman M, Ahmed T et al (2017) Antagonistic features displayed by plant growth promoting rhizobacteria (PGPR): a review. J Plant Sci Phytopathol 1:38–43. https://doi.org/10.29328/journal.jpsp.1001004
Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by Rhizosphere bacteria. Annu Rev Phytopathol 36:453–483. https://doi.org/10.1146/annurev.phyto.36.1.453
Van Loon LC, Glick BR (2004) Increased plant fitness by rhizobacteria. In: Sandermann H (ed) Ecological studies, Molecular ecotoxicology of plants, vol 170. Springer Verlag, Berlin, pp 177–205. https://doi.org/10.1007/978-3-662-08818-0_7
Vasudevan P, Kavitha S, Priyadarisini VB et al (2002) Biological control of rice diseases. p 11–32. In: Gnanamanickam SS (ed) Biological control of crop diseases. Marcel Dekker Inc., New York, p 468
Vejan P, Abdullah R, Khadiran T et al (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules 21(5):573. https://doi.org/10.3390/molecules21050573
Viveros OM, Jorquera MA, Crowley DE et al (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10:293–319
Walsh UF, Morrissey JP, O'Gara F (2001) *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Cur Opinion Biotechnol 12:289–295
Wang Y, Ohara Y, Nakayashiki H et al (2005) Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. Mol Plant Microbe Inte 18:385–396

Wani SP, Gopalakrishnan S (2019) Plant growth-promoting microbes for sustainable agriculture. In: Sayyed RZ et al (eds) Plant growth promoting rhizobacteria (PGPR): prospects for sustainable agriculture. Springer, Singapore, pp 19–45. https://doi.org/10.1007/978-981-13-6790-8_219

Wu G, Liu Y, Xu Y et al (2018) Exploring elicitors of the beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 to induce plant systemic resistance and their interactions with plant signaling pathways. Mol Plant-Microbe Interact 31(5):560–567. https://doi.org/10.1094/MPMI-11-17-0273-R

Xun F, Xie B, Liu S et al (2015) Effect of plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. Environ Sci Poll Res Int 22:598–608. https://doi.org/10.1007/s11356-014-3396-4

Zahir ZA, Arshad M, Frankenberger WT (2004) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agronomy 81:97–168. https://doi.org/10.1016/S0065-2113(03)81003-9

Zehnder GW, Murphy JF, Sikora EJ et al (2001) Application of rhizobacteria for induced resistance. European J Plant Pathol 107:39–50. https://doi.org/10.1023/A:1008732400383

The Role of Arbuscular Mycorrhiza in Sustainable Agriculture

4

Mehdi Sadravi

Abstract

Eighty percent of plants, including field crops, vegetables, fruit trees, and ornamental and medicinal plants, have arbuscular mycorrhiza. Arbuscular mycorrhizal fungi form arbuscules in the endodermis of root tissue, and an extramatrical fine hyphal net. Arbuscular mycorrhizal fungi help in the management of diseases caused by fungi, fungal-like organisms, nematodes, bacteria, phytoplasmas, and physiological disorders by increasing the absorption of water and nutrient elements for plants, competing with pathogens for nutrients and establishment site, making changes in chemical constituents of plant tissues, changing the root structure, alleviating the environmental stresses, and increasing the population of useful bacteria in soil. They also contribute to optimum plant growth and improved nutrient absorption in heavy metal-contaminated soils. As a result, in disturbed lands, arbuscular mycorrhizal fungi are powerful biological restoratives. They will help to minimize the use of chemical fertilizers and pesticides, which are both detrimental to the environment and agricultural product consumers. The use of these beneficial fungi can increase crop production and establish sustainable nonchemical agriculture.

Keywords

Funneliformis · Glomeromycota · *Glomus* · Phosphorous

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_4

4.1 Introduction

Arbuscular mycorrhiza is found in 80% of plants, including field crops, fruit trees, vegetables, and ornamental and medicinal plants (Avazzadeh-Mehrian and Sadravi 2017; Błaszkowski et al. 2010; Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) spores usually have plentiful storage lipid, some carbohydrate, and thick walls with chitin and at some instances with β1–3-glucan (Driver et al. 2005). The growth of hyphae as spore germination involves some reserves of carbohydrates and lipids, nuclear division, and production of limited amounts of branching coenocytic mycelium. Signal molecules from the roots of an associated plant will stimulate hyphal branching. Low soil phosphorus concentrations increase the growth and branching of the hyphae as well as induce plant exudation. The hyphae penetrate into the hairy and lateral roots and grow between epidermal cells. Most of these fungi appear between the cells of the root parenchyma, producing oval or ovoid "vesicles" with a thin wall in the middle or at the end of the hyphae (Fig. 4.1a). Due to the fact that these vesicles are rich in fat and their number increases in old roots, they are considered as a source of food storage and energy of the fungus and are durable after plant death in the soil. In this way, the fungus can survive in the root tissue for a long time if the roots are not removed from the soil. All of these fungi, inside the cells of endodermis, produce a shrub-like structure called "arbuscule" between the plasma membrane and the cell wall, which is rich in nuclei, glycogen particles, fat globules, and vacuoles. Arbuscules are the specific structures of arbuscular mycorrhizal fungi and the sites for the interchange of nutrients such as phosphorus, water, and carbohydrate (Fig. 4.1b). AMF produce a delicate network of "extraradical hyphae" on the root surface, after establishment in the root tissue. This hyphal network absorbs water and nutrients and transfers them to the root tissues and arbuscles. Spores are produced at the tips of extraradical hyphae branches, and after being released from the mother cell and germinating, they mutualize with other parts of the same plant's root or adjacent plants (Peterson et al. 2004).

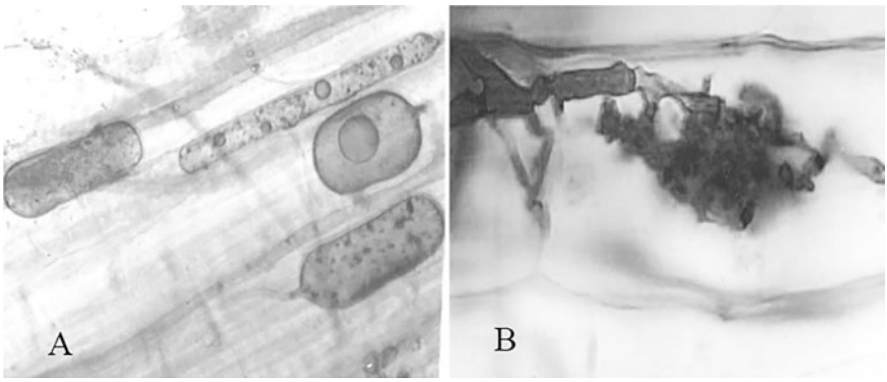

Fig. 4.1 Arbuscular mycorrhizal structure: (**A**) vesicles between the cells of the root parenchyma, (**B**) arbuscule inside the cell of root endodermis

Arbuscular mycorrhiza or endomycorrhiza is associated with mutual benefits, which means that the fungus provides more water and nutrients, and in return the plant supplies necessary carbohydrates for the fungus. AMF increase plant resistance to drought, environmental stresses, soil and water salinity, and soilborne pathogens. They also increase the activity of nitrogen-fixing bacteria leading to increase in agricultural yield and also uplift the efficiency of plants to grow in deserts, sand dunes, and contaminated soils (Sadravi 2000, 2005; Ray 2020).

4.2 The Range of Symbiotic Plants

The range of symbiotic plants of AM fungi is extremely wide, viz. wheat, barley, corn, sorghum, alfalfa, soybean, sunflower, sesame, cotton, apple, grape, olive, jujube, peace lily, ceriman, syngonium, pothos, sansevieria, asparagus fern, and spineless yucca (Avazzadeh-Mehrian and Sadravi 2017; Błaszkowski et al. 2010; Sadravi 2002, 2003, 2004, 2006a, b, c, d, 2007, 2010; Sadravi and Gharacheh 2015; Sadravi et al. 1999, 2000; Sadravi and Moshiri-Rezvany 2019; Sadravi and Seifi 2002). Moreover, arbuscular mycorrhiza has been reported in most herbaceous plants, and some valuable trees such as *Acer*, *Araucaria*, *Podocarpus*, and *Agathis*, as well as all members of *Taxodiaceae*, *Cupressaceae*, *Cephalotaxaceae*, and *Taxaceae* in addition to most tropical hardwoods. Reports available on plants in several major families, including *Brassicaceae*, *Chenopodiaceae*, *Polygonaceae*, *Caryophyllaceae*, *Proteaceae*, and *Juncaceae*, do not show any mycorrhizal symbiosis (Smith and Read 2008).

4.3 Taxonomy of Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhiza is thought to have formed about 1000 million years ago, at the same time as plants began to emerge on land (Fortin et al. 2005). AM fungi were probably important in plant colonization of land due to their roles in nutrient uptake. The first species of AM fungi were introduced in the genus *Glomus* in 1845, followed by the genera *Sclerocystis*, *Acaulospora*, *Gigaspora*, *Entrophospora*, *Scutellospora*, and others (Schenck and Perez 1990). Arbuscular mycorrhizal fungi were placed in the *Endogonales* (*Zygomycota*) due to their aseptate hyphae and similar spores to the zygospores of *Endogone* species, more than two decades ago (Morton and Benny 1990; Morton and Redecker 2001). The first attempt at representing phylogenetic relationships was made using cladistic tools and assuming a new monophyletic order *Glomales* (*Zygomycota*), containing only those fungi for which carbon is acquired obligately from their host plants via arbuscules. Then by genetic analysis, arbuscular mycorrhizal fungi were placed in the independent new phylum *Glomeromycota* (Schüβler et al. 2001). Since then, the taxonomy of arbuscular mycorrhizal fungi has greatly progressed (Oehl et al. 2008; Krüger et al. 2012; Wijayawardene et al. 2020). The latest taxonomy of AMF is given in Table 4.1.

Table 4.1 Taxonomy of arbuscular mycorrhizal fungi (Phylum: *Glomeromycota*)

Class	Order	Family	Genus	Species (type)
Glomeromycetes	*Glomerales*	*Glomeraceae*	*Glomus*	*G. macrocarpum*
			Funneliformis	*F. mosseae*
			Dominikia	*D. minuta*
			Halonatospora	*H. pansihalos*
			Kamienskia	*K. bistrata*
			Oehlia	*O. diaphana*
			Rhizophagus	*R. populinus*
			Sclerocystis	*S. coremioides*
			Sclerocarpum	*S. amazonicum*
		Claroideoglomeraceae	*Claroideoglomus*	*C. claroideum*
	Diversisporales	*Acaulosporaceae*	*Acaulospora*	*A. laevis*
			Entrophospora	*E. infrequens*
		Diversisporaceae	*Diversispora*	*D. spurca*
			Corymbiglomus	*C. corymbiforme*
			Desertispora	*D. omaniana*
			Otospora	*O. bareai*
			Redeckera	*R. megalocarpa*
			Tricispora	*T. nevadensis*
		Gigasporaceae	*Gigaspora*	*G. gigantea*
			Scutellospora	*S. calospora*
			Bulbospora	*B. minima*
			Cetraspora	*C. gilmorei*
			Dentiscutata	*D. nigra*
			Intraornatospora	*I. intraornata*
			Paradentiscutata	*P. baiana*

			Racocetra	*R. coralloidea*
		Pacisporaceae	*Pacispora*	*P. scintillans*
		Sacculosporaceae	*Sacculospora*	*S. baltica*
Archaeosporomycetes	*Archaeosporales*	*Archaeosporaceae*	*Archaeospora*	*A. trappei*
			Palaeospora	*P. spainiae*
		Ambisporaceae	*Ambispora*	*A. fennica*
		Geosiphonaceae	*Geosiphon*	*G. pyriformis*
Paraglomeromycetes	*Paraglomerales*	*Paraglomeraceae*	*Paraglomus*	*P. occultum*
			Innospora	*I. majewskii*
		Pervetustaceae	*Pervetustus*	*P. simplex*

4.4 The Role of AM Fungi in the Management of Plant Diseases

4.4.1 Impact on Fungal and Fungal-Like Diseases

Symbiosis of *Funneliformis mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler with barley root provides significant protection against infection with *Gaeumannomyces graminis* (Sacc.) vonArex and Olivier var. *tritici* Walker, the cause of take-all disease (Castellanos-Morales et al. 2011). *Rhizophagus intraradices* (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler symbiosis with chickpea root increased growth, number of pods, nitrogen, potassium, and phosphorus content, stem dry weight, and number of nitrogen-fixing bacterial nodules in the root, and reduced root rot caused by *Macrophomina phaseolina* (Tassi) Goidanich (Akhtar and Siddiqui 2010). In greenhouse, inoculating a mixture of several arbuscular mycorrhizal fungi into cucumber roots increases growth and controls vascular wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* J.H. Owen (Hu et al. 2010). The combination of a mixture of two AM fungi, and *Pseudomonas fluorescens* (Flugge 1886) Migula 1895, significantly reduces root rot of French bean (*Phaseolus vulgaris* L.) caused by *Rhizoctonia solani* J.G. Kühn and increases growth and yield (Neeraj 2011). The mixture of *F. mosseae* and *R. intraradices* significantly protected strawberry against wilt disease caused by *Verticillium dahliae* Klebahn, as well as showed a 60% increase in yield (Tahmatsidou et al. 2006). *R. intraradices*, *Trichoderma harzianum* Rifai, and *P. fluorescens* were inoculated alone or in combination with tomato in greenhouse and field conditions, to control Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici*. All treatments significantly reduce disease severity, but the combination of these fungi and bacterium has the best effect, as this treatment reduces the disease severity by 74% and provides a 20% increase in yield (Srivastava et al. 2009). Cucumber inoculation with *F. mosseae*, *Penicillium simplicissimum* (Oudem.) Thom, and *T. harzianum*, alone or in combination with control seedling damping-off by *R. solani*, showed that although each of these fungi can reduce disease severity, the best effect has been the mixture treatment of *F. mosseae* + *T. harzianum* (Chandanie et al. 2009). Inoculation of pea root with *R. intraradices* increased phosphorus in plant tissues and significantly reduced the severity of root rot caused by *Aphanomyces euteiches* Drechsler (Bodker et al. 1998). Inoculation of three citrus cultivars' seedling roots with the inoculum of *Acaulospora tuberculata* Janos and Trappe, and *Claroideoglomus etunicatum* (WN Becker and Gerd.) C. Walker and A. Schüßler for root rot disease control, caused by *Phytophthora nicotianae* Breda de Haan, significantly decreased dieback and increased the amount of phosphorus in leaves (Watanarojanaporn et al. 2011).

4.4.2 Impact on Plant Parasitic Nematodes

A review of 65 research articles on the effect of AM fungi on plant parasitic nematodes has shown that among the AM fungi *R. intraradices*, *C. etunicatum*,

and *F. mosseae* have the ability to reduce damage of root-knot nematodes (*Meloidogyne* sp.) and *Tylenchorhynchus* sp. (Gera Hol and Cook 2005). Cherry seedlings inoculated with *R. intraradices* showed significantly higher weight (in wet) and stem diameter than non-mycorrhizal seedlings and were more resistant to *Pratylenchus vulnus* Allen and Jensen (Pinochet et al. 1995). Apple seedlings inoculated with *F. mosseae* showed more resistance to *P. vulnus*, and their wet root weight and branch length were significantly higher than non-mycorrhizal seedlings (Pinochet et al. 1993). Symbiosis of *F. mosseae* with citrus roots has protected the roots against *Radopholus similis* Cobb and reduced the nematode population by 50% (Elsen et al. 2001). While stimulating plant growth, inoculation of tomato rootstocks with *Funneliformis coronatus* (Giovann.) C. Walker and A. Schüßler significantly reduced infection with the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood (Diedhiou et al. 2003).

4.4.3 Impact on Plant Bacterial Diseases

Tomato plants colonized with an arbuscular mycorrhizal fungus, that were inoculated by the wilt-causing bacterium *Pseudomonas syringae* pv. *syringae* van Hall 1902, after 3 weeks showed higher growth than non-mycorrhizal plants (Garcia-Garrido and Ocampo 1989).

4.4.4 Impact on Phytoplasmas

Inoculation of tobacco root with an arbuscular mycorrhizal fungus to investigate its effect on aster yellows disease has also showed that this symbiosis significantly increases root length and photosynthesis of diseased plants (Kaminska et al. 2010).

4.4.5 Impact on Plants' Physiological Disorders

In a greenhouse experiment, inoculating tomato seedlings with *F. mosseae* significantly improved their growth and yield in saline soil compared to uninoculated plants (Zhong Qun et al. 2007). Inoculation of the roots of coffee seedlings with *Glomus* sp. increased their resistance to drought and salinity of water and soil (Andrade et al. 2009).

4.5 Arbuscular Mycorrhizal Fungi's Mode of Action

4.5.1 Greater Water and Nutrient Uptake, and Minimizing Environmental Stresses

Arbuscular mycorrhizal fungi increase water uptake and transfer into the roots of symbiotic plants due to an increase in root absorption area by an extramatrical hyphal net. Also, the penetration of their arbuscules to endodermis cells provides a suitable path across the root for water to move and reach the woody vessels. These fungi also increase root growth, which in turn provides an extensive root system for water uptake. These fungi increase the uptake of inactive nutrients such as phosphorus from the soil, by secretion of enzyme phosphatase. Increased phosphorus absorption by these fungi for plants contributes to increased growth rate and faster passage of the plant through the critical stage of youth, as well as the ability of cells to proliferate and repair damaged tissues from soilborne pathogens. Arbuscular mycorrhizal fungi increase the absorption of water and nutrients, making plants more resistant to environmental stresses such as drought and nutrient deficiency (Pfleger and Linderman 1994).

4.5.2 Changes in Plant Tissue Chemicals

Colonization of peanut (*Arachis hypogaea* L.) and leek (*Allium porrum* (L.) J. Gay) roots by an arbuscular mycorrhizal fungus increased levels of ortho-dihydric phenol, a potent inhibitor of soilborne pathogens (Mahadevan 1991). An increase in isoflavonoid phytoalexin-like substances has been reported in soybean arbuscular mycorrhizal roots, which have shown resistance to infection by pathogenic fungi and nematodes (Pfleger and Linderman 1994).

4.5.3 Compete with Pathogens for Location and Nutrients

Plant parasitic nematodes usually attack roots and require plant-produced nutrients for growth and reproduction. Earlier establishment of arbuscular mycorrhizal fungi prevents nematodes from developing and absorbing nutrients (Gera Hol and Cook 2005).

4.5.4 Structural Changes in Roots

Increased lignification has been observed in mycorrhizal root cells of cucumber and tomato, which is considered to be the main factor in their resistance to vascular Fusarium wilt (Pfleger and Linderman 1994; Tahat et al. 2010).

4.5.5 Increasing the Population of Beneficial Soil Bacteria

Mycorrhizal roots have richer exudates that provide a suitable environment for the growth of beneficial soil bacteria. The extramatrical hypha of AM fungi also causes fine soil particles to aggregate and improve airflow in the soil, which is essential for the growth and propagation of soil bacteria. As a consequence, the population of nitrogen-fixing bacteria, plant growth-promoting rhizobacteria (PGPR), and a number of gram-positive bacteria in the rhizosphere increases, and the population of pathogens from the genus *Fusarium* or *Phytophthora* decreases significantly (Tarkka and Frey-Klett 2008).

4.6 AM Fungi's Importance in Phytoremediation of Polluted Soils

The expansion of cities and industrial factories has increased the contamination of limited agricultural lands with harmful substances from industrial wastewaters or urban wastes, posing a serious threat to society's sustainable production of agricultural products and food security. Toxic elements such as lead, copper, zinc, mercury, arsenic, cadmium, and nickel can enter water or soil through industrial and urban wastewater or through the extensive use of chemical fertilizers or herbicides, and pesticides. These heavy metals also increase the risk of oxidation of plant tissues, resulting in symptoms of root rot, plant yellowing, and stunted growth. The presence of these heavy metals in water and soil resource reduces the activity of beneficial microorganisms such as PGPRs and soil fertility, ultimately leading to significant yield reduction. Cadmium, one of these toxic heavy metals, prevents the growth of roots and stems, affects the absorption of essential nutrients from the soil, and often accumulates in the product. Refining soils contaminated with heavy metals requires a lot of energy and money. Methods such as excavation or soil leaching can damage the structure and soil fertility and transfer contaminants to groundwater. Green refining has been proposed as a low-cost, environmental-friendly alternative technology. Phytoremediation is a technology that uses plants to remove, decompose, or produce less hazardous materials in soil and water. This method uses plants that have the ability to tolerate or store large amounts of metal in the rhizosphere and their tissues. Plants that are symbiotic with AM fungi show a greater ability to regenerate contaminated soil (Schutzenduble and Polle 2001; Gaur and Adholeya 2004). AM fungi cause selective permeability of roots by forming extraradical hypha (Sudova and Vosatka 2007). The mechanisms that these fungi use to reduce the stress of heavy metals on plants include chelating of heavy metals, improving mineral nutrition (especially phosphorus) for the plant and accelerating growth, changing the pH of the rhizosphere, and regulating the expression of metal transporter genes (Gonzalez-Guerrero et al. 2005). The cell wall materials of AM fungi have compounds (e.g., hydroxyl and carboxyl and free amino acid groups) which can bind to toxic heavy metals and immobilize them. Cell wall proteins of AM fungi such as glomalin also show the ability to combine with heavy metals and inactivate

them. In a cadmium-contaminated soil, *F. mosseae* symbiosis with clover has inhibited its impact on optimal plant growth and production (Biro and Takacs 2007). Arbuscular mycorrhiza of sorghum in lead-contaminated soil stabilizes and inactivates this heavy metal in fungal organs by polyphosphate granules (Wong et al. 2007).

4.7 Conclusion

The increased use of chemical fertilizers and pesticides presents significant risks to human society and the environment. Arbuscular mycorrhizal fungi have symbiosis with most field crops, fruit trees, vegetables, and ornamental and medicinal plants. Some of the AMF can increase water and nutrient uptake for plants and resistance to pathogens and decrease soil pollution. Therefore, through sufficient inoculation with efficient AM fungus, plants can be well protected against diseases, environmental stresses, and soil contamination with toxic elements. The following factors should be considered when using them: (a) efficiency of the arbuscular mycorrhizal fungus used; (b) sufficient amount of inoculation of these fungi; (c) suitability of the symbiotic plant genotype (although these fungi do not have a specific host, in terms of root tissue establishment and multiplication in it, there may be differences between different cultivars of a plant, which affects their efficiency); (d) inoculation and their establishment before the attack of pathogens; and (e) suitability of physical and chemical conditions of soil and environmental conditions for their maximum efficiency and deterrence of pathogens. Also, for the survival of AM fungi in the soil, need to be performed agricultural operations with minimal tillage after harvesting, and set up a crops rotation with well symbiotic plants including cereals and legumes to increase their population. In this way, while increasing their efficiency, their inhibitory power from pathogens will be stable. The use of these beneficial fungi can increase crop production and establish sustainable nonchemical agriculture.

References

Akhtar MS, Siddiqui ZA (2010) Effect of AM fungi on the plant growth and root-rot disease of chickpea. Am Eurasian J Agric Environ Sci 8:544–549

Andrade SAL, Mazzafera P, Schiavinato MA et al (2009) Arbuscular mycorrhizal association in coffee. J Agric Sci 147(2):105–115. https://doi.org/10.1017/S0021859608008344

Avazzadeh-Mehrian Z, Sadravi M (2017) The effect of chemical properties of soil on symbiosis of apple and grape with arbuscular mycorrhizal fungi. J Soil Manage Sust Prod 7(1):115–125

Biro I, Takacs T (2007) Effects of *Glomus mosseae* strains of different origin on plant macro and micronutrient uptake in Cd polluted and unpolluted soils. Acta Agron Hung 55(2):1–10. https://doi.org/10.1556/AAgr.55.2007.2.6

Błaszkowski J, Kovacs GM, Balazs TK et al (2010) *Glomus africanum* and *G. iranicum*, two new species of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycologia 102(6):1450–1462. https://doi.org/10.3852/09-302

Bodker L, Kjoller R, Rosendah S (1998) Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza 8:169–174. https://doi.org/10.1007/s005720050230

Castellanos-Morales V, Keiser C, Cárdenas-Navarro R et al (2011) The bio protective effect of AM root colonization against the soil-borne fungal pathogen *Gaeumannomyces graminis* var. *tritici* in barley depends on the barley variety. Soil Biol Biochem 43(4):831–834. https://doi.org/10.1016/j.soilbio.2010.12.020

Chandanie WA, Kubota M, Hyakumachi M (2009) Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl Soil Ecol 41(3):336–341. https://doi.org/10.1016/j.apsoil.2008.12.006

Diedhiou PM, Hallmann J, Oerke EC et al (2003) Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. Mycorrhiza 13(4):199–204. https://doi.org/10.1007/s00572-002-0215-4

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biol Biochem 37(1):101–106. https://doi.org/10.1016/j.soilbio.2004.06.011

Elsen A, Declerck S, de Waele D (2001) Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture. Mycorrhiza 11:49–51. https://doi.org/10.1007/s005720100100

Fortin JA, Declerck S, Strullu D-G (2005) In vitro culture of mycorrhizas. In: Declerck S, Fortin JA, Strullu D-G (eds) Vitro culture of mycorrhizas. Soil biology, vol 4. Springer, Berlin, Heidelberg, pp 3–16. https://doi.org/10.1007/3-540-27331-X_1

Garcia-Garrido JM, Ocampo JA (1989) Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biol Biochem 21:165–167. https://doi.org/10.1016/0038-0717(89)90027-8

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86:528–534

Gera Hol WH, Cook R (2005) An overview of arbuscular mycorrhizal fungi-nematode interactions. Basic Appl Ecol 6:489–503. https://doi.org/10.3389/fmicb.2015.01280

Gonzalez-Guerrero M, Azcon-Aguilar C, Mooney M et al (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42(2):130–140. https://doi.org/10.1016/j.fgb.2004.10.007

Hu JL, Lin XG, Wang JH et al (2010) Arbuscular mycorrhizal fungal inoculation enhances suppression of cucumber Fusarium wilt in greenhouse soils. Pedosphere 20(5):586–593. https://doi.org/10.1016/S1002-0160(10)60048-3

Kaminska M, Klamkowski K, Berniak H et al (2010) Effect of arbuscular mycorrhizal fungi inoculation on aster yellows phytoplasma-infected tobacco plants. Sci Hortic 125:500–503

Krüger M, Krüger C, Walker C et al (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193(4):970–984. https://doi.org/10.1111/j.1469-8137.2011.03962.x

Mahadevan A (1991) Biochemical Aspects of Disease Resistance. Part II. Post Infectional Defense Mechanisms in Plants. Today and Tomorrows Publishers, New Delhi, 871p

Morton JB, Benny GL (1990) Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales*, two new suborders, *Glomineae* and *Gigasporineae*, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. Mycotaxon 37:471–491

Morton JB, Redecker D (2001) Two new families of *Glomales, Archaeosporaceae* and *Paraglomeraceae*, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characteristics. Mycologia 93:181–195. https://doi.org/10.1080/00275514.2001.12063147

Neeraj KS (2011) Organic amendments to soil inoculated arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* treatments reduce the development of root-rot disease and enhance the yield of *Phaseolus vulgaris* L. Eur J Soil Biol 47:288–295. https://doi.org/10.1016/j.ejsobi.2011.07.002

Oehl F, de Souza FA, Sieverding E (2008) Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular-forming *Glomeromycetes*. Mycotaxon 106:311–360

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: Anatomy and Cell Biology. NRC Research Press, Ottawa, 182p

Pfleger FL, Linderman RG (1994) Mycorrhizae and Plant Health. APS Press, St. Paul, MN, 85p

Pinochet J, Calvet C, Camprubi A et al (1995) Interaction between the root-lesion nematode *Pratylenchus vulnus* and the mycorrhizal association of *Glomus intraradices* and Santa Lucia 64 cherry rootstock. Plant Soil 170:323–329. https://doi.org/10.1007/BF00010485

Pinochet J, Camprubi A, Calvet C (1993) Effects of the root-lesion nematode *Pratylenchus vulnus* and the mycorrhizal fungus *Glomus mosseae* on the growth of EMLA-26 apple rootstock. Mycorrhiza 4:79–83. https://doi.org/10.1007/BF00204062

Ray P (2020) Mycorrhizae and its scope in agriculture. In: Nayak SK, Mishra BB (eds) Frontiers in soil and environmental microbiology, 1st edn. CRC Press, Boca Raton, pp 73–80. https://doi.org/10.1201/9780429485794-8

Sadravi M (2000) Role of mycorrhizal fungi in the stabilization of running sands and desert revegetation. In: Abstracts of Regional Congress of Durable Extention in Deserts Restoration, Yazd, Iran, p 63

Sadravi M (2002) Five Glomus species of arbuscular mycorrhizal fungi from Iran. J Agric Sci Nat Resour 9:15–30

Sadravi M (2003) Mycorrhizal fungi of soybean, sunflower and sesame in Golestan Province of Iran. In: Abstracts of First National Oilseeds Congress. Gorgan, Iran, p 6

Sadravi M (2004) Seven fungi new for Iran. J Agric Sci Nat Resour 11(3):71–78

Sadravi M (2005) Role of mycorrhizal fungi in increasing growth, yield and resistance in pulse crops. In: Abstracts of First Pulse Crops Congress of Iran. Ferdowsi University of Mashhad, Mashhad, Iran, p 132

Sadravi M (2006a) Arbuscular mycorrhizal fungi of wheat in northeast Iran. In: Abstracts of 8th International Mycological Congress. Cairns, QLD, Australia, p 86

Sadravi M (2006b) Comparison population and species richness of arbuscular mycorrhizal fungi between six field crops. In: Abstracts of 17th Plant Protection Congress of Iran. University of Tehran, Karaj, Iran, p 180

Sadravi M (2006c) Effect of crops rotation on population of mycorrhizal fungi of wheat. J Agric Sci Nat Resour 13:90–98

Sadravi M (2006d) Establishment of four arbuscular mycorrhizal fungi in tomato's root on minimal M medium. In: Abstracts of 17th Plant Protection Congress of Iran. University of Tehran, Karaj, Iran, p 200

Sadravi M (2007) Arbuscular mycorrhizal fungi of wheat fields in the Golestan Province. Rostaniha 7:129–140

Sadravi M (2010) Couch grass new host for arbuscular mycorrhizal fungus *Gigaspora gigantea*. In: Abstracts of 19th Iranian Plant Protection Congress. Iranian Institute of Plant Protection Researches, Tehran, Iran, p 111

Sadravi M, Gharacheh N (2015) Arbuscular mycorrhizal fungi of pulse crops in southwest Iran. In: Abstracts of Asian Mycological Congress, 7–10 October 2015. Goa University, Goa, India, p 172

Sadravi M, Moshiri-Rezvany R (2019) Biodiversity of arbuscular mycorrhizal fungi of seven ornamental plants in northeast Iran. In: Abstracts of 4th Iranian Mycological Congress. Sari Agricultural Sciences and Natural Resources University, Sari, Iran, p 10

Sadravi M, Seifi E (2002) Mycorrhizal fungi of olive trees in Golestan province. In: Abstracts of Second Golestan Province Researches Projects Congress, Gorgan, Iran, p 60

Sadravi M, Mohamadi-Goltapeh E, Blaszkowski J, Minasian V, Alizadeh A (1999) Four vesicular-arbuscular mycorrhizal fungi of Iran. Seed Plant 15:9–24

Sadravi M, Mohamadi-Goltapeh E, Blaszkowski J et al (2000) Seven cereal vesicular–arbuscular mycorrhizal fungi of Iran. In: Abstracts of 14th Plant Protection Congress of Iran. University of Tehran, Karaj, Iran, p 27

Schenck NC, Perez Y (1990) Manual for the Identification of VA Mycorrhizal Fungi. Synergistic Publications, Gainesville, FL, 286p

Schutzenduble A, Polle A (2001) Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105(12):1413–1421

Smith SE, Read D (2008) Mycorrhizal Symbiosis, 3rd edn. Elsevier, London, 815p

Srivastava R, Khalid A, Singh US et al (2009) Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol Control 53:24–31. https://doi.org/10.1016/j.biocontrol.2009.11.012

Sudova R, Vosatka M (2007) Differences in the effects of three arbuscular mycorrhizal fungal strains on P and Pb accumulation by maize plants. Plant Soil 296(1):77–83. https://doi.org/10.1007/s11104-007-9291-8

Tahat MM, Sijam K, Othman R (2010) Mycorrhizal fungi as a biocontrol agent. Plant Pathol J 9(4):198–207. https://doi.org/10.3923/ppj.2010.198.207

Tahmatsidou V, O'Sullivan J, Cassells AC et al (2006) Comparison of AMF and PGPR inoculants for the suppression of Verticillium wilt of strawberry (*Fragaria ananassa* cv. *selva*). Appl Soil Ecol 32(3):316–324. https://doi.org/10.1016/j.apsoil.2005.07.008

Tarkka MT, Frey-Klett P (2008) Mycorrhiza helper bacteria. In: Varma A (ed) Mycorrhiza, state of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics. Springer-Verlag, Berlin Heidelberg, Germany, pp 113–134. https://doi.org/10.1007/978-3-540-78826-3_6

Watanarojanaporn N, Boonkerda N, Wongkaewb S et al (2011) Selection of arbuscular mycorrhizal fungi for citrus growth promotion and *Phytophthora* suppression. Sci Hortic 128(4):423–433. https://doi.org/10.1016/j.scienta.2011.02.007

Wijayawardene NN, Hyde KD, Al-Ani LKT et al (2020) Outline of Fungi and fungus-like taxa. Mycosphere 11(1):1060–1456. https://doi.org/10.5943/mycosphere/11/1/8

Wong CC, Wu SC, Kuek C et al (2007) The role of mycorrhizae associated with Votive grown in Pb-Zn contaminated soils: greenhouse study. Restor Ecol 15:60–67. https://doi.org/10.1111/j.1526-100X.2006.00190.x

Zhong Qun H, Chao Xing H, Zhi Bin Z et al (2007) Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. Colloids Surf B-Biointerfaces 59(2):128–133. https://doi.org/10.1016/j.colsurfb.2007.04.023

5 Biocontrol Efficacy of Biomass and Secondary Metabolites of *P. fluorescens* Against Predominant Pest Affecting Agricultural Fields

C. Elizabeth Rani and S. Anusha Vijayan

Abstract

The constant increase in population has led to drastic increase in demand for all common necessities. Overexploitation of land resources and industrialization have led to new interventions in agriculture. Thus as to meet the ever-increasing needs of man, usage of unconventional methods has been adapted; this includes the latest practice of using chemical fertilizers and pesticides in agricultural fields, and also utilization of genetically modified plants has contributed largely for the same. Loss of natural fertility and indigenous microbial flora has decreased soil productivity and soil health. Modern agricultural practices have taken its toll on natural sustainability of land and nature, leading to slow, inconspicuous degradation of all natural processes that have continued to replenish soil fertility. Studies to replace chemical fertilizers have been going on for a decade now and there has been no valid advancement. Many biological organisms have been studied for the same. *Pseudomonas fluorescens* is one such biological organism that has been researched for its variety of beneficial properties.

Keywords

Pseudomonas fluorescens · Biocontrol · Plant growth-promoting rhizobacteria (PGPR) · Secondary metabolite · Bioformulation

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_5

5.1 Introduction

Introduction of chemical fertilizers, pesticides, and herbicides has drastically altered natural and age-old agricultural practices and has led to severe consequences. Their improper and continued excessive usage may cause irrevocable changes to the natural terrain. Excessive use of such chemical pesticides results in environmental pollution through contamination of groundwater by crop products and heavy metals present in such fertilizers and pesticides. Contamination of agricultural land by such heavy metals may eventually lead to medical damages, like cancer in humans. Apart from causing such medical damages, they also result in other agricultural repercussion, like degradation of natural nutrients in soil and depletion of nutrient replenishment. To avoid further damage alternative effective replacements are explored. The use of microorganisms called plant growth-promoting rhizobacteria (PGPR) is a largely opted replacement for chemical pesticides (Agbodjato et al. 2015). Among rhizobacteria, fluorescent pseudomonads are largely exploited alternates. They have been described as potent biocontrol agents against plant diseases. *Pseudomonas fluorescens, P. aeruginosa,* and *P. putida* are known to possess varying degrees of antifungal activity. *Pseudomonas* sp. includes common, nonpathogenic saprophytic organisms that colonize plant roots, soil, etc. They are very common soilborne gram-negative, rod-shaped rhizobacterium. *Pseudomonas* sp. includes ubiquitous bacteria and are preferred in agriculture as they possess many traits that make them effective PGPR. Among all Pseudomonas strains, *Pseudomonas fluorescens* is considered the most effective PGPR.

Research to understand and explore the potential ability of bacteria that belong to Pseudomonas species is advancing to a great extent to achieve plausible results. Deshwal et al. (2011) reported that *Pseudomonas* strains isolated from Mucuna induced production of hydrocyanic acid (HCN). Gupta et al. (2002) isolated the indole acetic acid (IAA)-producing *Pseudomonas fluorescens* bacterium from the potato plant rhizosphere. Alongside usage of intrusive modern-day agricultural practices another major challenge humankind face is the threat to food security due to emerging invasive pests. Invasive pests adversely affect agricultural biodiversity. Each year pest invasion in agricultural lands alone causes huge loss of agricultural yield and causes adverse economic losses. Invasive pests are exotic, non-native insects with high dispersal potential and invade natural habitats of native pest by eliminating them in the processes. Deliberate or unintentional introduction of invasive insects outside their habitats may cause them to invade and outcompete naturally occurring native insects, as they tend to express their capability to establish themselves in the new habitat. Increase in global trade in agriculture is one of the major reasons for the introduction of exotic insect pests. Cotton mealybug, coconut mite, and papaya mealybug are some examples of such exotic pests.

5.2 Fluorescent Pseudomonas

Pseudomonads belong to the class gamma proteobacteria, family Pseudomonades, and order Pseudomonadales. The genus *Pseudomonas* contains about 236 species (http://www.bacterio.net/pseudomonas.html). All bacteria that belong to this species are nonspore forming and catalase positive, possess one or more polar flagella, and are aerobic microorganisms. They are saprophytic microbes that inhabit plant root rhizosphere. *Pseudomonads* species are considered as the one of the most effective PGPR organisms and *Pseudomonads fluorescence* that belongs to this class is considered to possess antagonistic activity against most pathogens and pests (Weller et al. 2002). They are one of the most important groups of bacteria that colonize plant rhizosphere and also exhibit a set of beneficial mechanisms such as HCN production, antibiotic activity, competition, synthesis of siderophore, and fluorescent pigments and also possess antifungal properties (Singh and Mallick 2008). Thus they are globally studied for their potential properties and exploited accordingly.

5.3 Mode of Action of Fluorescent Pseudomonas

5.3.1 Biofertilizers

Plant growth-regulating rhizobacteria like fluorescent Pseudomonas are novel biofertilizers that are commercialized in large-scale fertilizer production. PGPR inoculants regulate plant growth via direct and indirect mechanisms or by a combination of direct and indirect mechanisms.

The direct mechanism involves

- Nitrogen fixation by bacterium
- High availability of nursing in rhizosphere area, increased solubilization of phosphate, and iron absorption by siderophore production
- Increased production of phytohormones as auxin, gibberellins, and cytokinins (Patten and Glick 2002)

The indirect mechanism involves increased resistance against plant pathogens by synthesizing inhibitors and also by increasing natural resistance of the host plant assisted by rhizobacteria (Handelsman and Stabb 1996).

The indirect mechanism of plant growth promotion involves

- Production of antifungal metabolites
- Synthesis of fungal cell wall-lysing enzymes
- Production of antibiotics
- Induced pathogenic resistance

5.3.2 Biocontrol Agents

Plant growth-regulating bacteria produce secondary metabolites that exhibit antimicrobial activity. Such secondary metabolites possess biocontrol properties and can protect the host plant against devastating phytopathogens. Fluorescent Pseudomonas produces secondary metabolites like siderophores and antibiotic elements that are plant growth hormones and mitigate the phytopathogen invasion. Secondary metabolites originate sideways of primary metabolites and are not essential because they are neither an important energy source nor used as reserve substances (Budzikiewicz 1993). Fluorescent Pseudomonas produces secondary metabolites such as hydrocyanic acid (HCN), pyoluteorin, phenazines, phloroglucinol, and cyclic lipopeptide (Mishra and Arora 2017).

5.3.3 Microbial Activity of Secondary Metabolites

Secondary metabolites produced by *Pseudomonas fluorescens* act as antagonistic agents in a microbial consortium. Secondary metabolites that consist of bacteriocins, siderophores, and certain antibiotic elements synthesized by *P. fluorescens* encompass their ability to work as an efficient PGPR; this has been the main focus in investigating and understanding PGPR (Maksimov et al. 2011). The antagonistic activities exhibited by such rhizobacteria are as follows:

- They can synthesize enzymes with hydrolytic properties, proteases, chitinases, etc. and can lyse fungal cell walls of pathogens (Maksimov et al. 2011).
- They regulate concentrations of ethylene in the host plant via ACC deaminase enzyme that can control the ethylene level in the host plant when exposed to stress due to infections (Glick and Bashan 1997).
- Rhizobacteria show the ability to compete for minerals and suitable colonization niches in root surfaces (Validov 2007).

Beattie (2007) stated that biocontrol agents are microbes that possess the ability to reduce the severity of diseases on host plants and antagonists exhibit antagonistic activity against pathogens. Riley and Wertz (2002) determined that bacteria synthesize a wide variety of antimicrobial compounds, like lytic agents, antibiotic agents, and exotoxins along with the synthesis of secondary metabolites. Rhizobacteria contribute to PGPR activity by production of auxin as they play a direct role in growth regulation of the host plant (Patten and Glick 2002). Plant growth-promoting rhizobacteria are exploited commercially to create sustainable agriculture. They were studied in association with many plants such as wheat, maize, peas, oat, and soy (Gray and Smith 2005). Juneius and Jenisha (2016) carried out studies to assess the efficiency of *Pseudomonas fluorescence* against rice (PAPTATLA) leaf folder pest (*Cnaphalocrocis medinalis*) and *Pythium* spp. MTCC 10247 and the samples were obtained from the area of Thiruporur, Chennai. *Pseudomonas fluorescence*

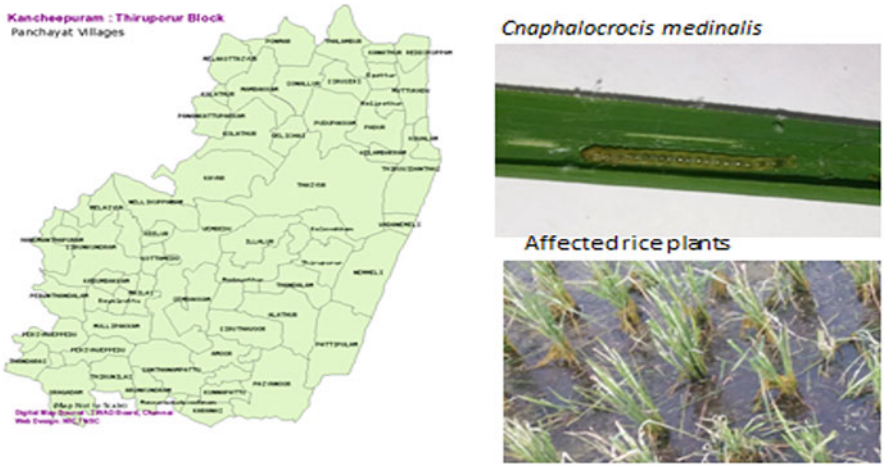

Fig. 5.1 Study site showing biopesticide efficiency of *P. fluorescens* secondary metabolites against chosen paddy pest

possesses a diversity of promising properties, which makes it an improved biocontrol agent.

A proportional study was conducted using formulated, wet biomass and a broth containing secondary metabolites of *Pseudomonas fluorescence*. The effectiveness of secondary metabolites comprising broth was very high when correlated to other preparations and they could kill the rice leaf folder pest larvae within 7 h. A histopathological investigation of the pest larvae treated with formulated biomass showed that it collapsed the epithelial cells of internal organs. The outcome exposed that there were noteworthy histological variations in the treated pests when matched to the control. Production of secondary metabolites of *Pseudomonas fluorescence* was carried out and the compounds were characterized by TLC, SDS-PAGE, and GC-MS. Seven peak compounds were found: 1,4-diaza-2,5-dioxobicyclo [4.3.0] nonane (13.69%), 3-isobutylhexahydropyrrolo [1,2-a]pyrazine-1,4-dione (10.11%), pyrrolo [1,2-a] pyrazine 1,4dione,hexahydro-3-(2-methylpropyl (17.79%) l-leucine, N-cyclopylcarbonyl-pentadecyl ester (7.09%), 3,6-diisobutyl-2,5-piperazinedione (37.62%), 3-benzylhexahydropyrrolo [1,2-A]pyrazine-1,4-dione (8.52%), L-prolinamide, and 5-oxo-l-prolyl-l-phenylanyl-4-hydroxy (5.19%). Antifungal activity of the ethyl acetate extract of the secondary metabolites was examined against *Pythium* spp. MTCC 10247 and result revealed that the lag period of the fungi was double-fold higher than the control and the compounds were fungistatic because there was a 57% of growth reduction after seventh day of incubation period. All the seven compounds were used for molecular docking study and result showed higher score value with L-prolinamide and 5-oxo-l-prolyl-l-phenylanyl-4-hydroxy against cell wall protein (3GNU receptor) *Pythium* spp. MTCC 10247 (Fig. 5.1).

5.4 Conclusion

P. fluorescens and its family strains need to be studied and investigated for their ability to synthesize siderophores and other plant growth-promoting phytohormones and also antibiotic compounds that possess antagonistic properties. They could be significant in maintaining sustainable agricultural process. Plant growth-regulating rhizobacteria require new systemic designs that will help for their potential and profitable usage. Bioformulation of such strains could be an effective and resourceful alternative for chemical pesticides. New inoculants that possess antagonistic and pest resistance properties can be formulated and can be used to replace harmful chemical pesticides and fertilizers. Combination of different strategies to apply such rhizobacteria to agricultural fields can increase agricultural outputs, increase crop yield, and reduce pest invasions all while avoiding chemical fertilizers.

References

Gupta A, Meyer JM, Goel R (2002) Development of heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization. Curr Microbiol 45(5): 323–327. https://doi.org/10.1007/s00284-002-3762-1

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15(2):353–378. https://doi.org/10.1016/s0734-9750(97)00004-9

Beattie GA (2007) Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances. In: Gnanamanickam SS (ed) Plant-associated bacteria. Springer, Dordrecht, pp 1–56. https://doi.org/10.1007/978-1-4020-4538-7_1

Budzikiewicz H (1993) Secondary metabolites from fluorescent *pseudomonads*. FEMS Microbiol Rev 10(3–4):209–228. https://doi.org/10.1111/j.1574-6968.1993.tb05868.x

Juneius CER, Jenisha E (2016) Comparative studies on the biocontrol efficiencies of wet biomass, dry biomass and secondary metabolites of *Pseudomonas fluorescence* on *Cnaphalocrocis medinalis*. Int Res J Eng Technol 3(4):2518–2530

Deshwal VK, Vig K, Amisha et al (2011) Synergistic effects of the inoculation with plant growth-promoting Rhizobium and Pseudomonas on performance of Mucuna. Ann Forest 19(1):13–20

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. https://doi.org/10.1016/j.soilbio.2004.08.030

Handelsman J, Stabb EV (1996) Biocontrol of soil borne plant pathogens. Plant Cell 8(10): 1855–1869. https://doi.org/10.1105/tpc.8.10.1855

Mishra J, Arora NK (2017) Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture. Appl Soil Ecol 125:35–45. https://doi.org/10.1016/j.apsoil.2017.12.004

Maksimov IV, Abizgil'dina RR, Pusenkova LI (2011) Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (review). Appl Biochem Microbiol 47(4):333–345. https://doi.org/10.1134/S0003683811040090

Agbodjato NA, Noumavo PA, Baba-Moussa F et al (2015) Characterization of Potential Plant Growth Promoting Rhizobacteria isolated from Maize (*Zea mays* L.) in Central and Northern Benin (West Africa). Appl Environ Soil Sci 2015:901656. https://doi.org/10.1155/2015/901656

Patten CL, Glick BR (2002) Role of Pseudomonas putida indole acetic acid in development of the host plant root system. Appl Environ Microbiol 68(8):3795–3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002

Riley MA, Wertz JE (2002) Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56:117–137. https://doi.org/10.1146/annurev.micro.56.012302.161024

Singh AK, Mallick N (2008) Enhanced production of SCL-LCL-PHA co-polymer by sludge-isolated *Pseudomonas aeruginosa* MTCC 7925. Lett Appl Microbiol 46(3):350–357. https://doi.org/10.1111/j.1472-765X.2008.02323.x

Validov S (2007) Biological control of tomato foot and root rot by *Pseudomonas* bacteria in stone wool. Leiden University, Leiden, The Netherlands

Weller DM, Raaijmakers JM, McSpadden Gardner BB et al (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348. https://doi.org/10.1146/annurev.phyto.40.030402.110010

Exopolysaccharide-Producing *Azotobacter* for Bioremediation of Heavy Metal-Contaminated Soil

6

Reginawanti Hindersah

Abstract

High content of heavy metal (HM) in arable soils is harmful to the food web in soil and probably menaces the human being. Nowadays, bioremediation techniques using microbes and plants are suggested as effective and clean technologies to overcome the HM contamination problem persisting in soil. Among the rhizobacteria, *Azotobacter* is a candidate for HM bioremediation due to their ability to synthesize exopolysachharide (EPS). This review aims to discuss mainly the *Azotobacter* resistance on toxic HM, as well as their formation of EPS that has a role in HM mobilization and accumulation in phytoremediation plant. This concise chapter also reports current knowledge on the source of heavy metal contamination in agricultural soil, and the ability of plants to prevent HM toxicity by synthesizing polypeptide phytochelatin in cytosol. A proposed theory of mechanism of *Azotobacter* involved in bioremediation of HM is also elaborately explained.

Keywords

Azotobacter · Exopolysaccharide (EPS) · Metal mobility · Metal availability · Phytoremediation

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_6

6.1 Introduction

Increasing toxic heavy metal (HM) content in agricultural soil due to anthropogenic activities paved the way for hazardous impact on living beings. Highly toxic HMs in soil are harmful to the complex food web in soil and a threat to animal as well as human health. Moreover, metals cannot undergo biodegradation; in soil several metals have more than one oxidation state (charge) that describes their mobility and bioavailability. Excessive HM mobilization increases their absorption by plant roots and induces HM toxicity.

In the agricultural field, increment of HM in soil is possibly caused by utilization of sewage and industrial sludge, irrigation water, manure, and rock phosphate-based fertilizer. In some mineral mining, tailing deposition in agricultural area can increase HM level in soil. Bioremediation technology that utilizes living and dead cell as well as microbial metabolites and HM accumulator plants (well known as phytoremediation) are suggested to control metal mobility in soil. Moreover, bioremediation process does not generate another contaminant that the chemical and physical processes do (Gupta and Diwan 2017).

Bioremediation enables to resolve the issues on soil contamination by low HM concentration (Iram et al. 2013; Patel et al. 2016). The plant growth-promoting rhizobacteria (PGPR) including *Erwinia* sp., *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Serratia* sp., *Chromobacterium* sp., *Caulobacter* sp., *Azospirillum* sp., *Azotobacter* sp., and *Agrobacterium* sp. have the ability to produce plant growth-promoting substances under HM stress which is further helpful in the alleviation of the abiotic stresses (Bhattacharyya and Jha 2012; Patel et al. 2016).

In sustainable agriculture, PGPR *Azotobacter* is a well-known biofertilizer to improve nitrogen and phosphor status in plant and soil through nitrogen fixation and phosphate solubilization (Nosrati et al. 2014). It is broadly reported that increment in plant growth and yield was observed due to *Azotobacter* inoculation (Namvar and Khandan 2013; Sumbul et al. 2020). Moreover, researchers demonstrated the ability of *Azotobacter* to protect the environment by alleviating soil abiotic stress especially HM stresses (Nanda and Abraham 2011; Hindersah et al. 2017).

The mechanism by which *Azotobacter* remediates HM-contaminated soil is by excreting the exopolysaccharide (EPS) polymer that was synthesized in the cytosol (Sabra et al. 2000; Gauri et al. 2012). The physicochemical properties of bacterial EPS result in net anionic that allows positively charged HM attachment by coordinative covalent bond (Ozdemir et al. 2003; Gupta and Diwan 2017). The EPS of *Azotobacter* has been reported to adsorb HM (Emtiazi et al. 2004; Rasulov et al. 2013). Also it is noted that bacterial polymer is soluble in water and weakly bound by the soil matrix, and after absorbing the metal it is not easily mineralized (Chen et al. 1995a; Czajka et al. 1997).

A major constraint to clean up the soil from excessive toxic metal is their low availability. Biosorption through EPS is a passive process by which HM is adsorbed, removed, and replaced due to increase in their mobility (Czajka et al. 1997; Das et al. 2008). The formation of heavy metal-exopolysachharide (HM-EPS) complex has a potency to increase the bioavailability of HM for plant uptake (Chen et al. 1995b;

Petry et al. 2000). This chapter aims to discuss mainly the *Azotobacter* resistance on toxic HMs and their formation of EPS by which *Azotobacter* mobilizes the HM and increases the HM accumulation in phytoremediation by plants. The review also reports current knowledge of the source of heavy metal contamination in agricultural soil, and the ability of plants to prevent HM toxicity by synthesizing polypeptide phytochelatin in cytosol.

6.2 Source of Heavy Metal Contamination in Agricultural Soil

In general, HMs are natural elements with a specific gravity higher than 5000 kg/m^3 and are associated with potential toxicity. Naturally, soil contains metals and HMs from the parent material and the soil genesis (Kabata-Pendias and Mukherjee 2007). Arsenic (Ar), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are the most toxic HMs with a significant phytotoxicity to living organisms and food crops (Keyster et al. 2020). The Indonesian Government has several regulations to control the five most toxic HMs in agricultural soils, fertilizers, and soil amendments. The World Health Organization classifies HMs antimony (Sb), As, Cd, Cr, copper (Cu), inorganic Pb, and Hg as essential trace elements while Ni as metal trace element whose presence in water must be controlled (Durand et al. 2015).

The natural concentration of the trace element in different soils varies (Table 6.1) and depends mainly on soil texture, pH, organic matter, presence of lime, as well as soil genesis (Kabata-Pendias and Mukherjee 2007).

Increased levels of HM in soil up to the minimum threshold are commonly caused by anthropogenic activities. Mineral mining, metal smelting, industry, and even agriculture might increase the concentration of metals in the soil through soil amendments, chemical fertilizers, and irrigation water. In China, Cu, As, and Cd were detected in either animal manure or their feeds (Zhang et al. 2012). Also composted cattle, horse breeding, pig, and poultry manure contains Fe, Mn, Zn, Cu, Ni, and Mo (Vukobratović et al. 2014). Phosphate fertilizers made from rock phosphate commonly contain HM impurities, and possibly increase the HM level in soil and plants (Thomas and Omueti 2012; Azzi et al. 2017). Sludge from industries is the source of HM for agricultural soil, i.e., the pollution load index higher than 1.0

Table 6.1 Average concentration of important heavy metals in soil

Heavy metal	Level in natural soil	Average
Antimony	0.05–1.33	–
Arsenic	<0.1–67	–
Cadmium	0.01–2.5	0.5
Chromium	1–1100	54
Copper	1–140	20
Lead	2–90	15–28
Mercury	0.01–1.12	0.07
Nickel	4.92	19–22

Source: Kabata-Pendias and Mukherjee (2007)

for Cr, Ni, Cu, As, Cd, and Pb, and was detected from tannery, dye, metal processing, and battery manufacturing industries (Islam et al. 2017).

6.3 Heavy Metal Toxicity to Plants

In general, HMs have not played any essential role in plant metabolism. However, HM phytotoxicity occurs when soil contains an excessive amount of mobile and available HMs for root uptake (Kalaivanan and Ganeshamurthy 2016). High concentration of HM is reported to cause permanent genetic changes and induce chromosomal damage in plants (Reutova 2017). Heavy metals are responsible for oxidative stress due to free radical and reactive oxygen species generation resulting in disproportion of antioxidants and prooxidants (Fryzova et al. 2017). The toxicity of HM is caused by the formation of radical compounds in cells, complexation of HM with cellular components containing thiol (S), and competition between structurally similar essential elements in the case of cadmium and zinc (Rouch et al. 1995). Phytotoxicity of HM in plants results in physiology and metabolism disturbance (Kalaivanan and Ganeshamurthy 2016). Furthermore HMs are divided into two groups: active redox HMs include Fe, Cu, Cr, and Co and inactive redox HMs include Cd, Zn, Ni, and Al (Hossain et al. 2012). Active redox HMs are directly involved in cell redox reactions and produce O_2^- and subsequently H_2O_2 and OH^- radicals (Schützendübel and Polle 2002). These radicals can cause oxidative damage and senescence in plants (Kalaivanan and Ganeshamurthy 2016; Keyster et al. 2020). Plants exposed to inactive redox HMs experience oxidative stress through indirect mechanisms such as interactions with antioxidant defense systems, electron transport chain disruption, or lipid peroxidation induction (Hossain et al. 2012).

Naturally, plants have a metal detoxification mechanism through phytochelatin (PCs) proteins which sequestrate metals in the vacuole (Cobbett 2000; Gupta and Diwan 2017). The PCs are polypeptides with cysteine residues, synthesized from glutathione catalyzed by phytochelatin synthases (Keyster et al. 2020). Plants detoxify the HM by forming PC-HM-S complex in cytosol in order to prevent HMs from entering the metabolic system in cytosol (Cobbett 2000; Pal and Rai 2010; Gupta et al. 2013). The intracellular sequestration of HM is one of the principles of phytoremediation of metal-contaminated soil using accumulator plants (Mohsenzadeh and Mohammadzadeh 2018). Therefore, a number of plants are tolerant to HMs and can continue to grow without toxicity symptoms at a certain limit of HM level.

6.4 Composition of Exopolysaccharide of Azotobacter

The aerobic-heterotrophic *Azotobacter* is Gram negative and pleomorphic in morphology (Fig. 6.1). The natural property of *Azotobacter* is the formation of EPS, a slime layer loosely attached on the cell surface, easily released and generally secreted to the surrounding environment (Cescutti 2010; Whitfield et al. 2020).

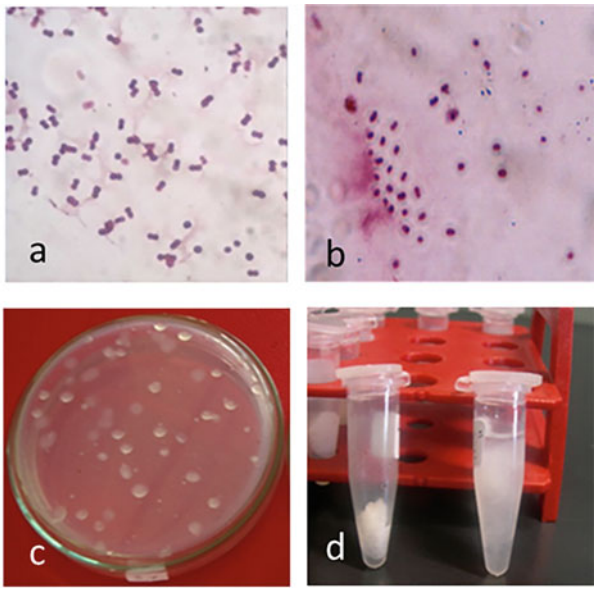

Fig. 6.1 (**a**) The cell morphology of *Azotobacter chroococcum* and (**b**) *A. vinelandii*; (**c**) the colony of EPS-producing *Azotobacter*; and (**d**) the crude exopolysaccharide extracted by cold acetone

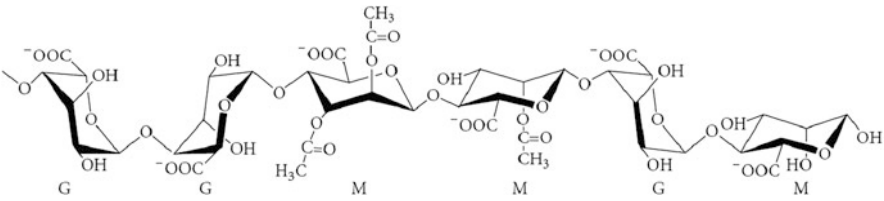

Fig. 6.2 Representation of the chemical structure from acetylated alginates produced by *Azotobacter vinelandii*. Mannuronic (M) and guluronic (G) acid residues (reproduced from Pacheco-Leyva et al. 2016)

Naturally, the roles of EPS of *Azotobacter* are to protect nitrogenase from oxygen (Sabra et al. 2000) and defense against abiotic stress such as drought and toxic substances (Gauri et al. 2012).

The EPS of *Azotobacter* is similar to alginate (Sabra et al. 2000; Vargas-Garcia et al. 2003) which is composed of D-mannuronic and L-guluronic acids (Remminghorst and Rehm 2006). Three species of *Azotobacter*, *A. beijerinckii*, *A. vinelandii*, and *A. chroococcum*, have been reported to produce alginate (Likhosherstov et al. 1991; Sabra et al. 2000; Pandurangan et al. 2012; Pacheco-Leyva et al. 2016). Gauri et al. (2012) stated that EPS of *Azotobacter* is composed of (1–4)-linked β-D-mannuronic acid and its C_5-epimer α-L-guluronic acid (Fig. 6.2). Purified EPS from *A. beijerinckii* is composed of D-galactose:L-rhamnose:pyruvic

Table 6.2 The composition of EPS extracted from some liquid culture of *Azotobacter* isolates in the presence of cadmium chloride

	Azotobacter BS3		Azotobacter LKM6		Azotobacter LH15	
Components	$-CdCl_2$	$+CdCl_2$	$-CdCl_2$	$+CdCl_2$	$-CdCl_2$	$+CdCl_2$
Fructose	21.2	110.1	36.0	96.3	39.2	123.4
Glucose	46.5	75.4	70.1	47.7	81.2	119.4
Mannose	18.4	5.5	21.8	30.5	29.6	56.3
Rhamnose	27.7	25.3	29.3	21.8	24.7	45.0
Galactose	11.2	12.3	10.6	10.5	2.1	14.6
Acetic acid	1.0	0.0	1.0	0.0	1.0	0.0
Lactic acid	26.1	1.5	27.2	0.0	29.1	0.0
Pyruvic acid	39.1	10.4	40.5	3.5	49.4	14.0
Mannuronic acid	40.0	22.5	19.5	16.0	39.4	25.5
Guluronic acid	15.8	25.4	11.4	15.0	20.9	31.0

Source: Hindersah (2015)

acid of 2:1:1, while mannuronic and guluronic acids are detected in minor fraction in EPS (Likhosherstov et al. 1991). The said composition of *Azotobacter* EPS partly agrees with that of *Azotobacter* isolated from Cd-contaminated soil (Table 6.2). It contains simple sugars, organic acids, as well as mannuronic and guluronic acids.

Organic acids contain carboxyl, hydroxyl, and ketone functional groups; simple sugar mainly contains hydroxyl and carbonyl (aldehyde or ketone) groups, while hydroxyl and carboxyl are the functional groups of mannuronic and guluronic acid (Fig. 6.2). All functional groups can bind cationic HMs to form EPS-HM complex. The Gram-negative and Gram-positive bacteria form EPS-HM complex by ionized functional groups in the cell wall or capsule, namely hydroxyl, carboxyl, phosphoryl, and amide groups, through a passive mechanism (Jiang et al. 2004; Hasan et al. 2017). In *Azotobacter nigricans* NEWG-1 cells, the functional groups for Cu metal biosorption include hydroxyl, methylene, carbonyl, and carboxylate (Ghoniem et al. 2020).

6.5 Azotobacter Resistance to Heavy Metal

The rhizobacterium *Azotobacter* has been found in the environment with certain species and levels of HM. The EPS-producing *Azotobacter* has been isolated from Cd-contaminated soil and Hg-contaminated tailing of gold mining (Hindersah et al. 2006, 2017). A total of 64 *Azotobacter* isolates were obtained from Zn^{2+}-, Cd^{2+}-, Cu^{2+}-, Pb^{2+}-, and Mn^{2+}-polluted soil.

In many studies *Azotobacter* resistance to HMs has been evaluated. The addition of 0.01 mM of $CdCl_2$ induces the growth of *Azotobacter* cells even though it inhibits cell proliferation (Hindersah et al. 2009). *Azotobacter* isolates obtained from HM-contaminated soil were resistant to various concentrations of Cd^{2+}, Cu^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} (Narula et al. 2011; Abo-amer et al. 2014). In

Table 6.3 Biosorption capacity of EPS of *Azotobacter* on some heavy metals in laboratory experiment using liquid culture

Azotobacter species/ strain	Biosorption capacity on heavy metals	References
Azotobacter AC2	Cu, Zn, and Fe: 15.5, 20, and 25 mg/g EPS; initial pH 7.2	Emtiazi et al. (2004)
Azotobacter LKM6	Cd: 21.5–23.08 mg/kg EPS; initial pH 7.0	Erni and Hindersah (2011)
Azotobacter	Pb: 0.1–4 mg/g EPS; initial pH 5.0	Soltani et al. (2011)
A. chroococcum XU1	Pb and Hg: 40.48% and 47.87%; initial pH 5.0	Rasulov et al. (2013)
Azotobacter salinestris	Pb: 50–250 mg/l; initial pH 4.0–8.0	Dhevagi et al. (2021)

liquid culture, *Azotobacter* LKM6 exhibited better cell viability and exopolysaccharide production in the presence of low content of lead (Hindersah and Kamaluddin 2014).

Indigenous bacteria can adapt to the ionic HMs through five main mechanisms, namely extracellular barrier, efflux pump, extracellular sequestration, intracellular sequestration, and reduction of metal ions (Mishra et al. 2021). Metal homeostasis in cell maintains the metal balance to keep the HM below nontoxic concentration (Bondarczuk and Piotrowska-Seget 2013). Extracellular barrier is related to metal biosorption process by cell wall or exopolymers such as EPS. The ability of *Azotobacter* cell wall to bind the metal cations, e.g., UO_2^{2+}, Cr^{2+}, Cr^{3+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}, has been reported by many researchers (Cotoras et al. 1992; Kurniawan et al. 2019; Ghoniem et al. 2020; Rizvi et al. 2020). However, the ability of EPS extracted from *Azotobacter* for heavy metal biosorption has been reported in limited references (Table 6.3).

The bacterial EPS is a promising chelating agent for heavy metal discharge (Rasulov et al. 2013). Biosorption of HMs by functional groups present in EPS is an independent metabolism (Hasan et al. 2017) but involves physicochemical adsorption processes such as acidity, temperature, time, ionic strength, and metal concentration (Joo et al. 2010). The biosorption of HM (and metals) is independent of metabolism (it does not require energy from ATP hydrolysis). The biosorption capacity also depends on the outer structure of cell, e.g., cell wall structures and charged sites on exopolysaccharide components related to anionic functional group that bind heavy metal cations and compounds (Gadd 2004; Ahluwalia and Goyal 2007). The mechanism of HM biosorption by EPS is a way to mobilize HMs and then being taken up by hyperaccumulator plants in phytoremediation process.

Another strategy for controlling intracellular metal levels in prokaryotes is active excretion of metal ions by the plasma membrane known as the efflux pump (Nies 2003). These systems involve transporters such as P-type ATPase or cation/H+ antiporter which depend on the energy derived from ATP hydrolysis (Silver 1996). Heavy metal pumping systems and their protein involved in HM efflux pump have been studied for beneficial rhizobacteria such as *Rhizobium* sp., *Bacillus*

Table 6.4 Change in composition and function of *A. chroococcum* CAZ3 cell due to 100, 1000, and 1200 μg/mL cadmium, chromium, and nickel exposure, respectively

Feature	Cell responses on HM exposure
Cell morphology	Cells distorted and shrank to 1.7 μm at 50 μg/mL Cd; 1.3 μm at 100 μg/mL Ni; and 1.9 μm at 100 μg/mL Cr. Normal cell size is 1.8 μm
EPS production	Secretion of 320, 353 d, and 133 μg EPS/mL on exposure to 100 μg/mL Cd, Cr, and Ni, respectively The Cd levels in EPS were 0.4%, 0.07%, and 0.24% on exposure to Cd, Cr, and Ni, respectively
Dark brown (melanin) pigmentation	Melanin secretion which accumulates 0.53%, 0.22%, and 0.12% Cd, Cr, and Ni, respectively
Functional groups	Increase the functional groups of CHO, alkyl, carboxylate, and alkene
Metallothionein	Metallothionein excretion when exposed to HM

Source: Rizvi et al. (2019)

sp., and *Pseudomonas* sp. (Kahn et al. 1989; Silver and Phung 1996; Muneer et al. 2016; Delmar et al. 2015). The metal pumping system of *Azotobacter* has not yet been studied extensively although the protein of heavy metal efflux pump of CzcA family from *Azotobacter vinelandii* (strain DJ/ATCCBAA-1303) is recorded in UniProt gallery (https://www.uniprot.org/uniprot/C1DDU8).

Excess HM results in toxicity due to cell metabolism disruption. However, *Azotobacter* has developed a system for lowering the negative effects of HM. *Azotobacter* develops a resistance system to HM by producing EPS and melanin pigment to prevent the entry of HM into cells including EPS production (Table 6.4). Both compounds play a role in HM sequestration via adsorption process.

Melanin efficiently chelates metals while simultaneously protecting bacterial cells from various biochemical stresses (El-Naggar and El-Ewasy 2017). The ability of melanin to chelate HM ions is very high that can be used for detoxification/removal of metal ions from HM-contaminated area (Thaira et al. 2019). Intracellular sequestration by the cysteine-rich protein, bacterial metallothionein (MT), has a similar role of PC in plants. The MT sequestrates and buffers the metals already present in the cell, where the thiol groups of cysteine residues on MT bind HM through a coordination bond (Robinson et al. 2001).

6.6 Azotobacter Mechanisms for Metal Bioremediation

Dealing with an eco-friendly and sustainable way to remediate HM-contaminated soil, bioremediation by using soil microbes is a way to reduce the level of toxic metals in soil. Some rhizobacteria naturally produce extracellular EPS. Bacterial EPS is commonly found in soil and acts as a ligand to bind Cu and Pb and increase their mobility in the soil and the EPS-metal complex becomes difficult to degrade (Czajka et al. 1997; Jensen-Spaulding et al. 2004). The EPS-metal complex possibly becomes a vehicle that enters the rhizosphere area and causes the metal to be taken

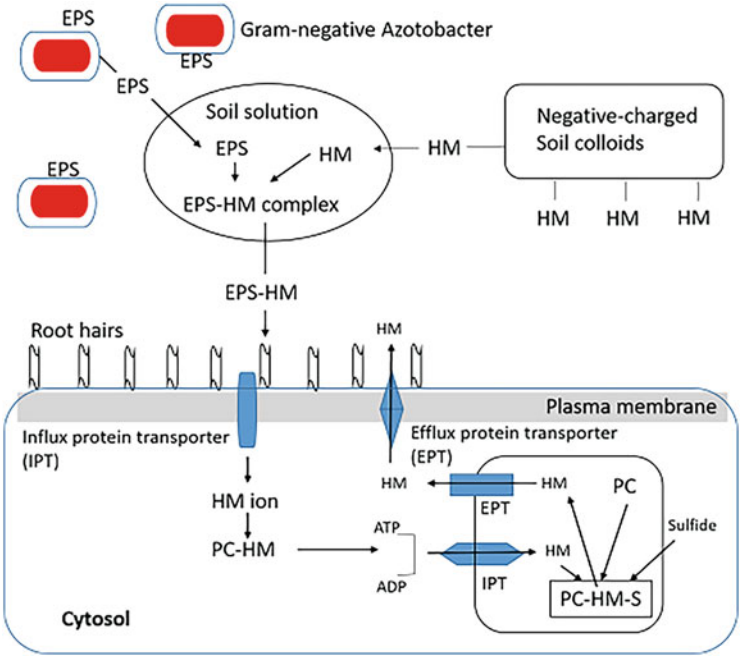

Fig. 6.3 Schematic diagram that relates *Azotobacter* and heavy metal bioremediation. The HM sequestration in vacuole adapted from Cobbett (2000); Colangelo and Guerinot (2006); and Delmar et al. (2015)

up by plants. This EPS might have a similar role with siderophore for iron mobilization in iron-limited soil (McRose et al. 2017).

The constraint of phytoremediation of HM-contaminated soil is the low availability of HM in the soil. Metal mobilization by EPS, an organic chelate, might have similar function with metal uptake facilitated by inorganic chelate, e.g., ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) widely used as "fertilizer" in agriculture (Bloem et al. 2017; López-Rayo et al. 2015). The schematic diagram of heavy metal biosorption and mobilization by *Azotobacter* EPS and sequestration in the vacuole of phytoremediation plant is shown in Fig. 6.3. The release of EPS and exchangeable HM to the soil solution induced EPS-HM complex which resulted in HM mobility in the rhizosphere and increase in the HM availability for plant uptake. Jain et al. (2018) reviewed that root secretions (RS) are able to chelate ionic metal (M) in order to protect the metal before entering the root cell. This RS-M complex moves towards the root and stops once they are inside root hairs. This process might be possible for EPS-HM complex.

Once inside root hairs, metals enter the cell roots through the protein transporter system in which the metal uptake protein is specific for certain metals, tissue expression, and cellular localization (Colangelo and Guerinot 2006). Once entering the cytosol, the metal is bound by PC protein (Cobbett 2000). If the plant takes up an

excessive amount of toxic metal, then the metals enter the vacuole through protein transporter system and bind to PC and S to become PC-HM-S complex (Cobbett 2000; Colangelo and Guerinot 2006; Jain et al. 2018). The metal sequestrations in the vacuoles limit metal toxicity in plant metabolisms basically carried out in cytosols. However, too high concentration in plant cell results in efflux pump activation to release metal to outer cell environment facilitated by metal efflux protein (Delmar et al. 2015; Colangelo and Guerinot 2006). Metal accumulator plants used in phytoremediation enable to accumulate higher level of metals without toxicity symptoms compared to non-accumulator ones. Sharma et al. (2020) verified that *Chenopodium album* L. leaves accumulate 69.38 mg/kg of Mn, 25.75 mg/kg of Cu, 23.20 mg/kg of As, and 20.90 mg/kg of Fe, while *Ricinus communis* L. leaves accumulate 22.41 mg/kg of Pb.

It is a possible approach/strategy for improving the effectivity of HM phytoextraction by increasing HM mobility in rhizosphere in order to enhance their uptake by phytoaccumulator plants. *Azotobacter* as PGPR provides available N and P in soil by N_2 fixation and P solubilization. Moreover, *Azotobacter* synthesizes and secretes phytohormones (Rubio et al. 2013; Vikhe 2014) that regulate and coordinate plant cell development and growth. Both mechanisms induce phytoextraction by MH-accumulated plants due to the positive effect of *Azotobacter* activities on plant growth and thus biomass. In this situation, *Azotobacter* has dual roles: to increase HM mobility and plant biomass. Both functions can enhance HM uptake by phytoaccumulator plants.

Although the information about the efficiency of phytoextraction by *Azotobacter* EPS in real condition remains limited, HM accumulation in plant shoots due to EPS-producing *Azotobacter* has been reported. The use of sludge containing Pb combined with *Azotobacter* inoculation increases the accumulation of Cd and Pb in lettuce shoot (Kurniawati et al. 2006; Hindersah and Kalay 2006).

In contrast, different effects of EPS on HM bioavailability have also been noted. The EPS produced by the *Azotobacter* sp. limits the uptake of Cd and Cr by wheat plants cultivated in HM-contaminated soil (Joshi and Juwarkar 2009). Inoculation of *Azotobacter* CAZ3 decreases the metal concentrations of Pb and Cu in roots, shoots, and kernels of maize (Rizvi and Khan 2018). It is possibly because the EPS forms a biofilm in soil and immobilizes the HM within the biofilm (François et al. 2012) to reduce their availability. This difference in effects shows that *Azotobacter* might either mobilize or immobilize the heavy metal. Further research is needed to verify the effect mechanisms of *Azotobacter* EPS to control the mobility as well as availability of HM in soil.

6.7 Conclusion

The increase of HM level in soil caused by organic as well as inorganic fertilizer is reported elsewhere. *Azotobacter* is a plant growth-promoting rhizobacterium found not only in plant rhizosphere of arable soil but also in HM-contaminated soil due to resistance for toxic heavy metals. *Azotobacter* produces EPS to avoid HM toxicity

through extracellular sequestration. The EPS of *Azotobacter* consists of mainly simple sugar, organic acid, and alginate that contain functional group with negative charges. Since the EPS is a biopolymer and the positively charged of HMs bind the organic substance by coordinative covalent bond. The EPS-HM complex is mobile and might move towards the root hair and further competes with the metals for uptake by root cells via influx protein transporter system. This mechanism is proposed as a way of *Azotobacter* EPS to involve in the bioremediation of HM-contaminated soil by using accumulator plants. Furthermore, *Azotobacter* activities as a PGPR affect plant growth and increase biomass. This leads to enabling of the phytoaccumulator plants to accumulate more HMs after presence/implementation of EPS-producing *Azotobacter* in the rhizosphere. However, intensive research is needed to prove that capacity for the same with an array of *Azotobacter* strains in different agricultural environments.

Acknowledgements The researches of *Azotobacter* as a biological agent for bioremediation were funded by Universitas Padjadjaran and General Directorate of Higher Education of the Republic of Indonesia.

References

Abo-amer AE, Abu-gharbia MA, Soltan EM et al (2014) Isolation and molecular characterization of heavy metal-resistant *Azotobacter chroococcum* from agricultural soil and their potential application in bioremediation. Geomicrobiol J 31(7):551–561. https://doi.org/10.1080/01490451.2013.850561

Ahluwalia S, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals form wastewater. Bioresour Technol 98:2243–2257. https://doi.org/10.1016/j.biortech.2005.12.006

Azzi V, El Samrani A, Lartiges B et al (2017) Trace metals in phosphate fertilizers used in Eastern Mediterranean countries. CLEAN-Soil Air Water 45(1):1500988. https://doi.org/10.1002/clen.201500988

Bhattacharyya PN, Jha DK (2012) Review plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350. https://doi.org/10.1007/s11274-011-0979-9

Bondarczuk K, Piotrowska-Seget Z (2013) Molecular basis of active copper resistance mechanisms in Gram-negative bacteria. Cell Biol Toxicol 29(6):397–405. https://doi.org/10.1007/s10565-013-9262-1

Bloem E, Haneklaus S, Haensch R et al (2017) EDTA application on agricultural soils affects microelement uptake of plants. Sci Total Environ 577:166–173. https://doi.org/10.1016/j.scitotenv.2016.10.153

Cescutti P (2010) Bacterial capsular polysaccharides and exopolysaccharides. In: Holst O, Brennan PJ, von Itzstein M, Moran AP (eds) Microbial glycobiology: structures, relevance and applications. Academic Press, pp 93–108. https://doi.org/10.1016/B978-0-12-374546-0.00006-7

Chen JH, Lion LW, Ghiorse WC et al (1995a) Mobilization of adsorbed cadmium and lead in aquifer material by bacterial extracellular polymers. Water Res 29:421–430. https://doi.org/10.1016/0043-1354(94)00184-9

Chen JH, Czajka DR, Lion LW et al (1995b) Trace metal mobilization in soil by bacterial polymers. Environ Health Perspect 103:53–58

Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport throughout plants. Curr Op Pl Biol 9:322–330

Czajka DR, Lion LW, Shuler ML et al (1997) Evaluation of the utility of bacterial extracellular polymers for treatment of metal-contaminated soils: polymer persistence, mobility, and the influence of lead. Water Res 31:2827–2839. https://doi.org/10.1016/S0043-1354(97)00129-2

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Pl Physiol 123: 825–832

Cotoras D, Millar M, Viedma P et al (1992) Biosorption of metal ions by *Azotobacter vinelandii*. World J Microbiol Biotechnol 8:319–323

Das N, Vimala R, Karthika P (2008) Biosorption of heavy metal-an overview. Indian J Biotechnol 7:159–169

Delmar JA, Su C-C, Yu EW (2015) Heavy metal transport by the CusCFBA efflux system. Protein Sci 24(11):1720–1736

Dhevagi P, Priyatharshini S, Ramya A et al (2021) Biosorption of lead ions by exopolysaccharide producing *Azotobacter* sp. J Environ Biol 42:40–50. https://doi.org/10.22438/jeb/42/1/MRN-1231

Durand MJ, Hua A, Jouanneau S et al (2015) Detection of metal and organometallic compounds with bioluminescent bacterial bioassays. Adv Biochem Eng Biotechnol 154:77–99. https://doi.org/10.1007/10_2015_332

El-Naggar NEA, El-Ewasy SM (2017) Bioproduction, characterization, anticancer and antioxidant activities of extracellular melanin pigment produced by newly isolated microbial cell factories *Streptomyces glaucescens* NEAE-H. Sci Rep 7:42129. https://doi.org/10.1038/srep42129

Emtiazi G, Ethemadifar Z, Habibi MH (2004) Production of extra-cellular polymer in *Azotobacter* and biosorption of metal by exopolymer. Afr J Biotechnol 3(6):330–333. https://doi.org/10.5897/AJB2004.000-2060

Erni, Hindersah R (2011) Biosorpsi kadmium dan komposisi eksopolisakarida *Azotobacter* sp pada dua konsentrasi $CdCl_2$ (Cadmium biosorption and exopolysachharides composition of *Azotobacter* sp. on the level of $CdCl_2$). Agri 1(1):33–37. (Abstract in English)

François F, Lombard C, Guigner J-M et al (2012) Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. Appl Environ Microbiol 78(4): 1097–1106. https://doi.org/10.1128/AEM.06522-11

Fryzova R, Pohanka M, Martinkova P et al (2017) Oxidative stress and heavy metals in plants. In: de Voogt P (ed) Reviews of environmental contamination and toxicology, vol 253. Springer, Cham, pp 129–156. https://doi.org/10.1007/398_2017_7

Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119. https://doi.org/10.1016/j.geoderma.2004.01.002

Gauri SS, Mandal SM, Pati BR (2012) Impact of *Azotobacter* exopolysaccharides on sustainable agriculture. Appl Microbiol Biotechnol 95(2):331–338. https://doi.org/10.1007/s00253-012-4159-0

Ghoniem AA, El-Naggar NEA, Saber WIA et al (2020) Statistical modeling-approach for optimization of Cu^{2+} biosorption by *Azotobacter nigricans* NEWG-1; characterization and application of immobilized cells for metal removal. Sci Rep 10(1):9491. https://doi.org/10.1038/s41598-020-66101-x

Gupta PA, Diwan B (2017) Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. Biotechnol Rep 13:58–71

Gupta DK, Vandenhove H, Inouhe M (2013) Role of phytochelatins in heavy metal stress and detoxification mechanisms in plants. In: Gupta D, Corpas F, Palma J (eds) Heavy metal stress in plants. Springer, Heidelberg, pp 73–94. https://doi.org/10.1007/978-3-642-38469-1_4

Hasan MK, Cheng Y, Kanwar MK et al (2017) Responses of plant proteins to heavy metal stress—a review. Front Plant Sci 8:1492. https://doi.org/10.3389/fpls.2017.01492

Hindersah R, Arief DH, Soemitro S et al (2006) Exopolysaccharide Extraction from Rhizobacteria *Azotobacter* sp. In: Proceeding of International Seminar IMTGT. Medan, 22nd–23rd June 2006, p 50–55

Hindersah R, Kalay AM (2006) Lead and cadmium accumulation on lettuce shoot following *Azotobacter* and domestic sludge application. J Budidaya Pertanian 2:1–5. (Abstract in English)

Hindersah R, Arief DH, Soemitro S et al (2009) Pengaruh CdCl2 terhadap Produksi Eksopolisakarida dan Daya Hidup *Azotobacter*. J Natur Indonesia 12(1):34–37. (Abstract in English)

Hindersah R, Kamaluddin NN (2014) Pengaruh timbal terhadap kepadatan sel dan kadar eksopolisakarida kultur cair *Azotobacter*. Bionatura J Ilmu-ilmu Hayati dan Fisik 16(1):1–5. (Abstract in English)

Hindersah R (2015) Growth and exopolysachharide composition of nitrogen fixing bacteria *Azotobacter* spp. in the presence of cadmium. Pros Sem Nas Masy Biodiv Indon 1(7):1644–1648. (Abstract in English)

Hindersah R, Mulyani O, Osok R (2017) Proliferation and exopolysaccharide production of *Azotobacter* in the presence of mercury. Biodiversity J 8(1):21–26

Hossain MA, Piyatida P, da Silva JAT et al (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Botany 2012:872875. https://doi.org/10.1155/2012/872875

Iram S, Uzma, Sadia GR et al (2013) Bioremediation of heavy metals using isolates of filamentous fungus collected from polluted soil of Kasur. Pakistan Int Res J Biol Sci 2(12):66–73

Islam MS, Ahmed MK, Raknuzzaman M et al (2017) Heavy metals in the industrial sludge and their ecological risk: a case study for a developing country. J Geochem Explor 172:41–49. https://doi.org/10.1016/j.gexplo.2016.09.006

Jensen-Spaulding A, Shuler ML, Lion LW (2004) Mobilization of adsorbed copper and lead from naturally aged soil by bacterial extracellular polymers. Water Res 38(5):1121–1128. https://doi.org/10.1016/j.watres.2003.11.015

Jiang W, Saxena A, Song B et al (2004) Elucidation of functional groups on gram-positive and gram-negative bacterial surfaces using infrared spectroscopy. Langmuir 20(26):11433–11442

Joo J-H, Hassan SHA, Oh S-E (2010) Comparative study of biosorption of Zn^{2+} by *Pseudomonas aeruginosa* and *Bacillus cereus*. Int J Biodeter Biodeg 64(8):734–741

Joshi PM, Juwarkar AA (2009) In vivo studies to elucidate the role of extracellular polymeric substances from *Azotobacter* in immobilization of heavy metals. Environ Sci Technol 43(15):5884–5889. https://doi.org/10.1021/es900063b

Kabata-Pendias A, Mukherjee AB (2007) Trace elements from soil to human. Springer, Berlin, Heidelberg

Kahn D, David M, Domergue O et al (1989) *Rhizobium meliloti* fixGHI sequence predicts involvement of a specific cation pump in symbiotic nitrogen fixation. J Bacteriol 171(2):929–939

Kalaivanan D, Ganeshamurthy AN (2016) Mechanisms of heavy metal toxicity in plants. In: Rao NKS, Shivashankara KS, Laxman RH (eds) Abiotic stress physiology of horticultural crops. Springer, pp 85–102. https://doi.org/10.1007/978-81-322-2725-0_5

Keyster M, Niekerk L-E, Basson G et al (2020) Decoding heavy metal stress signalling in plants: towards improved food security and safety. Plan Theory 9:1781

Kurniawan SB, Imron MF, Purwanti IF (2019) Biosorption of chromium by living cells of *Azotobacter* S8, *Bacillus subtilis* and *Pseudomonas aeruginosa* using batch system reactor. J Ecol Eng 20(6):184–189. https://doi.org/10.12911/22998993/108629

Kurniawati F, Hindersah R, Joy B (2006) Studi pengaruh aplikasi kadmium (Cd), inokulan dan eksopolisakarida *Azotobacter* sp UT1 terhadap Cd tanah, dan akumulasi Cd pada tajuk bibit selada (*Lactuca sativa* L.). J Pertanian Tropika 9(2):52–59. (Abstract in English)

Likhosherstov LM, Senchenkov SN, Shashkov AS et al (1991) Structure of the major exopolysaccharide produced by *Azotobacter beijerinckii* B-1615. Carbohydr Res 222:233–238. https://doi.org/10.1016/0008-6215(91)89021-7

López-Rayo S, Nadal P, Lucena JJ (2015) Reactivity and effectiveness of traditional and novel ligands for multi-micronutrient fertilization in a calcareous soil. Front Plant Sci 6:752. https://doi.org/10.3389/fpls.2015.00752

McRose DL, Baars O, Morel FMM et al (2017) Siderophore production in *Azotobacter vinelandii* in response to Fe-, Mo- and V-limitation. Environ Microbiol 19(9):3595–3605. https://doi.org/10.1111/1462-2920.13857

Mishra BB, Nayak SK, Mohapatra S et al (eds) (2021) Environmental and agricultural microbiology: applications for sustainability. Wiley-Scrivener Publisher, Beverly

Mohsenzadeh F, Mohammadzadeh R (2018) Phytoremediation ability of the new heavy metal accumulator plants. Environ Engineer Geosci 24(4):441–450. https://doi.org/10.2113/EEG-2123

Muneer B, Iqbal MJ, Shakoori FR et al (2016) Isolation, identification and cadmium processing of *Pseudomonas aeruginosa* (EP-Cd1) isolated from soil contaminated with electroplating industrial wastewater. Pak J Zool 48(5):1495–1501

Nanda S, Abraham J (2011) Impact of heavy metals on the rhizosphere microflora of *Jatropha multifida* and their effective remediation. Afr J Biotechnol 10(56):11948–11955

Namvar A, Khandan T (2013) Response of wheat to mineral nitrogen fertilizer and biofertilizer (*Azotobacter* sp. and *Azospirillum* sp.) inoculation under different levels of weed interference. Ekologija 59(2):85–94

Narula N, Behl RK, Kothe E (2011) Heavy metal resistance among *Azotobacter* spp. and their survival in HM contaminated soil using Indian mustard. IUP J Genetics Evol 4(2):55–62

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microb Rev 27(2–3):313–339. https://doi.org/10.1016/S0168-6445(03)00048-2

Nosrati R, Owlia P, Saderi H et al (2014) Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iran J Microbiol 6(4):285–295

Ozdemir G, Ozturk T, Ceyhan N et al (2003) Heavy metal biosorption by biomass of *Ochrobactrum anthropi* producing exopolysaccharide in activated sludge. Bioresour Technol 90:71–74. https://doi.org/10.1016/s0960-8524(03)00088-9

Pal R, Rai JPN (2010) Phytochelatins: peptides involved in heavy metal detoxification. Rev Appl Biochem Biotechnol 160(3):945–963. https://doi.org/10.1007/s12010-009-8565-4

Pandurangan G, Subbiah J, Thiyagarajan K et al (2012) Small scale production and characterization of alginate from *Azotobacter chroococcum* using different substrates under various stress conditions. Int J Appl Biol Pharm Technol 3:40–45

Patel PR, Shaikh SS, Sayyed RZ (2016) Dynamism of PGPR in bioremediation and plant growth promotion in heavy metal contaminated soil. Indian J Exp Biol 54(4):286–290

Pacheco-Leyva I, Pezoa FP, Díaz-Barrera A (2016) Alginate biosynthesis in *Azotobacter vinelandii*: overview of molecular mechanisms in connection with the oxygen availability. Int J Polymer Sci 2016:2062360

Petry S, Furlan S, Crepeau MJ et al (2000) Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* grown in a chemically defined medium. Appl Environ Microbiol 66(8):3427–3431. https://doi.org/10.1128/AEM.66.8.3427-3431.2000

Rasulov BA, Yili A, Aisa HA (2013) Biosorption of metal ions by exopolysaccharide produced by *Azotobacter chroococcum* XU1. J Environ Protection 4(9):989–993. https://doi.org/10.4236/jep.2013.49114

Remminghorst U, Rehm BHA (2006) Bacterial alginates: from biosynthesis to applications. Biotechnol Lett 28:1701–1712. https://doi.org/10.1007/s10529-006-9156-x

Reutova N (2017) Mutagenic potential of some heavy metals. Russian J Genetics: Appl Res 7(2):175–179

Rizvi A, Ahmed B, Zaidi A et al (2019) Bioreduction of toxicity influenced by bioactive molecules secreted under metal stress by *Azotobacter chroococcum*. Ecotoxicology 28(3):302–322. https://doi.org/10.1007/s10646-019-02023-3

Rizvi A, Ahmed B, Zaidi A et al (2020) Biosorption of heavy metals by dry biomass of metal tolerant bacterial biosorbents: an efficient metal clean-up strategy. Environ Monit Assess 192(12):801. https://doi.org/10.1007/s10661-020-08758-5

Rizvi A, Khan MS (2018) Heavy metal induced oxidative damage and root morphology alterations of maize (*Zea mays* L.) plants and stress mitigation by metal tolerant nitrogen fixing *Azotobacter*

chroococcum. Ecotoxicol Environ Saf 157(1):9–20. https://doi.org/10.1016/j.ecoenv.2018.03.063

Robinson NJ, Whitehall SK, Cavet JS (2001) Microbial metallothioneins. Adv Microb Physi 44: 183–213. https://doi.org/10.1016/s0065-2911(01)44014-8

Rouch DA, Lee BTO, Morby AP (1995) Understanding cellular responses to toxic agents: a model for mechanism-choice in bacterial metal resistance. J Industrial Microb 14(2):132–141

Rubio EJ, Montecchia MS, Tosi M et al (2013) Genotypic characterization of Azotobacteria isolated from Argentinean soils and plant-growth-promoting traits of selected strains with prospects for biofertilizer production. Sci World J 2013:519603. https://doi.org/10.1155/2013/519603

Sabra W, Zeng A-P, Lünsdorf H et al (2000) Effect of oxygen on formation and structure of alginate and its role in protecting nitrogenase. Appl Environ Microbiol 66(9):4037–4044. https://doi.org/10.1128/AEM.66.9.4037-4044.2000

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53(372):1351–1365

Sharma P, Tripathi S, Chandr R (2020) Phytoremediation potential of heavy metal accumulator plants for waste management in the pulp and paper industry. Heliyon 6(7):e04559. https://doi.org/10.1016/j.heliyon.2020.e04559

Jain S, Muneer S, Guerriero G (2018) Tracing the role of plant proteins in the response to metal toxicity: a comprehensive review. Plant Signal Behav 13(9):e1507401. https://doi.org/10.1080/15592324.2018.1507401

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50: 753–789. https://doi.org/10.1146/annurev.micro.50.1.753

Silver S (1996) Bacterial resistances to toxic metal ions—a review. Gene 179(1):9–19. https://doi.org/10.1016/s0378-1119(96)00323-x

Soltani SH, Emtiazi G, Sataee Mokhtari T (2011) Study of biosorption of heavy metals (lead and zinc) by bacterial exopolymer. Microb Biotechnol 2(7):23–28

Sumbul A, Ansari RA, Rizvi R et al (2020) *Azotobacter*: a potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci 27(12):3634–3640. https://doi.org/10.1016/j.sjbs.2020.08.004

Thaira H, Raval K, Manirethan V et al (2019) Melanin nano-pigments for heavy metal remediation from water. Separation Sci Technol 54(2):265–274. https://doi.org/10.1080/01496395.2018.1443132

Thomas E, Omueti JA (2012) The effect of phosphate fertilizer on heavy metal in soils and *Amaranthus caudatus*. Agric Biol J North Am 3(4):145–149. https://doi.org/10.5251/abjna.2012.3.4.145.149

Vargas-Garcia MC, Lopez MJ, Elorrieta MA et al (2003) Properties of polysaccharide produced by *Azotobacter vinelandii* cultured on 4-hydroxybenzoic acid. J Appl Microbiol 94(3):388–395. https://doi.org/10.1046/j.1365-2672.2003.01842.x

Vikhe PS (2014) *Azotobacter* species as a natural plant hormone synthesizer. Res J Rec Sci 3 (IVC-2014):59–63

Vukobratović M, Vukobratović Z, Lončarić Z et al (2014) Heavy metals in animal manure and effects of composting on it. In: ISHS Acta Horti 1034: International Symposium on Growing Media and Soilless Cultivation. https://doi.org/10.17660/ActaHortic.2014.1034.75

Whitfield C, Wear SS, Sande C (2020) Assembly of bacterial capsular polysaccharides and exopolysaccharides. Ann Rev Microbiol 74:521–543. https://doi.org/10.1146/annurev-micro-011420-075607

Zhang F, Li Y, Yang M et al (2012) Content of heavy metals in animal feeds and manures from farms of different scales in Northeast China. Int J Environ Res Public Health 9(8):2658–2668. https://doi.org/10.3390/ijerph9082658

Utilization of Arbuscular Mycorrhizal Fungi to Boom the Efficiency and Product Nature of Horticultural Crops

7

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Abstract

Improvement in the yield of many horticultural crops is due to the potential arbuscular mycorrhizal fungi (AMF) association with the host plant. AMF not only revamp the supplement and water supply or instigate resilience of natural pressure but also incite protection from root illnesses and pests to their respective host plants. Hence, inoculation of horticultural crops with AMF can be cost effective and commercial inoculation products are available. AMF are essential biotic soil segments which, while absent or ruined, can prompt a less proficient biological system functioning. The procedure of restoring the normal degree of AMF wealth can speak to a valid alternative to traditional fertilizer practices, with a view to manageable farming. The principal methodology that can be embraced to accomplish this objective is the immediate representation of AMF propagules (inoculum) into an objective soil. Vegetable yields with high mycorrhizal development reliance are from *Allium cepa*, *Allium ampeloprasum*, *Daucus carota*, *Lactuca sativa*, *Cucumis sativus*, *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum*, *Capsicum annuum*, etc. Genomics and transcriptomics have prompted a mammoth advance in the exploration field of AMF, with ensuing significant advances in the present information on the procedures engaged in their

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_7

communication with the host plant and other soil biota. The historical backdrop of AMF applications in controlled and open-field conditions is currently long. This chapter briefly identifies several future research areas relevant to AMF to exploit and improve the biostimulant effects of AMF in horticultural crops.

Keywords

Arbuscular mycorrhizal fungi (AMF) · Biotic stress · Abiotic stress · Mycorrhiza · Vegetables · Horticultural crop

7.1 Introduction

A preliminary issue for contemporary agriculture is confronting two opposing goals, similar to the need to supply nourishment for the requesting populace and to constrict harm to the biological system, which can in turn negatively impact agriculture (Duhamel and Vandenkoornhuyse 2013). Inside the most recent decade, a few mechanical advancements were proposed to build up the manageability of creation frameworks. Among valuable microorganisms, arbuscular mycorrhizal fungi (AMF) play a significant milestone in plant execution while within the host plants (Smith and Read 2008; Swain and Abhijita 2013; Owen et al. 2015). The possibility of this beneficial interaction is that the limit of AMF to build up a network of outer hyphae is fit for expanding the zone and furthermore the explorable soil volume for supplement uptake (Giovannetti et al. 2001). AMF can secrete the chemical like phosphatases to hydrolyze organic phosphate to a useable form (Swain et al. 2021), thus improving crop productivity (Swain et al. 2021). AMF are shown not exclusively to improve plant sustenance (biofertilizers); however they likewise meddle with the phytohormone equilibrium of the plant, consequently called as bioregulators and bioprotectors. During another review, Sbrana et al. (2014) reported that AMF advantageous cooperation could impel changes in plant auxiliary digestion achieving the improved biosynthesis of phytochemicals with prosperity-propelling properties. Furthermore to the advantages referred to above, AMF bestow other significant advantages like resistance to dry season (Jayne and Quigley 2014) and salt stress (Porcel et al. 2012; Sahoo et al. 2013), supplement insufficiency, weighty metal tainting (Garg and Chandel 2010), and antagonistic soil pH conditions (Rouphael et al. 2015a). For instance, furrowing and utilization of high compost can diminish AMF bounty and colonization (Lehmann et al. 2014). Different variables which will effectively affect AMF beneficial interaction include the use of explicit biocides (Swain and Abhijita 2013). This review revolves around the new advances of the exercises of AMF on plant prosperity, food, and nature of farming yields. The agronomical and physiological cycles introducing tolerance to abiotic stresses in AMF plants and farm management will also be covered. The review closes by recognizing a few opportunities for future examinations to improve the usage of AMF.

7.2 Arbuscular Mycorrhizal Fungi

7.2.1 Taxonomy

AMF are shaped among roots and a specific gathering of fungi, which are systematically isolated from any remaining. It is therefore impossible to recommend particular AM fungal strains unquestionably responsible for green yields. Nonetheless, on the grounds that types of the genera *Gigaspora* and *Scutellospora* could likewise be unsafe to the soil construction, most business inocula contain types of the genera *Rhizophagus* and *Funneliformis*. The abovementioned species are present in almost all dirt under a fair extent of all climate zones (Smith and Read 2008) and can, therefore, be applied in agricultural production in all geographical regions.

7.2.2 Life Cycle and Arrangement of the Beneficial Interaction of AMF

The existence pattern of AMF starts with the asymbiotic stage by the germination of agamically shaped chlamydospores in the dirt, which relies only upon actual factors like temperature and dampness. Upon actual contact with the root, the living being constructs hyphopodia on the root area. They structure the purported pre-entrance contraption, a transitory intracellular design utilized by the organism to infiltrate the root. At the point when the growth arrives at the internal cortex, it changes the kind of colonization and constructs profoundly fanned hypha-like designs, called as eponymous arbuscules. Corresponding to the root colonization, the growth investigates the encompassing soil with its hyphae, where it can ingest supplements, cooperate with different microorganisms, and colonize the underlying foundations of adjoining plants or something similar belonging to various species. Consequently, plants and their AM parasites are associated in an organization of roots and hyphae where they can trade supplements. Ultimately, new chlamydospores are structured at the extraradicular mycelium and the life cycle is closed.

7.2.3 Creation of Inocula and Quality Viewpoints

Agricultural harvests inoculated with AMF are turning out to be normal practice, particularly in intensive agricultural cropping frameworks because of the decrease in native AMF populaces in the soil. In any case, an excellent inoculum is fundamental for effective root colonization with AMF and ought to include:

(a) Blends of AMF (for example two or more mycorrhizal species are superior to one)
(b) High quantities of infective AMF propagules
(c) Absence of plant microorganisms and bugs

(d) Presence of helpful microorganisms and added substances which promotes root mycorrhizal colonization and movement
(e) Dry strong inoculum (long timeframe of realistic usability)

Field propagation is the affordable way to propagate AMF. Briefly, immunized host plants are cultivated in sandy soil, allowing the AMF to develop and propagate by themselves. Regardless of the effortlessness of this propagation method, there are few disadvantages like irregular production, difficulty of spore collection, and a high risk of inoculum contamination by pests, pathogens, and weeds. Many of these issues could be solved by the soilless creation of AMF inocula in greenhouses by utilizing sterile substances, such as vermiculite, to develop host plants. The in vitro culture framework combines a few favorable circumstances, for example, (a) an unadulterated and non-tainted item (sterile conditions), (b) simple recognizability and follow-up, (c) simplicity to focus, and (d) possibility to create mycorrhizal propagules lasting through the year. Nonetheless, in vitro spread is simply relevant to *Rhizophagus* and the short timeframe of realistic usability of inoculum, because of its fluid structure, could likewise restrict the business application. In addition, there are as yet very few long-term consideration and direct connections of things from unsterile or sterile creation structures; anyway negative impacts of sterile creation methodologies have been represented (Calvet et al. 2013). Finally, a couple of challenges arise at this point, for instance, the desperate prerequisite for business things having a high gathering of infective propagules, and advanced inoculum structures (for instance tablets, gel) to smooth out the application in farming yields.

7.3 Benefits of AMF Inoculants for Creation of Green Harvest

The AMF structure's worthwhile relationship with vascular plants is scattered in an arrangement of natural frameworks (Sadhana 2014). The mechanisms used by AMF include improved plant supplement uptake, extended cell support system, adjustment of root designing, and enzyme production (Nadeem et al. 2014). Additionally, extended plant strength to push, battling out of the microorganism for food and space (Schouteden et al. 2015), and establishment of shield frameworks due to phytochemical gathering (Olowe et al. 2018) are segments of the important systems of AMF.

Beyond improving the yield of agrarian harvests, mycorrhizal inoculation has moreover seemed to improve their wholesome quality (Rouphael et al. 2015b). It adjusts essential and optional metabolism systems of the host plant through exometabolites. These auxiliary metabolites are known to upgrade both the taste- and well-being-advancing properties of yields (Swain and Mukherjee 2020). Tomato is one of the well-being-advancing green yields that are broadly devoured. It contains cell reinforcements and so on that kill perilous free revolutionaries in the blood and decrease cholesterol (Bhowmik et al. 2012). Tomato inoculated with AMF demonstrated higher groupings of carbohydrates (Copetta et al. 2011). Essentially, AMF vaccination expanded the content of tomato organic products like phosphorus, zinc, and lycopene up to 50–60%, 25–30%, and 15–20%, respectively.

Another advantageous impact of AMF is expanded seedling endurance and foundation (Swain et al. 2018) though many of the crops are brought up in the nursery where AMF immunization is completed prior to relocating to the field. Expanded uptake of supplements encouraged by AMF underpins the seedling endurance and foundation. Quality seedling is an essential factor for a sound harvest and great yield. Bettoni et al. (2014) announced expanded development of proteins, proline, and dissolvable sugars on leaves of onion plants inoculated with AMF, which additionally upgraded the seedlings of onion for resistance of ecological stresses and further development. AMF upgraded the organic product yield and quality, consistency of natural product crops, and early and better blossoming (Mukherjee et al. 2018). Early blossoming and developing guarantee early collect which orders a higher market cost as detailed in tomato creation (Ortas et al. 2013).

AMF implementation may not generally be related with positive results. The AMF vaccination did not significantly affect biomass yield and uptake of nutrients in cashew seedlings. Indeed, they were accounted for to have even stifled nitrogen and phosphorus uptake in one of the considered soils (Ibiremo et al. 2012). Hitherto, the current outcomes on the utilization of AMF for enduring natural product trees are promising (Ananthakrishnan et al. 2004; Sarangi et al. 2021); however, further long-haul studies are required to recognize extra impacts.

AMF likewise by implication add to the nourishing nature of harvests by decreasing the requirement for pesticide applications since they improve plant resistance to microorganisms and infections (Adak et al. 2020). The well-being chances presented by lingering impacts of pesticides in plant crops are disposed of or possibly diminished to the barest least. Arbuscular mycorrhizal growth vaccination upgraded the actual quality by expanding the timeframe of realistic usability of onions (Makus 2020). The expanded solidness of the onions by AMF was likely prompted by the arrangement of calcium pectate which favors cells in establishing activity. Previously, inoculant creation was essential for PGPB; however, in present years there has been commercial mycorrhizae inoculant creation.

7.4 Effect of AMF on Harvest Resistance to Abiotic Stresses

Late investigations demonstrate that wellness benefits given by mutualistic organisms add to or are liable for plant variation to stretch (Swain and Abhijita 2013). Altogether, mutualistic organisms may give resistance to dry spell, metals, illness, heat, and herbivory as well as advance development and supplement obtaining. It has gotten clear that probably a few plants cannot endure natural surroundings' forced abiotic and biotic burdens without parasitic endophytes. Abiotic stresses, for example, dry spell, saltiness, outrageous temperatures (warmth and cold), substantial metal harmfulness, and oxidative pressure, are not small dangers to farming and result in the weakening of the climate (Wang et al. 2003). Abiotic stress prompts a progression of morphological, physiological, biochemical, and subatomic changes that unfavorably influence plant development and efficiency (Wang et al. 2001). Dry season, saltiness, extraordinary temperatures, and oxidative pressure are

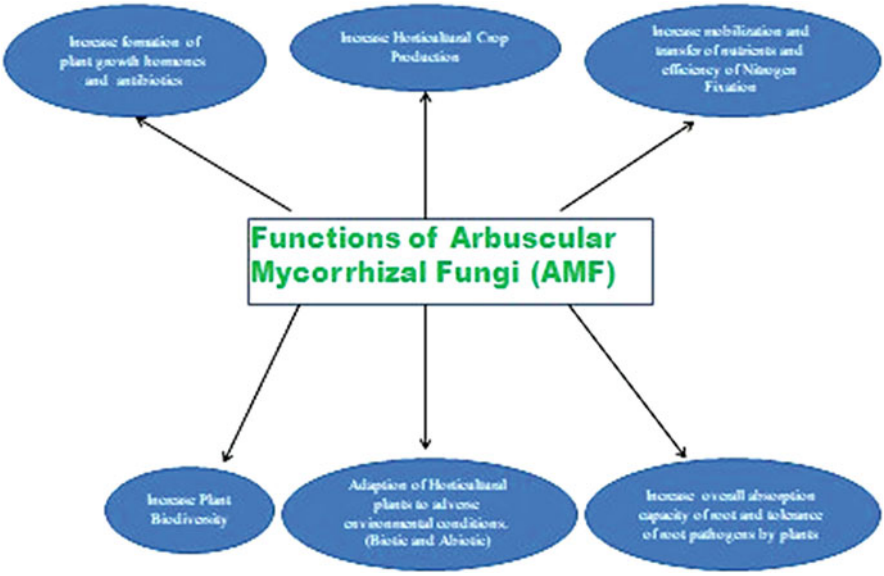

Fig. 7.1 Functional diversity of arbuscular mycorrhizal fungi (AMF) symbiosis

regularly interconnected and may prompt comparative cell harm (Wang et al. 2011). AMF have been generally investigated as yield development enhancers while improving plants' capacities to endure pressure by expanding water uptake and changing supplements from the dirt which are ordinarily unattainable to the plant (Fig. 7.1). This upgraded supplement uptake additionally permits AMF to brace the harvests against not exactly ideal developing conditions. Unequivocal assessments have exhibited that particular unfathomable kinds of the Glomus family, expressly *Glomus intraradices*, may help crops better withstand oil, considerable metals, pungency, lack of supplements, and drought-started edaphic stresses (Lenoir et al. 2016).

7.5 Effect of AMF on Yield Resistance to Biotic Anxieties

Biocontrol organisms are advantageous, noninfectious microbes which can diminish the negative impacts of contagious and bug-based plant microbes while advancing positive reactions in the plant itself (Mukherjee et al. 2018; Swain et al. 2021). Helpful contagious biocontrol instruments incorporate the guideline of antimicrobial creation, building up parasitic and supplement cooperation with the objective organic entities in the rhizosphere (Swain et al. 2018).

A few reports have shown that advantageous parasites may provoke fundamental protection of endophytes by upregulating explicit qualities in the plant itself identified with infection (Shoresh et al. 2010). Often plants are infected with a consortium of microbes and pests which triggers an array of reactions that might

be helpful or harmful to the host. Induced resistance is a plant response system which is activated when defied with certain abiotic or biotic components to create an active resistance state. This cycle includes redistributing carbon and nitrogen assets from plant development and propagation, and all things considered, making enduring and foundational obstruction of the plant to an expansive cluster of microbes and hindering bugs (Walling 2001). Localized and systemic induced resistance has been exhibited in many plants in light of assault by harmful organisms, physical damage due to insects or other factors, chemical treatments, and presence of non-pathogenic root-dwelling microorganisms (Harman et al. 2004). Other eminent examples of developments which have been used as biocontrol are *Trichoderma* sp. Species inside both parasitic genera have shown different capacities to control various foliar, root, and natural microorganisms, close by gutless animals (Davies and Spiegel 2011).

Although some contagious species have exhibited model potential for more extensive use in a variety of biocontrol applications, they are not used to their fullest degree. This is mostly because of indistinct principles with respect to their utilization, application, security, and specialized difficulties related with applying and keeping a particular, solitary strain of parasites in the dirt. To beat a portion of these difficulties, one would have to address the following: (1) know about components influencing their practicality rates in soils; (2) comprehend which contagious strains were best for explicit harvests and ecological conditions; (3) be learned on the most fitting definition, ranch the board, and application rehearses; (4) instruct the cultivators on the best utilization of this innovation; and (5) conquer tough and generally costly administrative boundaries in objective business sectors to guarantee items as biopesticides (FAO 2018).

7.6 Impact of AMF on Horticultural Crops

In the cultivation business, the spotlight has generally been on yield. Be that as it may, shoppers' advantage worldwide in the nature of agricultural items, and here particularly in the nature of palatable items like products of the soil, has expanded in the new past and will turn into the main impetus later on (Fig. 7.1) (Gruda 2009).

In bean stew (*Capsicum annuum* L.) inoculation with AMF (*Glomus fasciculatum*) expanded the ascorbic acid substance of green organic products (Gruda 2009). In yam (*Ipomoea batatas* L.) the ß-carotene concentrations increased after being treated with AMF (*G. intraradices* and *Glomus mosseae*) (Tong et al. 2013). In leaves of lettuce AMF initiated the aggregation of photosynthetic pigments and some mineral supplements along these lines improving its wholesome quality (Baslam et al. 2013). It is also found that mycorrhizal advantageous interaction can invigorate the amalgamation of primary metabolites, which may improve the collection of cancer prevention agents conceivably useful to human well-being. Their outcomes indicated that mycorrhizal symbiosis interaction improved the aggregation of antioxidant compounds and less significantly chlorophylls and phenolics in lettuce plant. These enhancements were higher under water deficiency than under

ideal water system, suggesting that mycorrhizal symbiosis interaction can improve the nature of lettuce. Baslam et al. (2012) demonstrated that AMF can improve the development and nourishing nature of nursery-developed lettuces grown in controlled CO_2 conditions. They additionally showed that mycorrhizal beneficial interaction is anticipated to be significant in characterizing plant reactions to raised climatic CO_2. Adjacent to its immediate impact on the plant's compound creation, AMF can likewise in a roundabout way add to an improved synthetic nature of vegetables by a decreased need of pesticide applications. For instance, one generally utilized organophosphate bug spray in vegetable cultivation; phoxim is regularly found as deposits in the yields and hence it poses a possible danger to the general well-being and environment.

7.7 Environmental and Management Impact on the Effects of AMF for the Host Crop

Development or advancement or quality improvement of crop yields by AMF inoculum is constrained by biotic and abiotic connections. Accordingly, the personalities of the AMF detach and natural and mineral preparation is uncovered to control the effect of AMF on vegetable harvests (Linderman and Davis 2004).

Notwithstanding concentrated exploration with mycorrhiza during the most recent couple of years, the reproducibility of examination discoveries under viable field conditions actually remains fairly troublesome. Moreover, it is not generally conceivable to duplicate the beneficial outcomes of mycorrhiza from year to year. These impacts are because of the way that energetic advancement of mycorrhiza relies upon a huge number of components, for example, soil type and surface, developing media, supplement content (especially P supply), and pH esteem, just as on development measures, which impact dampness and temperature in the root zone (Gruda 2009).

7.8 The Role of Mycorrhizae in Sustainable and Regenerative Agriculture

The advantageous interaction among plants and AMF started around 500 million years back and is thought to have assumed a significant role in the shift of plants surviving outside of water to becoming terrestrial. The beneficial interaction among AMF and a larger part of plant kingdom is accepted to be a chief driver of the biodiversity, biological system inconstancy, and profitability of plant networks (Chagnon et al. 2013). A new investigation of worldwide biogeography of AM fungi inspected DNA from 1014 plant root samples gathered from around the world. Despite the fact that AM fungal networks reflected neighborhood natural conditions and spatial distance between locales, 93% of AMF taxa were found on various landmasses and 34% on every one of the six mainlands studied. This proposes that AMF are viably and productively scattered all through the world. In this manner,

much exploration has been devoted to understanding the components through which AMF impact a wide scope of plant reactions in various ecological niches. At present it is broadly acknowledged that the strategies utilized and soil conditions common in economical agribusiness are probably going to be greater for AMF than those under ordinary horticulture (Nayak and Mishra 2020). The following segments sum up 30 years of exploration on mycorrhizal advancements, items, and undertakings. It features significant difficulties, recognizes holes in the exploration, and proposes systems to upgrade mycorrhizal immunization, quantifiable profit openings, and effective showcasing and advantages of mycorrhizal advancements. The significant points identified with the utilization of mycorrhizal inoculants that are examined and need to be checked for future endeavors are (a) local and worldwide business sectors; (b) techno-monetary examinations; (c) business techniques of effective organizations; (d) overview of worldwide mycorrhizal guideline; and (e) challenges for new mycorrhizal adventures.

7.9 Conclusions and Possibilities

The utilization of AM symbionts as a biostimulant in agricultural harvests has significantly expanded over the most recent 20 years, generally because of their capacity to get creation and yield strength in a naturally manageable manner. All through the survey, we have inspected the promising biostimulant impacts of AMF to upgrade the root framework and in this manner, full scale and micronutrient uptake by means of expanded supplement transport and additionally solubilization. Greatest advantages will be just accomplished by embracing valuable homestead the board rehearses, by vaccination with effective AMF strains, and furthermore by a precise determination of plants host/parasite interactions. Inoculation with chosen AMF can help plant's optional digestion prompting improved nutritional compounds and can likewise present resistance to dry spell and antagonistic synthetic soil conditions. Another significant angle is the assessment of the capacity of AMF in improving harvest profitability under field conditions. Be that as it may, a large portion of the investigations detailed in the logical writing were led under controlled conditions and the reaction of AMF may shift altogether in the common habitat, since a number of biotic and abiotic stresses can associate with these organisms and may influence their presentation. At last researchers, horticulturists, and enterprises need to work together to incorporate this modernized rural practice as a viable and economical apparatus for improving yield and item nature of agricultural harvests. Future explorations ought to be centered around (a) understanding the AMF strains/crop species/conditions associated with choosing the best blends; (b) improvement of great inocula having a high centralization of infective propagules, long time span of usability, and "simple-to-utilize" formulations; (c) recognizable proof of the mix of microscopic organisms/AMF strains that communicate synergistically to amplify the advantages; (d) surveying of the productivity of AMF vaccination under field conditions, and various pressure variables; and (e) distinguishing of the atomic components responsible for the upgrade of well-

being-advancing photochemicals in agricultural items prompted by AMF inoculation.

Acknowledgements Authors are immensely thankful to the Department of Science and Technology (DST), Govt. of India, for providing DST-INSPIRE Fellowship to Harekrushna Swain having Fellowship No. IF140749. Authors are also thankful to the Director, ICAR-NRRI, Cuttack-753006, and HOD, Department of Botany and Biotechnology, Ravenshaw University, Cuttack-753003, for providing necessary library and technical support.

References

Adak T, Mahapatra B, Swain H et al (2020) Indigenous biobed to limit point source pollution of imidacloprid in tropical countries. J Environ Manag 272:111084. https://doi.org/10.1016/j.jenvman.2020.111084

Ananthakrishnan G, Ravikumar R, Girija S et al (2004) Selection of efficient arbuscular mycorrhizal fungi in the rhizosphere of cashew and their application in the cashew nursery. Sci Hortic 100(1–4):369–375. https://doi.org/10.1016/j.scienta.2003.07.008

Baslam M, Esteban R, Garcia-Plazaola JI et al (2013) Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. Appl Microbiol Biotechnol 97(7):3119–3128. https://doi.org/10.1007/s00253-012-4526-x

Baslam M, Garmendia I, Goicoechea N (2012) Elevated CO_2 may impair the beneficial effect of arbuscular mycorrhizal fungi on the mineral and phytochemical quality of lettuce. Ann Appl Biol 161:180–191. https://doi.org/10.1111/j.1744-7348.2012.00563.x

Bettoni MM, Mogor ÁF, Pauletti V et al (2014) Growth and metabolism of onion seedlings as affected by the application of humic substances, mycorrhizal inoculation and elevated CO_2. Sci Hortic 180:227–235. https://doi.org/10.1016/j.scienta.2014.10.037

Bhowmik D, Kumar KS, Paswan S et al (2012) Tomato-a natural medicine and its health benefits. J Pharmacogn Phytochem 1(1):33–43

Calvet C, Camprubi A, Pérez-Hernández A et al (2013) Plant growth stimulation and root colonization potential of *in vivo* versus *in vitro* arbuscular mycorrhizal inocula. HortScience 48(7):897–901. https://doi.org/10.21273/HORTSCI.48.7.897

Chagnon PL, Bradley RL, Maherali H et al (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends Plant Sci 18:484–491. https://doi.org/10.1016/j.tplants.2013.05.001

Copetta A, Bardi L, Bertolone E et al (2011) Fruit production and quality of tomato plants (*Solanum lycopersicum* L.) are affected by green compost and arbuscular mycorrhizal fungi. Plant Biosyst 145(1):106–115. https://doi.org/10.1080/11263504.2010.539781

Davies KG, Spiegel Y (eds) (2011) Biological control of plant-parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Dordrecht

Duhamel M, Vandenkoornhuyse P (2013) Sustainable agriculture: possible trajectories from mutualistic symbiosis and plant neodomestication. Trends Plant Sci 18(11):597–600. https://doi.org/10.1016/j.tplants.2013.08.010

FAO (2018) Successful soil biological management with beneficial microorganisms. http://www.fao.org/agriculture/crops/thematic-sitemap/theme/spi/soil-biodiversity/case-studies/soil-biological-management-with-beneficial-microorganisms/en/

Garg N, Chandel S (2010) Arbuscular mycorrhizal networks: process and functions. A review Agron Sustain Dev 30:581–599. https://doi.org/10.1051/agro/2009054

Giovannetti M, Fortuna P, Citernesi AS et al (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 151(3):717–724. https://doi.org/10.1046/j.0028-646x.2001.00216.x

Gruda N (2009) Do soilless culture systems have an influence on product quality of vegetables? J Appl Bot Food Qual 82:141–147

Harman GE, Howell CR, Viterbo A et al (2004) *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56. https://doi.org/10.1038/nrmicro797

Ibiremo O, Olubamiwa O, Agbeniyi S et al (2012) Response of cashew seedlings from different nut sizes to phosphate fertilizer and Arbuscular mycorrhizal inoculation in two soils in Nigeria. Int J Plant Anim Environ Sci 2:147–158

Jayne B, Quigley M (2014) Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: a meta-analysis. Mycorrhiza 24:109–119. https://doi.org/10.1007/s00572-013-0515-x

Lehmann A, Veresoglou SD, Leifheit EF et al (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants: a meta-analysis. Soil Biol Biochem 69:123–131. https://doi.org/10.1016/j.soilbio.2013.11.001

Lenoir I, Fontaine J, Lounès-Hadj Sahraoui A (2016) Arbuscular mycorrhizal fungal responses to abiotic stresses: a review. Phytochemistry 123:4–15. https://doi.org/10.1016/j.phytochem.2016.01.002

Linderman RG, Davis EA (2004) Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by *Glomus intraradices*. Hort Technol 14(2):196–202. https://doi.org/10.21273/HORTTECH.14.2.0196

Makus D (2020) Mycorrhizal inoculation of tomato and onion transplants improves earliness. Acta Hortic 631:275–281

Mukherjee AK, Swain H, Adak T et al (2018) Evaluation of *Trichoderma* based product 'RiceVit' in farmers' field of Chandol, Kendrapada, Odisha. NRRI NEWSLETTER 39(4):20–21

Nadeem SM, Ahmad M, Zahir ZA et al (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32(2):429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Nayak SK, Mishra BB (eds) (2020) Frontiers in soil and environmental microbiology. CRC Press, Boca Raton

Olowe OM, Olawuyi OJ, Sobowale AA et al (2018) Role of arbuscular mycorrhizal fungi as biocontrol agents against *Fusarium verticillioides* causing ear rot of *Zea mays* L. (Maize). Curr Plant Biol 15:30–37. https://doi.org/10.1016/j.cpb.2018.11.005

Ortas I, Sari N, Akpinar C et al (2013) Selection of arbuscular mycorrhizal fungi species for tomato seedling growth, mycorrhizal dependency and nutrient uptake. Eur J Hortic Sci 78(5):209–218

Owen D, Williams AP, Griffith GW et al (2015) Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. Appl Soil Ecol 86:41–54. https://doi.org/10.1016/j.apsoil.2014.09.012

Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. Agron Sustain Dev 32:181–200. https://doi.org/10.1007/s13593-011-0029-x

Rouphael Y, Cardarelli M, Colla G (2015a) Role of arbuscular mycorrhizal fungi in alleviating the adverse effects of acidity and aluminium toxicity in zucchini squash. Sci Hortic 188:97–105. https://doi.org/10.1016/j.scienta.2015.03.031

Rouphael Y, Franken P, Schneider C et al (2015b) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic 196:91–108. https://doi.org/10.1016/j.scienta.2015.09.002

Sadhana B (2014) Arbuscular mycorrhizal fungi (AMF) as a biofertilizer - a review. Int J Curr Microbiol App Sci 3(4):384–400

Sahoo HP, Sarangi PK, Swain H et al (2013) Biodiversity of mangroves in India: impact of climate change and their management. In: Kambaska Kumar Behera KK (ed) Advance frontier on biotechnology. Jaya Publisher, New Delhi, pp 297–310

Sarangi S, Swain H, Adak T et al (2021) *Trichoderma*-mediated rice straw compost promotes plant growth and imparts stress tolerance. Environ Sci Pollut Res Int. https://doi.org/10.1007/s11356-021-13701-3

Sbrana C, Avio L, Giovanetti M (2014) Beneficial mycorrhizal symbionts affecting the production of health-promoting phytochemicals. Electrophoresis 35:1535–1546

Schouteden N, De Waele D, Panis B et al (2015) Arbuscular mycorrhizal Fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. Front Microbiol 6:1280. https://doi.org/10.3389/fmicb.2015.01280

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Swain H, Abhijita S (2013) Nitrogen fixation and its improvement through genetic engineering. J Global Biosci 2(5):98–112

Swain H, Adak T, Mukherjee AK et al (2018) Novel *Trichoderma* strains isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. Microbiol Res 214:83–90. https://doi.org/10.1016/j.micres.2018.05.015

Swain H, Adak T, Mukherjee AK et al (2021) Seed biopriming with Trichoderma strains isolated from tree bark improves plant growth, antioxidative defense system in rice and enhance straw degradation capacity. Front Microbiol 12:633881. https://doi.org/10.3389/fmicb.2021.633881

Swain H, Mukherjee AK (2020) Host–Pathogen–*Trichoderma* interaction. In: Sharma A, Sharma P (eds) Trichoderma. Rhizosphere biology. Springer, Singapore, pp 149–165. https://doi.org/10.1007/978-981-15-3321-1_8

Tong Y, Gabriel-Neumann E, Ngwene B et al (2013) Effects of single and mixed inoculation with two arbuscular mycorrhizal fungi in two different levels of phosphorus supply on ß-carotene concentrations in sweet potato (*Ipomoea batatas* L.) tubers. Plant Soil 372:361–374. https://doi.org/10.1007/s11104-013-1708-y

Walling LL (2001) Induced resistance: from the basic to the applied. Trends Plant Sci 6:445–447. https://doi.org/10.1016/S1360-1385(01)02046-5

Wang FY, Tong RJ, Shi ZY et al (2011) Inoculations with arbuscular mycorrhizal Fungi increase vegetable yields and decrease phoxim concentrations in carrot and green onion and their soils. PLoS One 6(2):e16949. https://doi.org/10.1371/journal.pone.0016949

Wang WX, Barak T, Vinocur B et al (2003) Abiotic resistance and chaperones: possible physiological role of SP1, a stable and stabilizing protein from Populus. In: Vasil IK (ed) Plant biotechnology 2002 and beyond. Springer, Dordrecht, pp 439–443. https://doi.org/10.1007/978-94-017-2679-5_91

Wang WX, Vinocur B, Shoseyov O et al (2001) Biotechnology of plant osmotic stress tolerance physiological and molecular considerations. Acta Hortic 560:285–292. https://doi.org/10.17660/ActaHortic.2001.560.54

Microbial Remediation of Persistent Agrochemicals

8

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Abstract

Agrochemicals are an integral part of the agricultural ecosystem as it contributes significantly to improving the crop yield through pest management. The chemically synthesized products such as insecticides, herbicides, and fungicides exhibit harmful effects on living organisms and few of them are characterized as resistant to degradation. Besides being persistent in nature, they may leach into groundwater and run off to surface water. Thus, to degrade the persistent agrochemicals, bioremediation with the help of microbes is one of the best options. This approach is environmentally friendly, effective, and less expensive with the least adverse effects. Microbes such as bacteria, actinobacteria, fungi, and cyanobacteria are reported of having the exclusive trait of degradation. The microbial world consumes persistent toxic chemicals as the source of their growth by facilitating the mineralization of those chemicals. This detoxification process is carried out with the help of microbial enzymes. Some efficient and potential bioremediation agents are *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Anabaena* sp.,

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_8

Leptolyngbya sp., *Nostoc* sp., *Spirulina* sp., etc. This chapter discusses the extent of the use of persistent agrochemicals and key biodegradation pathways. The chapter also discusses on the advantages and disadvantages of microbial remediation and the scope of commercial utilization of microbes for agrochemical degradation.

Keywords

Pesticides · Bioremediation · Enzymes · Persistent agrochemicals (PAs) · Bioconcentration factor (BCF)

8.1 Introduction

Use of agrochemicals has increased manyfold from the period of "Green Revolution." Agrochemicals, more particularly pesticides, are applied to improve crop yield with better quality of product through the management of pest (insecticides), disease (fungicides), and weed (herbicides). Synthetic pesticides are semi-volatile, toxic, and persistent in nature and trigger harmful effects on humans, environment, and wildlife. These chemicals take decades of time to degrade significantly in natural environment. During such longer period of time they may get transported to groundwater, surface water, surface, and core part of soil. From soil and water, they are accumulated in food crops and enter into the food cycle (El-Bestawy et al. 2007). Apart from this, beneficial microbes and nontarget organisms are affected by the indiscriminate use of synthetic chemicals in the agroecosystem. Aquatic flora, fauna, and microorganisms are affected by the discharges of agrochemical manufacturing factories as well as by unintended spills. Persistent organic pollutants in agriculture (persistent agrochemicals, PAs) can be degraded by various mechanisms such as photodegradation (Bustos et al. 2019), bioadsorption (Mishaqa 2017), bioaccumulation (Xu and Huang 2017), and biodegradation (Bhadouria et al. 2020).

The term bioremediation comprises two words, i.e., "bios" (Greek) means life and "remedium" (a Latin term) means to take out an evil. So, bioremediation is a process that eradicates, degrades, and detoxifies the persistent pollutants by living beings. The two highlighted classes of bioremediation are phytoremediation and microbial remediation. In ex situ bioremediation, the contaminants are removed from its native place to another place and treated with microbes. In in situ bioremediation, the microbes are directly inoculated at the contamination site. For certain microorganisms, PAs are the source of nutrients and act as electron donors. Hence, they can be used to manage the PAs in polluted areas.

Some of the important microbial genera efficient in bioremediating agrochemicals are described here. Bacterial strains having degrading capacity of PAs belong to the genera of *Bacillus* sp., *Arthrobacter* sp., *Rhodococcus* sp., *Alcaligenes* sp., *Flavobacterium* sp., *Yersinia* sp., *Pseudomonas* sp., *Acetobacter* sp., *Burkholderia* sp., *Weeksella virosa* sp., *Stenotrophomonas* sp., etc. (Padmanabhan et al. 2003). Among the actinobacteria group, reports suggest that

Streptomycetes can significantly detoxify PAs. Cyanobacteria with pesticide degradation prospective include *Nostoc* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., and *Spirulina* sp. Among fungi, *Fusarium* sp., *Aspergillus niger*, *Penicillium* sp., *Lecanicillium* sp., and *Oxysporum* sp. are known to be the most potent degraders of agrochemicals. Enzymes released by these microorganisms, namely, oxygenase, phosphotriesterase, hydrolases, peroxidases, dehydrogenase, dehalogenase, lignin-modifying enzymes, organophosphorus acid anhydrolase, and laccase, play a crucial role in PA degradation.

This chapter discusses the extent of use of persistent agrochemicals and key biodegradation pathways. It also focuses on the pros and cons of microbial remediation of persistent agrochemicals, and successful and commercial level utilization of microbes for agrochemical degradation. Mechanisms, genes, and enzymes involved in the metabolism of agrochemicals are also discussed in this chapter.

8.2 Persistent Agrochemicals

An ideal agrochemical/pesticide may be defined as a noxious compound that is only harmful to targeted organisms. Unfortunately, this is not true; pesticides also have a negative effect on non-targeted organisms and human beings. Thus, persistent agrochemicals (PAs) can be defined as groups of synthetic and nonvolatile chemicals exposed intentionally or non-intentionally to targeted or non-targeted organisms and having toxic/adverse impacts on humans, environment, and wildlife. According to the sources only 0.1% of applied pesticides reach the targeted organism, whereas the remaining pesticides are deposited on non-targeted environmental compartments such as soil, water, and sediments. Thus, pesticides and their metabolites are the main factors for environmental pollution posing serious threat to the health of non-target organisms like humans and wildlife (Rani et al. 2020).

Nowadays, in international market more than 1000 pesticide compounds and their metabolites have been registered. Popp (2011) reported that the international market capital of agrochemical/pesticide per annum is valued at about USD 40 billion and the total consumption is three million tonnes. Recently, the practical usage of agrochemical covers 25% of the total cultivated land. In India, use of agrochemicals has immensely increased after independence. On international platform, India has become the fourth largest manufacturer of agrochemicals after the USA, Japan, and China (Nayak et al. 2018). The most common agrochemicals of India are organophosphate, neonicotinoids, organochlorines, etc. Among the total pesticide consumption, India has accounted for 50% of insecticides, 35% of fungicides, and 15% of herbicides.

Depending on the chemical structure and mode of action, PA can be divided into several forms such as organochlorine, organophosphate, carbamates, pyrethroids, nicotinic, pyrazole, phenolics, trizines, benzoics, sulfonylureas, bipyridilium, chloroacetamide, glycine, dinitroaniline, phenylpyrazoline, methyl benzimidazole carbamate, demethylation inhibitor, phenylamide, anilopyrimidine, quinone outside inhibitor, and phenylpyrrole. In organochlorine group, on the basis of chlorination

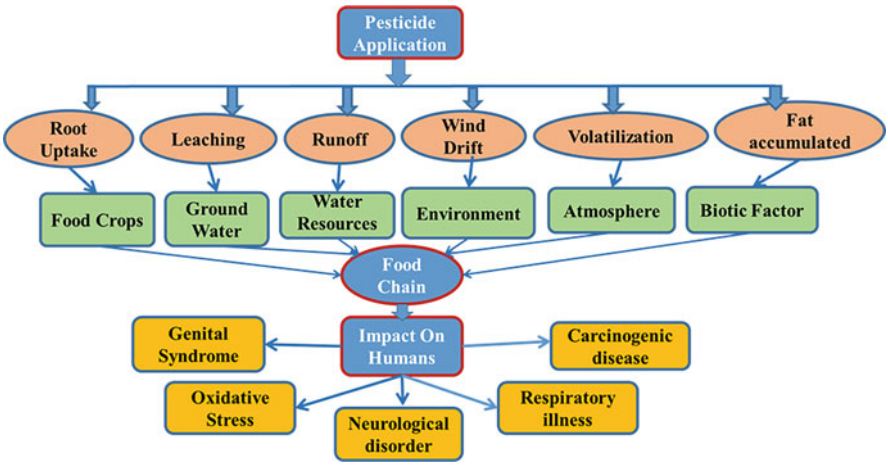

Fig. 8.1 Entry of pesticides into food chain through different ecological factors

number and substitution position, there may be 209 different polychlorinated biphenyls. In aromatics, the most persistent chlorine- and bromine-containing compounds are polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polybrominated diphenyl ethers, and organochloride pesticides (e.g., dichlorodiphenyl trichloroethane (DDT), toxaphene, chlordane) (Nayak et al. 2018). In Stockholm Convention, the United Nations Environment Programme (UNEP) motivates countries to get rid of 12 persistent organic pollutants that are termed as "dirty dozen" that constitutes 8 agrochemicals, 2 commercial enterprise chemical substances, and 2 accidental industrial intermediate products. However, these persistent virulent eight agrochemicals are prohibited by most of the developed countries, while in case of developing countries, it is being used till today due to their low cost.

The fate of PAs depends on three basic processes of transport, transfer, and transformation (Fig. 8.1). Throughout the mechanism of transport, the PA is departed from its original area of application to the surroundings, and thereafter dispersed throughout the surface water. In the process of transfer, various factors are involved in the distribution and dispersion of PAs in the environment. Last one is the transformation activity, which indicates the natural process along with chemical mechanisms that alters the PA into less complex form of chemicals or degrades it entirely. These persistent agrochemicals are considered as tolerant to degradation or it may take decades or even centuries to eradicate them successfully. These chemicals may get dispersed into extended areas that lead to environmental pollution and some of them get transported into food cycles and immensely affect humans.

8.2.1 Impact of Persistent Agrochemicals on Agriculture and Environment

According to the WHO report, more than three million people are suffering severely each year from exposure of pesticides. In India, the first agrochemical poisoning incident happened in 1958 in Kerala, where the death of over 100 people occurred by the consumption of wheat flour which was contaminated with parathion (Karunakaran 1958). Another terrible case was Bhopal Gas Tragedy of 1984, where the leakage of methyl isocyanate killed about 2259 people. Some studies also revealed that the accumulation of minute amount of persistent agrochemicals by a person can induce combined harmful effects on health conditions like induction of breast cancer, decrease in the number of sperms which results in male sterility, and suppression of immune response with hypersensitive response to some other agrochemicals/chemical antigens (Carvalho 2006). Pesticide application results in decrements in cell development, increment in mutagenesis condition, and nuclear anomalies (Iqbal Lone et al. 2013).

Moreover, agricultural step-up and excessively widespread usage of agrochemicals are responsible for the extinction of various indigenous flora and fauna, which causes a functional disorder in the agroecosystems. Through the long-term use, such persistent agrochemicals are either deposited in soils or leached into the groundwater, thereby dispersing to and polluting different land, marine, and fresh aquatic ecosystems (Nayak and Mishra 2020). These chemicals are decreasing the microbial population of soil and water. For example, the earthworm populations are negatively affected by PAs (Mahmood et al. 2016). By the excessive use of persistent agrochemicals, minor pests are turning into major pests. Natural predators and competitors are being eliminated by excessive use of insecticides. The agrochemical residues decrease the quality of groundwater. Another harmful effect is the leaf interception of agrochemicals, which causes several damages to the non-target plants. The air and other organisms may also be polluted by the excessive use of volatile agrochemicals.

8.3 Mechanism of Microbial Bioremediation of Persistent Agrochemicals

In the ecosystem, various mechanisms are put forward to make it pollutant/contaminant free. Bioremediation can be defined as a process to eradicate, degrade, and detoxify the persistent pollutants by using living beings. Bioremediation may be active or passive based upon the supply of energy, and various mechanisms of bioremediation are as follows:

8.3.1 Bioadsorption

Bioadsorption of PAs is categorized under passive process. Bioadsorption involves a number of mechanisms, i.e., electrostatic interaction, complexation of surface, exchange of ions, absorption, and precipitation (Bilal et al. 2018). Microbes like microalgae are more efficiently used as adsorbents. Mishaqa (2017) found that the cultured algae were able to get rid of 87–96% of pesticides (i.e., alachlor, atrazine, pendimethalin, propanil, simazine, isoproturon, molinate, and carbofuran) in aqueous phase. The efficiency of removal of pesticides was different depending upon the kinds of surface groups present in algae (Ata et al. 2012). The cell wall composition of microalgae plays an important role in PA biodegradation as it facilitates the adsorption of contaminants from polluted water (Qiu et al. 2017). *Gracilaria verrucosa* having hydroxyl, amine, and carboxyl as the surface groups was found to adsorb 2,4-dichlorophenoxyacetic acid (Ata et al. 2012). Several factors, i.e., optimal conditions of the biome, chemical composition and structure of organisms and pesticides, density of organism, pH, temperature, quality and strength of light, salinity, nutrients, water availability, organism (biological) and pesticide (substrate) contact, their surface bonding, redox potential, alternative substrates of carbon, oxygen tension, and electron accepter along with donor, are responsible for the completion of a suitable bioadsorption process.

8.3.2 Bioaccumulation

Bioaccumulation requires externally driven energy and is based on the bio-concentration factor (BCF). BCF reflects the concentration quotient of a contaminant of a certain organism with regard to its surroundings. The variation depends on different factors, i.e., bio-concentration activity differences, bioavailability of chemicals, physical barriers, dissolved organic matter, variation in interspecies, metabolism, and ionization of ionizable chemicals with certain ecological parameters. Reports suggested that the exposure of microbes to the pesticides (PA) produces reactive oxygen species (ROS) within the cell (Pérezgarcía et al. 2013) which is lethal as that causes functional damage leading to cell death. However, some microalgae manage to produce several antioxidants of the group, polyphenols, carotenoids, and sterols. These antioxidants are able to minimize the ROS effect on the matter of cell damage. In this way agrochemicals can induce the process of detoxification activity in microalgae and this reflects the possibility of biodegradation of PAs through bioaccumulation process. The combination of bioaccumulation and biodegradation process to detoxify agrochemicals rapidly is seen in microalgae group (Xu and Huang 2017). Biodegradation of triadimefon by green algae *Scenedesmus obliquus* through bioaccumulation has been successfully reported (Xu et al. 2007).

8.3.3 Biodegradation

The mechanism of catabolic activity to form simpler, nonhazardous, and smaller form of toxic PAs is termed as biodegradation. PA degradation can be done both by aerobic and anaerobic conditions. Under aerobic circumstances, the use of oxygenase enzyme on aromatic compound is generally initiated by electrophilic attack; however, it is delayed with the occurrence of various electron-withdrawing substituents like azo, nitro, and chloro groups. In anaerobic conditions the degradation is initiated via nucleophilic attack and these groups will favor preliminary reductive attack. For the agrochemicals like DDT and heptachlor, anaerobic degradation works better than aerobic degradation. The biodegradation of PAs refers to the chemical activities like reduction, ring cleavage dehydrogenation, dealkylation, oxidation, alkylation, and dehalogenation (Bhadouria et al. 2020).

Hatzios (1991) reported that pesticide degradation process is concluded under three stages. In stage I, the agrochemicals transform to less poisonous by-products by oxidation or hydrolysis. Oxidation, the essential step in the process of degradation, is controlled through the oxidative enzymes, e.g., peroxidase, dioxygenase, polyphenol oxidases, and cytochrome P450 polyphenol oxidases. The hydrolytic reaction plays a key role in some degradation processes. In the next stage, conjugation of PA metabolites occurs to amino acid, glutathione, or sugar. Pesticide or agrochemical conjugation can be defined as "a metabolic procedure where a natural compound is joined to an agrochemical or to its metabolite(s)/intermediate products facilitating sequestration, compartmentalization, detoxification, and/or mineralization."

8.4 Persistent Agrochemical-Degrading Microbes

8.4.1 Bacteria as PA-Degrading Agents

Bacteria can degrade diverse groups of pesticides (Table 8.1). A huge puddle of bacterial strains with degrading capacity include *Bacillus* sp., *Arthrobacter* sp., *Ralstonia* sp., *Rhodococcus* sp., *Yersinia* sp., and *Pseudomonas* sp. (Padmanabhan et al. 2003). The detoxification of PA is achieved by co-metabolism and it is further amplified through root fluids excreted in rhizosphere, because of the gross microbial interaction. In bacteria, PAs are generally taken as carbon and energy sources and get degraded to minerals (Fritsche and Hofrichter 2008). This degradation ability is influenced by several physiochemical factors like soil texture and water-holding capacity, pH, temperature, and availability of nutrients (Singh 2008).

Bacterial multiplication and growth are affected by pesticides because of the proficient absorption of PA in soil organic particles. Apart from this limitation, bacteria have exceptional significance to detoxify the PAs. Reports suggest that aerobic remediation is much faster than anaerobic; however, some exceptions are there such as DDT degradation that occurs ten times faster in anaerobic condition than in aerobic remediation. The most active prokaryotic genus for remediation

Table 8.1 Biodegradation of persistent agrochemicals by bacteria

Sl. No.	Pesticides	Bacteria	References
1.	Acetamiprid	*Ochrobactrum* sp. D-12	Wang et al. (2013)
2.	Alachlor	*Pseudomonas* sp. ADP, *Ancylobacter* sp. S15, *Agrobacterium* sp. CZBSA1	Katz et al. (2001); Ewida (2014)
3.	Aldrin	*Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp.	Sharma et al. (2016)
4.	Atrazine	*Arthrobacter* sp., *Clavibacter* sp.	Sharma et al. (2016)
5.	Cadusafos	*Pseudomonas putida*, *Flavobacterium* sp.	Karpouzas et al. (2005)
6.	Carbaryl	*Pseudomonas* sp., *Achromobacter* sp., *Arthrobacter* sp., *Xanthomonas* sp., *Pseudomonas cepacia*	Chapalamadugu and Chaudhry (1991); Gunasekara et al. (2008)
7.	Carbendazim	*Pseudomonas* sp., *Brevibacillus borstelensis*	Arya et al. (2017)
8.	Carbofuran	*Flavobacterium* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Achromobacterium* sp., *Sphingomonas* sp., *Arthrobacter* sp.	Sharma et al. (2016)
9.	Chlorpyrifos	*Achromobacter xylosoxidans* (JCp4), *Ochrobactrum* sp. (FCp1)	Akbar and Sultan (2016)
10.	Cyhalothrin	*Klebsiella* sp., *Pseudomonas oleovorans*	Thatheyus and Selvam (2013)
11.	Cypermethrin	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter asburiae*, *Pseudomonas stutzeri*	Thatheyus and Selvam (2013)
12.	DDT (dichlorodiphenyltrichloroethane)	*Klebsiella pneumonia*, *Bacillus* sp., *Pseudomonas putida*, *E. coli*, *Hydrogenomonas* sp.	Sharma et al. (2016)
13.	Diazinon	*Pseudomonas cepacia*	Tewari and Saini (2012)
14.	Dieldrin	*Pseudomonas* sp.	Sharma et al. (2016)
15.	Dimethoate	*Bacillus cereus*, *Bacillus subtilis*, *Bacillus safensis*	Ishag et al. (2016)

(continued)

Table 8.1 (continued)

Sl. No.	Pesticides	Bacteria	References
16.	Endosulfan (α- and β-endosulfan)	*Pseudomonas* sp., *Bacillus* sp., *Flavobacterium* sp.	Karpouzas et al. (2005)
17.	Ethoprophos	*Sphingomonas paucimobilis*	Karpouzas et al. (2005)
18.	Fenvalerate	*Bacillus cereus*, *Pseudomonas viridiflava*	Thatheyus and Selvam (2013)
19.	Glyphosate	*Clostridium* sp., *Arthrobacter* sp.	Tewari and Saini (2012)
20.	Imidacloprid	*Achromobacter* sp., *Pseudoxanthomonas* sp., *Sinorhizobium* sp., *Mesorhizobium* sp., *Microbacterium* sp.	Sharma et al. (2016)
21.	Iprodione	*Pseudomonas fluorescens*, *P. paucimobilis*, *Arthrobacter* sp. C1, *Achromobacter* sp. C2	Mercadier et al. (1997); Campos et al. (2015)
22.	Lindane	*Bosea thiooxidans*, *Sphingomonas paucimobilis*	Karpouzas et al. (2005)
23.	Malathion	*Pseudomonas aeruginosa* AA112	Abo-Amer (2007)
24.	Molinate	*Achromobacter xylosoxidans* subsp. *denitrificans*, *Stenotrophomonas maltophilia*, *Pseudomonas chlororaphis* IFO3904, *Pseudomonas nitroreducens* IAM 143, *Curtobacterium flaccumfaciens var*, *Flaccumfaciens* LMG 3645	Barreiros et al. (2003)
25.	Monocrotophos	*Rhodococcus* sp.	Tewari and Saini (2012)
26.	Pendimethalin	*Pseudomonas aeruginosa*, *Bacillus mycoides*, *Bacillus cereus*	Sharef Ibrahim et al. (2013)
27.	Pentachloronitrobenzene	*Cupriavidus* sp. BIS7	Teng et al. (2017)
28.	Pyridine	*Paracoccus* sp.	Qiao and Wang (2010)
29.	Rizolex	*Bradyrhizobium* sp.	Moawad et al. (2014)
30.	Strobilurin	*Stenotrophomonas maltophilia*, *Bacillus amyloliquefaciens*, *Bacillus flexus*, *Arthrobacter oxydans*	Clinton et al. (2011)

(continued)

Table 8.1 (continued)

Sl. No.	Pesticides	Bacteria	References
31.	Tetrachlorvinphos	*Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschnikovii*, *Serratia ficaria*, *Serratia* sp., *Yersinia enterocolitica*	Ortiz-Hernández and Sánchez-Salinas (2010)
32.	Thiamethoxam, clothianidin, dinotefuran	*Leifsonia* sp.	Sabourmoghaddam et al. (2015)
33.	Tetramethylthiuram disulfide	*Pseudomonas aeruginosa*	Ray and Mondal (2017)
34.	Triclosan	*Aspergillus versicolor*	Taştan and Dönmez (2015)
35.	Triazine (s) methylthio-s-triazines	*Rhodococcus* sp. strain FJ1117YT	Fujii et al. (2007)
36.	Tributyltin chloride (TBTCl)	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*	Ebah et al. (2016)
37.	Vitavax (37.5% thiram)	*Rhizobium leguminosarum*	Moawad et al. (2014)

purpose is *Pseudomonas* sp. and they are found universally. *Pseudomonas putida* is able to degenerate organophosphates (fenamiphos) and carbamate compounds (carbofuran) (Chanika et al. 2011). *Bacillus* sp. and *Pseudomonas* sp. have the capacity to degrade highly persistent substituents such as pyridine and their metabolites, triclopyridine, picloram, nitrapyrin, and fluridone aerobically (Sims and O'Loughlin 1989). Atrazine, a herbicide, breaks down by the excreted hydrolytic enzyme of *Pseudomonas* sp. and *Klebsiella pneumoniae* (Baishya and Sarma 2015).

8.4.1.1 Mechanism and Pathways of Remediation Process

The biodegradation or detoxification of PAs is a very complex process, involving numerous enzyme-controlled biochemical pathways. The thorough understanding of PA biodegradation pathway within bacteria enhances the capacity to modify microbes for bioremediation. PA biodegradation is based on various classes of enzymes, such as transferase, hydrolase, isomerase involved in redox reactions, conversion of amino to nitro group by oxidation, nitro group reduction, dehalogenation hydrolysis, insertion of O_2 to a double bond and a ^{-}OH group in benzene ring, and sulfur replacement (Megharaj et al. 2011). In aerobic conditions *Pseudomonas* species is able to degrade organochlorides as it has initially dechlorinated them and then converted to other forms by various reactions. For example, DDT is initially converted to less toxic dichlorodiphenyldichloroethane (DDD) which is then transformed to dihydroxy metabolites by dioxygenase enzyme (Nadeau et al. 1994).

The degradation process for 2,4-dichlorophenoxyacetic acid is carried out aerobically by an ortho-cleavage pathway by *Flavobacterium* sp., *Alcaligenes* sp., and *Pseudomonas* sp. finally yielding chloromaleylacetic acid along with its derivatives 2,4-dichlorophenol and 3,5-dichlorocatechol (Gibson and Sulflita 1990). Similarly, *Flavobacterium* sp. (ATCC 27551) is able to satisfy the need of carbon by breaking down the organophosphate compounds through phosphotriesterase enzyme. Atrazine (S-triazine group member) is degraded by dechlorination and hydrolysis. The bacteria (specially found in soil) have the ability to degrade atrazine moderately or completely with carbon dioxide and ammonia as the final yields (Singh et al. 2004).

Sims and O'Loughlin (1989) suggested that *Bacillus* sp. and *Pseudomonas* sp. carry out the metabolism of pyridine to produce hydropyridine and successive split into saturated aliphatic compounds. Association of *Burkholderia* sp. and *Ralstonia* sp. in the remediation of aromatic (unsaturated) hydrocarbons and degradation of n-hexadecanoic acid through intracellular β-oxidation (Yuan et al. 2013) was suggested. Glyphosate metabolism by the bacterium *Streptomyces lusitanus* was done by Lipok et al. (2009).

8.4.1.2 Bacterial Enzymes/Genes Involved in PA Degradation

Bacteria possess remediating genes in both chromosome and plasmid. Suenaga et al. (2001) reported that enzymes degrade PAs by considering them as their substrates. Evolution of microbial biodegrading gene gives a huge opportunity to use them as a bioremediating tool and also raise a ray of hope to deal with the challenge of agrochemical pollution. *Pseudomonas* sp., *Actinobacteria* sp., and *Klebsiella* sp. (Sayler et al. 1990) possess genes encoded for pesticide degradation and pollutant degradation within anticatabolic plasmids and transposons (Laemmli et al. 2000), respectively. The genes, i.e., *atzA*, *atzC* (*trzC*), and *atzB* (*trzB*), produce carbamate-degrading enzymes such as atrazine chlorohydrolase, N-isopropyl-ammelide isopropyl-amino-hydrolase, and hydroxy-atrazine ethylamino-hydrolase, respectively (Sadowski et al. 1998). These clusters of genes manage the successive conversion of atrazine to cyanuric acid after which it completely mineralizes into carbon dioxide and ammonia (Sene et al. 2010).

Degradation of organophosphate compounds by *Plesiomonas* sp. strain M6 is carried out through the enzyme methyl parathion hydrolase (MPH), encoded by mpd gene. Likewise, some cluster of genes have been identified from diverse bacterial species such as *Rhodococcus* sp. strain NI86/21 (Nagy et al. 1995) and *Achromobacter* sp. WMll (Tomasek and Karns 1989), which are able to degrade EPTC by the enzymes of aldehyde dehydrogenase, and P450 was responsible for the degradation of thiocarbamate. 2,4-D (2,4-dichlorophenoxyacetic acid) biodegradation is carried out via the plasmid pJP4 (entitled as *tfd* gene) of *Alcaligenes eutrophus* JMP134, 2,4-dichlorophenoxyacetate monooxygenase encoded by tfdA (Streber et al. 1987), 2,4-dichlorophenol hydroxylase encoded by *tfdB* (Kaphammer and Olsen 1990), and chlorocatechol-1,2-dioxygenase, chloromuconate cycloisomerase, chlorodienelactone isomerase, and chlorodienelactone hydrolase encoded by *tfdCDEF* (Kaphammer and Olsen 1990). Some transposons of *Ralstonia eutropha* (Tn4371), *Burkholderia cepacia* (Tn5530), *Alcaligenes* sp. (Tn5271), and

Pseudomonas putida (Tn4654) enable the degradation of biphenyl 4-chlorobi-phenyl molecules, 2,4-D, toluene, carbofuran, and 3-chlorobenzoate, respectively (Verma et al. 2014). Nagata et al. (1999) reported that *Sphingobium japonicum* UT26 has dechlorinase enzyme, LinA (γ-hexachlorocyclohexane dehydrochlorinase, EC 4.5.1), encoded by *linA* gene, which catalyzes a dehydrochlorination of two steps: γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-pentachlorocyclohexene (γ-PCCH).

8.4.2 Cyanobacteria as PA-Degrading Agents

Cyanobacteria are the largest group of gram-negative, oxygen-evolving photoautotrophic prokaryotes which belongs to the kingdom Eubacteria. The other well-known name of cyanobacteria is blue-green algae (BGA), named because of its diverse morphology (unicellular, filamentous, and colonial) and pigmentation (pigments like chlorophyll a, phycocyanin, allophycocyanin, phycoerythrin, carotenoids, and xanthophylls). They can easily accommodate in diverse ecosystems. Nowadays, cyanoremediation is a new term evolved to define the use of cyanobacteria to fulfil the purpose of degradation or detoxification of contaminants like PAs, heavy metal, and dye. There are frequent instances of successful bioremediation of PAs by cyanobacteria (Table 8.2).

8.4.2.1 Mechanism and Pathways of Remediation Process

Nostoc ellipsosporum and *Anabaena* sp. PCC7120 are able to degrade hexachlorocyclohexane to a combination of 1,2,3- and 1,2,4-trichlorobenzenes. According to some reports, cyanobacteria, namely, *Nostoc*, *Phormidium*, and *Oscillatoria*, can utilize methyl parathion by considering it as the solitary source of nitrate and organic phosphorus (Megharaj et al. 1994) for their growth and metabolism. In aerobic conditions, *Anabaena* sp. strain PCC 7120 is able to reduce the nitro group of methyl parathion to an amino group (Barton et al. 2004). One of the intermediate products of OP decomposition is para-nitrophenol which is more lethal than OP. Report suggests that cyanobacteria oxidize nitro group of para-nitrophenol and release nitrite. However, the biological mechanism of this process is still unknown. Nevertheless, further metabolism of released nitrite is carried out by "nir" operon which encodes nitrite reductase enzyme (Megharaj et al. 1994). *Phormidium valderianum* BDU 20041 is able to tolerate the exposure of chlorpyrifos by showing the enhancement activity of oxidoreductase enzymes for chlorpyrifos degradation (Palanisami et al. 2009). Thengodkar and Sivakami (2010) reported that *Spirulina platensis* is tolerant up to high concentration (80 ppm) of chlorpyrifos treatment by converting it to 3,5,6-trichloro-2-pyridinol by utilizing its alkaline phosphatase enzyme. Report suggests that *Nostoc muscorum*, *Spirulina platensis*, and *Anabaena oryzae* facilitate the degradation of malathion and high-concentration pesticides enhance the protein, carbohydrate, and biomass content in these cyanobacterial cells (Ibrahim et al. 2014). Forlani et al. (2008) reported that *Nostoc punctiforme*, *Anabaena* sp., and *Microcystis aeruginosa* have the potential of

Table 8.2 Biodegradation of persistent agrochemicals by cyanobacteria

Sl. No.	Pesticides	Cyanobacteria	References
1.	2,4-d (Dichlorophenoxyacetic acid)	*Anabaena fertilissima*, *Aulosira fertilissima*, *Westiellopsis prolifica*	Kumar et al. (2013)
2.	2,4-DNP (dinitrophenol)	*Anabaena variabilis*, *Anabaena cylindrica*	Hirooka et al. (2006)
3.	Anilofos	*Synechocystis* sp. PUPCCC 64	Singh et al. (2013)
4.	Acetochlor	Cyanobacterial mat consisting of *Phormidium* and *Oscillatoria*	El-Nahhal et al. (2013)
5.	Carbaryl	*Calothrix brevissima*	Habib et al. (2011)
6.	Carbendazim	*Oscillatoria* sp.	Ravindran et al. (2000)
7.	Carbofuran	*Anabaena sphaerica*, *Nostoc hatei*, *Westiellopsis prolifica*	Jha and Mishra (2005)
8.	Chlorpyrifos	*Phormidium valderianum*, *Spirulina platensis*, *Synechocystis* sp. PUPCCC64	Palanisami et al. (2009)
9.	Cypermethrin	*Oscillatoria* sp.	Thengodkar and Sivakami (2010)
10.	Endosulfan (α- and β-endosulfan)	*Anabaena* sp. PCC 7120, *Anabaena flosaquae*, *Aulosira fertilissima*	Singh et al. (2011a, b, c); Ravindran et al. (2000); Lee et al. (2003)
11.	Fenamiphos	*Nostoc muscorum*, *Anabaena* sp.	Cáceres et al. (2008)
12.	Glyphosate	*Spirulina platensis*, *Nostoc punctiforme*, *Microcystis aeruginosa*, *Leptolyngbya boryana*	Kumar et al. (2012); Cáceres et al. (2008); Forlani et al. (2008); Lipok et al. (2009)
13.	Isoproturon	*Anabaena inaequalis*	Arunakumara et al. (2013)
14.	Lindane	*Anabaena* sp. PCC7120, *Nostoc ellipsosporum*	González et al. (2012)
15.	Malathion	*Anabaena oryzae*, *Nostoc muscorum*, *Spirulina platensis*	Ibrahim et al. (2014)
		Anabaena sp. PCC7120	El-Bestawy et al. (2007)
16.	Methyl parathion	*Anabaena fertilissima*, *Aulosira fertilissima*, *Westiellopsis prolifica*, *Fischerella* sp., *Scytonema* sp. BHUS-5	Ibrahim et al. (2014); Tiwari et al. (2017)

glyphosate degradation and consume it as a prime source of phosphorus. *Trichodesmium erythraeum* has been reported to carry out the glyphosate transformation process for the utilization of phosphorus (Dyhrman et al. 2006).

8.4.2.2 Cyanobacterial Genes Involved in PA Degradation

The genetically manipulated cyanobacterial strains such as *Anabaena*, *Nostoc* sp. PCC7120 (Masukawa et al. 2007), *Anabaena variabilis* ATCC 29413 (Roessler et al. 2009), *Synechococcus elongatus* PCC 7942 (Kaczmarzyk and Fulda 2010), and *Synechococcus* sp. PCC 6301 (McNeely et al. 2010) have been tested for their bioremediating capacity. *Anabaena* sp. PCC 7120 and *Nostoc muscorum* FACHB244 were genetically modified by introducing a plasmid which contains opd (organophosphorus degradation) gene through conjugation gene transfer system. By the process of genetic engineering, fcABC was introduced into *Anabaena* sp. and *Nostoc ellipsosporum* for dechlorination of 4-chlorobenzoate. For the degradation of lindane, *linA* gene was introduced into *Anabaena* sp. and *Nostoc ellipsosporum* (Kuritz and Wolk 1995).

8.4.3 Fungus as PA-Degrading Agents

In the biogeochemical cycle, fungi play a significant role as they are responsible for degrading different kinds of xenobiotics including agrochemicals (Diez et al. 2012) (Table 8.3). Various fungal species are able to mineralize different groups of substances (Esterhuizen-Londt et al. 2016). Gianfreda and Rao (2004) reported that fungi are able to alter the structures of agrochemicals and other fractious compounds releasing biotransformed products. These biotransformed products are further broken down by other potential microbial strains.

Another strain, *Penicillium oxalicum*, showed 99.9% biodegradation of methamidophos within the incubation period of 12th day (Zhao et al. 2010). The phenylurea agrochemicals such as linuron, chlortoluron, isoproturon, and diuron were found to be degraded by *Mortierella* sp., *Bjerkandera adusta*, and *Rhizoctonia solani* (Khadrani et al. 1999). Several reports suggested that soil fungi such as *Penicillium* sp., *Eurotium* sp., and *Aspergillus* sp. have the capacity to degrade chlorpyrifos and its by-product TCP after 7 days of incubation (Maya et al. 2012). *Mucor racemosus* can degrade dieldrin (93%), DDE (79%), endosulfan sulfate (95%), heptachlor (94%), endosulfan (80%), heptachlor epoxide (67.5%), and DDT (49.3%) (Kataoka et al. 2010). Several white-rot fungal isolates including *Phanerochaete sordida*, *Trametes hirsutus*, and *Pleurotus ostreatus* have also revealed their potential to degrade diuron, lindane, and other fractious agrochemicals (Sagar and Singh 2011). Purnomo et al. (2014) suggested that *P. ostreatus*, a white-rot fungus, had the ability to eliminate around 89% of heptachlor and 32% of heptachlor epoxide after the incubation period of 28 days.

Current scenario of pesticide biodegradation is the utilization of fungal-bacterial co-culture because they frequently share the same niche (Warmink et al. 2009). Reports (Ellegaard-Jensen et al. 2014) suggested that the consortium of fungi (*Mortierella* sp. LEJ703 and LEJ702) and bacteria (*Arthrobacter globiformis*, *Sphingomonas* sp., and *Variovorax* sp.) has the ability of fast mineralization of the agrochemical diuron. Barathidasan et al. (2014) recorded a consortium of *Cellulomonas fimi* (bacteria) and *Phanerochaete chrysosporium* (fungi) able to

Table 8.3 Biodegradation of persistent agrochemicals by fungi

Sl. No.	Pesticides	Fungi	References
1.	Aldrin	*Phlebia acanthocystis*, *Phlebia brevispora*, *Phlebia aurea*, *Mucor racemosus*	Bhosle and Nasreen (2013); León-Santiesteban and Rodríguez-Vázquez (2017)
2.	Atrazine	*P. ostreatus* INCQS 40310, *Rhizopus stolonifer*, *Penicillium purpurogenum*	Pereira et al. (2013); Gonçalves et al. (2012)
3.	Bensulfuron-methyl	*Penicillium pinophilum*	Peng et al. (2012)
4.	Chlordane	*Boletus edulis*	Bhandari (2017)
5.	Chlorothalonil	*Pleurotus* ECS-0190	Camacho-Morales and Sanchez (2016)
6.	Chlorfenvinphos	*Trichoderma harzianum*	Oliveira et al. (2015)
7.	Chlorpyrifos	*Verticillium* sp.	Yu et al. (2006)
		Aspergillus sp., *Penicillium* sp., *Eurotium* sp., *Emericella* sp.	Maya et al. (2012)
		Cladosporium cladosporioides	Chen et al. (2012)
		Ganoderma sp.	Silambarasan and Abraham (2014)
		Acremonium sp. strain GFRC-1	Kulshrestha and Kumari (2011)
		Streptomyces sp. M7	Fuentes et al. (2013)
		Verticillium sp.	Fang et al. (2008)
8.	Cypermethrin	*Pseudomonas aeruginosa*	Bhosle and Nasreen (2013)
9.	β-Cypermethrin	*Aspergillus niger* YAT	Deng et al. (2015)
10.	DDT (dichlorodiphenyltrichloroethane)	*Laccaria bicolor*, *Boletus edulis*, *L. scabrum*, *Gymnopilus viscidus*, *P. ostreatus*, *G. trabeum*, *Daedalea dickinsii*, *Fomitopsis pinicola*, *Gomphidius viscidus*	Purnomo et al. (2010); Purnomo et al. (2011); Bhandari (2017)
11.	Dimethoate	*Phlebia acanthocystis*, *P. brevispora*, *Phlebia aurea*	Xiao et al. (2011)
12.	Diuron	*Phanerochaete chrysosporium*, *Cunninghamella elegans*, *Mortirella*	Fratila-Apachitei et al. (1999); Tixier et al. (2000, 2001); Badawi

(continued)

Table 8.3 (continued)

Sl. No.	Pesticides	Fungi	References
		isabellina, *Beauveria bassiana*, *Aspergillus niger*, *Mortierella isabellina*, *Mortierella* sp., *Aspergillus fumigatus*	et al. (2009); Oliveira et al. (2015)
13.	Endosulfan (α- and β-endosulfan)	*Aspergillus terricola*, *Aspergillus terreus*, *Trametes hirsute*, *Aspergillus niger*, *Aspergillus niger* ARIFCC 1053, *Mortierella* sp. Cm1–45, *Mortierella* sp. W8, *Aspergillus sydoni*, *Gloeophyllum trabeum*	Hussain et al. (2007); Kamei et al. (2011); Bhalerao and Puranik (2007); Bhalerao (2012); Kataoka et al. (2010); Goswami et al. (2009); Spina et al. (2018)
14.	Endrin	*Leccinum scabrum*	Bhandari (2017)
15.	Heptachlor	*Pleurotus ostreatus*	Purnomo et al. (2014)
		Phlebia acanthocystis, *Phlebia tremellosa*, *Phlebia brevispora*	Xiao et al. (2011)
16.	Isoproturon	*Mortierella* sp., *Mucor* sp., *Alternaria* sp., *Phoma eupyrena*, *Basidiomycete* Gr177, *Cunninghamella elegans*, *Penicillium melanoconidium*	Rønhede et al. (2005); Oliveira et al. (2015)
17.	Lindane	*Fusarium solani*, *Fusarium poae*, *Fusarium verticillioides*, *Irpex lacteus*, *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Phlebia radiata*, *Stereum hirsutum*, *Gloeophyllum trabeum*	Dritsa et al. (2009); Sagar and Singh (2011); Guillen-Jimenez et al. (2012); Quintero et al. (2008); Spina et al. (2018)
18.	Malathion	*Fusarium oxysporum* JASA1	Peter et al. (2015)
19.	Methamidophos	*Penicillium oxalicum*	Zhao et al. (2010)
20.	Monocrotophos	*Aspergillus flavus*, *Fusarium pallidoroseum*, *Macrophomina* sp.	Jain et al. (2014)
21.	Parathion	*Bjerkandera adusta* 8258, *P. ostreatus* 7989, *Phanerochaete chrysosporium* 3641	Jauregui et al. (2003)

(continued)

Table 8.3 (continued)

Sl. No.	Pesticides	Fungi	References
22.	Pendimethalin	*Aspergillus terreus*, *A. versicolor*	Caihong et al. (2011)
		Lentinula edodes	Pinto et al. (2016)
23.	Pentachlorophenol (PCP)	*T. harzianum* CBMAI 1677	Vacondio et al. (2015)
		Anthracophyllum discolor	Rubilar et al. (2007)
24.	Pyrene	*Pseudotrametes gibbosa*	Wen et al. (2011)
25.	Vydate	*Trichoderma viride*	Helal and Abo-El-Seoud (2015)

mineralize chlorpyrifos completely within 16 h. Abraham and Silambarasan (2014) reported a co-culture of bacterial strains such as *Enterobacter cloacae* JAS7 and *Klebsiella pneumoniae* JAS8 and fungal strains such as *Lasiodiplodia* sp. JAS12, *Aspergillus tamarii* JAS9, and *Botryosphaeria laricina* JAS6 which had the ability to degrade endosulfan completely in both aqueous and solid media.

8.4.3.1 Mechanism and Pathways of Remediation Process

The fungal strains follow various pathways during the degradation process. For example, during the biodegradation process of isoproturon (IPU) by *Mortierella* sp. Gr4, it was found that IPU undergoes two successive demethylation activities on urea chain and results in generating monodemethyl isoproturon and didemethyl isoproturon and then hydroxylation of isopropyl ring takes place which leads to the formation of 1-OH-IPU, 1-OH-monodemethyl isoproturon, and 1-OH-didemethyl isoproturon (Hussain et al. 2007). Another agrochemical β-cypermethrin, a pyrethroid insecticide, was esterified into two intermediates, i.e., permethric acid and α-cyano-3-phenoxy benzyl alcohol by *Aspergillus niger* (Deng et al. 2015). Kadimaliev et al. (2011) observed phenol degradation by *Lentinus tigrinus* in liquid medium via peroxidase and laccase enzymes.

León-Santiesteban and Rodríguez-Vázquez (2017) found that *Rhizopus oryzae* CDBB-H-1877 has the efficiency of pentachlorophenol biosorption. However, it has been notified that this agrochemical can be degraded through the process of methylation along with dechlorination. Two dark septate endophytes (DSEs) as *Alternaria alternata* and *Cochliobolus* sp. are able to degrade glyphosate, cypermethrin, and carbendazim by their intracellular enzymes (Spagnoletti and Chiocchio 2020).

8.4.3.2 Fungal Genes/Enzymes Involved in PA Degradation

Recently, few studies have revealed different fungal enzymes involved in the biodegradation of different types of agrochemicals (Jain et al. 2014). *Conidiobolus*

sp. (a fungal strain) was found to be capable of removing lindane from liquid medium by using its extracellular oxidative enzymes (lignin peroxidase and lignin-modifying enzymes). Similar enzymatic activity was observed during the degradation process of dieldrin, trifluralin, and simazine by *Trametes versicolor* and *Phanerochaete chrysosporium*, and the production of extracellular enzymes such as dehydrogenase/cellulase was enhanced in inoculated soil (Fragoeiro and Magan 2008). Likewise, Jain et al. (2014) documented the degradation of monocrotophos by three fungal isolates *Macrophomina* sp., *Fusarium pallidoroseum*, and *Aspergillus flavus* that were coupled with the release of extracellular enzymes like alkaline phosphatase, ammonia, and inorganic phosphates.

Nguyen et al. (2014) tested the efficiency of crude form of laccase extracted from *Trametes versicolor* to degrade the various agrochemicals such as fenoprop, ametryn, and atrazine. The genotype and growth conditions permit certain fungi to release specific enzymes such as manganese-dependent peroxidase (MnP) and lignin peroxidase (LiP) that play a significant role in pesticide degradation (Purnomo et al. 2010). Nowadays, the genetically transformed fungal strains are playing a vital role as they enhance the efficiency of pesticide degradation (Zhou et al. 2007; He et al. 2014).

8.5 Factors Affecting Biodegradation of PA

Bioremediation of PAs is affected by numerous chemical, physical, and environmental factors like chemical structure and concentration of PAs, soil moisture, soil pH, temperature, salinity, sustainable microbial population, aeration, and medium composition.

8.5.1 Chemical Structure and Concentration of PA

Chemical structure of PAs is a crucial factor in the biodegradation of PAs. The physiochemical properties of agrochemicals are varying from compound to compound. It was revealed that the polar group such as NH_2 and OH, of an agrochemical, is an easier site of attack by the microbial system (Cork and Krueger 1991). However, the presence of any substituent of alkyl or halogen in a pesticide makes it resistant to degradation. It was stated that minor differences in the structure and nature of substituent groups of same class can affect the rate of degradation. The amount of agrochemical significantly affects the biodegradation of agrochemicals. Reports suggested that some microbes can be able to degrade PA rapidly at high concentrations, whereas some can carry out degradation at low concentrations.

8.5.2 pH

pH is always an important factor in the environment and affects degradation of PAs by fungi and other microbes (Fang et al. 2008). It also affects the bioavailability, chemical speciation, and mobility of the chemical compounds. Racke et al. (1997) stated that biodegradation of an agrochemical depends on the soil pH. Any variation in pH from the optimum value adversely affects the biodegradation capacity of specific fungi. pH range of 4.0–8.0 showed good degradation rate for dieldrin by *Mucor racemosus*, a potential fungal strain (Kataoka et al. 2010). Yang et al. (2011) found that pH 7.5 was the optimum pH for the highest degradation rate of carbofuran by *Pichia anomala*. An optimal pH for the highest degradation rate of chlorpyrifos by a consortium of *Serratia* sp. and *Trichosporon* sp. was found to be 8 (Xu et al. 2007). However, several investigations also suggest that somewhat acidic pH is comparatively more desirable to carry out optimal fungal degradation of agrochemicals (Hussain et al. 2007). Caihong et al. (2011) observed that the maximum biodegradation of an agrochemical pendimethalin (belongs to dinitroaniline class) by *Aspergillus versicolor* was achieved at pH 6.5.

8.5.3 Temperature

Besides pH, temperature also has significant effects on the pesticide degradation. The optimum temperature is not fixed; it can be variable in certain conditions. For example, reports found that the optimal chlorpyrifos degradation by a bacterial strain, *Verticillium* sp., was attained at 35 °C (Fang et al. 2008). Derbalah and Belal (2008) reported the optimal degradation of cymoxanil by microbes to be 30 °C. A reliable temperature of 30 °C was reported in the degradation process of various pesticides—endosulfan, carbofuran, and pendimethalin—by the isolates of *Aspergillus terreus*, *Pichia anomala*, and *Aspergillus versicolor*, respectively (Hussain et al. 2007; Yang et al. 2011). Reports of Dritsa et al. (2009) suggested the optimal temperature to degrade lindane by *Ganoderma australe* to be 18 °C.

8.5.4 Moisture and Water Availability

Moisture is a considerable factor that affects the biodegradation rate by facilitating water as the medium for mobility and diffusion of agrochemicals as well as essential for making agrochemicals available for microbes. For a blooming degradation process, the moisture content of soil should be in a range of 25–85% of the water-holding capacity. However, the optimum range varies between 50 and 80%. Water availability has an impact on oxygen supply that later impacts the growth of fungus and production of enzymes (Philippoussis et al. 2001). It also impacts the agrochemical binding patterns and its dispersion in soil by affecting the accessibility of compounds to the soil microbiota. Bastos and Magan (2009) investigated the biodegradation of atrazine in a period of 24 weeks by *Trametes versicolor* and

indicated that 98% and 85% degradation took place at water potential of −0.7 MPa and −2.8 MPa, respectively.

8.5.5 Salinity

There is less information on the effect of salinity on the rate of biodegradation process. But salinity is a hurdle in varied regions like coastal, arid, and semiarid. Thus, it may influence the degradation rate of PAs. In nonsaline soil condition, the rate of parathion degradation is much faster than saline condition. The stability of agrochemicals is affected by the degree of salinity. For example, high salinity may cause an obstacle for biodegradation of agrochemicals as it inhibits degradation process.

8.5.6 Nutrients

The optimal biodegradation takes place by the occurrence of high nitrogen content along with 1% of glucose. Zhao et al. (2010) reported that only 1% of glucose is needed for the biodegradation of methamidophos by *Penicillium oxalicum* ZHJ6. Likewise, Kataoka et al. (2010) found *Mucor racemosus* to have better efficiency in degrading dieldrin in the presence of nitrogen and glucose. Dritsa et al. (2009) reported the media composition of 1.28 g/L of nitrogen to be the optimal condition for lindane biodegradation by the fungus *Ganoderma australe*. Hussain et al. (2007) reported that the rate of endosulfan degradation by fungi *Aspergillus terreus*, *Aspergillus terricola*, and *Chaetosartorya stromatoides* is considerably higher in agitation incubation than in static incubation. Similarly, Xu et al. (2007) suggested that the addition of sucrose with a little higher concentration was able to enhance chlorpyrifos mineralization by a consortium of *Trichosporon* sp. and *Serratia* sp. Likewise, Caihong et al. (2011) reported that *Aspergillus versicolor* showed enhancement in the rate of pendimethalin degradation by adding 1–2% of sucrose. In case of soil, oxyfluorfen degradation by fungus is affected by both temperature and mineral fertilizers.

8.6 Advantages and Limitations of Bioremediation

For a successful bioremediation process, microbes with specific quantity and correct timing under correct place and environment are required. The definition of a perfect microbe regarding this context is having the potential to degrade, detoxify, or remove contaminants including agrochemicals and other persistent pollutants. Thus, bioremediation is such a process that helps in keeping the environment clean by removing contaminants through biological aspects such as microorganisms and plants. In nature, every aspect has its own pros and cons; however, bioremediation technique offers numerous benefits with little limitations. Bioremediation can be

carried out in contamination sites, termed as in situ bioremediation. In this context, the substrates or nutrients are added to that particular contaminated site which stimulates the growth of indigenous microbes to enhance the degradation rate. This process is often less expensive as it minimizes the site disruption which leads to non-disturbance of soil and ensures soil fertility and integrity. Additionally, it helps to get rid of waste permanently and eradicates long-term liability. After the destruction of pollutants, the land is allowed to use. In some cases, the contaminated or polluted material is collected from the site and supplied with essential microbes or microbial consortium at an organized site, called as ex situ bioremediation. This process is found successful in wastewater management. The latter technique is more controlled than the former one. The bioremediation method can easily be coupled with other treatment (chemical and physical) methods. It has more public acceptance with proper regulatory encouragement. In the context of performance efficiency, there is no such kind of universal guidelines to define degradation efficiency as standard. Thus, there is always a variation in performance. The bioremediation process needs microorganisms along with a suitable environmental condition to keep them growing, which might not be always possible in in situ bioremediation. In case of certain uncontrolled remediation processes, the partial destruction might produce more poisonous and ambulant products than its native form. Uninterrupted observations are essential to check the status and know the speed of degradation of the persistent agrochemicals. In ex situ process, the organic compounds which have volatile property are challengeable to control. As the process is dependent on biological and physiological activity of microbes, its duration is slightly longer than that of chemical and physical processes. The genetically modified microbes are hard to take away from application sites and there is always a frightened possibility of causing more potential damage by these microbes than the original pollutants. Apart from all these limitations, bioremediation is considered as a significant tool in mitigating today's environmental issues.

8.7 Strategies to Enhance the Efficacy of PA Degradation

As contamination of environment is rising rapidly, to cope up this situation certain strategies are needed which enhance the efficacy of biodegradation. These processes are described below:

8.7.1 Immobilization

The immobilization concept may be defined as the act of restricting the movement of molecules/cellular organelles/enzymes/cells/microbes in a matrix. This concept is developed from the attachment nature of microbes onto a surface, thereafter growing on them (Robinson et al. 1986). In this method the accurate positioning of microbes takes place in such a manner that they are active in biodegradation (Mohamad et al. 2015). This process needs high biomass (mass culture) of microbes with proper

catalytic activity, simple separation, and reusability. This technique can be divided into four types, i.e., surface adsorption, embedding, covalent bonding, and cross-linking (Vasilieva et al. 2018). The surface adsorbent is the most affordable and simple process. The adsorbent materials/carriers play vital roles with reversible route as it convenes the prospect of sustaining the catalytic activity for a longer duration (Chen and Georgiou 2002). A suitable carrier should have the following criteria: (1) affordable price, (2) nonhazardous, (3) non-pollutant, (4) easy physical structure, and (5) lightweight. For passive immobilization, natural carrier and polyvinyl and polyurethane (synthetic carrier) are mostly used. In active immobilization the carriers are flocculent agents (chitosan), chemical attachment (glutaraldehyde), and gel entrapment (natural polysaccharides and synthetic polymers, i.e., acrylamide; proteins, i.e., collagen) (Taha and Khateb 2013). Reports suggest that the immobilization technique has been widely employed in the bioremediation of pesticides and wastewater treatment (Cassidy et al. 1996). Immobilized microalgae like *Chlorella* were used for the removal of lindane (Kuritz and Wolk 1995). The combination of algae and bacteria can be used for the enhancement of pollutant removal. Thus, immobilization techniques are considered as an admirable way for removal of pesticides.

8.7.2 Acclimation

This process is defined as the continuous association of a population of microbes to a particular chemical, leading to quick degradation of that chemical. In this association, microbes produce enzymes that can provide them tolerance or degrading capacity against that particular chemical (Guo et al. 2017). In stress conditions, organisms always tend to retain their internal mechanism by altering gene expression (Borowitzka 2018). Reports suggest that lindane concentration between 5 and 120 mg/L could be tolerated by *Staphylococcus intermedius* under this acclimation process with 99% lindane-degrading efficiency (González et al. 2012). The extended acclimation period is the major obstacle on the way of achieving a potential microbial strain.

8.7.3 Co-cultivation

Co-cultivation is a process where the existence of more than one microbial group is found. Cyanobacteria and bacteria (Patel et al. 2017), bacteria and microalgae co-cultures remediate organic pollutants more efficiently. Cyanobacterium offers growth substrates and oxygen along with suitable environmental condition to bacteria for promoting their growth (Subashchandrabose et al. 2013). The bacteria produce carbon dioxide which is used as a carbon source by cyanobacteria and microalgae (Kumar and Singh 2017; Kumari et al. 2016). In certain cases, *Bacillus pumilus*, for example, promotes the growth of *Chlorella vulgaris* in a medium without nitrogen and inhibits the growth of nannochloropsis species (Fulbright

et al. 2016). The research on co-cultivation concept should be more focused to be a promising bioremediation tool.

8.7.4 Genetic Modification and Enzyme Application

The genetic alteration of microbes is an innovative strategy that inserts certain target genes into the chromosome of host cell or erases a particular chromosomal fragment, which can undergo successive screening and acclimation activity to instantly express the preferred form and intensify the metabolism of the cell (Poliner et al. 2018). A wild fungal strain with hygromycin B phosphotransferase (hph) gene insertion showed improved quality to decadent pesticide. In fungal species, cytochrome P450 monooxygenases induce the gene clusters to express deferentially, based on the availability of nutrients and xenobiotic compounds (Yadav et al. 2015). The CCA gene cluster consisting of copied carbonic anhydrase and cyanase in *Fusarium oxysporum* has the efficiency to detoxify an agrochemical, cyanate (Elmore et al. 2015).

8.8 Conclusion

The hazardous chemicals need a promising tool for detoxification and remediation of its toxicity from our environment. The environmental consciousness resulted in improved regulatory measures to remediate environmental pollution and defend our ecosystem from upcoming pollution and exploitation. Because of this rational motive, it is essential to make strategies for the bioremediation of contaminated environment. Nowadays bioremediation is a most active, innovative, fascinating, and multidisciplinary area of research. In developing countries, the microbial remediation can enhance soil quality by detoxifying the hazardous chemicals from soil. However, more research programs are required to improve the potential of bioremediation and restore the soil quality by applying microbes. Economically, the use of PAs is beneficial as it improves the crop production and controls the diseases and pests, while in the environmental context PAs are considered as the most harmful factor for the environment. Thus, from both the environmental and economic standpoints, biodegradation is a fruitful technology upon PA application. Currently, the usefulness of indigenous as well as genetically modified organisms in removing or detoxifying persistent agrochemicals has emerged as a potential in situ remediation method. Numerous research reports have been collected and presented here on various organisms like bacteria, blue-green algae, and fungus, used for the bioremediation of environmental pollutants. However, the large-scale utilization of microbes for the degradation of PA pollutants is still to be explored.

Acknowledgement Authors sincerely thank the Director, ICAR-National Rice Research Institute, Cuttack, for providing technical and financial support. The authors declare that they have no competing interests.

References

Abo-Amer AE (2007) Involvement of chromosomally-encoded genes in malathion utilization by *Pseudomonas aeruginosa* AA112. Acta Microbiol Immunol Hung 54(3):261–277. https://doi.org/10.1556/AMicr.54.2007.3.3

Abraham J, Silambarasan S (2014) Biomineralization and formulation of endosulfan degrading bacterial and fungal consortiums. Pestic Biochem Physiol 116:24–31. https://doi.org/10.1016/j.pestbp.2014.09.006

Akbar S, Sultan S (2016) Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. Braz J Microbiol 47(3):563–570. https://doi.org/10.1016/j.bjm.2016.04.009

Arunakumara KKIU, Walpola BC, Yoon MH (2013) Metabolism and degradation of glyphosate in aquatic cyanobacteria: a review. Afr J Microbiol Res 7(32):4084–4090

Arya R, Kumar R, Mishra NK et al (2017) Microbial flora and biodegradation of pesticides: trends, scope, and relevance. In: Kumar R, Sharma AK, Ahluwalia SS (eds) Advances in environmental biotechnology. Springer, Singapore, pp 243–263

Ata A, Nalcaci OO, Ovez B (2012) Macro algae *Gracilaria verrucosa* as a biosorbent: a study of sorption mechanisms. Algal Res 1(2):194–204. https://doi.org/10.1016/j.algal.2012.07.001

Badawi N, Rønhede S, Olsson S et al (2009) Metabolites of the phenylurea herbicides chlorotoluron, diuron, isoproturon and linuron produced by the soil fungus *Mortierella* sp. Environ Pollut 157(10):2806–2812. https://doi.org/10.1016/j.envpol.2009.04.019

Baishya B, Sarma HP (2015) Advances in biodegradation of organophosphorus pesticides. Arch Appl Sci Res 7(4):37–43

Barathidasan K, Reetha D, Milton DJ et al (2014) Biodegradation of chlorpyrifos by co-culture of *Cellulomonas fimi* and *Phanerochaete chrysosporium*. Afr J Microbiol Res 8(9):961–966. https://doi.org/10.5897/AJMR2013.6530

Barreiros L, Nogales B, Manaia CM et al (2003) A novel pathway for mineralization of the thiocarbamate herbicide molinate by a defined bacterial mixed culture. Environ Microbiol 5(10):944–953. https://doi.org/10.1046/j.1462-2920.2003.00492.x

Barton JW, Kurtiz T, O'Connor LE et al (2004) Reductive transformation of methyl parathion by cyanobacterium *Anabaena* sp., strain PCC 7120. Appl Microbiol Biotechnol 65:330–335. https://doi.org/10.1007/s00253-004-1557-y

Bastos AC, Magan N (2009) *Trametes versicolor*: potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions. Int Biodeterior Biodegradation 63(4):389–394. https://doi.org/10.1016/j.ibiod.2008.09.010

Bhadouria R, Mishra D, Singh VK et al (2020) Nanocatalyst types and their potential impacts in agroecosystems: an overview. In: Singh P, Borthakur A, Mishra PK, Tiwary D (eds) Nano-Materials as photocatalysts for degradation of environmental pollutants. Elsevier, Netherlands, pp 323–344. https://doi.org/10.1016/B978-0-12-818598-8.00016-X

Bhalerao TS, Puranik PR (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. Int Biodet Biodeg 59(4):315–321

Bhalerao TS (2012) Bioremediation of endosulfan-contaminated soil by using bioaugmentation treatment of fungal inoculant *Aspergillus niger*. Turk J Biol 36:561–567. https://doi.org/10.3906/biy-1112-44

Bhandari G (2017) Mycoremediation: an eco-friendly approach for degradation of pesticides. In: Prasad R (ed) Mycoremediation and environmental sustainability. Fungal Biology. Springer, Cham, pp 119–131. https://doi.org/10.1007/978-3-319-68957-9_7

Bhosle NP, Nasreen S (2013) Remediation of cypermethrin-25 EC by microorganisms. Eur J Exp Biol 3(1):144–152

Bilal M, Rasheed T, Sosahernández JE et al (2018) Biosorption: an interplay between marine algae and potentially toxic elements—a review. Mar Drugs 16(2):65. https://doi.org/10.3390/md16020065

Borowitzka MA (2018) The 'stress' concept in microalgal biology—homeostasis, acclimation and adaptation. J Appl Phycol 30(5):2815–2825. https://doi.org/10.1007/s10811-018-1399-0
Bustos N, Cruz-Alcalde A, Iriel A et al (2019) Sunlight and UVC-254 irradiation induced photodegradation of organophosphorus pesticide dichlorvos in aqueous matrices. Sci Total Environ 649:592–600. https://doi.org/10.1016/j.scitotenv.2018.08.254
Cáceres TP, Megharaj M, Naidu R (2008) Biodegradation of the pesticide fenamiphos by ten different species of green algae and cyanobacteria. Curr Microbiol 57(6):643–646
Caihong Y, Yingnan S, Ronghua L et al (2011) Biodegradation of Pendimethalin by two fungus strains isolated from soil in China. In: Bioinform and Biomed Eng, (iCBBE) 2011 5th International Conference, p 1–5. https://doi.org/10.1109/icbbe.2011.5781540
Camacho-Morales RL, Sanchez JE (2016) Biotechnological use of fungi for the degradation of recalcitrant agro-pesticides. In Mushroom. Biotechnology:203–214
Campos M, Perruchon C, Vasilieiadis S et al (2015) Isolation and characterization of bacteria from acidic pristine soil environment able to transform iprodione and 3,5-dichloraniline. Int Biodeterior Biodegrad 104:201–211. https://doi.org/10.1016/j.ibiod.2015.06.009
Carvalho FP (2006) Agriculture, pesticides, food security and food safety. Environ Sci Pol 9(7–8): 685–692. https://doi.org/10.1016/j.envsci.2006.08.002
Cassidy MB, Lee H, Trevors JT (1996) Environmental applications of immobilized microbial cells: a review. J Ind Microbiol Biotechnol 16(2):79–101. https://doi.org/10.1007/BF01570068
Chanika E, Georgiadou D, Soueref E et al (2011) Isolation of soil bacteria able to hydrolyze both organophosphate and carbamate pesticides. Bioresour Technol 102:3184–3192
Chapalamadugu S, Chaudhry GR (1991) Hydrolysis of carbaryl by a *Pseudomonas* sp. And construction of a microbial consortium that completely metabolizes carbaryl. Appl Environ Microbiol 57(3):744–775. https://doi.org/10.1128/aem.57.3.744-750.1991
Chen W, Georgiou G (2002) Cell-surface display of heterologous proteins: from high-throughput screening to environmental applications. Biotechnol Bioeng 79(5):496–503. https://doi.org/10.1002/bit.10407
Chen S, Liu C, Peng C et al (2012) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* Hu-01. PLoS One 7(10):e47205. https://doi.org/10.1371/journal.pone.0047205
Clinton B, Warden AC, Haboury S et al (2011) Bacterial degradation of strobilurin fungicides: a role for a promiscuous methyl esterase activity of the subtilisin proteases? Biocatal Biotransformation 29(4):119–129. https://doi.org/10.3109/10242422.2011.578740
Cork DJ, Krueger JP (1991) Microbial transformations of herbicides and pesticides. Adv Appl Microbiol 36:1–66
Deng W, Lin D, Yao K et al (2015) Characterization of a novel β-cypermethrin-degrading *Aspergillus niger* YAT strain and the biochemical degradation pathway of β-cypermethrin. Appl Microbiol Biotech 99(19):8187–8198. https://doi.org/10.1007/s00253-015-6690-2
Derbalah AS, Belal EB (2008) Biodegradation and degradation kinetics of cymoxanil in aquatic system. Chem Ecol 24:1–12. https://doi.org/10.1080/02757540802032173
Diez MC, Gallardo F, Tortella G et al (2012) Chlorophenol degradation in soil columns inoculated with *Anthracophyllum discolor* immobilized on wheat grains. J Environ Manag 95:S83–S87. https://doi.org/10.1016/j.jenvman.2010.09.024
Dritsa V, Rigas F, Doulia D et al (2009) Optimization of culture conditions for the biodegradation of lindane by the polypore fungus *Ganoderma australe*. Water Air Soil Pollut 204(1–4):19–27. https://doi.org/10.1007/s11270-009-0022-z
Dyhrman ST, Chapell PD, Haley ST et al (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71. https://doi.org/10.1038/nature04203
Ebah E, Ichor T, Okpokwasili GC (2016) Isolation and biological characterization of tributyltin degrading bacterial from onne port sediment. Open J Mar Sci 6:193–199
El-Bestawy EA, Abd El-Salam AZ, Mansy AERH (2007) Potential use of environmental cyanobacterial species in bioremediation on lindane-contaminated effluents. Int Biodeterior Biodegradation 59:180–192. https://doi.org/10.1016/j.ibiod.2006.12.005

Elmore MH, McGary KL, Wisecaver JH et al (2015) Clustering of two genes putatively involved in cyanate detoxification evolved recently and independently in multiple fungal lineages. Genom Biol Evol 7(3):789–800. https://doi.org/10.1093/gbe/evv025

Ellegaard-Jensen L, Knudsen BE, Johansen A et al (2014) Fungal–bacterial consortia increase diuron degradation in water-unsaturated systems. Sci Total Environ 466:699–705. https://doi.org/10.1016/j.scitotenv.2013.07.095

El-Nahhal Y, Awad Y, Safi J (2013) Bioremediation of acetochlor in soil and water systems by cyanobacterial mat. Int J Geosci 4:880–890. https://doi.org/10.4236/ijg.2013.45082

Esterhuizen-Londt M, Schwartz K, Pflugmacher S (2016) Using aquatic fungi for pharmaceutical bioremediation: uptake of acetaminophen by *Mucor hiemalis* does not result in an enzymatic oxidative stress response. Fungal Biol 120(10):1249–1257

Ewida AYI (2014) Biodegradation of alachlor and endosulfan using environmental bacterial strains. World Appl Sci J 32(4):540–547. https://doi.org/10.5829/idosi.wasj.2014.32.04.14530

Fang H, Xiang YQ, Hao YJ et al (2008) Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int Biodeterior. Biodegradation 61(4):294–303. https://doi.org/10.1016/j.ibiod.2007.10.001

Forlani G, Pavan M, Gramek M et al (2008) Biochemical basis for a wide spread tolerance of cyanobacteria to the phosphonate herbicide glyphosate. Plant Cell Physiol 49:443–456. https://doi.org/10.1093/pcp/pcn021

Fragoeiro S, Magan N (2008) Impact of *Trametes versicolor* and *Phanerochaete chrysosporium* on differential breakdown of pesticide mixtures in soil microcosms at two water potentials and associated respiration and enzyme activity. Int Biodeterior Biodegrad 62(4):376–383. https://doi.org/10.1016/j.ibiod.2008.03.003

Fratila-Apachitei LE, Hirst JA, Siebel MA et al (1999) Diuron degradation by *Phanerochaete chrysosporium* BKM-F-1767 in synthetic and natural media. Biotechnol Lett 21(2):147–154

Fritsche W, Hofrichter M (2008) Aerobic degradation by microorganisms. In: Rehm HJ, Reed G (eds) Biotechnology, 2nd edn. Wiley-VCH Verlag GmbH, Weinheim, pp 144–167. https://doi.org/10.1002/9783527620951.ch6

Fuentes MS, Briceño GE, Saez JM et al (2013) Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized *Streptomyces* strains. Biomed Res Int 2013: 392573. https://doi.org/10.1155/2013/392573

Fujii K, Takagi K, Hiradate S et al (2007) Biodegradation of methylthios-Triazines by *Rhodococcus* sp. strain FJ1117YT, and production of the corresponding methylsulfinyl, methylsulfonyl and hydroxyl analogues. Pest Manag Sci 63(3):254–260. https://doi.org/10.1002/ps.1331

Fulbright SP, Chisholm S, Reardon KF (2016) Growth inhibition of *Nannochloropsis* species by *Bacillus pumilus*. Algal Res 20:70–76. https://doi.org/10.1016/j.algal.2016.09.016

Gianfreda L, Rao M (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzym Microb Technol 35:339–354

Gibson SA, Sulflita JM (1990) Anaerobic biodegradation of 2,4,5-trichlorophenoxyoacetic acid in samples from a methanogenic aquifer: stimulation by short-chain organic acids and alcohols. Appl Environ Microbiol 56:1825–1832. https://doi.org/10.1128/aem.56.6.1825-1832.1990

González R, García-Balboa C, Rouco M et al (2012) Adaptation of microalgae to lindane: a new approach for bioremediation. Aquat Toxicol 109:25–32

Gonçalves MS, Sampaio SC, Sene L et al (2012) Isolation of filamentous fungi present in swine wastewater that are resistant and with the ability to remove atrazine. Afr J Biotechnol 11(50): 11074–11077. https://doi.org/10.5897/AJB11.4018

Goswami S, Vig K, Singh DK (2009) Biodegradation of α and β endosulfan by *Aspergillus sydoni*. Chemos 75(7):883–888

Guillen-Jimenez FDM, Cristiani-Urbina E, Cancino-Díaz JC et al (2012) Lindane biodegradation by the *Fusarium verticillioides* AT-100 strain, isolated from Agave tequilana leaves: kinetic study and identification of metabolites. Int Biodeterior Biodegrad 74:36–47. https://doi.org/10.1016/j.ibiod.2012.04.020

Gunasekara AS, Rubin AL, Goh KS et al (2008) Environmental fate and toxicology of carbaryl. Rev Environ Contam Toxicol 196:95–121. https://doi.org/10.1007/978-0-387-78444-1_4

Guo Y, Yuan Z, Xu J et al (2017) Metabolic acclimation mechanism in microalgae developed for CO 2 capture from industrial flue gas. Algal Res 26:225–233

Habib K, Kumar S, Manikar N et al (2011) Biochemical effect of carbaryl on oxidative stress, antioxidant enzymes and osmolytes of cyanobacterium *Calothrix brevissima*. Bull Environ Contam Toxicol 87(6):615–620. https://doi.org/10.1007/s00128-011-0410-0

Hatzios KK (1991) Biotransformation of herbicides in higher plants. Environ Chem Herb 2:141–185

Helal IM, Abo-El-Seoud MA (2015) Fungal biodegradation of pesticide vydate in soil and aquatic system. In: 4th international Conference on Radiation Sci Appl, p 13–17

He X, Wubie AJ, Diao Q et al (2014) Biodegradation of neonicotinoid insecticide, imidacloprid by restriction enzyme mediated integration (REMI) generated Trichoderma mutants. Chemos 112: 526–530. https://doi.org/10.1016/j.chemosphere.2014.01.023

Hirooka T, Nagase H, Hirata K et al (2006) Degradation of 2,4-dinitrophenol by a mixed culture of photoautotrophic microorganisms. Biochem Eng J 29:157–162

Hussain S, Arshad M, Saleem M et al (2007) Screening of soil fungi for in vitro degradation of endosulfan. World J Microbiol Biotechnol 23(7):939–945

Ibrahim WM, Karam MA, El-Shahat RM et al (2014) Biodegradation and utilization of organophosphorus pesticide malathion by cyanobacteria. Biomed Res Int 2014:392682. https://doi.org/10.1155/2014/392682

Iqbal Lone M, Nazam N, Shaikh S et al (2013) Genotoxicity of an organochlorine pesticide dichlorophene by micronucleus and chromosomal aberration assays using bone marrow cells of *Rattus norvegicus*. Caryologia 66(4):296–303. https://doi.org/10.1080/00087114.2013.852344

Ishag AESA, Abdelbagi AO, Hammad AMA et al (2016) Biodegradation of chlorpyrifos, malathion, and dimethoate by three strains of bacteria isolated from pesticide-polluted soils in Sudan. J Agric Food Chem 64:8491–8498. https://doi.org/10.1021/acs.jafc.6b03334

Jain N, Bhargava A, Panwar J (2014) Enhanced photocatalytic degradation of methylene blue using biologically synthesized "protein-capped" ZnO nanoparticles. Chemic Eng J 243:549–555. https://doi.org/10.1016/j.cej.2013.11.085

Jauregui J, Valderrama B, Albores A et al (2003) Microsomal transformation of organophosphorus pesticides by white rot fungi. Biodegradation 14(6):397–406

Jha MN, Mishra SK (2005) Biological responses of cyanobacteria to insecticides and their insecticide degrading potential. Bull Environ Contam Toxicol 75(2):374–381

Kadimaliev DA, Revin VV, Atykyan NA et al (2011) The role of laccase and peroxidase of *Lentinus (Panus) tigrinus* fungus in biodegradation of high phenol concentrations in liquid medium. Appl Biochem Microbiol 47(1):66–71

Kamei I, Takagi K, Kondo R (2011) Degradation of endosulfan and endosulfan sulfate by white-rot fungus *Trametes hirsute*. J Wood Sci 57:317–322

Kaczmarzyk D, Fulda M (2010) Fatty acid activation in cyanobacteria mediated by acyl-acyl carrier protein synthetase enables fatty acid recycling. Plant Physiol 152:1598–1610

Kaphammer B, Olsen RH (1990) Cloning and characterization of tfdS, the repressor-activator gene of tfdB, from the 2, 4-dichlorophenoxyacetic acid catabolic plasmid pJP4. J Bacteriology 172(10):5856–5862. https://doi.org/10.1128/jb.172.10.5856-5862.1990

Karpouzas DG, Fotopoulou A, Menkissoglu-Spiroudi U et al (2005) Non-specific biodegradation of the organophosphorus pesticides, cadusafos and ethoprophos, by two bacterial isolates. FEMS Microbiol Ecol 53(3):369–378. https://doi.org/10.1016/j.femsec.2005.01.012

Karunakaran CO (1958) The Kerala food poisoning. J Indian Med Assoc 31:204–205

Kataoka R, Takagi K, Kamei I et al (2010) Biodegradation of dieldrin by a soil fungus isolated from a soil with annual endosulfan applications. Environ Sci Technol 44(16):6343–6349

Katz I, Dosoretz CG, Mandelbaum RT et al (2001) Atrazine degradation under denitrifying conditions in continuous culture of Pseudomonas ADP. Water Res 35:3272–3275

Khadrani A, Seigle-Murandi F, Steiman R et al (1999) Degradation of three phenylurea herbicides (chlortoluron, isoproturon, and diuron) by micromycetes isolated from soil. Chemos 38(13): 3041–3050. https://doi.org/10.1016/s0045-6535(98)00510-4

Kulshrestha G, Kumari A (2011) Fungal degradation of chlorpyrifos by *Acremonium* sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil. Biol Fertil Soils 47 (2): 219-225. https://doi.org/10.1007/s00374-010-0505-5

Kumar N, Bora A, Kumar R et al (2012) Differential effects of agricultural pesticides endosulfan and tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, filamentous cyanobacteria. J Biol Environ Sci 6(16):67–75

Kumar NJI, Amb MK, Kumar RN et al (2013) Studies on biodegradation and molecular characterization of 2,4-D ethyl ester and pencycuron induced cyanobacteria by using GC-MS and 16S r DNA sequencing. Proc Int Acad Ecol Environ Sci 3(1):1–24

Kumar A, Singh JS (2017) Cyanoremediation: a green-clean tool for decontamination of synthetic pesticides from agro-and aquatic ecosystems. In: Singh J, Seneviratne G (eds) Agro-Environmental sustainability. Springer, Cham, pp 59–83

Kumari M, Ghosh P, Thakur IS (2016) Landfill leachate treatment using bacto-algal co-culture: an integrated approach using chemical analyses and toxicological assessment. Ecotoxicol Environ Saf 128:44–51. https://doi.org/10.1016/j.ecoenv.2016.02.009

Kuritz T, Wolk CP (1995) Use of filamentous cyanobacteria for biodegradation of organic pollutants. Appl Environ Microbiol 61:234–238. https://doi.org/10.1128/aem.61.1.234-238.1995

Laemmli CM, Leveau JHJ, Zehnder AJB et al (2000) Characterization of a second tfd gene cluster for chlorophenol and chlorocatechol metabolism on plasmid pJP4 in *Ralstonia eutropha* JMP134 (pJP4). J Bacteriol 182:4165–4172. https://doi.org/10.1128/JB.182.15.4165-4172.2000

León-Santiesteban HH, Rodríguez-Vázquez R (2017) Fungal degradation of organochlorine pesticides. In: Singh S (eds) Microbe-induced degradation of pesticides. Environmental Science and Engineering, Springer, Cham, p131–149. Doi:https://doi.org/10.1007/978-3-319-45156-5_6

Lee SE, Kim JS, Kennedy IR et al (2003) Biotransformation of an organochlorine insecticide, endosulfan, by *Anabaena* Species. J Agric Food Chem 51:1336–1340

Lipok J, Wieczorek D, Jewginski M et al (2009) Prospects of in vivo 31P NMR method in glyphosate degradation studies in whole cell system. Enzym Microb Technol 44(1):11–16. https://doi.org/10.1016/j.enzmictec.2008.09.011

Mahmood I, Imadi SR, Shazadi K et al (2016) Effects of pesticides on environment. In: Hakeem K, Akhtar M, Abdullah S (eds) Plant soil and microbes. Springer, Cham, pp 253–269. https://doi.org/10.1007/978-3-319-27455-3_13

Masukawa H, Inoue K, Sakurai H (2007) Effects of disruption of homocitrate synthase genes on *Nostoc* sp. strain PCC 7120 photobiological hydrogen production and nitrogenase. Appl Environ Microbiol 73(23):7562–7570

Maya K, Upadhyay SN, Singh RS et al (2012) Degradation kinetics of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) by fungal communities. Bioresour Technol 126:216–223

McNeely K, Xu Y, Bennette N et al (2010) Redirecting reductant flux into hydrogen production via metabolic engineering of fermentative carbon metabolism in a cyanobacterium. Appl Environ Microbiol 76:5032–5038

Megharaj M, Madhavi DR, Sreenivasulu C et al (1994) Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. Bull Environ Contam Toxicol 53:292–297. https://doi.org/10.1007/BF00192047

Megharaj M, Ramakrishnan B, Venkateswarlu K et al (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37:1362–1375

Mercadier C, Vega D, Bastide J (1997) Iprodione degradation by isolated soil microorganisms. FEMS Microbiol Ecol 23(3):207–215. https://doi.org/10.1016/S0168-6496(97)00024-X

Mishaqa ESI (2017) Biosorption potential of the microchlorophyte *Chlorella vulgaris* for some pesticides. J Ferti Pestici 8(1):5. https://doi.org/10.4172/2471-2728.1000177

Moawad H, El-Rahim WMA, Shawky H et al (2014) Evidence of fungicides degradation by Rhizobia. Agric Sci 5:618–624. https://doi.org/10.4236/as.2014.57065

Mohamad NR, Marzuki NH, Buang NA et al (2015) An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. Biotechnol Biotechnol Equip 29(2):205–220. https://doi.org/10.1080/13102818.2015.1008192

Nadeau LJ, Menn F-M, Breen A et al (1994) Aerobic degradation of l,l,l-trichloro-2,2-bis (4-chloropheny1)ethane (DDT) by *Alcaligenes eutrophus* A5. Appl Environ Microbiol 60:51–55

Nagata Y, Miyauchi K, Takagi M (1999) Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. J Ind Microbiol Biotechnol 23(4):380–390. https://doi.org/10.1038/sj.jim.2900736

Nagy I, Schoofs G, Compernolle F et al (1995) Degradation of the thiocarbamate herbicide EPTC (8-ethyl dipropylcarbamothioate) and biosafening by *Rhodococcus* sp. Strain NI86/21 involve an inducible cytochrome P-450 system and aldehyde dehydrogenase. J Bacteriol 177:676–687. https://doi.org/10.1128/jb.177.3.676-687.1995

Nayak SK, Dash B, Baliyarsingh B (2018) Microbial remediation of persistent agro-chemicals by soil bacteria: an overview. In: Patra J, Das G, Shin HS (eds) Microbial biotechnology. Springer, Singapore, pp 275–301. https://doi.org/10.1007/978-981-10-7140-9_13

Nayak SK, Mishra BB (eds) (2020) Frontiers in soil and environmental microbiology. CRC Press, Boca Raton. https://doi.org/10.1201/9780429485794

Nguyen LN, Hai FI, Kang J et al (2014) Enhancement of trace organic contaminant degradation by crude enzyme extract from *Trametes versicolor* culture: effect of mediator type and concentration. J Taiwan Inst Chem Eng 45(4):1855–1862

Oliveira BR, Penetra A, Cardoso VV et al (2015) Biodegradation of pesticides using fungi species found in the aquatic environment. Environ Sci Pollut Res Int 22:11781–11791. https://doi.org/10.1007/s11356-015-4472-0

Ortiz-Hernández ML, Sánchez-Salinas E (2010) Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in Mexico. Rev Int Contam Ambient 26(1):27–38

Padmanabhan P, Padmanabhan S, De Rito C et al (2003) Respiration of 13 C-labelled substrates added to soil in the field and subsequent 16S rRNA gene analysis of 13 C labeled soil DNA. Appl Environ Microbiol 69:1614–1622

Palanisami S, Prabaharan D, Uma L (2009) Fate of few pesticide metabolizing enzymes in marine cyanobacterium *Phormidium valderianum* BDU 20041 in perspective with chlorpyrifos exposure. Pestic Biochem Physiol 94:68–72

Patel JG, Kumar NJI, Kumar RN et al (2017) Evaluation of nitrogen fixing enzyme activities in response to pyrene bioremediation efficacy by defined artificial microalgal-bacterial consortium of Gujarat. India Polycycl Aromat Compd 38(3):282–293. https://doi.org/10.1080/10406638.2016.1207684

Peng X, Huang J, Liu C et al (2012) Biodegradation of bensulphuron-methyl by a novel *Penicillium pinophilum* strain BP-H-02. J Hazard Mater 213:216–221. https://doi.org/10.1016/j.jhazmat.2012.01.077

Pereira PM, Teixeira RSS, de Oliveira MAL et al (2013) Optimized atrazine degradation by *Pleurotus ostreatus* INCQS 40310: an alternative for impact reduction of herbicides used in sugarcane crops. J Microbial Biochem Technol S12:006. https://doi.org/10.4172/1948-5948.S12-006

Pérezgarcía S, Lubián LM, Blasco J et al (2013) Detection of intracellular Reactive Oxygen Species (ROS) by flow cytometry on microalgae: studies on laboratory monoalgal and natural mixed cultures. In: The Iberian and 6th Iberoamerican Congress of Environmental Contamination and Toxicology. http://hdl.handle.net/10261/104150

Philippoussis A, Zervakis G, Diamantopoulou P (2001) Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp. World J Microbiol Biotechnol 17(2):191–200

Pinto AP, Rodrigues SC, Caldeira AT et al (2016) Exploring the potential of novel biomixtures and *Lentinula edodes* fungus for the degradation of selected pesticides. Evaluation for use in biobed systems. Sci Total Environ 541:1372–1381

Peter L, Gajendiran A, Mani D et al (2015) Mineralization of malathion by *Fusarium oxysporum* strain JASA1 isolated from sugarcane fields. Environ Prog Sust Ener 34(1):112–116. https://doi.org/10.1002/ep.11970

Poliner E, Farré EM, Benning C (2018) Advanced genetic tools enable synthetic biology in the oleaginous microalgae *Nannochloropsis* sp. Plant Cell Rep 37(10):1383–1399

Popp J (2011) Cost-benefit analysis of crop protection measures. J Verbr Lebensm 6:105–112. https://doi.org/10.1007/s00003-011-0677-4

Purnomo AS, Mori T, Kamei I et al (2010) Application of mushroom waste medium from *Pleurotus ostreatus* for bioremediation of DDT-contaminated soil. Int Biodeterior Biodegrad 64(5):397–402. https://doi.org/10.1016/j.ibiod.2010.04.007

Purnomo AS, Mori T, Kamei I et al (2011) Basic studies and applications on bioremediation of DDT: a review. Int Biodeterior Biodegrad 65(7):921–930. https://doi.org/10.1016/j.ibiod.2011.07.011

Purnomo AS, Putra SR, Shimizu K et al (2014) Biodegradation of heptachlor and heptachlor epoxide-contaminated soils by white-rot fungal inocula. Environ Sci Pollut Res 21(19):11305–11312. https://doi.org/10.1007/s11356-014-3026-1

Qiao L, Wang J (2010) Biodegradation characterization of a pyridine-degrading strain. Qinghua Daxue Xuebao 50(6):869–872

Qiu YW, Zeng E, Qiu H et al (2017) Bioconcentration of polybrominated diphenyl ethers and organochlorine pesticides in algae is an important contaminant route to higher trophic levels. Sci Total Environ 579:1885–1893. https://doi.org/10.1016/j.scitotenv.2016.11.192

Quintero JC, Moreira MT, Feijoo G et al (2008) Screening of white rot fungal species for their capacity to degrade lindane and other isomers of hexachlorocyclohexane (HCH). Cienc Inv Agr 35(2):159–167. https://doi.org/10.4067/S0718-16202008000200005

Racke KD, Skidmore MW, Hamilton DJ et al (1997) Pesticides report 38. Pesticide fate in tropical soils (technical report). Pure Appl Chem 69(6):1349–1372

Rani L, Thapa K, Kanojia N et al (2020) An extensive review on the consequences of chemical pesticides on human health and environment. J Clean Produc 283:124657. https://doi.org/10.1016/j.jclepro.2020.124657

Ray AS, Mondal J (2017) Potential applications of various fungal and bacterial agents in decontamination of agricultural soils: an overview. IOSR J Agric Vet Sci 10:40–47

Ravindran CRM, Suguna S, Shanmugasundaram S (2000) Tolerance of *Oscillatoria* isolates to agrochemicals and pyrethroid components. Indian J Exp Biol 38(4):402–404

Robinson PK, Goulding KH, Mak AL et al (1986) Factors affecting the growth characteristics of alginate-entrapped Chlorella. Enzym Microb Technol 8(12):729–733

Roessler PG, Chen Y, Liu B et al (2009) Secretion of fatty acids by photosynthetic microorganisms in Patent US, Ed. Synthetic Genomics, United States

Rønhede S, Jensen B, Rosendahl S et al (2005) Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. Appl Environ Microbiol 71(12):7927–7932

Rubilar O, Feijoo G, Diez MC et al (2007) Biodegradation of pentachlorophenol in soil slurry cultures by *Bjerkandera adusta* and *Anthracophyllum discolor*. Ind Eng Chem Res 46:6744–6751. https://doi.org/10.1021/ie061678b

Sabourmoghaddam N, Zakaria MP, Omar D (2015) Evidence for the microbial degradation of imidacloprid in soils of Cameron Highlands. J Saudi Soc Agric Sci 14(2):182–188. https://doi.org/10.1016/j.jssas.2014.03.002

Sadowski MJ, Tong Z, Souza MLD et al (1998) AtzC is a new member of the amino hydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. J Bacteriol 180: 152–158

Sagar V, Singh DP (2011) Biodegradation of lindane pesticide by non-white-rots soil fungus *Fusarium* sp. World J Microbiol Biotechnol 27(8):1747–1754

Sayler GS, Hooper SW, Layton AC et al (1990) Catabolic plasmids of environmental and ecological significance. Microbiol Ecol 19:1–20

Sene L, Converti A, Secchi GAR et al (2010) New aspects on atrazine biodegradation. Braz Arch Biol Technol 53(2):487–496. https://doi.org/10.1590/S1516-89132010000200030

Sharef Ibrahim B, Abdelbagi Azhari O, Elsheikh Elsiddig AE et al (2013) Biodegradation of pendimethalin by three strains of bacteria isolated from pesticide-polluted soils. U K J Agric Sci 21(2):233–252

Sharma A, Pankaj, Khati P, Gangola S, Kumar G (2016) Microbial degradation of pesticides for environmental cleanup. In: Saxena RNBG (ed) Bioremediation of industrial pollutants. Write & Print Publications, Lanham, pp p179–p205

Silambarasan S, Abraham J (2014) Efficacy of *Ganoderma* sp. JAS4 in bioremediation of chlorpyrifos and its hydrolyzing metabolite TCP from agricultural soil. J Basic Microbiol 54(1): 44–55. https://doi.org/10.1002/jobm.201200437

Sims GK, O'Loughlin EJ (1989) Degradation of pyridines in the environment. Crit Rev Environ Control 19:309–340

Singh DK (2008) Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Ind J Microbiol 48:35–40

Singh P, Suri CR, Cameotra SS (2004) Isolation of a member of *Acinetobacter* species involved in atrazine degradation. Biochem Biophys Res Commun 317:697–702

Singh JS, Abhilash PC, Singh HB et al (2011a) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480(1–2):1–9. https://doi.org/10.1016/j.gene.2011.03.001

Singh DP, Khattar JIS, Nadda J et al (2011b) Chlorpyrifos degradation by the cyanobacterium *Synechocystis* sp. strain PUPCCC 64. Environ Sci Pollut Res 18:1351–1359. https://doi.org/10.1007/s11356-011-0472-x

Singh JS, Singh DP, Dixit S (2011c) Cyanobacteria: an agent of heavy metal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher Co., New Delhi, pp 223–243

Singh DP, Khattar JIS, Kaur M et al (2013) Anilofos tolerance and its mineralization by the Cyanobacterium *Synechocystis* sp. strain PUPCCC 64. PLoS One 8(1):e53445

Spagnoletti FN, Chiocchio VM (2020) Tolerance of dark septate endophytic fungi (DSE) to agrochemicals in vitro. Rev Argent Microbiol 52(1):43–49. https://doi.org/10.1016/j.ram.2019.02.003

Spina F, Cecchi G, Landinez-Torres A et al (2018) Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water. Plant Biosyst 152(3):474–488

Streber WR, Timmis KN, Zenk MH (1987) Analysis, cloning, and high-level expression of 2,4-dichlorophenoxyacetate monooxygenase gene tfdA of *Alcaligenes eutrophus* JMP134. J Bacteriol 169(7):2950–2955. https://doi.org/10.1128/jb.169.7.2950-2955.1987

Subashchandrabose SR, Ramakrishnan B, Megharaj M et al (2013) Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environ Int 51(5): 59–72. https://doi.org/10.1016/j.envint.2012.10.007

Suenaga H, Mitsuoka M, Ura Y et al (2001) Directed evolution of biphenyl dioxygenase: emergence of enhanced degradation capacity for benzene, toluene and alkylbenzenes. J Bacteriol 183:5441–5444. https://doi.org/10.1128/JB.183.18.5441-5444.2001

Taha H, Khateb A (2013) Resolving the orthographic ambiguity during visual word recognition in Arabic: an event-related potential investigation. Front Hum Neurosci 7:821. https://doi.org/10.3389/fnhum.2013.00821

Taştan BE, Dönmez G (2015) Biodegradation of pesticide triclosan by *A. versicolor* in simulated wastewater and semi-synthetic media. Pest Biochem Physiol 118:33–37

Teng Y, Wang X, Zhu Y et al (2017) Biodegradation of pentachloronitrobenzene by *Cupriavidus* sp. YNS-85 and its potential for remediation of contaminated soils. Environ Sci Pollut Res 24(10):9538–9547

Tewari L, Saini J, Arti (2012) Bioremediation of pesticides by microorganisms: general aspects and recent advances. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publishing House Pvt. Ltd., New Delhi, p 25–48

Thatheyus AJ, Selvam ADG (2013) Synthetic pyrethroids: toxicity and biodegradation. Appl Ecol Environ Sci 1(3):33–36. https://doi.org/10.12691/aees-1-3-2

Thengodkar RRM, Sivakami S (2010) Degradation of Chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegradation 21:637–644. https://doi.org/10.1007/s10532-010-9331-6

Tiwari B, Chakraborty S, Srivastava AK et al (2017) Biodegradation and rapid removal of methyl parathion by the paddy field cyanobacterium *Fischerella* sp. Algal Res 25:285–296. https://doi.org/10.1016/j.algal.2017.05.024

Tixier C, Bogaerts P, Sancelme M et al (2000) Fungal biodegradation of a phenylurea herbicide, diuron: structure and toxicity of metabolites. Pest Manag Sci 56(5):455–462

Tixier C, Sancelme M, Bonnemoy F et al (2001) Degradation products of a phenylurea herbicide, diuron: synthesis, ecotoxicity, and biotransformation. Environ Toxicol Chem 20(7):1381–1389. https://doi.org/10.1897/1551-5028(2001)020<1381:dpoaph>2.0.co;2

Tomasek PH, Karns JS (1989) Cloning of a carbofuran hydrolase gene from *Achromobacter* sp. WMlll and its expression in Gram-negative bacteria. J Bacteriol 171:4038–4044

Vacondio B, Birolli WG, Ferreira IM et al (2015) Biodegradation of pentachlorophenol by marine-derived fungus *Trichoderma harzianum* CBMAI 1677 isolated from ascidian Didemnum ligulum. Biocat Agri Biotech 4(2):266–275

Vasilieva S, Shibzukhova K, Morozov A et al (2018) Immobilization of microalgae on the surface of new cross-linked polyethylenimine-based sorbents. J Biotechnol 281:31–38. https://doi.org/10.1016/j.jbiotec.2018.03.011

Verma JP, Jaiswal DK, Sagar R (2014) Pesticide relevance and their microbial degradation: a-state of-art. Rev Environ Sci Biotechnol 13(4):429–466301

Wang G, Yue W, Liu Y et al (2013) Biodegradation of the neonicotinoid insecticide Acetamiprid by bacterium *Pigmentiphaga* sp. strain AAP-1 isolated from soil. Bioresour Technol 138:359–368. https://doi.org/10.1016/j.biortech.2013.03.193

Warmink JA, Nazir R, van Elsas JD (2009) Universal and species-specific bacterial fungiphiles in the mycospheres of different basidiomycetous fungi. Environ Microbiol 11:300–312. https://doi.org/10.1111/j.1462-2920.2008.01767.x

Wen J, Gao D, Zhang B et al (2011) Co-metabolic degradation of pyrene by indigenous white-rot fungus *Pseudotrametes gibbosa* from the northeast China. Int Biodet Biodeg 65(4):600–604. https://doi.org/10.1016/j.ibiod.2011.03.003

Xiao P, Mori T, Kamei I et al (2011) Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus *Phlebia*. Chemosphere 85(2):218–224. https://doi.org/10.1016/j.chemosphere.2011.06.028

Xu G, Li Y, Zheng W et al (2007) Mineralization of chlorpyrifos by co-culture of *Serratia* and *Trichosporon* spp. Biotechnol Lett 29(10):1469–1473

Xu P, Huang L (2017) Stereoselective bioaccumulation, transformation, and toxicity of triadimefon in Scenedesmus obliquus. Chirality 29(2):61–69

Yadav IC, Devi NL, Syed JH et al (2015) Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. Sci Total Environ 511:123–137

Yang L, Chen S, Hu M et al (2011) Biodegradation of carbofuran by *Pichia anomala* strain HQ-C-01 and its application for bioremediation of contaminated soils. Biol Fertil Soils 47(8):917–923. https://doi.org/10.1007/s00374-011-0602-0

Yuan Y, Guo SH, Li FM et al (2013) Effect of an electric field on n-hexadecane microbial degradation in contaminated soil. Int Biodeterior Biodegrad 77:78–84

Yu YL, Fang H, Wang X et al (2006) Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation 17(5): 487–494. https://doi.org/10.1007/s10532-005-9020-z

Zhao RB, Bao HY, Liu YX (2010) Isolation and characterization of *Penicillium oxalicum* ZHJ6 for biodegradation of methamidophos. Agric Sci China 9(5):695–703. https://doi.org/10.1016/S1671-2927(09)60145-0

Zhou X, Xu S, Liu L et al (2007) Degradation of cyanide by *Trichoderma* mutants constructed by restriction enzyme mediated integration (REMI). Bioresour Technol 98(15):2958–2962. https://doi.org/10.1016/j.biortech.2006.09.047

Microbe-Based Pesticides for Insect Pest Control and Their Management

9

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Abstract

Microbial biocides include various microorganisms such as bacteria, nematode-related bacteria, and fungi. These microorganisms and their products have been proven to be very effective against pests. In all cases, these species are considered organic and are specific to pest control strategies. Today, microbial biological pesticides are replacing chemical pesticides to overcome the harmful effects of chemicals on non-target organisms. There are several reasons for the continued development of agriculture and health care, although there are also problems. This chapter elaborately describes the insecticidal properties of microorganisms and their potential uses in pest control, the ways of their production and development, and the challenges in microbial pesticide realm in the future.

Keywords

Insecticides · Biological pesticides · Microorganisms · Pests and pest control

9.1 Introduction

Due to new crops in addition to global warming, more and more pests are introduced or transferred to new locations, intentionally or unintentionally. It was introduced into the new territories due to the expansion in global trading. Since an array of chemical pesticides for crop protection are available in the market, fighting these invasive species is an unprecedented challenge worldwide; on the other hand pesticides are harmful to humans and animals through their original or transformed (fragments) form and have adverse effect on the environment. Owing to the

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_9

mammoth use of chemical pesticides, there is an urgent need to develop reliable, biodegradable, and environmentally friendly pesticides. Also in order to avoid the harmful effects priority has been shifted to the biological control of pests in the form of biological pesticides or biopesticides (Meissle et al. 2010; Nayak and Mishra 2020). The paramount advantages of these biological control agents include specificity to target pests, protection against organisms, harmlessness to the environment and/or human health, and combat with pesticide-resistant pests.

According to Flint and van den Bosch (1981), integrated pest control is an environment-friendly pest control way based on natural death factors and control devices that poses least threat. A comprehensive pest control plan analyzes the possible interactions between different pest control activities, various harvest processes, surrounding climate, other pests, and crop protection products along with all available pest control methods. They are ideal components for integrated pest control systems (IPM) and ecological cultivation systems. On the other hand, these biological control agents are classified as slow acting or deadly, and are sensitive to the environment, leading to inconsistencies and low success rates in field applications (Fedele et al. 2020; Zhang et al. 2017). At present, the concern is finding out highly effective and aggressive strains with inherent potential to improve field effectiveness along with operative formulations for increase in field resistance. In recent years, in order to eradicate insects in an economical and ecological way bioinsecticide has been a safe alternative. It is also a safe alternative to harmful chemical pesticides and has high efficacy. Currently more than 1.5% of microbial pesticides are available in the world market. This chapter provides an overview of the microbial pesticide manuals available in the world and the Indian conservation market.

9.2 Types of Microbiological Insecticides

There are three types of microbial insecticides or insecticides, namely biochemical insecticides, crop protection products, insecticides, and microbial insecticides. Microbial pesticides are naturally occurring or genetically modified bacteria, fungi, algae, protozoa, or viruses. They are very safe and can be used in place of chemical pesticides. Biotoxins produced by microorganisms such as bacteria or fungi are the microbial toxins employed for the same. The role of microbial insect pathogens is based on their penetration into the outer skin or intestines of insects, leading to the replication of pathogens and death of hosts such as insects (Butt et al. 2016; Narayanan 2004). Insecticidal toxins are produced by pathogens and play an important role in pathogenesis.

Moreover, pesticide substances from plant genetic material have been integrated. For example, scientists insert the gene for the pesticide protein Bt into the genome of plants. Now, genetically modified plants have replaced Bt bacteria and can produce pesticides. The Environmental Protection Agency (EPA) controls protein and its genetic material (Kachhawa 2017). Biochemical pesticides come from plants or microbes such as fatty acids and pheromones which are modified by human beings

to control pests by killing or shooting insects. Pheromones act as biochemical pesticides, and attract the insects that disrupt the growth or mating (Kumar et al. 2019; AL-Ahmadi 2019; Sherwani and Khan 2015).

9.2.1 Bacteria

Among others, *Bacillus* and *Pseudomonas* and their type strains are used as microbial pesticides to control pesticides and plant diseases. The most famous pesticides mainly come from different subspecies of *Bacillus thuringiensis* Berliner (Bt). *B. thuringiensis* sp. kurstaki and *B. thuringiensis* sp. *aizawai* are two members extremely toxic to lepidopterans (Perez and Shelton 1997; Federici et al. 2010). Commercially available Bacillus species such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* 2362 (Bs) were found notably effective against mosquito larvae and fungus gnats (Roh et al. 2007). *B. thuringiensis tenebrionis* is active against coleopteran adults and larvae, including the Colorado potato beetle (*Leptinotarsa decemlineata*). *B. thuringiensis* japonensis strain Buibui is active against soil-dwelling beetles *Calleida decora* (Bohorova et al. 1997; Senthil-Nathan 2015). Bt produces a crystalline protein that binds mostly to the intestinal receptors of target insects and kills certain pests (de Maagd et al. 2001; Roh et al. 2007).

The formation of spores may be very toxic to insects. Different types of Bt will produce different types of endotoxins. These endotoxins feed our body the larvae of various insects. As a result, insects suffer from intestinal paralysis (Thomas and Ellar 1983; Raymond et al. 2010). Infected larvae avoid food and eventually die from malnutrition and damage to the midgut epithelium. High temperature competes with dangerous creatures for resources. Other microbial pesticides work by competing with insect pest species for resources. Microbial pesticides should be closely monitored to ensure that they do not damage non-target species, such as humans (Sarwar 2015).

Bt toxin (bacteriocin) is an insecticidal toxin produced by rhizobia, which can trigger plant prosperity. It controls various plant pests around the world, especially caterpillars, mosquito larvae, and mosquitoes (Sanchis and Bourguet 2008; Sanahuja et al. 2011). Dust-containing dry spores and crystal contamination are industrialized Bt products. These can be used when the larvae feed on leaves or various habitats. Part of the flora has been genetically modified, including the Bt toxin gene (Sanahuja et al. 2011; Senthil-Nathan 2015). Under chicken farm conditions, the main decay controlled by *Pseudomonas maltophilia* accounts for 40.8% of the seed function of beans (*Cyamopsis tetragonoloba*) when co-inoculated with *Rhizoctonia bataticola*, *R. solani*, *Fusarium oxysporum*, and *Sclerotinia sclerotiorum* under screenhouse conditions (Ramanujam et al. 2014).

9.2.2 Fungi

More than 750 species of fungi (mainly filamentous and endophytic fungi) are insect pathogens, many of which have great pest control potential. Pathogenic fungi are an important community of microbial pests found in terrestrial and aquatic environments. If they are directly related to insects, they are classified as human pathogenic fungi (Sahid et al. 2017). Compared with Bt and VPN, handling bulk mushrooms is easier, faster, and cheaper. In contrast to bacteria and viruses, fungi that are pathogenic to insects directly infect insect epidermis, and insects that suck insects can also infect and/or kill sucking pests and their pathogenic behavior is established on contact (Khan et al. 2012).

Mycelial pathogenic fungi are opportunistic pathogens that kill insects due to malnutrition, destruction, and tissue release. Enzymes that destroy the epidermis of pathogenic insect fungi are chitinase, protease, and lipase and play an important role in the pathogenicity of these insects which further prevents insects from entering the epidermal space (Butt et al. 2016; Boucias et al. 2018; Nayak et al. 2021). The chitinase, protease, and lipase of pathogenic insect fungi and other skin-degrading enzymes play an important role in the pathogenicity of these species, so the fungal germinal tubules can enter the insect's body. Species that have had the most extensive research into mycoinsecticides for crop pest control include *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Metarhizium anisopliae* var. *acridium*, *Lecanicillium* sp. (previously *Verticillium lecanii*), *Hirsutella thompsonii*, *Nomuraea rileyi*, and *Isaria fumosorosea* (previously *Paecilomyces fumosoroseus*) (Wraight and Carruthers 1999; Charnley and Collins 2007).

Fungi infect almost all insect orders, with Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera, and Hymenoptera being the most common. In certain insect orders, nymphal or larval stages are more likely to be infected than adult stages, while in others, the opposite is true (Mousseau and Dingle 1991). Some fungi have a very narrow host range, such as *Aschersonia aleyrodis*, which only affects whiteflies, and *N. rileyi* that only infects Lepidopteran larvae, while other fungi *B. bassiana* and *M. anisopliae* infect a large number of insects and have several pathogenic types with high host specificity (Wraight and Carruthers 1999; Randolph 2004; Anselme et al. 2006).

The use of *M. anisopliae* to control spittlebugs (Cercopidae) in South American sugarcane and pastures is currently the largest single microbial control program using fungi. The use of *B. bassiana* to monitor the pine moth *Dendrolimus* sp. in China is likely one of the biggest uses of a biocontrol agent in the world, covering over one million hectares of pine forest (Roberts 1989; Feng et al. 1994). In Europe, *B. bassiana* strain Bb-147 is approved for use on maize to combat the European corn borer, *Ostrinia nubilalis*, and other pests (Narayanan 2004; Rosell et al. 2008; Maina et al. 2018). *Ostrinia furnacalis*, the GHA strain, has been approved to control whiteflies, thrips, aphids, etc., and ATCC 74040 has been approved to control several soft insects from the members of Homoptera, Heteroptera, and Coleoptera (Abrol and Shankar 2014; Charnley and Collins 2007). Several commercial EPF formulations for crop pest management have been developed. Among the 171 EPF

products produced, products based on *B. bassiana* account for 33.9% of total products, products based on *M. anisopliae* account for 33.9%, and products based on *I. fumosorosea* and *B. brongniartii* account for 5.8% and 4.1%, respectively. Approximately 90 genera and nearly 700 species have been considered as insects infecting fungi in recent times (Roberts 1989). Virulent isolates of *B. bassiana* (Bb-11, 47, and 49) and *M. anisopliae* (NBAII Ma-4 and 42) were described based on the toxicity of extracellular crude soluble proteins (CSPs) against *Spodoptera litura*. Promising isolates with higher chitinase, protease, and lipase activities were established based on their ability to produce cuticle-degrading enzymes (SabaHasan et al. 2013).

9.2.3 Virus

Viruses have unique activity against insects and can be extremely useful in controlling a variety of caterpillar pests, naturally. Epizootics are known to wipe out pest populations, particularly when insect populations are large. Baculoviruses are rod-shaped viruses with a specific target that can kill and infect a wide range of plant pests (Kachhawa 2017; Chaeychomsri et al. 2020). Since their large-scale development poses some challenges, their use has been limited.

Various researchers have documented viral products for the codling moth, *Heliothis zea*, and beet armyworm nuclear polyhedrosis virus for the control of pest Lepidoptera order, such as the cotton bollworm *Helicoverpa armigera* and budworm *Choristoneura* sp. (Abd-Alla et al. 2020; Rajput et al. 2020). Baculoviruses are effective against lepidopterous pests of cotton, rice, and vegetables (Lico et al. 2008; Thakur et al. 2020).

9.3 Protozoan as Microbial Insecticides

Entomopathogenic protozoans, also known as microsporidians, are a diverse group of organisms that attack invertebrates, including insects, and about 1000 species. *Nosema* sp. and *Vairimorpha necatrix* are the most well-known entomopathogenic protozoa. The only commercially viable species is the microsporidian *Nosema locustae*, which is used to track grasshoppers and crickets in countries such as the USA, Canada, China, and Brazil (Ramanujam et al. 2014). Another beneficial microsporidian is *Nosema pyrausta*, which reduces adult fecundity and survival while also killing European corn borer larvae (Lewis et al. 2009).

9.4 Present Prospectus of Microbial Pesticides in Global Context

In the past 10 years, the fields of molecular biology, protein engineering, and genetic engineering have achieved rapid development, which has promoted the development of microbial pesticides. Through research and applications, microbial pesticides are constantly replacing highly toxic pesticides in the market. The number of chemical pesticides is reduced by 2% every year (Marrone 2009).

The bacterium *Bacillus thuringiensis* (Bt) covers 2% of the total market of insecticidal which is known as the most successful insect pathogen used for insect control. The most commonly used bacterial pathogens are of *Bacillus thuringiensis* subspecies or strains. Each strain generates a unique set of toxins which kill one or a few closely related insect species (Bt subspecies *kurstaki* and *aizawai* for lepidopteran larvae; Bt subspecies *tenebrionis* for coleopteran larvae). Some of these strains are unique to mosquitoes, such as Bt subspecies *israelensis* (Kumar et al. 2019). Because of their specificity, baculoviruses (nuclear polyhedrosis virus (NPV) and granulosis virus (GV)) are the most promising insect viruses for insect control, especially in Lepidoptera and Diptera. NPVs have been used successfully in some countries to control devastating pests such as *Heliothis* sp. and *Spodoptera* sp. on cotton, fruit, and vegetable crops (Ramanujam et al. 2014). Entomopathogenic fungi such as *Beauveria* sp., *Metarhizium* sp., *Lecanicillium* sp., and *Isaria* sp. have been developed as effective mycoinsecticides for a variety of insect pests. Control of *Lymantria* sp. outbreaks in forest in Poland and North America with the products from *B. thuringiensis* subsp. *kurstaki* is one of the effective and large-scale pest management programs using microbial insecticides (Khan et al. 2015). *Beauveria* sp., *Metarhizium* sp., *Lecanicillium* sp., and *Isaria* sp. have all been produced as effective mycoinsecticides for a variety of insect pests (Gani et al. 2019). *B. bassiana* is used to monitor pine caterpillars in China (Wang et al. 2004; Lu et al. 2008); *Metarhizium anisopliae* var. *acridium* is used to control locusts in Africa, Australia, and China (Li et al. 2010); *Metarhizium anisopliae* var. *acridium* is used to control sugarcane spittlebugs in Brazil (YubakDhoj 2006; Ramanujam et al. 2014); *Sporothrix insectorum* is used to control rubber lace bugs (Zhang et al. 2019; Gani et al. 2019); and *B. bassiana* is used to control corn borer in Europe and North America (Feng et al. 1985). "H" serotyping, or the immune response to the bacterial flagellar antigen, has been used to classify Bt strains. At least 69 H serotypes and 82 serological varieties (serovars) of Bt have been identified worldwide, with distinct flagellin amino acid sequences linked to particular Bt H serotypes (Smith et al. 2003). It is reported by Qayyum et al. (2015) that the most common pesticides are Bt pesticides, viral pesticides (*Heliothis armigera* nuclear polyhedrosis viruses (NPV)), and fungal pesticides (*Trichoderma*, etc.). Bt product revenue peaked at $984 million in 1997 and increased to $3.6 billion in 2005. The global leading biopesticide organisms in 2006 were as follows: B.t. CryF1, NRRL 21882 (*Aspergillus flavus*), *Bacillus licheniformis* strain SB3086, and some of the other microbial products that have been successfully commercialized (Table 9.1).

Table 9.1 Commercial production of insecticides from microbes

Country	Target insects	Brand name	Microbes	References
USA	Whiteflies/ aphids/thrip	Naturalis	*Beauveria bassiana*	Miller et al. (1983); Starnes et al. (1993); Smith et al. (2003); Kesho (2020)
	Termites	Bio-Blast™	*Metarhizium anisopliae*	
	Whiteflies/ thrips	PFR-97™	*Isaria fumosorosea* (erstwhile *Paecilomyces fumosoroseus*)	
	Sucking insects	Mycotrol	*Beauveria bassiana*	
	Forest tent caterpillars; gypsy moth	Dipel 176	*Bacillus thuringiensis* subsp. *israelensis*	
	Fungus gnats	VectoBac	*Bacillus thuringiensis*	
	Colorado potato and elm leaf beetle	Novodor Flow Able	*Bacillus thuringiensis* subsp. *tenebrionis*	
UK	Locusts, grasshoppers	Green muscle	*Metarhizium flavoviride*	Allsopp (2020); Khater (2012)
Germany	Coffee berry borer	Conidia	*Beauveria bassiana*	Khan et al. (2012); Gani et al. (2019)
	Black vine weevil	BIO 1020	*Metarhizium anisopliae*	
France	Corn borer	Ostrinol	*Beauveria bassiana*	Karimi et al. (2019); Tripathi et al. (2020)
	Scarab beetle larvae	Betel	*Beauveria brongniartii*	
Australia	Scarab beetle larvae	Melocont	*Beauveria brongniartii*	Khun et al. (2021)
	Red-headed cockchafer	BioGreen	*Metarhizium flavoviride*	
Holland	Aphids and whiteflies	Vertalec	*Verticillium lecanii*	Feng et al. (1985); Smith et al. (2003)
	Thrips	Mycotal	*Verticillium lecanii*	
Canada	Gypsy moth; tent caterpillar and cabbage looper	BTK	*Berliner* subsp. *kurstaki*	Marrone (2009); Khan et al. (2015)
	Eastern and western spruce budworm	Bioprotec	*Bacillus thuringiensis*	

9.5 Present Prospectus of Microbial Insecticides in India

In India, 194 chemical pesticides had been reported by 2006, but just 12 biopesticides (including Bt, *Trichoderma*, *Pseudomonas*, and *Beauveria* species) had been evaluated. The number of newly developed and licensed microbial pesticides is increasing at a rate of 4% per year and in the present context biopesticides account for 30% of the market (Gani et al. 2019). A total of 17 *B. thuringiensis* var. *kurstaki* products, 58 *B. bassiana* products, 49 *V. lecanii* products, 11 *M. anisopliae* products, 18 HlNPV products, and 3 SlNPV products have been registered for the management of insect pests in India. Scientists from the NBAII, Bangalore, conducted surveys in 2010 to determine the status of microbial pesticide development in the region (Narladkar and Shivpuje 2014; Das et al. 2019). Entomofungal pathogen formulations based on talc are commonly used in pest control programs, which is a common practice in India. These talc formulations have a maximum shelf life of 3–4 months which is very short. As a result, formulations with a minimum shelf life of 18 months are required for better outcomes (Bhat et al. 2021). The effectiveness of entomofungal pathogens in the field is highly dependent on the availability of favorable climatic conditions. The NBAII, Bangalore, conducts routine quality analyses of the products of microbial pesticides produced in the region, and it has been discovered that 50–70% of samples do not meet the Central Insecticide Board requirements (Sharma et al. 2013).

At least 16 insect species have been reported as resistant to *B. thuringiensis* in the last few years. Endotoxins have been found in noctuids such as *Spodoptera frugiperda*, *Busseola fusca*, and *H. zea* in both laboratory and field conditions. Since the majority of microbial pesticides produced today are of low quality, government agencies should step in and enforce stringent quality standards to ensure that microbial BCA products do not harm the atmosphere, humans, or other living species, as well as to avoid the selling of low-quality products to farmers. Before commercial processing, import, or sale, government regulatory agencies (India's Central Insecticides Board, for example) have made microbial pesticide registration mandatory. Data on non-target organisms, toxicological reports on laboratory animals, eco-toxicity, and other effectiveness data must be provided at the time of registration (Białk-Bielińska et al. 2011; Bondarenko et al. 2013).

9.6 Conclusion

Microbial biopesticides are eco-friendly as well as not hazardous for human and animal health. So, it should be given priority over the chemical pesticides globally and in our country also. Remarkable progress has been made in molecular biology, protein engineering, and genetic engineering, which have aided in the development of microbial pesticides. Microbial pesticides have become a hot bed of study in biotechnology institutions and companies due to their superior characteristics. According to research and applications, microbial pesticides are gradually replacing highly toxic pesticides in the market. Despite the many benefits of biopesticides,

their use is not as widespread as it is for chemical pesticides. Short shelf life, sensitivity to environmental factors, costly manufacturing processes, and effectiveness issues are the key causes of it. Advanced research and optimized field trials are the need of the hour to resolve these problems.

References

Abd-Alla AM, Meki IK, Demirbas-Uzel G (2020) Insect viruses as biocontrol agents: challenges and opportunities. In: El-Wakeil N, Saleh M, Abu-hashim M (eds) Cottage industry of biocontrol agents and their applications. Springer, Cham, pp 277–295. https://doi.org/10.1007/978-3-030-33161-0_9

Abrol DP, Shankar U (2014) Pesticides, food safety and integrated pest management. In: Pimentel D, Peshin R (eds) Integrated pest management. Springer, Dordrecht, The Netherlands, pp 167–199. https://doi.org/10.1007/978-94-007-7796-5_7

Al-Ahmadi MS (2019) Pesticides, anthropogenic activities, and the health of our environment safety. In: Larramendy M, Soloneski S (eds) Pesticides-use and misuse and their impact in the environment. IntechOpen, London, pp 1–23. https://doi.org/10.5772/intechopen.84161

Allsopp PG (2020) IPM for Whitegrubs in Australian sugarcane: from a continuing success to regressing to the past. Sugar Tech 23:225–238. https://doi.org/10.1007/s12355-020-00904-01-14

Anselme C, Vallier A, Balmand S et al (2006) Host PGRP gene expression and bacterial release in endosymbiosis of the weevil *Sitophilus zeamais*. Appl Environ Microbiol 72(10):6766–6772. https://doi.org/10.1128/AEM.00942-06

Bhat AH, Chaubey AK, Shokoohi E et al (2021) Molecular and phenotypic characterization of *Heterorhabditis indica* (Nematoda: Rhabditida) nematodes isolated during a survey of agricultural soils in Western Uttar Pradesh, India. Acta Parasit 66:236–252. https://doi.org/10.1007/s11686-020-00279-y

Białk-Bielińska A, Stolte S, Arning J et al (2011) Ecotoxicity evaluation of selected sulfonamides. Chemosphere 85(6):928–933. https://doi.org/10.1016/j.chemosphere.2011.06.058

Bohorova N, Cabrera M, Abarca C et al (1997) Susceptibility of four tropical lepidopteran maize pests to Bacillus thuringiensis CryI-type insecticidal toxins. J Eco Entomol 90(2):412–415. https://doi.org/10.1093/jee/90.2.412

Bondarenko O, Juganson K, Ivask A et al (2013) Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch Toxicol 87(7):1181–1200. https://doi.org/10.1007/s00204-013-1079-4

Boucias DG, Zhou Y, Huang S et al (2018) Microbiota in insect fungal pathology. Appl Microbio Biotech 102(14):5873–5888. https://doi.org/10.1007/s00253-018-9089-z

Butt TM, Coates CJ, Dubovskiy IM et al (2016) Entomopathogenic fungi: new insights into host–pathogen interactions. Adv Genet 94:307–364. https://doi.org/10.1016/bs.adgen.2016.01.006

Chaeychomsri S, Chaeychomsri W, Siruntawineti J (2020) Evidence for covert baculovirus infections in the field-collected Spodoptera Litura larvae. J Adv Agri Technol 7(2):20–27. https://doi.org/10.18178/joaat.7.2.20-27

Charnley AK, Collins SA (2007) Entomopathogenic fungi and their role in pest control. In: Kubicek C, Druzhinina I (eds) Environmental and microbial relationships. The Mycota, vol 4. Springer, Berlin, Heidelberg, pp 159–187. https://doi.org/10.1007/978-3-540-71840-6_10

Das P, Hazarika LK, Kalita S et al (2019) Current state of fungal antagonists with special emphasis on Indian Scenario. In: Khan M, Ahmad W (eds) Microbes for sustainable insect pest management. Sustainability in plant and crop protection. Springer, Cham, pp 365–385. https://doi.org/10.1007/978-3-030-23045-6_14

de Maagd RA, Bravo A, Crickmore N (2001) How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet 17(4):193–199. https://doi.org/10.1016/s0168-9525(01)02237-5

Fedele G, Bove F, González-Domínguez E et al (2020) A generic model accounting for the interactions among pathogens, host plants, biocontrol agents, and the environment, with parametrization for *Botrytis cinerea* on grapevines. Agron 10(2):222. https://doi.org/10.3390/agronomy10020222

Federici BA, Park HW, Bideshi DK (2010) Overview of the basic biology of *Bacillus thuringiensis* with emphasis on genetic engineering of bacterial larvicides for mosquito control. Open Toxinology J 3:154–171

Feng MG, Poprawski TJ, Khachatourians GG (1994) Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Sci Tech 4(1):3–34. https://doi.org/10.1080/09583159409355309

Feng Z, Carruthers RI, Roberts DW et al (1985) Age-specific dose-mortality effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J Invert Patho 46(3):259–264. https://doi.org/10.1016/0022-2011(85)90067-9

Flint ML, Van den Bosch R (1981) A history of pest control. In: Introduction to integrated pest management. Springer, Boston, MA, pp 51–81

Gani M, Hassan T, Saini P et al (2019) Molecular phylogeny of entomopathogens. In: Khan M, Ahmad W (eds) Microbes for sustainable insect pest management. Sustainability in plant and crop protection. Springer, Cham, pp 43–113. https://doi.org/10.1007/978-3-030-23045-6_3

Kachhawa D (2017) Microorganisms as a biopesticides. J Entomol Zool Stud 5(3):468–473

Karimi J, Dara SK, Arthurs S (2019) Microbial insecticides in Iran: history, current status, challenges and perspective. J Invertebr Pathol 165:67–73. https://doi.org/10.1016/j.jip.2018.02.016

Kesho A (2020) Microbial bio-pesticides and their use in integrated pest management. Chem Biomol Engin 5(1):26–34. https://doi.org/10.11648/j.cbe.20200501.15

Khan S, Guo L, Maimaiti Y et al (2012) Entomopathogenic fungi as microbial biocontrol agent. Mol Plant Breed 3(7):85–91. https://doi.org/10.5376/mpb.2012.03.0007

Khan HA, Ahmad A, Mehboob R (2015) Nosocomial infections and their control strategies Asian pacific. J Tropical Biomed 5(7):509–514

Khater HF (2012) Prospects of botanical biopesticides in insect pest management. Pharmacologia 3(12):641–656. https://doi.org/10.7324/JAPS.2012.2546

Khun KK, Ash GJ, Stevens MM et al (2021) Compatibility of Metarhizium anisopliae and Beauveria bassiana with insecticides and fungicides used in macadamia production in Australia. Pest Manag Sci 77(2):709–718. https://doi.org/10.1002/ps.6065

Kumar M, Gautam V, Sharma RK et al (2019) Biopesticides: an alternate to chemical pesticides. Pharma Innov J 8(7):667–672

Lewis LC, Bruck DJ, Prasifka JR et al (2009) *Nosema pyrausta*: its biology, history, and potential role in a landscape of transgenic insecticidal crops. Biol Control 48(3):223–231. https://doi.org/10.1016/j.biocontrol.2008.10.009

Li Z, Alves SB, Roberts DW et al (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. Biocontrol Sci Tech 20(2):117–136. https://doi.org/10.1080/09583150903431665

Lico C, Chen Q, Santi L (2008) Viral vectors for production of recombinant proteins in plants. J Cell Physiol 216(2):366–377. https://doi.org/10.1002/jcp.21423

Lu D, Pava-Ripoll M, Li Z et al (2008) Insecticidal evaluation of Beauveria bassiana engineered to express a scorpion neurotoxin and a cuticle degrading protease. Appl Microbiol Biotechnol 81(3):515–522. https://doi.org/10.1007/s00253-008-1695-8

Maina UM, Galadima IB, Gambo FM et al (2018) A review on the use of entomopathogenic fungi in the management of insect pests of field crops. J Entomol Zoo Stud 6(1):27–32

Marrone PG (2009) Barriers to adoption of biological control agents and biological pesticides. In: Radcliffe EB, Hutchison WD, Cancelado RE (eds) Integrated pest management. Cambridge University Press, Cambridge, UK, pp 163–178. https://doi.org/10.1017/CBO9780511626463.014

Meissle M, Mouron P, Musa T et al (2010) Pests, pesticide use and alternative options in European maize production: current status and future prospects. J Appl Entomol 134(5):357–375. https://doi.org/10.1111/j.1439-0418.2009.01491.x

Miller LK, Lingg AJ, Bulla LA (1983) Bacterial, viral, and fungal insecticides. Science 219(4585): 715–721. https://doi.org/10.1126/science.219.4585.715

Mousseau TA, Dingle H (1991) Maternal effects in insect life histories. Annu Rev Entomol 36(1): 511–534. https://doi.org/10.1146/annurev.en.36.010191.002455

Narayanan K (2004) Insect defence: its impact on microbial control of insect. Cur Sci 86:800–814

Narladkar BW, Shivpuje PR (2014) Prevalence, population dynamics and host preferences of *Culicoides* spp. (Diptera: Ceratopogonidae) of livestock, Marathwada region of Maharashtra state. Veterinary World 7(9):45–52. https://doi.org/10.14202/vetworld.2014.717-726

Nayak SK, Mishra BB (eds) (2020) Frontiers in soil and environmental microbiology. CRC Press, Boca Raton

Nayak SK, Nayak S, Mohanty S et al (2021) Microbial chitinases and their applications: an overview. In: Mishra BB, Nayak SK, Mohapatra S, Samantaray D (eds) Environmental and agricultural microbiology: applications for sustainability. Wiley-Scrivener Publisher, Beverly. https://doi.org/10.1002/9781119525899.ch15

Perez CJ, Shelton AM (1997) Resistance of *Plutella xylostella* (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* Berliner in Central America. J Eco Entomol 90(1):87–93. https://doi.org/10.1093/jee/90.1.87

Qayyum MA, Wakil W, Arif MJ et al (2015) *Bacillus thuringiensis* and nuclear polyhedrosis virus for the enhanced bio-control of *Helicoverpa armigera*. Int J Agri Bio 17(5):62–69. https://doi.org/10.17957/IJAB/15.0025

Rajput VS, Jhala J, Acharya VS (2020) Biopesticides and their mode of action against insect pests: a review. Int J Chem Stud 8(2):2856–2862. https://doi.org/10.22271/chemi.2020.v8.i2ar.9184

Ramanujam B, Rangeshwaran R, Sivakmar G et al (2014) Management of insect pests by microorganisms. Proc Indian Natl Sci Acad 80(2):455–471

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129:S37–S65. https://doi.org/10.1017/s0031182004004925

Raymond B, Johnston PR, Nielsen-LeRoux C et al (2010) *Bacillus thuringiensis*: an impotent pathogen? Trends Microbiol 18(5):189–194. https://doi.org/10.1016/j.tim.2010.02.006

Roberts DW (1989) World picture of biological control of insects by fungi. Mem Inst Oswaldo Cruz 84(3):89–100. https://doi.org/10.1590/S0074-02761989000700018

Roh JY, Choi JY, Li MS et al (2007) *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17(4):547–559

Rosell G, Quero C, Coll J et al (2008) Biorational insecticides in pest management. J Pest Sci 33(2): 103–121. https://doi.org/10.1584/jpestics.R08-01

SabaHasan AA, Purwar A, Khan N et al (2013) Production of extracellular enzymes in the entomopathogenic fungus *Verticillium lecanii*. Bioinformation 9(5):238–242. https://doi.org/10.6026/97320630009238

Sanahuja G, Banakar R, Twyman RM et al (2011) *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotech J 9(3):283–300. https://doi.org/10.1111/j.1467-7652.2011.00595.x

Sanchis V, Bourguet D (2008) *Bacillus thuringiensis*: applications in agriculture and insect resistance management—a review. Agron Sustain Dev 28(1):11–20. https://doi.org/10.1051/agro:2007054

Sarwar M (2015) Usage of biorational pesticides with novel modes of action, mechanism and application in crop protection. Int J Mat ChemPhy 1(2):156–162

Senthil-Nathan S (2015) A review of biopesticides and their mode of action against insect pests. In: Thangavel P, Sridevi G (eds) Environmental sustainability. Springer, New Delhi, pp 49–63. https://doi.org/10.1007/978-81-322-2056-5_3

Sahid NSM, Rahman MM, Kadirgama K et al (2017) Experimental investigation on properties of hybrid nanofluids (TiO2 and ZnO) in water–ethylene glycol mixture. J MechEngSci 11(4): 3087–3094. https://doi.org/10.15282/jmes.11.4.2017.11.0277

Sharma OP, Bhagat S, Gopali JB (2013) Integrated pest management in indian agriculture: achievements, challenges and opportunities. Innov Agri Policy, p 113

Sherwani SI, Khan HA (2015) Modes of action of biopesticides. In: Nollet LML, Rathore HS (eds) Biopesticides handbook. CRC Press, Boca Raton, pp 68–87

Smith KD, Andersen-Nissen E, Hayashi F et al (2003) Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat Immunol 4(12): 1247–1253. https://doi.org/10.1038/ni1011

Starnes RL, Liu CL, Marrone PG (1993) History, use, and future of microbial insecticides. Am Entomol 39(2):83–91. https://doi.org/10.1093/ae/39.2.83

Thakur N, Kaur S, Tomar P et al (2020) Microbial biopesticides: current status and advancement for sustainable agriculture and environment. In: Rastegari AA, Yadav AN, Yadav N (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 243–282. https://doi.org/10.1016/B978-0-12-820526-6.00016-6

Thomas WE, Ellar DJ (1983) *Bacillus thuringiensis* var *israelensis* crystal delta-endotoxin: effects on insect and mammalian cells in vitro and in vivo. J Cell Sci 60(1):181–197

Tripathi AN, Meena BR, Pandey KK et al (2020) Microbial bioagents in agriculture: current status and prospects. In: Rakshit A, Singh H, Singh A, Singh U, Fraceto L (eds) New frontiers in stress management for durable agriculture. Springer, Singapore, pp 331–368. https://doi.org/10.1007/978-981-15-1322-0_20

Wang C, Fan M, Li Z et al (2004) Molecular monitoring and evaluation of the application of the insect-pathogenic fungus *Beauveria bassiana* in southeast China. J Appl Microbio 96(4): 861–870. https://doi.org/10.1111/j.1365-2672.2004.02215.x

Wraight SP, Carruthers RI (1999) Production, delivery, and use of mycoinsecticides for control of insect pests on field crops. In: Hall FR, Menn JJ (eds) Biopesticides: use and delivery. Methods in biotechnology, vol 5. Humana Press, pp 233–269. https://doi.org/10.1385/0-89603-515-8:233

YubakDhoj GC (2006) White grubs (Coleoptera: Scarabaeidae) associated with Nepalese agriculture and their control with the indigenous entomopathogenic fungus "*Metarhizium anisopliae*" (Metsch) Sorokin, Doctoral dissertation, University_of_Basel

Zhang H, Mahunu GK, Castoria R et al (2017) Augmentation of biocontrol agents with physical methods against postharvest diseases of fruits and vegetables. Trends Food Sci Technol 69:36–45. https://doi.org/10.1016/j.tifs.2017.08.020

Zhang S, Zhang YJ, Li ZL (2019) Complete mitogenome of the entomopathogenic fungus *Sporothrix insectorum* RCEF 264 and comparative mitogenomics in Ophiostomatales. Appl Microbiol Biotechnol 103(14):5797–5809. https://doi.org/10.1007/s00253-019-09855-3

In Silico Tools and Approach of CRISPR Application in Agriculture

10

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Abstract

The emergence of new gene editing technologies poses the ability to transform human healthcare, lifestyle, and agriculture. The groundbreaking implications of genome editing have already been showcased in crops and agricultural system. Its successful applications span from breeding of animals and plants to exhibiting resistance to pests and diseases. CRISPR being the pioneered product of research-academia has gained tremendous importance and dominance in the field of genetic engineering. Advances in clustered regularly interspaced short palindromic repeats (CRISPR) have provided platform for large-scale production of engineered products. However, successful application of CRISPR requires precise design and target strategy for Cas protein and guide RNA. The application of CRISPR/Cas systems is limited by the inconsistent efficiency of endonucleases and cleavage at off-targets. Computational tools, platforms, and programs have reduced the hindrance in achieving cleavage efficiency and specificity. This review provides information on tools and platforms that are available in designing of guide RNA, selecting target sites, analyzing output results and efficiency, etc. The updated information on online and off-line tools will prevent CRISPR/Cas off-targeting during in vivo application.

Keywords

CRISPR · Gene editing · Crop improvement · Protospacers · Guide RNA

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https://doi.org/10.1007/978-981-16-9682-4_10

10.1 Introduction

The burden of achieving global food security over the years is increasing and becoming a major challenge for many countries, which needs immediate attention. Limited arable land, crop loss by disease and pests, and maintaining of nutritional quality intensify the problem of food scarcity. Without hampering the natural resources and balance, the focus shifted to enhancing the inherent quality of the plants for increased food production. Traditionally, farmers have to rely on seeds from fewer varieties of crops with higher quality attributes and improved vigor. However, the solution to issues like marginal difference in the selected traits and longer duration of plant breeding programs may take 10–20 years and unpredictable weather change makes it more difficult to increase food production. On the other hand, introduction of new crop species, maximized use of degraded land for agricultural production, plants resisting stressful climate, or geographical areas with drastic climate change would meet the challenge of global food security (Zhang et al. 2018a). Currently, the genetic and species diversification in agricultural systems will solve diverse range of food production challenges (Fernie and Yan 2019).

In the field of biological research, genome editing or genome engineering had created a revolution by manipulating the genome of an organism either directly or indirectly through gene silencing. These genome alterations have proved to be effective in expressing the desirable trait within the organism to fulfil the desired purpose. Since 1960s, the discovery of restriction endonuclease has led the gene alteration and manipulation process. Moreover, metagenomics is also a key player in exploring hidden genetic features and advancing the application of biotechnology in finding novel bioactive compounds, improved biochemical functions, and gene of interest (Baliyarsingh 2020). Subsequently, advances in the recombinant DNA technology as well as traditional homologous recombination methods have quickened the genomics and genomic manipulation. But due to their minimized efficiency, at present new types of restriction endonuclease are being developed and designed like clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein, zinc-finger nucleases (ZNF), and transcription activator-like effector nucleases (TALEN).

Out of these new methods available, the focus here would be widely on clustered regularly interspaced short palindromic repeats/Cas method that is believed to show the best efficiency so far. This method is an easy, comfortable, user-friendly, and well-adopted genome editing tool using RNA-guided endonuclease for producing double-stranded break (DSB) (Khatodia et al. 2016). The CRISPR/Cas (CRISPR-associated protein) system has turned out to be an efficient technology that has the ability to bring transformation in the field of genome engineering. In the present scenario, the Cas9 nuclease is predominantly used when there is a need of target-specific DNA cleavage. In its natural settings, the CRISPR/Cas9 system provides adaptive immunity of bacteria and archaea against the introduced mobile genetic elements. The prokaryote keeps records of viral infection occurred in genome as CRISPR arrays. These CRISPR arrays consist of acquired viral DNA fragments

interspersed by palindromic repeats (called spacers). Majorly studied Cas 9 system is guided by single-guide RNA (sgRNA) or a hybrid of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) to target a specific DNA with sgRNA complementarity (Jinek et al. 2012). After the double-stranded break has been created the genome can be modified as per required, like gene addition, disruption, or correction (site directed) by activating recombinase repair activity (Kim et al. 2011). The whole principle of CRISPR/Cas technology is now adapted in computational tools (online/off-line) to help in designing experiment, finding target sites, constructing target-specific guide RNA, predicting off-target sites, etc. (Sangar et al. 2016). These software have eased the method of performing the experiment with predetermination of the approximate efficiency of result. There is a great need of editing plant genome (especially crops) to develop or to have improved varieties of them that were previously achieved by plant breeding process. Targeted genome editing by CRISPR/Cas is believed to have potential in crop improvement by modifying the plant genome to produce a more valuable product of interest and to meet the surge of food demand globally (Liu et al. 2013). This topic would summarize on the mechanism of CRISPR/Cas in genome editing of plant species and the online tools available for designing the experiment as well as on finding the targets.

10.2 Mechanism of Action

The CRISPR/Cas is now the most powerful technique of gene editing which was discovered from bacterial species back in 1987 as their defense mechanism. This is an important machinery of prokaryotic representation of adaptive immunity, containing Cas protein along with a pair of RNA fragments needed to guide for the specific cleavage of viral genome, ultimately providing the protection against infection. Different forms of Cas proteins are expressed in bacterial cells which perform with minor difference of functions from each other. Short palindromic repeat sequences generated from the foreign DNA get incorporated as spacers (approximately of 20 nucleotides) in the CRISPR array after fragmentation by nuclease activity of Cas (Mei et al. 2016). The spacers are known to help Cas9 protein in recognizing the same viral DNA (as template strand) if encountered again in future (immunity stage or expression) to perform specific fragmentation. During the condition when the cell confronts foreign genome for the first time, regarded as prokaryotic immunization stage or adaptation stage, a different form of Cas is activated and it results in integrating a short fragment of it into the host genome (CRISPR array). There are three different types of CRISPR/Cas editing, type I, II, and III, and these are distinguished mainly based on the type of Cas protein involved. The Cas 3/Cas 6, Cas 9, and Cas 10 are involved in type I, type II, and type III, respectively, having their own cascade protein system required for the activity (Barrangou and Horvath 2017).

Cas protein is a bilobed structural protein with nuclei acid sites, involved in RNA binding (DNA-binding motifs) and restricted core domain. These sites are responsible for forming recognition lobe (REC) followed by a nuclease lobe (NUC)

connected by a helix bridge (Song et al. 2016). Type II system (RNA-guided endonuclease) with Cas 9 cleaves double-stranded DNA with HNH (His-Asn-His) nuclease domain and RuvC-like domain cutting each strand (Wang et al. 2013). The Cas protein is composed mainly of six functional domains and these are REC-I, REC-II, HNH domain, RuvC, bridge helix, and protospacer adjacent motif (PAM) sequence. The main role of PAM sequence is to differentiate self-DNA from non-self-DNA, hence protecting CRISPR arrays from Cas protein's activity against its own genome (Sternberg et al. 2014).

Agricultural methods need a desired change in plant genome to introduce the desired trait into them. CRISPR/Cas and Cpf1 (centromere and promoter factor-1) system has been proved to be a revolutionary method in producing the variant types in plants (Zetsche et al. 2015). CRISPR is provided into plant cells as DNA, RNA, or protein that induces double-stranded break which is then repaired by the cells through annealing of DNA ends (gene KO). The strand joining can also involve inserting different gene sequences at DSB or by sequence replacement (Gao 2018).

10.3 CRISPR Role in Agriculture Advancement

10.3.1 Overview of CRISPR Application in Agriculture

CRISPR/Cas system is able to produce required plant germplasm by specific alteration of gene and developing mutated genome that showed gain of trait by insertion and/or loss of function of undesired gene of interest by deletion. Initial studies of crop improvement were focused around increasing the yield by manipulating factors affecting it (Zhu et al. 2020). Practically, expression of cytokinin is the likely target for improving the yield of cereals and the enzymes involved are cytokinin activation enzyme and cytokinin dehydrogenase (CKX). Thus, modifying the ends of cytokinin activation enzyme and knocking out CKX from the cell increase the yield in different environmental conditions (Zhang et al. 2019; Wang et al. 2020). Along with increasing yield, quality improvement is equally essential for a crop to be considered as healthy for consumption. For example, amylose content in crops is desired at different levels, where low amylose is better suited in grains and high amylose content in cereals is valuable to human health. The enzyme granule-bound starch synthase 1 (GBSS1) is important for amylose biosynthesis and CRISPR technique proven to be successful in targeting its pathway of biosynthesis (Sun et al. 2017).

Crops are threatened by many types of disease-causing organisms during development, mainly by bacteria and viruses. On the other hand, microbes of soil are key players in maintaining soil structural integrity, promoting plant growth, and thereby increasing food productivity (Baliyarsingh et al. 2017). CRISPR/Cas technique can help in reducing the biotic stress over crops. In case of viruses, Cas9 protein can be programmed to cleave the DNA of infecting virus and can also trigger transcription of certain genes whose products are required for inhibiting bacterial infection to confer virus and bacterial infection, respectively (Ji et al. 2018). Similarly inducing herbicide resistance in plant maintains and improves high crop productivity. The key

enzyme, acetolactate synthase (ALS) which is involved in amino acid synthesis, is the primary target of many herbicides (like sulfonylurea and imidazolinone). Thus studies on introduction of specific substitution of amino acid by CRISPR/Cas showed herbicide tolerance (Powles and Yu 2010).

Breeding technologies can be approached using CRISPR/Cas for add-on benefits to agricultural production. To fix the genetic background of hybrid plants haploid induction can be achieved in fewer generations with CRISPR than traditional methods. Targeting certain genes for mutation like MTL (coding phospholipase A1), CENH3, and DMP by CRISPR can cause haploid formation (Liu et al. 2017a; Zhong et al. 2020). Hybrid seeds are effectively produced by eliminating self-pollination of female organ, i.e., by inducing male sterility in maternal plants (Okada et al. 2019). Hybrid vigor can be fixed by eliminating meiosis recombination (by passing second meiosis) and keeping mitosis to develop clonal multiploidy gametes (Wang et al. 2019).

10.3.2 In Silico-Assisted Gene Editing Using CRISPR/Cas

The dependency of engineered nucleases and guide RNAs on gene editing processes is well established. However, its application is limited by their off-target DNA cleavage leading to cellular toxicity. Different organisms possess a variety of Cas9 proteins that utilize different PAM sequences. Moreover, evidence of RNA-guided endonucleases (RGENs) cleaving DNA at off-targets with several mismatches (Fu et al. 2013; Cho et al. 2014) or causing addition/deletion of nucleotides (Lin et al. 2014) has hindered the application in the healthcare to agriculture. In silico approach and tools have benefited in overcoming these issues. Although the CRISPRs' in silico analyses began in mid-1990s (Mojica et al. 1995), the progress in the development of CRISPR software tool has been slow. Initial software tools used to identify particular repeats had to screen and discard the background manually and sometimes short CRISPR clusters were missed or neglected. Since then many researchers have been developing and presenting computational tools that help in selecting appropriate targets, designing guide RNAs, PAMs, and output analysis (Sander et al. 2010; Bae et al. 2014; Heigwer et al. 2014; Naito et al. 2015) (Table 10.1).

10.4 Applications of CRISPR in Agriculture

Gene editing by CRISPR is being widely accepted for creating noble plant varieties with desired phenotype that further helps in yield improvement, quality improvement, and stress resistance to abiotic and biotic factors. The gene of interest is altered to generate In-Dels or to produce a desired type of crop variety by changing the level of expression. Online tools, software, and databases are providing the medium to access the gene and its target sequences for gene editing. With crop improvement being the major objective of genome editing process, the knocking-out and

Table 10.1 In silico tools that are helpful in designing and guiding CRISPR/Cas gene editing

Sl. no.	Software	Molecule involved	Target	Application	Reference
1.	CRISPR-GE	Cas9 or Cpf1	sgRNAs	Off-target site prediction and primer designing	Xie et al. (2017)
2.	PhytoCRISP-Ex	Cas9	PAM sequence	Searching target sites of Cas9	Rastogi et al. (2016)
3.	CRISPR-P	Cas9	Guide sequence	Searches for highly specific Cas9 targets in interested DNA sequence	Lei et al. (2014)
4.	GuideScan	PAM and gRNA	Guide sequence	Designing comprehensive guide RNA database	Perez et al. (2017)
5.	sgRNAcas9	Cas9	sgRNA	Quick designing of sgRNA with low off-target effects	Xie et al. (2014)
6.	PrimeDesign	pegRNA and ngRNA	Design of PE experiment	Automatic designing of pegRNA and ngRNA	Hsu et al. (2021)
7.	CRISPRseek	PAM and gRNA	gRNA	Constructing target-specific guide RNA with known PAM sequence	Zhu et al. (2014)
8.	CRISPRdirect	PAM	gRNA selection	Finding target sites with minimum off-target candidates and it is a repository of off-target sites from few organisms	Naito et al. (2015)
9.	CHOPCHOP	Genome sequence	Off-target sites	Prediction of binding off-target with TALENs	Montague et al. (2014)
10.	CRISPRTarget		Protospacers	Identification of protospacer targets	Biswas et al. (2013)
11.	CRISPRer	PAM and seed sequence	Protospacers	Selection of CRISPR/Cas protospacer by comparing with seed sequence	Sangar et al. (2016)
12.	E-CRISP	Cas9 nuclease	gRNA	Used to design gRNA and it is a fast approach to find the binding sites (complementary to gRNA)	Heigwer et al. (2014)
13.	CRISPR-ERA		sgRNA	Designing of sgRNA for editing	Liu et al. (2015)
14.	CRISPRfinder		CRISPR loci	A tool for detecting CRISPR and PAM sequence	Grissa et al. (2007)
15.	Cas-OFFinder		Off-target sites	Finds potential off-target sites in user-defined sequence	Bae et al. (2014)
16.	Cas-Designer	PAM sequence	gRNA	Used for gRNA selection and finding potential off-targets	Park et al. (2015)

(continued)

Table 10.1 (continued)

Sl. no.	Software	Molecule involved	Target	Application	Reference
17.	DESKGEN		Experiment design	Designing CRISPR experiment: setting up and analyzing the experiment	Hough et al. (2016)
18.	caRpools		Result screen analysis	Experimental data analysis and workflow analysis	Winter et al. (2016)

Cas9 CRISPR-associated protein 9; *Cpf1* CRISPR from *Prevotella* and *Francisella* 1; *sgRNA (gRNA)* single-guide RNA (guide RNA); *PAM* protospacer adjacent motif; *PE experiment* prime editing experiment; *pegRNA* prime editing guide RNA; *ngRNA* nicking single-guide RNA; *TALEN* transcription activator-like effector nuclease

knocking-in techniques are major players in achieving the quality improvement in crops over the wild variety. A notable example is targeting of GW5 protein (inhibiting the kinase activity of GSK2), a positive regulator of signaling pathway that controls grain width and weight of rice. The expression of GW5 gene can be altered by knockout-based method according to the yield requirement in crops (Liu et al. 2017b). In a similar study, by pedigree analysis, whole-genome sequencing (WGS), and CRISPR/Cas-based knockout a large gene in rice involved in high yield was identified. The genes essential for the production of rice also showed associated phenotype alteration at different loci such as plant height and flowering time (Huang et al. 2018).

Quantity improvement by gene editing is focused on altering nutritional value, storage capacity, and major content of the crop. With rice being the major dietary food of many countries, the starch content is targeted for reduction for improved cooking and rice eating. Waxy gene knockout led to the production of low amylose content in grains by CRISPR/Cas9 editing which did not affect any other trait in the crop (Zhang et al. 2018b). And same waxy gene is also deleted in case of corn line (six genes coding for polyphenol oxidase) for high yield and quality crop production (Waltz 2016). The alpha-gliadin genes in cereal are reduced to downregulate gluten protein (that causes celiac disease in humans). CRISPR is used to knock down alpha-gliadin in wheat with no off-target mutation other than the potential target observed (Sánchez-León et al. 2018). Seeds are edited with CRISPR/Cas9 for high oleic acid content to reduce steroid toxicity level and to increase the shelf life of camelina (Morineau et al. 2017) and tomatoes (mutation in lncRNA 14,559 gene) (Li et al. 2018a).

CRISPR is not limited to increasing the yield but to alleviate various other challenges of crop production and protection (Table 10.2). This site-specific gene editing technique is helpful in facing the biotic stress by inducing resistance to bacteria, viruses, fungus, and insects. Rice genes are targeted to produce resistance to fungal disease by knockout of OsERF922 transcriptional factor gene (Wang et al.

Table 10.2 Application of CRISPR tools in agriculture

Sl. no.	Online tool	Targeted crop	Desired effect	Reference
1.	CRISPR-GE	Maize	Comparison of gene editing efficiency with different Cas proteins to detect mutation at the desired site in *O2* gene	Gong et al. (2021)
2.	CRISPR-GE	Rice	Controlling amylose synthesis by editing *Waxy (Wx)* gene for quality improvement	Zeng et al. (2020)
3.	CRISPR RGEN and CRISPR-P	Grape	Deleting *VvWRKY52* gene (transcriptional factor) increases biotic stress resistance (resistance against pathogen)	Wang et al. (2018)
4.	Cas-OFFinder	Rice	Analysis of any off-target mutation in rice due to CRISPR editing	Liu et al. (2021)
5.	CRISPR-P	Chardonnay	Site-specific mutation in L-idonate dehydrogenase gene (*IdnDH*) to reduce synthesis and accumulation of tartaric acid	Ren et al. (2016)
6.	Guide design resources	Potato	Mutation in granule-bound starch synthase (*GBSSI*) gene to produce waxy potatoes having amylopectin	Andersson et al. (2017)
7.	CRISPR-P	Soybean	sgRNA to edit soybean hairy root and its gene function analysis (*GmFEI2* and *GmSHR* endogenous gene) by CRISPR	Cai et al. (2015)
8.	CRISPR-P	Tomato	Targeting insertion and deletion in *RIN* (MADS-box transcription factor) gene of tomato genome to state its role in fruit ripening	Ito et al. (2015)
9.	CRISPR-GE	Rice	Developing herbicide tolerance allele by generating In-Dels in acetolactate synthetase (*OsALS*) gene by CRISPR/Cas9 editing	Wang et al. (2021a)
10.	CRISPR-P	Tomato	*SBP-CNR* and *NAC-NOR* transcriptional factor gene editing which is involved in fruit ripening	Gao et al. (2019)
11.	CRISPR-P	Tomato	Knocking out *SGR1*, *Blc*, and *LCY* gene to reduce conversion of lycopene to carotene	Li et al. (2018b)
12.	SSFinder	Banana	Termination of *RAS-PDS1* and *RAS-PDS2* gene by inserting a stop codon in between resulting in carotenoid content	Kaur et al. (2018)
13.	CRISPR-P and CHOPCHOP	Maize	Targeting functional genes of maize to find its effectivity in editing	Hunter (2021)
14.	CRISPR RGEN and CRISPR-P	Grapevine	Targeting *VvbZIP36* gene (transcriptional factor) to find any off-target regions edited through WGS	Wang et al. (2021b)
15.	CRISPR-P	Cotton	Knocking out GhFAD2 gene to increase oleic acid content reducing linoleic acid to have better oxidative stability in cottonseed oil	Chen et al. (2021)

2016), bacterial blight by deleting OsSWEET13 gene (Zhou et al. 2015), and viral disease resistance rice varieties (Macovei et al. 2018). The CRISPR system has been used to make improved crops, for example cassava resistance to brown streak disease and mosaic virus, resistance in spinach to downy mildew, and resistance to fire blight diseases (Ricroch et al. 2016).

10.5 Future Prospects

In plant breeding process the commonly accepted practice is to use improved variations that have aroused from natural or induced mutagenesis. Both the traditional breeding strategies and gene modification technologies have facilitated the finding and generation of newer traits. Genome editing in crops not only holds the promise of speeding up plant breeding programs but also helps in achieving novel agro-traits like resistance against stress, pests, and diseases; improvement of food quality; increase of yield; and limited use of natural resources. Genome editing techniques like CRISPR that uses site-directed nucleases have proven to advance the crop improvement process by precisely editing the required gene of interest. This target-specific gene editing technique is well controlled than other gene alteration techniques as the risk of off-target/site mutations is minimal. The Cas protein has been very precise in cleaving gene at a particular site and has been in use in designing sgRNA for target-specific gene editing. Having such incomparable ability, they have been used to modify large crop and plant varieties as well as wild crop varieties are being targeted for manipulation in order to incorporate change to meet the current food demand of the society.

Moreover, a number of online tools or software have been developed to ease the process of guide sequence generation and identification of CRISPR targets. Apart from sgRNA, PAM sequence and protospacer recognition are also feasible using the online tools. Online tools are serving as a fast and accurate platform for CRISPR experiment designing with additional advantages of off-target prediction which may have partial dysfunctional effect over other exons, not intended for editing. All the potential parameters can be considered at once with online tools and it reduces the hit-and-trial success. Most of the CRISPR gene editing done with the help of online tools is showing effective results than traditional methods or manual designing of targets. CRISPR/Cas is evolving slowly and will tend to improve crops and plant biotechnology over the ages. Addressing food security and sustainability of world, genome editing holds promise in developing new plant and animal varieties that would meet the global challenge while preserving the environment and natural resources.

References

Andersson M, Turesson H, Nicolia A et al (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Rep 36:117–128. https://doi.org/10.1007/s00299-016-2062-3

Bae S, Park J, Kim J-S (2014) Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30:1473–1475. https://doi.org/10.1093/bioinformatics/btu048

Baliyarsingh B, Nayak SK, Mishra BB (2017) Soil microbial diversity: an ecophysiological study and role in plant productivity. In: Advances in soil microbiology: recent trends and future prospects. Springer, Singapore, pp 1–17. https://doi.org/10.1007/978-981-10-7380-9_1

Baliyarsingh B (2020) Metagenomics: the approach and techniques for finding new bioactive compounds. In: Frontiers in soil and environmental microbiology. CRC Press, pp 107–114. https://doi.org/10.1201/9780429485794-12

Barrangou R, Horvath P (2017) A decade of discovery: CRISPR functions and applications. Nat Microbiol 2:1–9. https://doi.org/10.1038/nmicrobiol.2017.92

Biswas A, Gagnon JN, Brouns SJ et al (2013) CRISPRTarget: bioinformatic prediction and analysis of crRNA targets. RNA Biol 10:817–827. https://doi.org/10.4161/rna.24046

Cai Y, Chen L, Liu X et al (2015) CRISPR/Cas9-mediated genome editing in soybean hairy roots. PLoS One 10:e0136064. https://doi.org/10.1371/journal.pone.0136064

Chen Y, Fu M, Li H et al (2021) High-oleic acid content, nontransgenic allotetraploid cotton (Gossypium hirsutum L.) generated by knockout of GhFAD2 genes with CRISPR/Cas9 system. Plant Biotechnol J 19(424). https://doi.org/10.1111/pbi.13507

Cho SW, Kim S, Kim Y et al (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res 24:132–141. https://doi.org/10.1101/gr.162339.113

Fernie AR, Yan J (2019) De novo domestication: an alternative route toward new crops for the future. Mol Plant 12:615–631. https://doi.org/10.1016/j.molp.2019.03.016

Fu Y, Foden JA, Khayter C et al (2013) High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol 31:822–826. https://doi.org/10.1038/nbt.2623

Gao C (2018) The future of CRISPR technologies in agriculture. Nat Rev Mol Cell Biol 19:275–276. https://doi.org/10.1038/nrm.2018.2

Gao Y, Zhu N, Zhu X et al (2019) Diversity and redundancy of the ripening regulatory networks revealed by the fruit ENCODE and the new CRISPR/Cas9 CNR and NOR mutants. Hortic Res 6:1–10. https://doi.org/10.1038/s41438-019-0122-x

Gong C, Huang S, Song R, Qi W (2021) Comparative study between the CRISPR/Cpf1 (Cas12a) and CRISPR/Cas9 systems for multiplex gene editing in maize. Agriculture 11:429. https://doi.org/10.3390/agriculture11050429

Grissa I, Vergnaud G, Pourcel C (2007) CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. https://doi.org/10.1093/nar/gkm360

Heigwer F, Kerr G, Boutros M (2014) E-CRISP: fast CRISPR target site identification. Nat Methods 11:122–123. https://doi.org/10.1038/nmeth.2812

Hough SH, Ajetunmobi A, Brody L et al (2016) Desktop genetics. Per Med 13:517. https://doi.org/10.2217/pme-2016-0068

Hsu JY, Grünewald J, Szalay R et al (2021) PrimeDesign software for rapid and simplified design of prime editing guide RNAs. Nat Commun 12:1–6. https://doi.org/10.1038/s41467-021-21337-7

Huang J, Li J, Zhou J et al (2018) Identifying a large number of high-yield genes in rice by pedigree analysis, whole-genome sequencing, and CRISPR-Cas9 gene knockout. Proc Natl Acad Sci 115:E7559–E7567. https://doi.org/10.1073/pnas.1806110115

Hunter CT (2021) CRISPR/Cas9 targeted mutagenesis for functional genetics in maize. Plan Theory 10:723. https://doi.org/10.3390/plants10040723

Ito Y, Nishizawa-Yokoi A, Endo M et al (2015) CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. Biochem Bioph Res Co 467:76–82. https://doi.org/10.1016/j.bbrc.2015.09.117

Ji X, Si X, Zhang Y et al (2018) Conferring DNA virus resistance with high specificity in plants using virus-inducible genome-editing system. Genome Biol 19:1–7. https://doi.org/10.1186/s13059-018-1580-4

Jinek M, Chylinski K, Fonfara I et al (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821. https://doi.org/10.1126/science.1225829

Kaur N, Alok A, Kaur N et al (2018) CRISPR/Cas9-mediated efficient editing in phytoene desaturase (PDS) demonstrates precise manipulation in banana cv. Rasthali genome. Funct Integr Genomic 18:89–99. https://doi.org/10.1007/s10142-017-0577-5

Khatodia S, Bhatotia K, Passricha N et al (2016) The CRISPR/Cas genome-editing tool: application in improvement of crops. Front Plant Sci 7:506. https://doi.org/10.3389/fpls.2016.00506

Kim H, Um E, Cho S-R et al (2011) Surrogate reporters for enrichment of cells with nuclease-induced mutations. Nat Methods 8:941. https://doi.org/10.1038/nmeth.1733

Lei Y, Lu L, Liu H-Y et al (2014) CRISPR-P: a web tool for synthetic single-guide RNA design of CRISPR-system in plants. Mol Plant 7:1494–1496. https://doi.org/10.1093/mp/ssu044

Li R, Fu D, Zhu B et al (2018a) CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. Plant J 94:513–524. https://doi.org/10.1111/tpj.13872

Li X, Wang Y, Chen S et al (2018b) Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front Plant Sci 9:559. https://doi.org/10.3389/fpls.2018.00559

Lin Y, Cradick TJ, Brown MT et al (2014) CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. Nucleic Acids Res 42:7473–7485. https://doi.org/https://doi.org/10.1093/nar/gku402

Liu C, Li X, Meng D et al (2017a) A 4-bp insertion at ZmPLA1 encoding a putative phospholipase A generates haploid induction in maize. Mol Plant 10:520–522. https://doi.org/10.1016/j.molp.2017.01.011

Liu G, Qi Y, Zhang T (2021) Analysis of off-target mutations in CRISPR-edited rice plants using whole-genome sequencing. In: Rice genome engineering and gene editing. Springer, pp 145–172. https://doi.org/10.1007/978-1-0716-1068-8_10

Liu H, Wei Z, Dominguez A et al (2015) CRISPR-ERA: a comprehensive design tool for CRISPR-mediated gene editing, repression and activation. Bioinformatics 31:3676–3678. https://doi.org/10.1093/bioinformatics/btv423

Liu J, Chen J, Zheng X et al (2017b) GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nat Plants 3:1–7. https://doi.org/10.1038/nplants.2017.43

Liu W, Yuan JS, Stewart CN Jr (2013) Advanced genetic tools for plant biotechnology. Nat Rev Genet 14:781–793. https://doi.org/10.1038/nrg3583

Macovei A, Sevilla NR, Cantos C et al (2018) Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant Biotechnol J 16:1918–1927. https://doi.org/10.1111/pbi.12927

Mei Y, Wang Y, Chen H et al (2016) Recent progress in CRISPR/Cas9 technology. J Genet Genomics 43:63–75. https://doi.org/10.1016/j.jgg.2016.01.001

Mojica FJM, Ferrer C, Juez G, Rodríguez-Valera F (1995) Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. Mol Microbiol 17:85–93. https://doi.org/10.1111/j.1365-2958.1995.mmi_17010085.x

Montague TG, Cruz JM, Gagnon JA et al (2014) CHOPCHOP: a CRISPR/Cas9 and TALEN web tool for genome editing. Nucleic Acids Res 42:W401–W407. https://doi.org/10.1093/nar/gku410

Morineau C, Bellec Y, Tellier F et al (2017) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant Biotechnol J 15:729–739. https://doi.org/10.1111/pbi.12671

Naito Y, Hino K, Bono H, Ui-Tei K (2015) CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off-target sites. Bioinformatics 31:1120–1123. https://doi.org/10.1093/bioinformatics/btu743

Okada A, Arndell T, Borisjuk N et al (2019) CRISPR/Cas9-mediated knockout of Ms1 enables the rapid generation of male-sterile hexaploid wheat lines for use in hybrid seed production. Plant Biotechnol J 17:1905–1913. https://doi.org/10.1111/pbi.13106

Park J, Bae S, Kim J-S (2015) Cas-Designer: a web-based tool for choice of CRISPR-Cas9 target sites. Bioinformatics 31:4014–4016. https://doi.org/10.1093/bioinformatics/btv537

Perez AR, Pritykin Y, Vidigal JA et al (2017) GuideScan software for improved single and paired CRISPR guide RNA design. Nat Biotechnol 35:347–349. https://doi.org/10.1038/nbt.3804

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347. https://doi.org/10.1146/annurev-arplant-042809-112119

Rastogi A, Murik O, Bowler C, Tirichine L (2016) PhytoCRISP-Ex: a web-based and stand-alone application to find specific target sequences for CRISPR/CAS editing. BMC Bioinform 17:1–4. https://doi.org/10.1186/s12859-016-1143-1

Ren C, Liu X, Zhang Z et al (2016) CRISPR/Cas9-mediated efficient targeted mutagenesis in Chardonnay (*Vitis vinifera* L.). Sci Rep 6:1–9. https://doi.org/10.1038/srep32289

Ricroch A, Harwood W, Svobodová Z et al (2016) Challenges facing European agriculture and possible biotechnological solutions. Crit Rev Biotechnol 36:875–883. https://doi.org/10.3109/07388551.2015.1055707

Sánchez-León S, Gil-Humanes J, Ozuna CV et al (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16:902–910. https://doi.org/10.1111/pbi.12837

Sander JD, Maeder ML, Reyon D et al (2010) ZiFiT (Zinc Finger Targeter): an updated zinc finger engineering tool. Nucleic Acids Res 38:W462–W468. https://doi.org/10.1093/nar/gkq319

Sangar VC, Samant L, Pawar S et al (2016) Online and offline tools: Crispr/Cas off-target detection. Int J Pharm Sci Res 7:1889. https://doi.org/10.13040/IJPSR.0975-8232.7(5).1889-95

Song G, Jia M, Chen K et al (2016) CRISPR/Cas9: a powerful tool for crop genome editing. Crop J 4:75–82. https://doi.org/10.1016/j.cj.2015.12.002

Sternberg SH, Redding S, Jinek M et al (2014) DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature 507:62–67. https://doi.org/10.1038/nature13011

Sun Y, Jiao G, Liu Z et al (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Front Plant Sci 8:298. https://doi.org/10.3389/fpls.2017.00298

Waltz E (2016) CRISPR-edited crops free to enter market, skip regulation. Nat Biotechnol 34:582. https://doi.org/10.1038/nbt0616-582

Wang C, Liu Q, Shen Y et al (2019) Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nat Biotechnol 37:283–286. https://doi.org/10.1038/s41587-018-0003-0

Wang C, Wang G, Gao Y et al (2020) A cytokinin-activation enzyme-like gene improves grain yield under various field conditions in rice. Plant Mol Biol 102:373–388. https://doi.org/10.1007/s11103-019-00952-5

Wang F, Wang C, Liu P et al (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS One 11:e0154027. https://doi.org/10.1371/journal.pone.0154027

Wang F, Xu Y, Li W et al (2021a) Creating a novel herbicide-tolerance OsALS allele using CRISPR/Cas9-mediated gene editing. Crop J 9:305–312. https://doi.org/10.1016/j.cj.2020.06.001

Wang H, Yang H, Shivalila CS et al (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell J 153:910–918. https://doi.org/10.1016/j.cell.2013.04.025

Wang X, Tu M, Wang D et al (2018) CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. Plant Biotechnol J 16:844–855. https://doi.org/10.1111/pbi.12832

Wang X, Tu M, Wang Y et al (2021b) Whole-genome sequencing reveals rare off-target mutations in CRISPR/Cas9-edited grapevine. Hortic Res 8:1–11. https://doi.org/10.1038/s41438-021-00549-4

Winter J, Breinig M, Heigwer F et al (2016) caRpools: an R package for exploratory data analysis and documentation of pooled CRISPR/Cas9 screens. Bioinformatics 32:632–634. https://doi.org/10.1093/bioinformatics/btv617

Xie S, Shen B, Zhang C et al (2014) sgRNAcas9: a software package for designing CRISPR sgRNA and evaluating potential off-target cleavage sites. PLoS One 9:e100448. https://doi.org/10.1371/journal.pone.0100448

Xie X, Ma X, Zhu Q et al (2017) CRISPR-GE: a convenient software toolkit for CRISPR-based genome editing. Mol Plant 10:1246–1249. https://doi.org/10.1016/j.molp.2017.06.004

Zeng D, Liu T, Ma X et al (2020) Quantitative regulation of Waxy expression by CRISPR/Cas9-based promoter and 5'UTR-intron editing improves grain quality in rice. Plant Biotechnol J 18:2385–2387. https://doi.org/10.1111/pbi.13427

Zetsche B, Gootenberg JS, Abudayyeh OO et al (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell J 163:759–771. https://doi.org/10.1016/j.cell.2015.09.038

Zhang H, Li Y, Zhu J-K (2018a) Developing naturally stress-resistant crops for a sustainable agriculture. Nat Plants 4:989–996. https://doi.org/10.1038/s41477-018-0309-4

Zhang J, Zhang H, Botella JR, Zhu J-K (2018b) Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. J Integr Plant Biol 60:369–375. https://doi.org/10.1111/jipb.12620

Zhang Z, Hua L, Gupta A et al (2019) Development of an Agrobacterium-delivered CRISPR/Cas9 system for wheat genome editing. Plant Biotechnol J 17:1623–1635. https://doi.org/10.1111/pbi.13088

Zhong Y, Chen B, Li M et al (2020) A DMP-triggered in vivo maternal haploid induction system in the dicotyledonous Arabidopsis. Nat Plants 6:466–472. https://doi.org/10.1038/s41477-020-0658-7

Zhu H, Li C, Gao C (2020) Applications of CRISPR–Cas in agriculture and plant biotechnology. Nat Rev Mol Cell Biol 21:661–677. https://doi.org/10.1038/s41580-020-00288-9

Zhu LJ, Holmes BR, Aronin N, Brodsky MH (2014) CRISPRseek: a bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. PLoS One 9:e108424. https://doi.org/10.1371/journal.pone.0108424

Zhou J, Peng Z, Long J, Sosso D, Liu B, Eom JS, Huang S, Liu S, Vera Cruz C, Frommer WB (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J 82(4):632–643. https://doi.org/10.1111/tpj.12838

Application of Bioinformatics in the Plant Pathology Research

11

Raghunath Satpathy

Abstract

Bioinformatics is the study of molecular biological data using techniques of computer sciences and statistical analysis to solve the biological problem. The main task of bioinformatics is to store, process and analyse the huge biological data obtained from the experimental research. Similarly, plant pathology involves the study of the basis of plant disease resistance, identification of the pathogens, disease aetiology, disease cycles, genetics of pathology and management of plant diseases. Therefore, to understand the molecular mechanism of pathogenesis of plant pathogens is a major aspect of plant biology. So various bioinformatics-based methods and tools have been developed for comparative genomic analysis, evolutionary analysis, genome-wide association study (GWAS), molecular modelling methods and so on. The specific analysis includes illustrating the mechanism behind the plant-pathogen interaction and predictions of the accurate location of the disease-causing genes on the genome that lead to the development of the disease-free transgenic plant. Comparative genomics of plant pathogens of an emerging crop is one of the effective approaches of bioinformatics-based analysis. Similarly, the development of unique user-friendly bioinformatics database resources of different aspects of plant pathology will facilitate the sharing of information among the scientific communities and will ultimately be beneficial to plant breeders and farmers. This chapter focuses on the recent challenges and opportunities in plant pathology research by narrating the literature and how the bioinformatics methods have been used to solve the problems.

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_11

Keywords

Comparative genomics · GWAS · Plant genomics · Database · Plant-pathogen interaction · Molecular modelling

11.1 Introduction

Plants are the major sources of food, fibre and fuel in the agriculture sector and hence play a dominant role in the world economy. Plant pathogens cause a major threat to and are responsible for the huge loss in crops by causing diseases in plants. Also, plant pathogens spread very quickly while infecting a healthy plant from a diseased plant. So the primary challenge associated with a plant pathologist is to minimise crop loss by eradiating the plant pathogen (Mack et al. 2000; Mitra 2021). Plants are persistently under the threat of several pathogens like bacteria, viruses, fungi, nematodes and others. However, molecular complicacy in plant-pathogen interactions makes it difficult to interpret. The plant pathogens that cause disease in plants are directly responsible for food security and scarcity and ultimately even threaten human health. However, plants also contain a specific immune system that provides resistance to the pathogen. Plants have evolved highly sophisticated mechanisms to resist pathogens by using different barriers and induction of specific signalling pathways. The induction of several metabolic pathways in the plant system also requires the recognition of the pathogen by pathogen-derived factors and by specific proteins (effector molecules) that are encoded by pathogens. However, if the pathogen is suppressed, these factors enable them to infect and cause diseases in plants. Due to recent developments in the fundamental biological research, many of the interesting molecular mechanisms regarding infection of pathogen, effector molecule and modulator activity of the immune systems are known (Kachroo et al. 2017; Zhang et al. 2013; de Wit 2007). In addition to the fundamental molecular biological research, due to the advancement of genomic technologies, there is a flooding of a huge amount of genomic information for the analysis by in silico methods. However, challenges exist to validate the biological data as well as for proper prediction and interpretation. In this aspect, *bioinformatics*-based analysis plays a major role in the management of data. Bioinformatics is generally defined as the application of computational techniques to the storing, processing and managing of the biological data that are usually generated from molecular biological experiments. The ultimate objective of bioinformatics is the functional and statistically reliable prediction from the given biological data. To facilitate this, several categories of databases, web servers as well as executable software are being developed and many are currently available with a suitable interface for analysis and interpretation of these data. Bioinformatics-based analysis facilitates to open the door to understand the complex biological processes by implementing the genomic and protein sequence analysis, advanced data mining and machine learning algorithms on biological data and molecules (Fig. 11.1). So, the new knowledge can be suitably used for several aspects of biotechnological

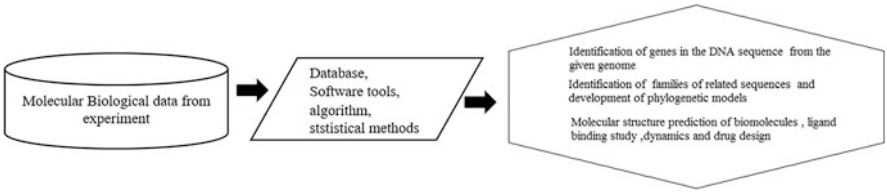

Fig. 11.1 Basic tasks of bioinformatics

research (Untergasser et al. 2007; Mishra et al. 2016; Singh et al. 2011; Satpathy 2014; Satpathy et al. 2015).

The challenge in controlling plant diseases lies upon the molecular basis of identification of the key pathogenic factors that are responsible for spreading in case of a specific plant pathogen. Many of these molecular pieces of information are available that can be suitably analysed by in silico methods. This chapter provides a specific report on the application of specific computational tools and the methods for plant pathogen analysis such as the study of host-pathogen interaction, molecular modelling studies and whole-genome analysis (WGA) used for plant pathology research (Alemu 2015).

11.2 Applications of Bioinformatics in the Plant Pathological Study

Biological databases are a repository of several molecular biological data that are stored in a consistent manner. For example, the database might contain a single file containing several records but each of which having the same set of information. Similar several tools are available to understand the mechanism and function of metabolites and compounds and their pathway details involved in the phytopathological mechanism. Plants having the potential to resists themselves from the infection of a pathogen are known as resistant plants and in this case the host-pathogen interaction is considered as incompatible. Despite the economic impact of plant pathology, the fundamental molecular mechanisms underlying the pathogenicity of pathogens are still poorly understood, which opens the door for implementation of bioinformatics methods (Andersen et al. 2018; Scholthof 2001; Narayanasamy 2008).

From the biological point of view the in-depth study of plant pathogenesis processes includes four different approaches: (a) gene expression analysis, (b) structural and comparative genomics, (c) molecular modelling study and (d) GWAS analysis. Currently, the databases contain many numbers of molecular data of host plants, as well as the information of plant pathological aspects of specific pathogens provides a strong platform to analyse the data (Fig. 11.2). Many of the databases and tools have been developed to perform a thorough analysis specifically in the plant pathology area (Tables 11.1 and 11.2).

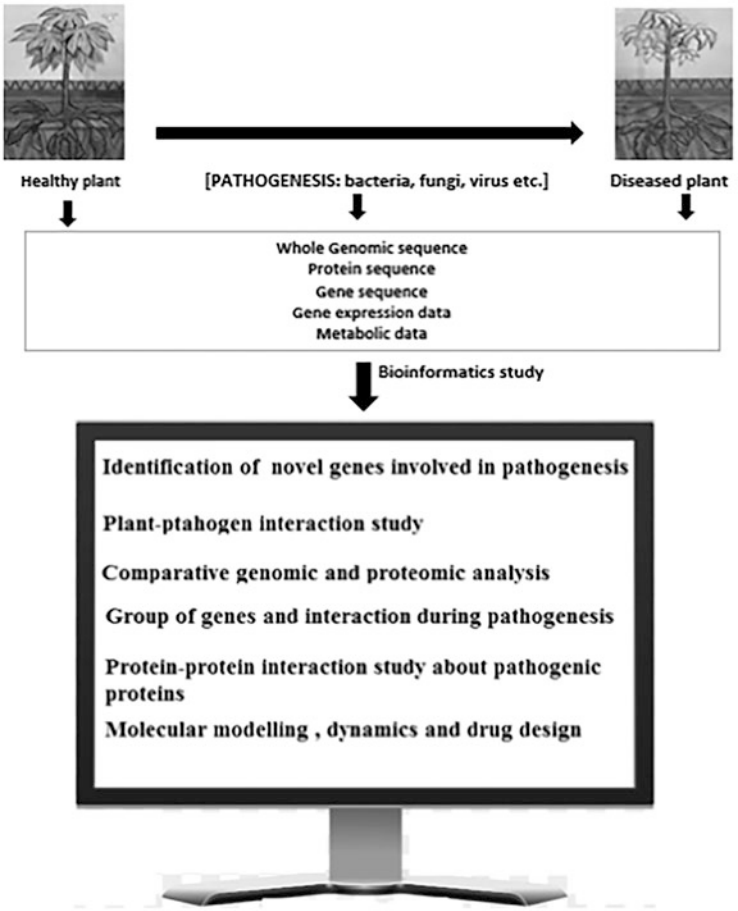

Fig. 11.2 Bioinformatics-based methods to study plant pathology

Some of the specific implementations of the bioinformatics applications for the plant pathology study are described under the following sections:

11.2.1 Plant-Pathogen Interaction Study

Plant-pathogen interactions exhibit several important molecular responses based on which pathogens can colonise and spreading of the disease occurs. For example, some fungi produce secondary metabolites that control a wide range of molecular functions such as the production of virulence factors siderophores and phytotoxins that lead to the establishment of the disease. Shi-Kunne et al. carried out in silico analysis to identify the 25 potential secondary metabolites producing gene clusters in case of *Verticillium dahliae* (Shi-Kunne et al. 2019). Graham-Taylor et al. described

Table 11.1 Database and resources of plant pathology study

Sl. no.	Name	Availability	Description
1	Vegetable MD Online	http://vegetablemdonline.ppath.cornell.edu/	The database provides access to many vegetable diseases
2	Plant Viruses Online	http://bio-mirror.im.ac.cn/mirrors/pvo/vide/refs.htm	Contains the information on most species of virus known to infect plants
3	MIGREW	https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-018-2569-4	A database on molecular identification of genes for resistance in wheat
4	PlaD	http://systbio.cau.edu.cn/plad/index.php	A transcriptomics database for plant defence responses to pathogens
5	PathoPlant	http://www.pathoplant.de/	A database on plant-pathogen interactions as well as the components of signal transduction pathways related to plant pathogenesis process
6	EXPath	http://expath.itps.ncku.edu.tw/	A database of comparative expression analysis of the metabolic pathways for plants
7	Description of plant virus	https://www.dpvweb.net/seqs/plantviruses.phpc	Contains the source of information about viruses, viroids and satellites of plants, fungi and protozoa
8	GenFam	http://mandadilab.webfactional.com/home/	A Web application and database for gene family-based classification and functional enrichment analysis
9	RiceMetaSysB	http://14.139.229.201/RiceMetaSysB/	A database of blast and bacterial blight-responsive genes in rice and its utilisation in identifying key blast-resistant WRKY genes
10	*Phytophthora database*	http://www.phytophthoradb.org/welcome.php?a=intro	Information for the *Phytophthora*, an oomycete plant pathogen
11	*Common Names of Plant Diseases*	https://www.apsnet.org/edcenter/resources/commonnames/Pages/default.aspx	The database contains lists of the given plants, along with the associated pathogens or causes
12	Integrated Microbial Genomes and Microbiomes	https://img.jgi.doe.gov/	Supports the annotation, analysis and distribution of microbial genome and microbiome datasets sequenced at DOE's Joint Genome Institute (JGI)
13	VBI Microbial Database (VMD)	http://phytophthora.vbi.vt.edu/	Contains genome sequence and annotation data of two plant pathogens *Phytophthora sojae* and *Phytophthora ramorum*

(continued)

Table 11.1 (continued)

Sl. no.	Name	Availability	Description
14	*Magnaporthe grisea Oryza sativa* interaction database	www. mgosdb.org	Web-based database contains data from *Oryza sativa* and *Magnaporthe grisea* interaction experiments in which *M. grisea* is the fungal pathogen that causes the rice blast disease
15	MIPS *Fusarium graminearum* Genome Database (FGDB)	http://mips.gsf.de/genre/proj/fusarium/	Genome database on one of the most devastating fungal plant pathogens of wheat and barley
16	OmicsDB:: Pathogens	https://pathogens.omicsdb.org/	Database for exploring functional networks of plant pathogens

the number of gene clusters with a potential role in virulence in *Sclerotinia sclerotiorum* (Graham-Taylor et al. 2020). Computational analysis by Kamal et al. described the identification of interacting regions in *Begomovirus*-encoded βC1 protein with cotton plant (*Gossypium hirsutum*) SnRK1 protein by using computational approaches including sequence recognition, and binding site and interface prediction methods followed by experimental analysis (Kamal et al. 2019). Pavlopoulou described the interacting molecules that are involved in plant defence by building a protein-protein interaction (PPi) network, and provided evidence for prominent crosstalk between the various defence mechanisms to several stresses including pathogen infection (Pavlopoulou et al. 2019). Kaur et al. (2017) analysed the expression pattern and role of pathogenesis-related (PR) proteins (possess antifungal activities such as PR-1, PR-2, PR-5, PR-9, PR-10 and PR-12) in case of *Arabidopsis thaliana* and *Oryza sativa* by using computational analysis. The in silico study about the plant cell wall-degrading enzymes (PCWDEs) has been carried out by Chang et al. (2016). As plant pathogens secrete PCWDEs for the degradation of plant cell walls, to counter this, plants also release some PCWDE inhibitor proteins (PIPs) to reduce the infection. However, some of the species of the pathogen *Fusarium* can escape this PIP inhibition. So in silico study has been performed to understand this resistance mechanism by analysing the genomic structure of the pathogen.

11.2.2 Gene Expression, Structural and Comparative Genomics Study

Study about the expression pattern of pathogenesis-related genes is important to build the computational model of the establishment process of plant diseases. The gene expression analysis also leads to identifying the pathogenic genes and expression profile in different host systems. This finally provides insights into the possible ways of attack and resistance mechanisms involved in the pathogenesis process. In

Table 11.2 Some of the major bioinformatics resources, availability and their application in the plant pathological study

S. no.	Name of the tool/ database	Availability	Domain application
1	Phred/Phrap/Consed	http://www.phrap.org	Sequence assembly
2	Arachne	http://www.broad.mit.edu/wga/	
3	GAP4	http://staden.sourceforge.net/overview.html	
4	TIGR-AMOS	http://www.tigr.org/software/AMOS/	
5	Genscan	http://genes.mit.edu/GENSCAN.html	Gene prediction
6	GeneMarkHMM	http://opal.biology.gatech.edu/GeneMark/	
7	GRAIL	http://compbio.ornl.gov/Grail-1.3/	
8	Genie	http://www.fruitfly.org/seq tools/genie	
9	Glimmer	http://www.tigr.org/softlab/glimmer	
10	FASTA	http://fasta.bioch.virginia.edu/	Homology searching algorithm
11	BLAST	http://www.ncbi.nlm.nih.gov/blast/	
12	GeneSpring Affymetrix	http://www.agilent.com/chem/genespring	Genomics and transcriptomics analysis
13	Open Biological Ontologies	http://obo.sourceforge.net/	Gene ontology
14	The Gene Ontology (GO)	www.geneontology.org	
15	GenBank	https://www.ncbi.nlm.nih.gov/genbank/	Gene and genomic sequence database
16	UniProt	https://www.uniprot.org/	Protein sequence information database
17	Protein Data Bank	https://www.rcsb.org/	Protein three-dimensional (3D) structure information
18	Array Express	https://www.ebi.ac.uk/arrayexpress/	Microarray data repository
19	Gene Expression Omnibus (GEO)	https://www.ncbi.nlm.nih.gov/geo/	
20	AutoDock Vina	http://vina.scripps.edu/	Molecular docking study
21	Gromacs	http://www.gromacs.org/	Molecular dynamics simulation
22	Rasmol	http://www.openrasmol.org/	Molecular visualisation
23	Marvin sketch	https://chemaxon.com/	Chemical drawing
24	Modeller	https://salilab.org/modeller/	Homology modelling software

addition to this, comparative genomics about the different pathogens and among host and pathogens is essential to identify the region of the gene responsible for pathogenesis and resistance. Also, it is possible to explore the distribution of homologous genes and their locus in several pathogenic genomes. The gene expression pattern as well as structural and comparative genomics-based study uncovers the path to gain deeper knowledge about the relationship between the host plant and pathogens. Pinzón et al. studied the gene expression of *Phytophthora infestans* in host cells and identified the favourable and non-favourable patterns of gene interaction. Further sequence-level analysis about resistance genes has been proposed to identify virulence gene pathogens and gene families (Pinzón et al. 2009). Comparative genomics analysis by Klosterman et al. (2011) established a set of proteins that are shared among three selected fungal pathogens which cause the wilt disease. A homologue of a bacterial homologous gene glucosyltransferase that synthesises virulence-related osmoregulated periplasmic glucans to adopt the pathogen in osmotic stress has been identified. Valero-Jiménez et al. (2019) used comparative genomics methods to determine the function of 7668 protein families of selected 9 numbers of *Botrytis* species. These families of proteins were observed in two distinct phylogenetic clades that contain unique genes for secondary metabolite synthesis. Benevenuto et al. implemented the comparative genomics approach to analyse the genetic basis of invading the smut fungi that infect different host systems. Different types of genes such as positively selected genes, gain or loss of effector genes, orphan genes and a genomic signature have been studied in terms of their host specialisation (Benevenuto et al. 2018). Adhikari et al. (2013) reported the sequencing, assembly and annotation study of given six *Pythium* genomes with other plant pathogenic oomycetes such as *Phytophthora* species. The comparative genomic analysis established the close relationship between the oomycetes and *Phytophthora* species based on the involvement of different protein families with diverse functions. Different proteins such as proteolytic enzymes, effector molecules and cell wall-degrading enzymes were found to be associated according to the trophic behaviour of the pathogen. Trantas et al. conducted extensive comparative genomics of the pathogens *Pseudomonas corrugata* and *Pseudomonas mediterranea* to identify the gene clusters for the biosynthesis of siderophores and other metabolites (Trantas et al. 2015). Chen et al. (2019) studied the genomic assembly of *Puccinia hordei* (*Ph*), which is a damaging pathogen of barley, and identified three candidate genes that can be investigated further for their biological properties, to uncover the mechanism of pathogen virulence. Genomic analyses by Méndez et al. showed the phylogenetic relationships among three Chilean strains of Clavibacter and identified the unique virulence factors responsible for virulence activity in tomato plants (Méndez et al. 2020).

11.2.3 Molecular Modelling Study

Progress in computational molecular modelling studies in the last 20 years about plant-pathogen analysis has revealed some of the key mechanisms of this complex

process. Due to the availability of the genomic and protein sequence information as well as the three-dimensional (3D) structures, it is possible to use several molecular modelling approaches to deduce basic molecular phenomena associated with it. Molecular modelling analysis depicts different processes such as the interaction of pathogen-secreted molecules with host target molecules followed by their responses. It is also essential to study different molecules and the metabolic pathways in the case of the plants that play an important role in establishing the diseases. Apart from this, the activity, affinity and specificity of specific agrochemicals towards the pathogenic target can be obtained by applying computer-aided drug design (CADD) methods in the plant pathology area. After choosing specific target molecules in the database such as Protein Data Bank (PDB), specific chemical molecules can be docked to identify the binding site, energy as well as position of chemicals by the process of molecular docking.

A review by Shanmugam and Jeon (2017) described two major categories of computer-based drug discovery strategies, such as structure-based drug design (SBDD) and ligand-based drug design (LBDD) as shown in Fig. 11.3. Several methods such as structure prediction, molecular docking, de novo ligand design, pharmacophore modelling and quantitative structure-activity relationship modelling are used to facilitate the drug design process as described in Fig. 11.3. Shanmugam et al. (2019) studied the essential enzyme such as MoRPD3, a histone deacetylase (HDAC), that causes histone protein acetylation and deacetylation, which helps in the growth and development of rice blast fungus, *Magnaporthe oryzae*. So considering the protein as the drug target to which several compounds were virtually screened by molecular docking method followed by in vitro study and 3D QSAR analysis suggested that [2-[[4-(2-methoxyethyl) phenoxy] methyl] phenyl] boronic

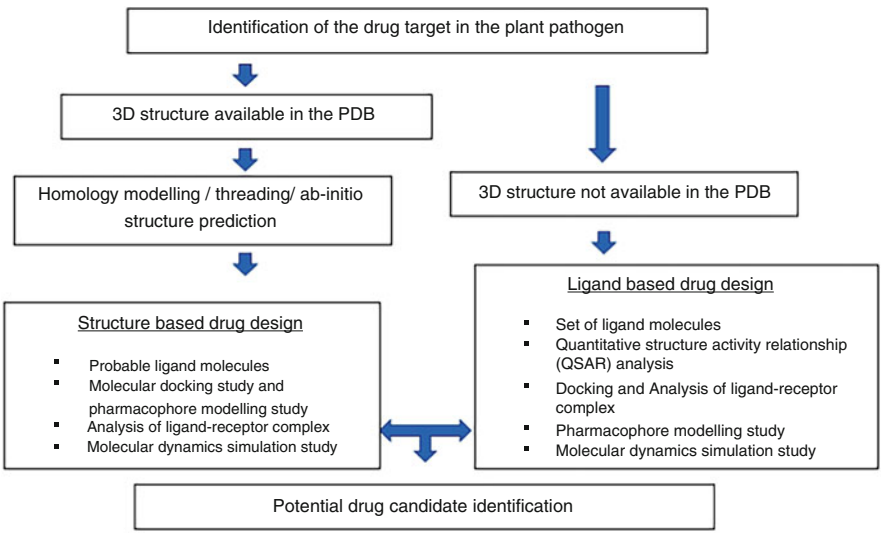

Fig. 11.3 Basic strategies of drug design approaches against plant pathogens

acid compound is a good hit as a HDAC inhibitor. Kumar et al. (2020) used the molecular docking (protein-protein) method between the polygalacturonase inhibitor protein of banana and polygalacturonase (PG) of the pathogen *Erwinia carotovora.* Further, in silico site-directed mutagenesis, docking and molecular dynamics simulation results revealed that particularly the residues at the active sites and the structural changes are responsible for the inhibition of enzyme activity. System biological computational model has been utilised by Islam et al. (2020), who identified three potential antifungal compounds from *Bacillus subtilis* that can be suitably used for suppression of *Rhizoctonia solani* mycelium growth. In silico analysis was performed by using homology modelling and molecular docking followed by molecular dynamics simulation and ADMET analysis. Imran and Ravi (2020) predicted 3D structures of potential drug target proteins of the plant pathogen *Colletotrichum falcatum* that causes 'red rot' disease of sugar cane. This study was conducted by using online resources to construct homology models of drug target proteins against which the suitable drug molecule can be designed. Mishra et al. (2019) used virtual screening and molecular docking strategies to find the lead compounds against fungal diseases such as Fusarium wilt, rice blast, late blight of potato, necrotrophic, early blight of Solanaceae members, flax rust to eradicate these. In the study, seven different antifungal ligand molecules were docked into the selected target proteins of six different fungal pathogens and it showed that several hydrophobic and polar contacts are responsible for binding of the ligand molecule. Pathak et al. (2016) considered molecular targets such as ABC transporter, Amr1, beta-tubulin, cutinase, fusicoccadiene synthase and glutathione transferase of *Alternaria brassicicola* in order to study the binding affinity with phytoalexin. Molecular modelling and docking confirmed that the compound spirobrassinin can be used for the protection of Brassica plants against infection by *Alternaria* sp. In the work by Prajapat et al. (2011) the homology modelling method was followed to deduce the 3D structure of coat protein of mimosa yellow vein virus. The subsequent molecular docking study was performed on the modelled structure of coat protein with α-lactalbumin and further binding pattern was analysed. A recent molecular modelling and protein-protein docking study of pepper yellow leaf curl virus (PepYLCV) pathogenicity protein BC1 and pepper SnRK1 protein revealed the involvement of domain-level interaction in pathogenicity (Nova and Jamsari 2020). The in silico approach for the domain arrangement study of several R-proteins belonging to 33 plant organisms was analysed by Sanseverino and Ercolano (2012). Detailed analysis performed on conserved profiles revealed that specific domain features and several atypical domain associations were also obtained from a diverse set of R-proteins.

11.2.4 GWAS Study in Plant Pathology

Genome-wide association studies (GWAS) are an effective tool widely used for mapping multiple traits in case of wild-type genome. The advancement in genomic sequencing technologies with reduced cost of genotyping, enhanced computational

efficiency and development of improved algorithms has made the genome-wide association study more perfect to explore the position of several essential traits. The basic objective of the GWAS is to identify single-nucleotide polymorphisms (SNP) in the given population, so that any other trait can be measured that is associated with it. Hence, it is expected that such associations may provide variants in specific genes that play a crucial role in the phenotype of interest. Presently, this method is suitable for identifying important genes in natural populations and is being widely used in case of plants for traits as crop yield, crop quality, disease resistance and abiotic stress tolerance (Skøt et al. 2005; Quesada et al. 2010; Rosenberg et al. 2010). The basic steps followed for the GWAS analysis have been described by Marees et al. (2018) and are outlined as below:

DNA sample (from cases and controls) → Hybridise DNA to the array → Identify the genotypes → Find additional SNPs → Find the hotspot for the disease resistance gene → Compute the association of SNP markers with disease resistance genes → Perform statistical analysis → Interpret findings

Alqudah et al. (2020) conducted the genome-wide association study (GWAS) with the aim to map the stem rust resistance loci of barley plant genome by identifying single-nucleotide polymorphic (SNP) markers. Bartoli and Roux (2017) described the importance of GWA mapping tool for the detection of genomic regions associated with disease resistance that predicts the pathogenicity in plant pathogens. Shrestha et al. (2019) reviewed the implementation of GWAS analysis in five major disease resistance varieties of maize plant along with novel SNPs and identification of novel disease resistance genes associated with it. Sánchez-Vallet et al. (2018) used both GWAS and classic linkage mapping methods to establish the function of the avirulence effector of *Zymoseptoria tritici* that is recognised by the resistance genes of wheat. A GWAS study by Volante et al. (2017) identified two regions (*qBK1_628091* of chromosome 1 and *qBK4_31750955* of chromosome 4) in the genome of *Oryza japonica* rice plant that are associated with the single-nucleotide polymorphism (SNP) marker and proposed to be involved in *bakanae* disease resistance mechanism.

11.3 Future Aspects

Despite many advancements in research in the area of plant pathology, molecular basis of various functions is still poorly understood. Hence it becomes essential to study the complex mechanism using bioinformatics-based tools and methods. Some of the opportunities for the application of bioinformatics in plant pathology are as follows:

- Exploring the phylogenetic as well as the structural basis for the study of biomolecules associated with the plant immune system and their distribution across taxonomical diverse species.
- Analysing the plant pathological system and understanding the mechanism of resistance against virulence factors acquired by diverse host plants.

- Understanding the structural features of specific plant proteins to predict the pathological phenomena like how pathogens cause disease in plants and how plants defend themselves against pathogens.
- Development of a unique database of the plant pathogenic target is essential to discover the role of new agrochemicals as the effective drug molecule.
- Use of system biological study by using the available multiomics data is an important aspect, in which it will enable to develop new sophisticated models relating to the phenomena like plant-pathogen-disease establishment-environmental factors/parameters.
- The next-generation sequencing data from the database can be conveniently used for the analysis of plant genome and pathogens to elucidate the key genomic features associated with the pathogenesis.

11.4 Conclusion

Plant diseases cause significant destruction of crop plants ultimately leading to huge economic loss worldwide, especially in the food production sector. So it is crucial to study the disease-causing mechanism related to physiological systems in case of plants. Research on plant-pathogen as well as the molecular basis of the study is interesting as well as complex too while conducting experiment and interpreting the result. However in the recent age, implementation of bioinformatics-based application makes the prediction task easy. The availability of sophisticated software tools and databases for the biological information about the plant pathology enables researchers to focus on in silico studies of individual components in which genes and proteins can be investigated. In this chapter a recent view of different bioinformatics-based methods that are being used by researchers has been provided. In addition to this, major bioinformatics resources have been listed out that can be implemented to retrieve and analyse plant pathological data. However, in the upcoming years, one of the major challenges for the scientific community of plant pathology is more utilisation of the genomics data and tools in model plants so that it can be extrapolated to the disease management aspects. Ultimately this will lead to the enhancement of productivity. Therefore, *bioinformatics*-based findings would provide a deeper understanding and insights into plant pathogen-host protein interactions and will ultimately lead to understanding of the complex plant pathological system.

References

Adhikari BN, Hamilton JP, Zerillo MM et al (2013) Comparative genomics reveals insight into virulence strategies of plant pathogenic oomycetes. PLoS One 8(10):e75072. https://doi.org/10.1371/journal.pone.0075072

Alemu K (2015) The role and application of bioinformatics in plant disease management. Adv Life Sci Technol 28:28–33

Alqudah AM, Sallam A, Baenziger PS et al (2020) GWAS: fast-forwarding gene identification and characterization in temperate Cereals: lessons from Barley—a review. J Adv Res 22:119–135. https://doi.org/10.1016/j.jare.2019.10.013

Andersen EJ, Ali S, Byamukama E et al (2018) Disease resistance mechanisms in plants. Genes 9: 339. https://doi.org/10.3390/genes9070339

Bartoli C, Roux F (2017) Genome-wide association studies in plant pathosystems: toward an ecological genomics approach. Front Plant Sci 8:763. https://doi.org/10.3389/fpls.2017.00763

Benevenuto J, Teixeira-Silva NS, Kuramae EE et al (2018) Comparative genomics of smut pathogens: insights from orphans and positively selected genes into host specialization. Front microbial 9:660. https://doi.org/10.3389/fmicb.2018.00660

Chang HX, Yendrek CR, Caetano-Anolles G et al (2016) Genomic characterization of plant cell wall degrading enzymes and *in-silico* analysis of xylanses and polygalacturonases of *Fusarium virguliforme*. BMC Microbiol 16(1):1–12. https://doi.org/10.1186/s12866-016-0761-0

Chen J, Wu J, Zhang P et al (2019) De novo genome assembly and comparative genomics of the barley leaf rust pathogen *Puccinia hordei* identifies candidates for three avirulence genes. G3 (Bethesda) 9:3263–3271

de Wit PJGM (2007) How plants recognize pathogens and defend themselves. Cell Mol Life Sci 64: 2726–2732. https://doi.org/10.1007/s00018-007-7284-7

Graham-Taylor C, Kamphuis LG, Derbyshir MC (2020) A detailed in silico analysis of secondary metabolite biosynthesis clusters in the genome of the broad host range plant pathogenic fungus *Sclerotinia sclerotiorum*. BMC Genomics 21:1–20. https://doi.org/10.1186/s12864-019-6424-4

Imran S, Ravi L (2020) Elucidation of computational 3D models of protein drug targets for *Colletotrichum falcatum* a fungal plant pathogen causing red rod of sugarcane. Biomed Pharmacol J 13:627–633. https://doi.org/10.13005/bpj/1926

Islam MS, Mahmud S, Sultana R et al (2020) Identification and in silico molecular modelling study of newly isolated *Bacillus subtilis* SI-18 strain against S9 protein of *Rhizoctonia solani*. Arab J Chem 13:8600–8612. https://doi.org/10.1016/j.arabjc.2020.09.044

Kachroo A, Vincelli P, Kachroo P (2017) Signaling mechanisms underlying resistance responses: what have we learned, and how is it being applied? Phytopathology 107:1452–1461. https://doi.org/10.1094/PHYTO-04-17-0130-RVW

Kamal H, Minhas FUAA, Farooq M (2019) In silico prediction and validations of domains involved in *Gossypium hirsutum* SnRK1 protein interaction with cotton leaf curl Multan betasatellite encoded βC1. Front Plant Sci 10:656. https://doi.org/10.3389/fpls.2019.00656

Kaur A, Pati PK, Pati AM et al (2017) In-silico analysis of cis-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. PLoS One 12: e0184523. https://doi.org/10.1371/journal.pone.0184523

Klosterman SJ, Subbarao KV, Kang S et al (2011) Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. PLoS Pathog 7(7):e1002137. https://doi.org/10.1371/journal.ppat.1002137

Kumar S, Dehury B, Tandon G et al (2020) An insight into molecular interaction of PGIP with PG for banana cultivar. Front Biosci (Landmark Ed) 25:335–362. https://doi.org/10.2741/4809

Mack RN, Simberloff D, Lonsdale WM et al (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710

Marees AT, de Kluiver H, Stringer S et al (2018) A tutorial on conducting genome-wide association studies: quality control and statistical analysis. Int J Methods Psychiatr Res 27(2):e1608. https://doi.org/10.1002/mpr.1608

Méndez V, Valenzuela M, Salvà-Serra F et al (2020) Comparative genomics of pathogenic *Clavibacter michiganensis* subsp. michiganensis strains from Chile reveals potential virulence features for tomato plants. Microorganisms 8(11):1679. https://doi.org/10.3390/microorganisms8111679

Mishra P, Eswaran M, Raman NM et al (2019) Probing of phytofungal proteins for fungicidal activity by molecular docking. J Proteomics Bioinform 12:079–084. https://doi.org/10.35248/0974-276X.19.12.499

Mishra VK, Mishra RR, Singh A et al (2016) Importance of bioinformatics for development of neglected and underutilized (Orphan) crops in India. Agri 5:20–29

Mitra D (2021) Emerging plant diseases: research status and challenges. In: Singh KP, Jahagirdar S, Sarma BK (eds) Emerging trends in plant pathology. Springer, Singapore, pp 1–17. https://doi.org/10.1007/978-981-15-6275-4_1

Narayanasamy P (2008) Molecular biology in plant pathogenesis and disease management: disease management, vol 3. Springer Science & Business Media

Nova B, Jamsari J (2020, April) In silico analysis of PepYLCV-βC1 protein interaction with pepper-SnRK1 for pathogenicity prediction. In: IOP conference series: earth and environmental science, vol 497, IOP Publishing, p 012027

Pathak RK, Taj G, Pandey D (2016) Molecular modeling and docking studies of phytoalexin (s) with pathogenic protein (s) as molecular targets for designing the derivatives with anti-fungal action on *'Alternaria' spp.* of '*Brassica*'. Plant Omics 9:172–183. https://doi.org/10.21475/poj.16.09.03.p7654

Pavlopoulou A, Karaca E, Balestrazzi A et al (2019) In silico phylogenetic and structural analyses of plant endogenous danger signaling molecules upon stress. Oxidative Med Cell Longev 2019: 8683054. https://doi.org/10.1155/2019/8683054

Pinzón A, Barreto E, Bernal A et al (2009) Computational models in plant-pathogen interactions: the case of *Phytophthora infestans*. Theor Biol Medical Model 6:1–11. https://doi.org/10.1186/1742-4682-6-24

Prajapat R, Marwal A, Sahu A et al (2011) Phylogenetics and in silico docking studies between coat protein of Mimosa yellow vein virus and whey α-lactalbumin. Am J Biochem Mol Biol 1:265–274

Quesada T, Gopal V, Cumbie WP et al (2010) Association mapping of quantitative disease resistance in a natural population of loblolly pine (*Pinus taeda* L.). Genetics 186:677–686

Rosenberg NA, Huang L, Jewett EM et al (2010) Genome-wide association studies in diverse populations. Nat Rev Genet 11:356–366

Sánchez-Vallet A, Hartmann FE, Marcel TC et al (2018) Nature's genetic screens: using genome-wide association studies for effector discovery. Mol Plant Pathol 19:3–6. https://doi.org/10.1111/mpp.12592

Sanseverino W, Ercolano MR (2012) In silico approach to predict candidate R proteins and to define their domain architecture. BMC Res 5:1–11

Satpathy R, Konkimalla VB, Ratha J (2015) Application of bioinformatics tools and databases in microbial dehalogenation research: a review. Appl Biochem Microbiol 51:11–20. https://doi.org/10.7868/s0555109915010146

Satpathy R (2014) Bioinformatics resources for plant sciences. In: Biotechnology: an over view. Daya Publication, pp 53–65. ISBN: 978-93-5124-333-5

Scholthof HB (2001) Molecular plant-microbe interactions that cut the mustard. Plant Physiol 127: 1476–1483

Shanmugam G, Jeon J (2017) Computer-aided drug discovery in plant pathology. Plant Pathol J 33: 529–542

Shanmugam G, Kim T, Jeon J (2019) In silico identification of potential inhibitor against a fungal histone deacetylase, RPD3 from *Magnaporthe Oryzae*. Molecules 24(11):2075. https://doi.org/10.3390/molecules24112075

Shi-Kunne X, Jové RDP, Depotter JR et al (2019) In silico prediction and characterisation of secondary metabolite clusters in the plant pathogenic fungus *Verticillium dahliae*. FEMS Microbiol Lett 366:fnz081. https://doi.org/10.1093/femsle/fnz081

Shrestha V, Awale M, Karn A (2019) Genome Wide Association Study (GWAS) on disease resistance in Maize. In: Wani S (ed) Disease resistance in crop plants. Springer, Cham, pp 113–130. https://doi.org/10.1007/978-3-030-20728-1_6

Singh VK, Singh AK, Chand R et al (2011) Role of bioinformatics in agriculture and sustainable development. Int J Bioinform Res 3:221–226

Skøt L, Humphreys MO, Armstead I et al (2005) An association mapping approach to identify flowering time genes in natural populations of *Lolium perenne* (L.). Mol Breed 15:233–245

Trantas EA, Licciardello G, Almeida NF et al (2015) Comparative genomic analysis of multiple strains of two unusual plant pathogens: *Pseudomonas corrugata* and *Pseudomonas mediterranea*. Front Microbiol 6:811. https://doi.org/10.3389/fmicb.2015.00811

Untergasser A, Nijveen H, Rao X et al (2007) Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 35:W71–W74

Valero-Jiménez CA, Veloso J, Staats M et al (2019) Comparative genomics of plant pathogenic *Botrytis* species with distinct host specificity. BMC Genomics 20:1–12. https://doi.org/10.1186/s12864-019-5580-x

Volante A, Tondelli A, Aragona M et al (2017) Identification of bakanae disease resistance loci in japonica rice through genome wide association study. Rice (NY) 10(29):1–16. https://doi.org/10.1186/s12284-017-0168-z

Zhang Y, Lubberstedt T, Xu M (2013) The genetic and molecular basis of plant resistance to pathogens. J Genet Genomics 40:23–35. https://doi.org/10.1016/j.jgg.2012.11.003

New-Age Genomic Measures for Uncovering Plant-Microbiome Interactions: Tools, Pipelines and Guidance Map for Genomic Data Mining

12

Balaram Mohapatra, Swati Pattnaik, and Abhishek Gupta

Abstract

In the current post-genomic era, advancement in high-throughput genome sequencing and associated computational tools for genomic analyses have enabled researchers to gain new insights into molecular details of microbe-microbe/microbe-host/microbe-environment interactions. The last decade has also witnessed the onset of other 'OMICS', i.e. (meta)genomic, (meta)-transcriptomic, (meta)proteomic, metabolomic, fluxomic, and mobilomic to gain system-level understanding of microbial physiology for novel biotechnological interventions. Simultaneously, over the years, various databases, prediction softwares, algorithms, and pipelines have been developed to mine and interpret these OMICS data for getting useful information on a spectrum of microbial processes. But systematic use and assessment of such software/database tools/algorithms without a standardized framework, adequate guidance, proper selection criteria, and ideal computational background remain to be a major challenge. OMICS and associative computational tools have widely been applied for understanding plant-microbiome interaction or crosstalk, especially plant diseases; pest-, fertilizer-, pesticide-, water- and nutrient- management; agricultural production; climate change, etc. But unsystematic use and assessment of such tools/techniques with lack of proper workflow (a guidance map) have been

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_12

encountered, which has led to a developer-researcher (end-user) gap. Hence, genomic benchmarking measures would greatly help research communities in using computational tools for selecting appropriate software/tools/methods based on specific data and major research aim. In this context, this chapter describes the use of various curated databases, open-source tools, software and pipelines associated with genomic data mining (other OMICS as well) for uncovering plant-microbiome interactions. The information would help to rationalize the host's metabolic engineering to better optimize the analysis framework for gaining system-level understanding of plant-microbiome communications.

Keywords

Plant-microbiome interaction · Genomics · OMICS · Computational tools · Databases · Pipelines · Methodological workflow · System biology

12.1 Introduction

Plant, soil and associated microflora form one of the most dynamic tripartite interrelationships in and around the plant parts, e.g. rhizosphere. The different physico-chemical and biological attributes of the root/associated bulk soil influence the overall changes in microbial diversity and metabolic activity of microorganisms in the rhizospheric soil microenvironment, thus affecting the overall plant health (Brimecombe et al. 2001; Berendsen et al. 2012). Amongst various microbial influencers, plant growth-beneficial/-promoting microbes/rhizobacteria (PGPM/R), i.e. symbiotic/free-living rhizobia, actinomyces, mycorrhizal fungi and bacteria, influence plant growth and crop yield significantly either by increasing the availability of nutrients or by producing growth substances or by suppressing pathogens, thereby contributing to disease suppression and better yield (Persello-Cartieaux et al. 2003; Dutta and Podile 2010; Etesami and Adl 2020). These microbes are often referred to as nodule-promoting or plant health-beneficial rhizobacteria with multifarious plant-microbe interactions (Hayat et al. 2010). Kloepper and Schroth (1978) coined the term 'PGPR' to denote rhizospheric bacteria having multipartite beneficial effects on plant growth including increased vigour, enhanced nutrient uptake, higher biomass and yield, early seedling emergence, and increased root proliferation (Kloepper 1993; Tan et al. 2014). Owing to such activities, PGPR has been a suitable substitute of chemical fertilizers and preferred as a safe and cost-effective biocontrol agent (Bloemberg and Lugtenberg 2001; Jiao et al. 2021). PGPR effect on plant is mostly ascribed to the microbial potency of colonizing the root/surface or tissue and the effective signalling between roots of specific host plant/genotypes and PGPR taxa. Usually, plants secrete various chemical compounds (high and low molecular weight, volatile or non-volatile/soluble) from their roots because of secretions from root tissues, microbial degradation products and protozoan grazing as root exudates. These compounds constitute acetoin, alcohol, organic acids, amino acids, enzymes, sugars, nucleotides and polysaccharides and act as signal molecules or C/N/S

sources for microbial nutrition (Bashan and de-Bashan 2005; Antoun and Prévost 2005; Santoyo et al. 2021). PGPR organisms belong to broad phylogenetic lineages within the domain Eubacteria, which include Gram-positive *Bacilli* and *Actinobacteria* and Gram-negative *Proteobacteria* members. The major PGPR taxa are *Bacillus*, *Clostridium*, *Arthrobacter*, *Rhodococcus*, *Pseudomonas*, *Burkholderia*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Xanthomonas*, *Enterobacter*, etc. (Pattnaik et al. 2019). The mechanisms of plant growth promotion by PGPR candidates are specific to plant species/host genotypes and microbes in the bulk soil/vicinity of plant. Hence, no single mechanism is accountable for plant growth promotion but can be of direct or indirect mechanisms or a combination of both (Fig. 12.1; Glick 1995; Gupta et al. 2000; Kumar et al. 2016). Direct mechanisms facilitate better resource acquisition/utilization and modulating phytohormone levels which affect plant's metabolism and growth regulatory process, thus leading to an increase in its adaptive capacity to specific environment. Alternatively, indirect mechanisms occur outside the plant, with the involvement of plants' defensive development, mostly against phytopathogens (biotic stress) and climatic extremities (abiotic stress) (Goswami et al. 2016; Pattnaik et al. 2019). Although till date various PGPR strains have been characterized and currently used for agricultural production at laboratory scale or pot-trial/greenhouse basis, many of them yielded failed results under field conditions (at larger scale) and still have not been used widely. Several hurdles like isolating and characterizing efficient PGPR strains, understanding its molecular basis of growth promotion, environmental adaptability/competitive survival, screening by pot and field trial conditions, mass production, suitable formulation strategies, toxicological aspects, field efficacy and viability and quality control amongst others are delimiting its use (John et al. 2020; Basu et al. 2021). Most importantly, with the advent of new-age next-generation sequencing strategies and community/metagenomic analytical skills, combined with multiple 'OMICS' tools like whole-genome sequencing (functional and comparative genomics), proteomics (whole-cell and membrane associated), transcriptomics (total RNA pool sequencing) and high-throughput metabolomics, the associated questions/hurdles on PGPR effectiveness at field conditions can be answered. Simultaneously, these OMICS techniques include association of various tools, databases, software and pipelines, which becomes a technological barrier for many of the non-computational personnel/researchers, thus making PGPR-OMICS study more challenging. For more than a decade, many researchers have been highlighting the PGPR taxa, their mode of actions and plant growth attributes associated with them, but could not highlight the use of new/high-throughput OMICS-based technological details, i.e. how to use or integrate various tools, software, databases, pipelines and computational programs to better understand the genomic and metabolic portrait of PGPR taxa. This review comprehends technological details and optimization for studying genomic portrait of PGPR taxa including computational tools, databases, software and pipelines to uncover plant-microbiome interactions. The review describes the methodological workflow (a guidance map) for analysing genomic data by any science enthusiast or

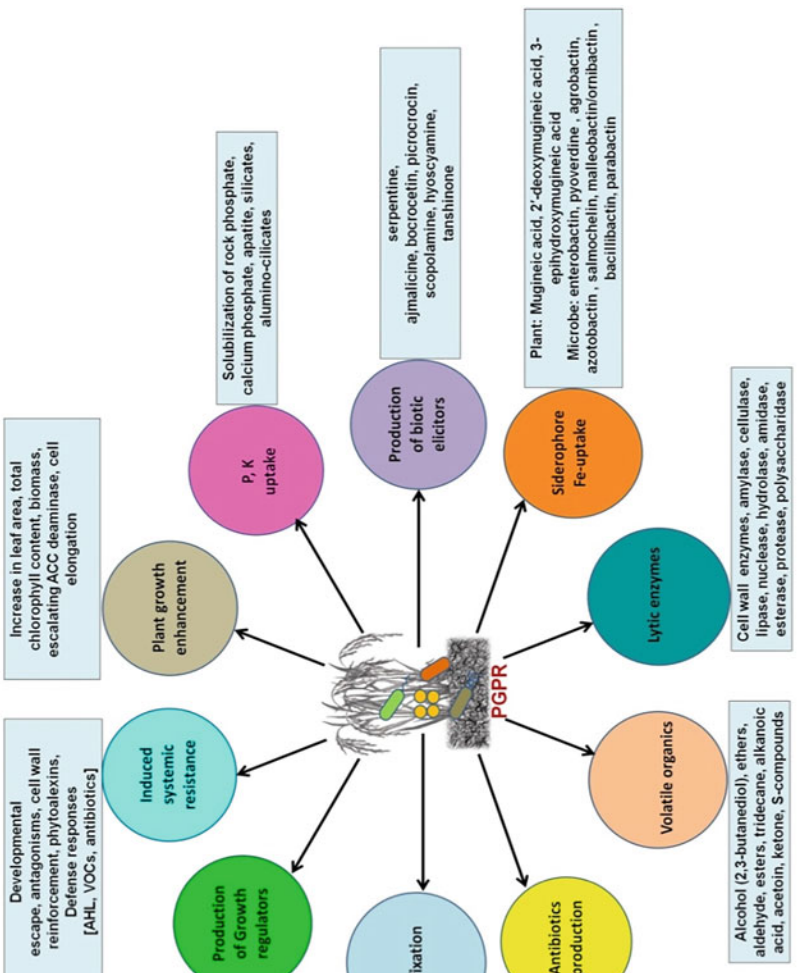

Fig. 12.1 Various plant growth-promoting attributes of PGP microbes for enhancing crop yield and productivity. The circles depict various aspects of plant growth activities, while rectangular boxes indicate the details of each beneficial trait

microbiologists (end users), starting from the 'reads' obtained to mine the physiological functionality in various PGP microbes.

12.2 OMICS, System Biology and PGPR

Recent advancement in 'OMICS' techniques in combination with systems biology has contributed significantly to understand the molecular details of plant-microbiome interaction and rationalize the selection of suitable hosts for metabolic engineering as well as field application. The big data generated from next-generation sequencing platforms together with new algorithms and high-throughput computational tools/pipelines are aiding researchers to understand dynamic interactions between microbes, microbe and plant, and microbe and soil/environment signalling and crosstalk (Vilchez-Vargas et al. 2010; Bouhajja et al. 2016; Dvorak et al. 2017). Both functional and comparative genomic approaches together with functional gene microarray data have enabled us to gain new insights into the evolution of new/conventional microbial metabolism/pathways (Shapir et al. 2007). The genome-scale reconstruction of metabolic models, balancing of metabolic flux and fluxomic analyses in association with transcriptomic and proteomic studies during in vitro or microcosm/megacosm experiments have changed the paradigmatic view of microbe-host communication/interaction mechanisms.

12.3 Genomics: Sequencing the Organism's Genetic Code

Genomics deals with studying the complete set of genes of an organism, mapping, functionally annotating and mining the metabolic portrait of the organism from genome sequence datasets. After the first genome sequencing of bacteriophage Φ-X174 (5386 bp size) in 1977 occurred (Sanger et al. 1977), sequencing technology has become automated and cost effective. *Haemophilus influenzae* (1.8 Mb size) was the first bacterium to be sequenced followed by *Mycoplasma genitalium* (Fleischmann et al. 1995). The advent of shotgun genome sequencing has helped researchers to better understand how bacteria function, evolve and interact with each other with their ecosystems, thus providing critical insights into newer genomic functions and molecular mechanisms (Muller et al. 2007; Vanlnsberghe et al. 2020). With reference to plant-microbe interactions, a combined genome sequencing and computation-driven analysis of sequence data (bioinformatics) has transformed our understanding on the mechanism of bacterial crosstalk with plant host and microbial genomic plasticity for better niche colonization. Genomes of myriad of plant-beneficial taxa have been sequenced till date, predominantly belonging to *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Cyanobacteria* (Fig. 12.2a). Based on the colonization abilities or rhizosphere competence and host tissue invasion traits, members of *Pseudomonas*, *Burkholderia*, *Rhizobia* (*Rhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*), *Agrobacterium*, *Azotobacter*, *Azospirillum*, *Flavobacterium*, *Bacillus*, *Arthrobacter*, *Micrococcus*, etc. amongst

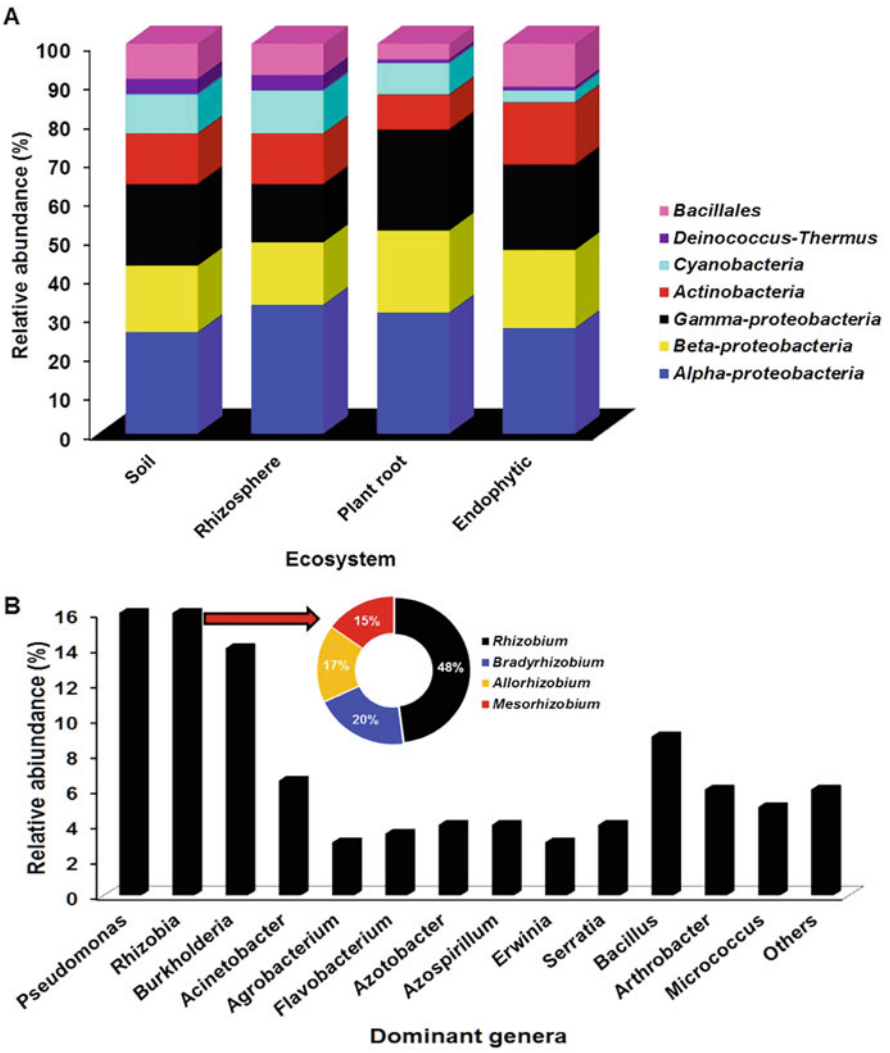

Fig. 12.2 Distribution (% abundance) of predominant PGP microbial groups: (**a**) Relative abundance of major (top) PGP bacterial phyla and (**b**) percentage distribution of dominant PGP taxa (genus level) for which genome sequences (draft or complete) are available in NCBI Genome database. The distribution of rhizobial members (including *Rhizobium* and others) is further presented as pie chart

others are predominantly studied and their dominance is reflected in the NCBI genome database as many of the species members of these groups are sequenced and submitted as genome projects (Fig. 12.2b; Gray and Smith 2005; Gupta et al. 2015; Pattnaik et al. 2019).

12.3.1 Sequencing Platforms and Processes

Compared to traditional sequencing projects which used to take years and were highly expensive and labour intensive, new-age sequencing has become cost effective and time saving with an output of larger dataset (statistically significant) for various analyses (Sharma et al. 2008). Through various sequencing platforms (next-generation sequencing platforms, NGS) researchers are able to perform multiple projects like amplicon sequencing, metagenome sequencing, whole-genome sequencing, targeted gene sequencing/exome and total/meta-RNA sequencing (RNA-Seq) (Shokralla et al. 2012; Sar et al. 2017). In the commercial market, many short-read (approx. 75–300 bp length) sequencers are available amongst which Roche 454 (GS Junior and FLX Titanium) was the first NGS technology based on pyrosequencing (Margulies et al. 2005). Subsequently, Illumina (developed by Solexa: MiSeq, HiSeq, NextSeq) replaced Roche system which is based on bridge amplification (Bentley 2006) and comes as bench-top versions. Parallel to this, Ion Torrent (by Life Technologies: Ion PGM, Proton PGM, Ion S5) employed emulsion PCR for template amplification and uses semiconductor method to detect change in pH as signal during sequencing (Merriman et al. 2012). Long-read sequence technology has been termed as third-generation technologies, which are provided by Pacific Biosciences (PacBio, RS II) and Oxford Nanopore Technologies (NanoPore, MinIon) and are becoming widely used. PacBio employs single-molecule real-time sequencing (SMRT) inside a zero-mode waive-guide after-bell template library preparation with long sequencing reads (2–20 Kb) (Eid et al. 2009). With an accuracy of 99%, these NGS platforms provide a massive amount of data (in million base (Mb) or gigabase (Gb)) as FASTQ files at low cost. Each technology has developed the mode/way of data filtering (quality filtering) like trimming various adapters/index/primers and low-quality sequences (as quality score, Q score), which are then processed to give good-quality nucleotides as FASTA file. Based on the sequencing projects and its aim, data are analysed through specific protocols/pipelines. For example in whole-genome sequence analysis, metabolic functions (functional genomics), evolutionary demarcations (phylo-genomics), comparison with other members (comparative genomics), gene sets/pool, its flux in the community, unique functions (pan-genomics), etc. are interpreted through various databases/servers, described in subsequent sections. The basic methodological workflow is presented in Fig. 12.3.

12.3.2 Sequence Data Assembly, Correctness and Interpretation

Different sequencing techniques/platforms output various sequence reads (pair-end/single-end reads or mate-paired reads) which need to be aligned/mapped and assigned to their exact position in the genome. One of the most difficult tasks in genome sequence analysis is genome assembly. In genome assembly, all the reads generated from NGS sequencer are assembled together to generate a representation of the original chromosomes from which the DNA originated (Sharma et al. 2008).

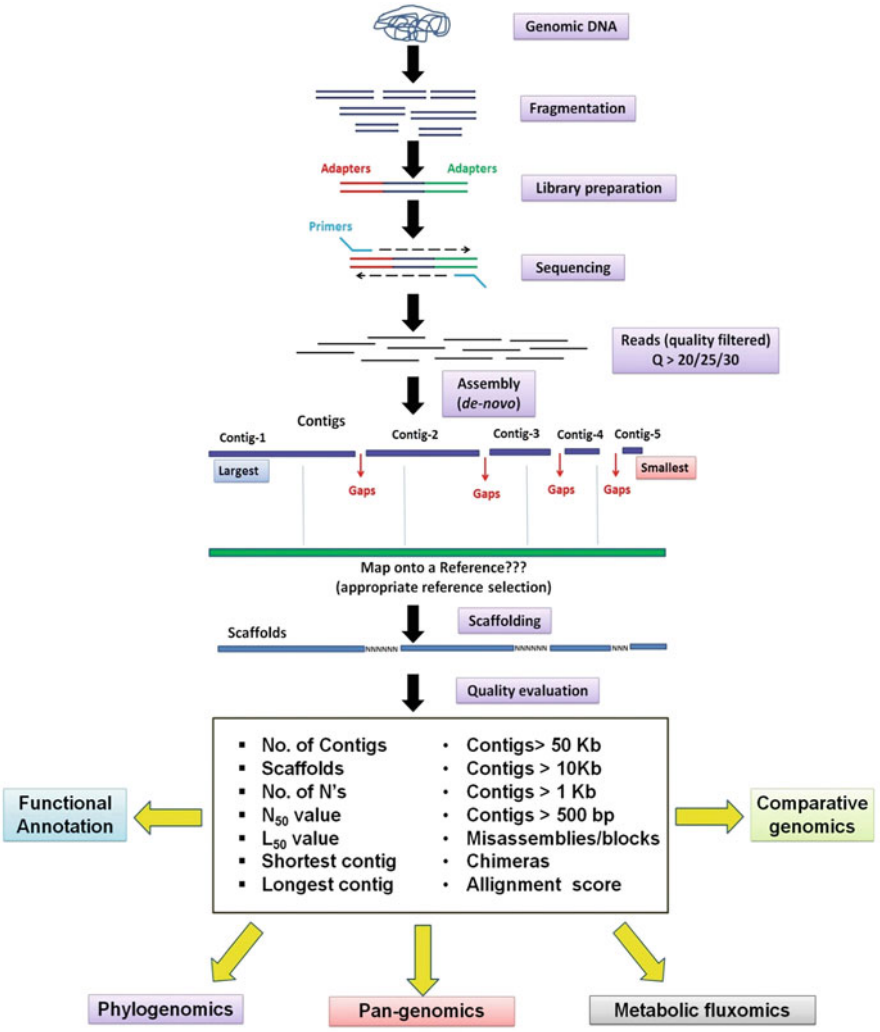

Fig. 12.3 Sequencing, assembly, quality evaluation and functional annotation pipeline workflow for analysing whole-genome sequences of PGP bacteria

In order to assemble DNA sequence data, many genome assembly programs (called assemblers) have been developed like GAP (Genome Assembly Program), Celera Assembler, PCAP (Parallel Contig Assembly Program), ARACHNE, RePS (repeat-masked Phrap with scaffolding), TIGR (The Institute for Genomic Research) Assembler, AMOS (A Modular, Open-Source assembler), Velvet, SOAPDENOVO, SPAdes, IDBA-UD, MIRA, ABYSS, Phred, Phrap and Consed (Bonfield et al. 1995; Sutton et al. 1995; Myers et al. 2000; Batzoglou et al. 2002; Wang et al. 2002; Huang et al. 2003; Zerbino 2010; Prjibelski et al. 2020). But reconstruction of

full chromosomes, resolving of errors (chimeras, mis-assemblies, frame shifts, etc.) and large repeats in the genome are the major challenges, thus posing the question of how to assess the quality of an assembly and compare different assemblies produced from different assemblers for correct genome annotation. Various web-based or command-line based assembly platforms like Plantagora, Assemblathon competition, GAGE, CheckV, CheckM, BUSCO and GenomeQC are available that evaluate a set of metrics, including different types of mis-assembly/errors (inversions, relocations and translocations). Most widely used platforms QUAST and CheckM consider a full range of metrics with ease of working through interface and visualizations and are able to evaluate assembly quality even without a reference genome, i.e. assessment of genome of new species (not having a finished reference genome) (Gurevich et al. 2013; Parks et al. 2015). In addition, QUAST is rather fast and effectively runs on multicore processors. In addition, QUAST uses the Nucmer aligner from MUMmer v3.23 (Kurtz et al. 2004) to align assemblies to a reference genome and evaluate metrics depending on alignments. Upon uploading the user's genome files (contigs/scaffolds), it assesses various parameters like contig size, number of contigs, largest-smallest contigs, total length and Nx ($0 < x < 100$, using contigs of length L accounts for at least x% of the bases of the assembly). It also describes the structural errors, variations, mis-assemblies including number of mis-assemblies, mis-assembled contigs, its length, unaligned contigs and ambiguously mapped contigs (Gurevich et al. 2013). In addition, it evaluates genome representation in contigs, viz. genome fraction percentage (total number of aligned bases in the reference, divided by the genome size), duplication ratio (total number of aligned bases/total number of aligned bases in the reference), GC percentage (total GC nucleotides/total length of assembly), number of mismatches, 'in-dels' (insertion/deletion), total number of genes, and operons, along with the predicted functional genes by GeneMark and Glimmer HMM (Gurevich et al. 2013). QUAST also presents a number of statistics (sample plots) in graphical form; supports SVG, PNG and PDF formats like Nx plot, cumulative plots, GC content/skew and contig alignment; and hosts a web-based viewer, Icarus-Contig viewer, for closer look at the contig's positions and length-wise map. Alternatively, the genome of any pure culture (axenic) can be assessed for any possible contamination (of external DNA sequence/reads or handling error/lab contamination), strain purity/heterogeneity and completeness. The stepwise flow of analysing the genome assembly is depicted in Fig. 12.4. The contamination level in genome (in %) can be analysed using the number and types of 16S rRNA gene using ContEst16S tool of EzBiocloud-EzTaxon server (EzBiocloud-eztaxon.net/tool), which constitutes screening of 69,745 genomes from NCBI Genome assembly database (Lee et al. 2017) as well as other tools like CheckM (Parks et al. 2015). Similarly, strain heterogeneity can be assessed by using annotation services of the Microscope-Genoscope platform (Vallenet et al. 2009). The most ideal way of assessing genome completeness is to count and identify universal single-copy genes (SCG: ribosomal genes and housekeeping genes), which are predominantly (core genes) present in all bacterial taxa and can be retrieved by using AmphoraNet/AMPHORA or CheckM servers as well as Public MLST databases (Kerepesi et al. 2014; Parks et al. 2015).

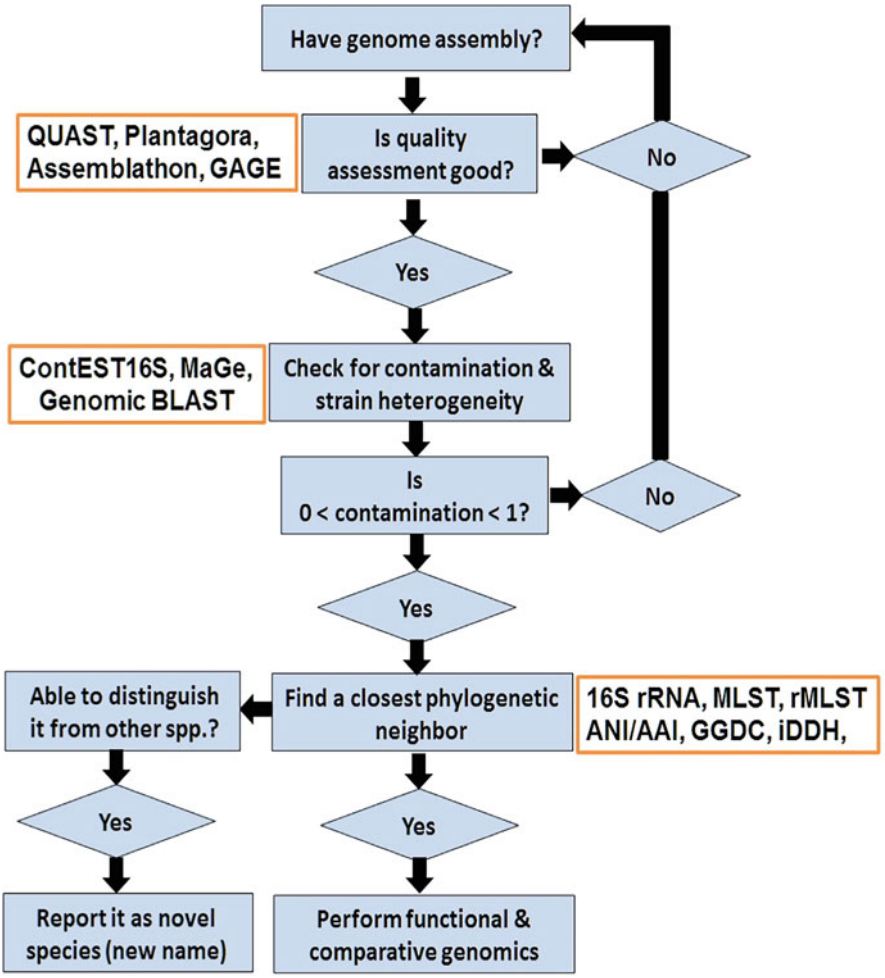

Fig. 12.4 Flow steps for analysing the genome assembly with required computational tools/methods (unfilled rectangles in orange colour)

Completeness is calculated as the number of SCGs present in de novo bacterial genome assembly as compared to complete reference gnome. Alternatively, from these SCGs, the number of multiple copies of SCGs gives an idea of the level of contamination as only one copy should be present in each genome. Once the genome assembly is curetted to be perfect, it must be analysed for functional annotation.

12.3.3 Gene Function Assignments: Annotation and Genetic Analyses

After assessing the correctness of the assembly, the contigs can be assigned to their functions (annotation): (a) structural (coding/non-coding RNAs, *trans*-acting elements, ORFs, etc.) and (b) functional annotation (gene prediction by similarity searches against entries of proteins in reference database) (Sharma et al. 2008; Sar et al. 2017; Lobb et al. 2020). Many open-source annotation pipelines (stepwise integration of various software modules/algorithms) have been developed based on various gene calling algorithms and requirement of users (Table 12.1). But in recent times, many integrated computation tools and pipelines have been developed for annotating genes in a stepwise ease-to-use manner. The notable tools that are popularly used include GLIMMER (Gene Locator and Interpolated Markov ModelER, Delcher et al. 1999), AMIGene (Annotation of MIcrobial Genes by Microscope platform, Bocs et al. 2003), Integrated Microscope-Genoscope (MaGe, Vallenet et al. 2009), MetaCyc-BioCyc (Caspi et al. 2010), *Pseudomonas* Genome database (Winsor et al. 2011), RAST (Rapid Annotation using Sub-system Technology, Aziz et al. 2008; Brettin et al. 2015), IMG-ER (Integrated Microbial Genomics-Expert Review, Markowitz et al. 2012), PATRIC (Wattam et al. 2014), AmyloWiki (Fan et al. 2019), etc. Some of the tools and pipelines that have improved data integration system and ease of analysing data are discussed in the following sections.

12.3.3.1 RAST

Since genome sequencing has started, automated annotation of genome assemblies has become a major step in genomic science and growing number of efforts focusing on different aspects of automated annotation have emerged (Aziz et al. 2008). One of the fully automated annotation services for complete or near-complete genomes is RAST (Rapid Annotation using Subsystem Technology), initially planned for use by the National Microbial Pathogen Data Resource (NMPDR) community, but it is widely used by all researchers now. RAST analysis for genome rapidly produces high-quality assessments of gene functions and metabolic reconstruction of the organism. Users can upload a genome (as assembled contigs/scaffold in FASTA format) and get to know the comparative annotation features against hundreds of existing genomes integrated within SEED environment, produced within 12–24 h with throughput of 50–100 genomes per day having a high accuracy, consistency and completeness (Aziz et al. 2008). It employs two classes of asserted gene functions: (a) subsystem-based (based on the recognition of functional variants of subsystems) and (b) non subsystem-based assertions (common approaches based on the integration of evidence from a number of tools). RAST annotation uses categorizing gene functions based on protein families (as FIGfams). The FIGfams proteins are believed to be globally similar (homologous) and the members all share a common function. So, after input of a query sequence (from genome), the decision about whether or not the protein could be added to the family (i.e. whether or not the protein is globally similar to the members and shares the common function) is resulted showing its subsystem belongingness. Once the neighbouring genomes

Table 12.1 Open-source computational tools and databases available for various bioinformatics analyses

Tools	Functions	Domain link	Use
ArrayExpress	Functional genomics data storage	http://www.ebi.ac.uk/arrayexpress/	Sequence analysis
BLAST	Calculates the statistical significance of nucleotide and protein based on identity	https://blast.ncbi.nlm.nih.gov/Blast.cgi	Sequence analysis
FASTA	To infer functional and evolutionary relationships between sequences	https://fasta.bioch.virginia.edu/fasta_www2/fasta_list2.shtml	Sequence analysis
DNA Databank of Japan	Nucleotide sequence data and supercomputer system	http://www.ddbj.nig.ac.jp/	Sequence analysis
Global Align	Compare two sequences across their entire span (Needleman-Wunsch)	https://blast.ncbi.nlm.nih.gov/Blast.cgi	Sequence analysis
Primer-BLAST	Primer designing specific to PCR template	https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi	Sequence analysis
Lotus japonicus genome assembly build 3.0	Draft of genome sequences for legumes, available	http://www.kazusa.or.jp/lotus/	Sequence analysis
ORF Finder	To find open reading frame (ORF)	http://www.ncbi.nlm/	Sequence analysis
Arabidopsis	Small RNA database	http://asrp.danforthcenter.org/	Sequence analysis
European Nucleotide Archive (EMBL-EBI)	Nucleotide sequencing information	http://www.ebi.ac.uk/ena	Sequence analysis and functional annotation
GenePattern	Information on genomes including sequences, maps, chromosomes, assemblies and annotations	http://software.broadinstitute.org/cancer/software/genepattern/	Sequence analysis
Joint Genome Institute Data and Tools	For genome and metagenome studies	http://jgi.doe.gov/data-and-tools/	Sequence analysis
Nucleotide	Genome database	https://www.ncbi.nlm.nih.gov/nuccore/	Sequence analysis
UCSC Genome Browser	Genome database	http://genome.ucsc.edu/index.html	Sequence analysis
VISTA	Genome database	http://genome.lbl.gov/vista/aboutus.shtml	Sequence analysis
Gene (NCBI)	Links to genome-, phenotype- and locus-specific resources	https://www.ncbi.nlm.nih.gov/gene	Sequence analysis

(continued)

Table 12.1 (continued)

Tools	Functions	Domain link	Use
GEO DataSets (NCBI)	Genome database including cluster tools	https://www.ncbi.nlm.nih.gov/gds	Sequence analysis
Cereal Small RNA Database	Small RNA database	http://sundarlab.ucdavis.edu/smrnas/	Sequence analysis
Plant Small RNA Target Analysis Server	Provides large information of plant mRNA data	http://plantgrn.noble/	Sequence analysis
Plant mRNA Database (PMRD)	miRNA database	http://bioinformatics.cau.edu.cn/PMRD/	Sequence analysis
UEA snRNA Toolkit	Small RNA database	http://srna.tools.cmp.uea.ac.uk/plant/cgi-bin/srna-tools.cgi	Sequence analysis
PsRobot:Plant Small RNA Analysis Toolbox	Small RNA database	http://omicslab.genetics.ac.cn/psRobot/	Sequence analysis
MiSolRNA	Provide tomato miRNA data	http://misolrna.org/about	Sequence analysis
Phytophthora	Small RNA database	http://phytophthora-smallrna-db.cgrb.oregonstste.edu/	Sequence analysis
Abalone	Biomolecular dynamics simulations of proteins, DNA, ligands	http://www.biomolecular-modeling.com/Abalone/index.html	Molecular dynamics simulation tool
Ascalaph	Molecular modelling tool for model development	http://www.biomolecular-modeling.com/Products.html	Molecular dynamics simulation tool
Discovery Studio	Protein-ligand docking, protein homology modelling, sequence analyses, protein-protein docking	http://accelrys.com/products/discovery-studio/	Molecular dynamics simulation tool
FoldX	Advanced protein design features	http://foldx.crg.es/	Molecular dynamics simulation tool
SMART	Information about the protein query	http://smart.embl-heidelberg.de/	Molecular interactions
GeneMarkHMM	Identifies protein-coding genes	http://opal.biology.gatech.edu/GeneMark/	Genome annotation
Genie	Identifies protein-coding genes	http://www.fruitfly.org/seqtools/genie.html	Genome annotation

(continued)

Table 12.1 (continued)

Tools	Functions	Domain link	Use
GRAIL	Identifies protein-coding genes	http://compbio.ornl.gov/Grail-1.3/	Genome annotation
Glimmer	Identifies protein-coding genes	http://www.tigr.org/softlab/glimmer	Genome annotation
SynBrowse	Enhances gene identification accuracy	http://www.synbrowser.org/	Genome annotation
VISTA	Enhances gene identification accuracy	http://genome.lbl.gov/vista/index.shtml	Genome annotation
Phred/Phrap/Consed	For assembly of the genome	http://www.phrap.org	Genome sequencing
Arachne	For assembly of the genome	http://www.broad.mit.edu/wga/	Genome sequencing
GAP4	For assembly of the genome	http://staden.sourceforge.net/overview.html	Genome sequencing
Pfam	Provides a complete and accurate classification of protein families	http://pfam.xfam.org/	Protein sequence analysis
PROSITE	Describes protein domains, families and functional sites along with associated patterns	https://prosite.expasy.org/	Protein sequence analysis
Protein	Determinants of biological structure and function	https://www.ncbi.nlm.nih.gov/protein/	Protein sequence analysis
Protein Data Bank	3D structures of proteins, nucleic acids and complex assemblies	https://www.wwpdb.org/	Protein sequence analysis
Reactome	Genome analysis, modelling	https://reactome.org/	Protein sequence analysis
UniProt	Protein sequence and functional information	http://www.uniprot.org/	Protein sequence analysis

(phylogenetic context) are determined, RAST forms a set of FIGfams that are present in these genomes, which are likely to be found in the new genome The gene calling process is based on GLIMMER2 (Delcher et al. 1999), and based on subsystem-based gene ordering, metabolic reconstruction is performed. To start the analysis, the new user must register (rast.nmpdr.org) for the service and must create a framework to have access to the genomes that have been submitted by users. After the annotation is complete, the user can choose to download the annotated genome in a variety of export formats (GenBank, FASTA, GFF3, Excel) and browse the genome in the SEEDViewer, where the subsystem features (functional categories, its distribution, gene involved, number of genes, its order, sets of orthologues) get displayed as pie chart (Fig. 12.5a). Interestingly, the user can data mine everything from the Excel file

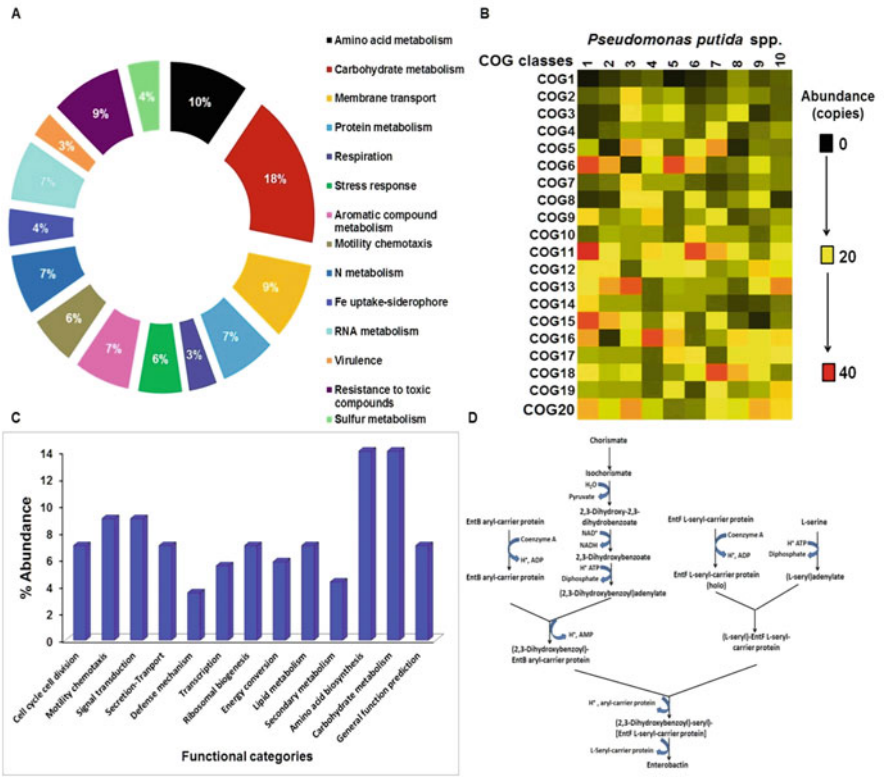

Fig. 12.5 Functional and comparative genome annotation results involving the genome of *Pseudomonas putid*a KM 1081 and *Enterobacter cloacae* (type member) obtained through various functional genomic pipelines like RAST (**a**, pie chart of gene distribution into subsystem functional categories), IMG-ER (**b**, abundance distribution of COG categories as heat map), MaGe (**c**, distribution of core genes/essential genes into various COG categories) and MetaCyc-BoCyc (**d**, metabolic pathway for synthesis of siderophore enterobactin)

(annotation details) for further analyses like operon mapping, phylogenetic analysis, gene/protein studies, and analysis of regulators, promoters, transporters, etc.

12.3.3.2 IMG-ER

The Integrated Microbial Genomes (IMG) server hosts publicly available draft and complete microbial genomes, plasmids and viruses and employs NCBI's RefSeq resource for prediction of genes and protein products (Markowitz et al. 2012). The pipeline links to genomic data with metadata from GOLD (Genome OnLine Database) and makes use of RefSeq for finding CRISPR repeats, signal peptides from SignalP server and transmembrane helices using TMHMM, RNAs by tRNAS-canSE-1.23 for tRNA and in-house HMMs for rRNAs, Rfam and INFERNAL v1.0 for other small RNAs (Markowitz et al. 2012). The annotations of protein products are performed using COG clusters, Pfam, TIGRfam, TIGR, InterPro, Gene Ontology

(GO), Transporter by Transport Classification Database based on their assignment to COG, Pfam or TIGRfam, and KEGG Ortholog (KO) terms and pathways. For each set of gene, specific homologue, paralogue and orthologue (with % identity, bit score and E value) are based on sequence similarities computed in NCBI BLASTP and BLASTN. The 'Expert Review' (ER) version of IMG enables researcher to review and curate the functional annotation of microbial genomes with reference to publicly available genomes. Data and studies on proteomic, transcriptomic, metabolomic and interactomic are also integrated with genomics data to refine the understanding of gene functions (Markowitz et al. 2012). Genome-based prediction of phenotypes (observable characteristic) is one of the interesting functions enabled by the IMG-ER system. According to IMG-ER the example is described as follows: 'if an organism degrades cellulose to cellobiose outside the cell, it can only utilize cellulose as a carbon source if it also has a transport pathway for the uptake of cellobiose and, within the cell, a metabolic pathway to gain energy from cellobiose. If all three steps are present, then the organism has the phenotype of growth on cellulose via cellobiose'. Such prediction is based on AND–OR combinations of IMG pathway assertions (56 rules). IMG-ER also includes many comparative tools where genomes can be compared in terms of gene content using Phylogenetic Profiler which allows users to identify genes in a query genome in terms of presence or absence of homologues in other genomes. With respect to phylogenetic analysis, 'Phylogenetic Distance Tree' and 'Radial Phylogenetic Tree' can be computed for the genomes, where phylogenetic distance is calculated based on the 16S alignment derived from the SILVA database and the distance tree is displayed using the Archaeopteryx tool integrating phyloXML for data exchange. The Abundance Profile Overview and Function Profile tools help users to compare the relative abundance of protein families (COGs, Pfams, TIGRfams) and enzymes (functions) across user-selected genomes and results are displayed either as a heat map or a matrix (Fig. 12.5b). In addition to this, the metabolic capabilities of genomes can be compared using KEGG pathways. In addition to these, IMG-ER enables users for identifying and correcting annotation anomalies like dubious protein product names and annotation gaps using comparative analysis tools (Markowitz et al. 2012).

12.3.3.3 Microscope-Genoscope (MaGe)

In 2006, French National Sequencing Center (CEA/DSV/Institut de Genomique) developed MicroScope to support microbial genome (re)annotation and comparative analysis. It enables curation in a rich comparative genomic context focusing on (re)annotation projects and initially dedicated for analysing *Acinetobacter baylyi* APD1. The annotation service makes use of primary databanks like UniProt, NCBI RefSeq microbial genomes and Enzyme, combining programs such as AMIGene, tRNAscan-SE server, RNAmmer 2.0 and Rfam scan to predict genomic objects like CDSs and RNA genes (Vallenet et al. 2009). Many bioinformatics methods including homology search in UniProt COG, InterPro and PRIAM for enzymatic classification, protein localization using TMHMM, SignalPPsortB-based signal recognition, gene synteny, proteomic comparison and metabolic network reconstruction are enabled for detailed genomic analysis. The MaGe web interface

(http://www.genoscope.cns.fr/agc/mage) allows experts for performing annotation using gene annotation editor, synteny results and metabolic network predictions for gaining insights into biological function of genes and gene pool of species members through pan-genomic content analysis (Fig. 12.5c; Vallenet et al. 2009).

12.3.3.4 MetaCyc-BioCyc

The MetaCyc database is a comprehensive and freely accessible resource for almost 1400 metabolic pathways and enzymes, where pathways are experimentally determined, linked to one or more well-characterized enzymes, and are curated from the primary scientific literatures (reviews, evidence codes and citations) (Krieger et al. 2004). It has been used for genome analysis, metabolism and metabolic engineering. In addition to metabolic pathways, it is used as a reference database to computationally predict the metabolic network [in the form of a Pathway/Genome Database (PGDB)] of any organism's sequenced and annotated genome (Fig. 12.5d; Caspi et al. 2010). In connection to this, BioCyc includes 500 organism-specific PGDBs, in which users can integrate genomic data with metabolism, regulation and genetics. It also enables users to analyse omics datasets from experiments related to gene transcription, metabolomics, proteomics, ChIP-chip analysis, etc. With regard to MetaCyc, metabolic pathways for biosynthesis ($n = 902$ base pathways) are predominant, consisting of secondary metabolite biosynthesis ($n = 351$); cofactors, prosthetic groups and electron carrier biosynthesis ($n = 160$); amino acid biosynthesis ($n = 105$); and fatty acid biosynthesis ($n = 101$). Other major classes include assimilation/degradation (aromatic compounds, amino acids, inorganic nutrients, secondary metabolites), and energy transduction (fermentation, respiration, autotrophy) (Caspi et al. 2010). Users can also link the pathway data to various other databases like IUBMB (International Union of Biochemistry and Molecular Biology) for enzyme EC numbers/classification, NCBI taxonomy (organism's classification string), GO (Gene Ontology: biological process, molecular function), PubChem (compound entries), and KEGG (compounds, pathways, reaction).

12.3.3.5 *Pseudomonas* Genome Database

Owing to the versatile metabolic capacity and ubiquity towards wide environmental settings, *Pseudomonas* has been of enough interest with potential plant growth-beneficial traits and free-living or serious opportunistic pathogens; ability to degrade synthetic organics, transform metals and produce industrially relevant biomolecules, etc. (Dvorak et al. 2017). To study *Pseudomonas* genomic content, the *Pseudomonas* Community Annotation Project (PseudoCAP) was originally formed to meet the need of providing a conservative, peer-reviewed annotation of the *Pseudomonas aeruginosa* PAO1 genome. This subsequently led to the development of the *Pseudomonas* genome database (http://www.pseudomonas.com). It endows users with improved comparative analysis and population genomics capability for *Pseudomonas* genomes. It enables users to view polymorphisms in closely related *P. aeruginosa* genome sequences, strain-specific portals for accessing whole-genome data (including experimental data) and updated high-precision computational predictions and comparative genome analyses based on robust methods for

predicting and clustering orthologues (Winsor et al. 2011). Likewise, *Burkholderia* genome database hosts several integrated pipelines for comparative genomic analyses of *Burkholderia* spp. (Winsor et al. 2008).

Considerable progress has been made in studying the genomics of PGP microbes to decipher the eco-physiology and genetics but the complex interplay of gene transcription, expression pattern, proteomic networking and phenotypic/metabolomics dynamics plays a significant role in imparting plant-beneficial attributes in rhizosphere ecosystem. Hence, the use of interactomics (proteomics, transcriptomics, metabolomics, fluxomic, ionomics and phenomics) must be conducted as a complementary study with the genomics.

12.3.4 Functional and Comparative Genomic Analyses of PGP Taxa

Data mining of genomes of PGP taxa can be performed at various levels, i.e. gene functions and its comparison with reference sequences from already sequenced genomes, often termed as functional and comparative genomics. Various open-source tools and pipelines are developed to assess the genomic data and are described in subsequent sections.

12.3.4.1 CARD (Antibiotic Resistance)

Increased incidence of antimicrobial resistance (AMR) by various microbial taxa as a result of antibiotic misuse and poor stewardship has resulted in global health crisis. Genome-enabled surveillance of resistance determinants in non-pathogenic to pathogenic taxa has become an urgent need to understand the evolution of new resistance traits (McArthur and Tsang 2017). Comprehensive Antibiotic Resistance Database (CARD 2020: https://card.mcmaster.ca/analyze), a web-based tool, uses a built-in BLAST instance for comparing genome and protein sequences to CARD reference database for resistome prediction with data visualization (Alcock et al. 2020). All curated data within CARD are organized using four central ontologies, i.e. the ARO, the CARD Model Ontology (MO), the CARD Relations Ontology (RO) and NCBI-Taxon (a curated subset of the NCBI Organismal Taxonomy Ontology) (Sayers et al. 2019). The ARO includes molecular basis for antibiotic resistance as AMR determinants like acquired resistance genes, resistant mutations of housekeeping genes, efflux overexpression, drug targets, antibiotic molecules, drug classes and molecular mechanisms of resistance and is organized into Determinant of Antibiotic Resistance, Antibiotic Molecule and Mechanism of Antibiotic Resistance. From whole-genome data, RGI (Resistance Gene Identifier) predicts AMR genes (ORF) through Prodigal followed by sequence alignment using BLAST/DIAMOND. There are three cut-offs for determining AMR traits by (a) perfect, (b) strict and (c) loose hit method (Jia et al. 2017). In perfect hit, exact (100%) matched AMR is determined while strict hit is flexible allowing variation from the CARD reference sequence as long as the sequence falls within the curated BLAST score. The loose hit includes AMR gene outside of the detection model cut-offs to provide detection of new, emergent threats and more distant AMR homologues.

12.3.4.2 VFDB (Virulence Factors/Pathogenic Markers)

Pathogenicity of microbial members (virulence) to their host plants and understanding at molecular level are of utmost important to reduce biotic stress in field crops. The likelihood of a microbe causing infection (by virulence factors, VFs) is the result of virulence causing gene products enabling the microbe to colonize host niche (Weiss 2002). Virulence factors are forms of secreted proteins, protein toxins and enzymes, cell-surface structures (capsular polysaccharides, lipopolysaccharides and membrane proteins), siderophores, catalases, regulators, etc., which directly or indirectly contribute to the pathogenesis. The Virulence Factor Database (VFDB: http://www.mgc.ac.cn/VFs/) is a comprehensive and user-friendly database with browse and search option enabled with information of VFs from pathogens (includes virulence-associated genes, protein structural features, functions and mechanisms) and pathogenicity islands (PAIs: clusters of virulence genes) (Chen et al. 2005). It enables users to extract information from the database quickly through three different ways: (1) text search, (2) BLAST search and (3) VF function search. The stand-alone BLAST program integrated into VFDB allows users to compare and align sequences against all reference nucleotides and/or amino acids with pairwise comparison using PSI/PHI BLAST programs. On the other hand, users can also browse reference genome sequences for possible VFS and pathogenicity islands where the detailed information, i.e. VFS for motility, adherence, invasion, toxins, regulators and location of associated genes in the genome. In PAIs, species descriptions, start-end of PAIs, size, operonic structures, phenotypic details and other remarks (like functions) can be mapped by the user (Chen et al. 2005).

12.3.4.3 antiSMASH 5.0 (Secondary Metabolites)

Microbial secondary metabolites have found to be an important source of antimicrobials and bioactive compounds and act as signalling molecules for microbial communication and ecological interactions (van der Meij et al. 2017). Genome mining for the presence of biosynthetic pathways/gene clusters (BGCs) for secondary or specialized metabolites is one of the interesting approaches of identifying novel PGP traits in soil-rhizospheric microbes (Ziemert et al. 2016). The tool antiSMASH 5.0 version (antibiotic and secondary metabolite analysis shell developed in 2011) is preferred over many tools like CLUSEAN and PRISM. In connection to antiSMASH, several tools like mass spectrometry-guided peptide mining tool Pep2Path (Bouhajja et al. 2016), the 'Antibiotic Resistance Target Seeker' (ARTS), the sgRNA design tool CRISPy-web, a reverse-tailoring tool to match finished non-ribosomal peptides (NRPS)/polyketides (PKS) and BGC clustering and classification platform BiG-SCAPE are developed to better interpret the combined result for identifying secondary metabolite clusters (Blin et al. 2019). BGCs are identified based on identifying co-occurring conserved core enzymes in the genome using HMM profiles derived from Pfam, SMART or BAGEL. With 52 different BGCs including N-acyl amino acids, L-lactones, polybrominated diphenyl ethers, C-nucleosides, pseudopyronines, fungal RiPPs, RaS-RiPPs, nrps-like, Trans-AT type I PKS and type II PKS, this server is comprehensive and accurate. antiSMASH 5 also uses the hidden Markov models (pHMMs) from Resfams to annotate potential

resistance genes within biosynthetic clusters. The antiSMASH 5.0 provides the analysis results in an interactive web page (as user interface, UI) as annotated GenBank format files (.gbk) for the whole genome and individual clusters that can be analysed by third-party tools like BLAST, BLAST2GO and Genome mapper, thus enabling the user to analyse genomes of PGP microbes at a multiscale level (Blin et al. 2019).

12.3.5 Mobile Genomics: Regions of Genomic Plasticity

Mobile genetic elements (MGEs: genomic islands, integrative conjugative elements, plasmids, etc. as regions of genomic plasticity) transmitted through horizontal gene transfer (HGT) events have become the major driver of genome evolution and provide adaptive traits that enhance the fitness of PGP bacteria and archaea in heterogeneous environmental network (Wozniak and Waldor 2010; Aminov 2011; Phale et al. 2019). These elements often code novel gene functions like resistance to metals, antimicrobials, degradation of hydrocarbons, elemental cycling (N_2 fixation), motility and pathogenicity. Understanding of genomic localization, structures and mode of transmission of these MGEs through various database tools would greatly help in identifying the evolutionary fitness and physiological advantages of PGP microbes.

12.3.5.1 ICEberg 2.0

Amongst all MGEs, integrative and conjugative elements (ICEs) are integrative to the bacterial chromosome having intact conjugation machinery and are self-transmissible between bacterial cells (Wozniak and Waldor 2010; Johnson and Grossman 2015). Typically, ICEs harbour two to three core functional modules. The first module includes an integrase gene (*xer*C) for its excision from the host genome, forming a circular intermediate, and is often assisted by an excisionase or recombination directionality factors (rdfs) (Burrus et al. 2002; Wozniak and Waldor 2010). The second module transfers this circular intermediate by conjugation through Type IV secretion system (T4SS) by intimate contact between the ICE donor and recipient for its transfer. The third core module is found to be involved in ICE maintenance and regulation with toxin-antitoxin/partition systems, ensuring its vertical transmission within prokaryotic lineage (Delavat et al. 2017; Liu et al. 2019). Interestingly, besides core modules, ICEs harbour an extensive array of 'cargo genes' encoding diverse metabolic function confirming a host's survival advantages (Wozniak and Waldor 2010). In addition, homologous recombination between ICEs (inter-ICE mismatch) often generates hybrid ICEs, which contribute to the dynamicity of ICEs (Wozniak and Waldor 2010). Unlike conjugative transposons, ICEs get inserted at a specific site (*att*B), i.e. usually a tRNA flanked by specific direct repeats (*att*R and *att*L). Recently, an increasing number of ICEs (200 T4SS-type ICEs) have been identified from many draft/complete bacterial chromosomes. In connection to this, ICEberg 2.0 (http://db-mml.sjtu.edu.cn/ICEberg/) database is stand-alone in providing ICE types, numbers, locations,

structure and family which includes data from both experimental supports and bioinformatics predictions (Liu et al. 2019). Besides ICEs, integrative and mobilizable elements (IMEs) and cis-mobilizable elements (CIMEs) can be predicted through ICEfinder in ICEberg 2.0 from the user's bacterial genomes (input as FASTA or .gbk file format). With the recent updates, ICEberg 2.0 might provide better support for understanding ICEs encoding traits and their interaction with cognate mobilizable elements to better understand the horizontal gene flow (Liu et al. 2019).

12.3.5.2 IslandViewer 4.0 (for Larger Scale Datasets)

Similarly, large number of methods have been developed to predict and visualize GIs from genome sequences to identify GI features (mobility genes, phage-related genes, direct repeats) and skewness in nucleotide composition (atypical GC content) (Langille et al. 2008). IslandViewer (latest version 4.0) is the first web server integrating three of the most accurate and integrated GI prediction tools, i.e. IslandPath-DIMOB (nucleotide bias and presence of mobility genes), SIGI-HMM (codon usage bias with a hidden Markov model) and IslandPick (comparative genomics approach), with flexible visualization interface (Bertelli et al. 2017). IslandPath-DIMOB uses updated Pfam profiles for the identification of mobility genes and more stringent significance cut-offs to avoid false positives (based on the biased dinucleotide score). The large number of complete reference genomes available in IslandViewer has significantly increased the time to compute pairwise genomic distance calculations using CVTree which enabled the user-custom selection of genomes for IslandPick to perform comparative genomic analysis (Bertelli et al. 2017).

12.4 Conclusion and Future Prospects

PGP microbes with multipartite eco-physiological traits like production of pigments, alkaloids, antimicrobials, polymers, organics (volatiles), hormones and acids are important not only for agricultural application but also in biomedical, pharmaceutical and food applications. Our review provides details of PGP microbial traits and stepwise flow on how to handle the genomic datasets of PGP microbes to better understand the genomic architecture, physiological portrait and systems biology that might be helpful for genetic engineering approaches. This genomic comprehension might assist users for gene editing methods of PGP taxa, thus providing successful platforms for strain improvement strategies to increase the market demands of specific PGP microbial applications. However, despite their promising potential, various challenges associated with the successful application of such PGP microbes delimit their study at larger scale. A single technique (like genomics) must be assisted with other high-throughput OMICS tools for detailed characterization of PGP microbes. In the near future, the combination of all OMICS for PGP taxa will provide a detailed and comprehensive study of PGP microbe's behaviour. Further, the use of proteomic and metabolomic approaches with the development of high-end

computational tools for large-scale screening is still rare for many potential PGP taxa. The metagenome-assisted genome assemblies (MAGs) and metagenome-based genome reconstruction from metagenome binning are the need of the hour to identify the least represented PGP taxa (rare microbiome member) at the plant-microbe continuum.

References

Alcock BP, Raphenya AR, Lau TTY et al (2020) CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/10.1093/nar/gkz935

Aminov RI (2011) Horizontal gene exchange in environmental microbiota. Front Microbiol 2:158. https://doi.org/10.3389/fmicb.2011.00158

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38. https://doi.org/10.1007/1-4020-4152-7_1

Aziz RK, Bartels D, Best AA (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75

Bashan Y, de-Bashan LE (2005) Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination. Soil BiolBiochem 37:1795–1804. https://doi.org/10.1016/j.soilbio.2005.02.013

Basu A, Prasad P, Das SN et al (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability 13(3):1140. https://doi.org/10.3390/su13031140

Batzoglou S, Jaffe DB, Stanley K et al (2002) ARACHNE: a whole-genome shotgun assembler. Genome Res 12:177–189. https://doi.org/10.1101/gr.208902

Bentley DR (2006) Whole-genome re-sequencing. Curr Opin Genet Dev 16:545–552. https://doi.org/10.1016/j.gde.2006.10.009

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Bertelli C, Laird MR, Williams KP et al (2017) IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res 45:W30–W35. https://doi.org/10.1093/nar/gkx343

Blin K, Shaw S, Steinke K (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10.1093/nar/gkz310

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. CurrOpin Plant Biol 4:343–350. https://doi.org/10.1016/s1369-5266(00)00183-7

Bocs S, Cruveiller S, Vallenet D et al (2003) AMIGene: annotation of MIcrobial genes. Nucleic Acids Res 31:3723–3726. https://doi.org/10.1093/nar/gkg590

Bonfield JK, Smith K, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Res 23:4992–4999

Bouhajja E, Agathos SN, George IF (2016) Metagenomics: probing pollutant fate in natural and engineered ecosystems. Biotechnol Adv 34:1413–1426. https://doi.org/10.1016/j.biotechadv.2016.10.006

Brettin T, Davis JJ, Disz T et al (2015) RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:1–6. https://doi.org/10.1038/srep08365

Brimecombe MJ, De Lelj FA, Lynch JM (2001) The effect of root exudates on rhizosphere microbial populations. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizo-sphere, biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp 95–140

Burrus V, Pavlovic G, Decaris B et al (2002) Conjugative transposons: the tip of the iceberg. MolMicrobiol 46(3):601–610. https://doi.org/10.1046/j.1365-2958.2002.03191.x

Caspi R, Altman T, Joseph MD et al (2010) The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res 38: D473–D479. https://doi.org/10.1093/nar/gkp875

Chen L, Yang J, Yu J et al (2005) VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res 33:D325–D328. https://doi.org/10.1093/nar/gki008

Delavat F, Miyazaki R, Carraro N et al (2017) The hidden life of integrative and conjugative elements. FEMS Microbiol Rev 41:512–537. https://doi.org/10.1093/femsre/fux008

Delcher AL, Harmon D, Kasif S et al (1999) Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636

Dutta S, Podile AR (2010) Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Crit Rev Microbiol 36:232–244. https://doi.org/10.3109/10408411003766806

Dvorak P, Nikel PI, Damborsky J et al (2017) Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. Biotechnol Adv 35:845–866. https://doi.org/10.1016/j.biotechadv.2017.08.001

Eid J, Fehr A, Gray J et al (2009) Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986

Etesami H, Adl SM (2020) Plant growth-promoting rhizobacteria (PGPR) and their action mechanisms in availability of nutrients to plants. In: Kumar M, Kumar V, Prasad R (eds) Phyto-microbiome in stress regulation. Environmental and microbial biotechnology. Springer, Singapore, pp 147–203. https://doi.org/10.1007/978-981-15-2576-6_9

Fan B, Wang C, Ding X et al (2019) AmyloWiki: an integrated database for Bacillus velezensis FZB42, the model strain for plant growth-promoting *Bacilli*. Database (Oxford) 2019:baz071. https://doi.org/10.1093/database/baz071

Fleischmann RD, Adams MD, White O et al (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269:496–512

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117

Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2:1–19. https://doi.org/10.1080/23311932.2015.1127500

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412. https://doi.org/10.1016/j.soilbio.2004.08.030

Gupta A, Gopal M, Tilak KV (2000) Mechanism of plant growth promotion by rhizobacteria. Indian J Exp Biol 38:856–862

Gupta G, Parihar SS, Ahirwar NK et al (2015) Plant growth promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microbiol Biochem 7: 96–102

Gurevich A, Saveliev V, Vyahhi N et al (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086

Hayat R, Ali S, Amara U et al (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598. https://doi.org/10.1007/s13213-010-0117-1

Huang X, Wang J, Aluru S et al (2003) PCAP: a whole-genome assembly program. Genome Res 13:2164–2170

Jia B, Raphenya AR, Alcock B et al (2017) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/nar/gkw1004

Jiao X, Takishita Y, Zhou G et al (2021) Plant associated rhizobacteria for biocontrol and plant growth enhancement. Front Plant Sci 12:420. https://doi.org/10.3389/fpls.2021.634796

John CJ, Kumar S, Ge M (2020) Probiotic prospects of PGPR for green and sustainable agriculture. Arch Phytopathol Plant Protect 1-16. https://doi.org/10.1080/03235408.2020.1805901

Johnson CM, Grossman AD (2015) Integrative and Conjugative Elements (ICEs): what they do and how they work. Ann Rev Genet 49:577–601. https://doi.org/10.1146/annurev-genet-112414-055018

Kerepesi C, Banky D, Grolmusz V (2014) AmphoreNet: the web server implementation of the AMPHORA2 metagenomic workflow suite. Gene 533:538–540. https://doi.org/10.1016/j.gene.2013.10.015

Kloepper JW (1993) Plant growth promoting rhizobacteria as biological control agents. In: Metting FB Jr (ed) Soil microbial ecology-applications in agricultural and environmental management. Mercel Dekker, New York, pp 255–274

Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes. In: Proceedings of the fourth international conference on plant pathogen bacteria, vol 2. INRA, p 879

Krieger CJ, Zhang P, Mueller LA et al (2004) MetaCyc: a multi-organism database of metabolic pathways and enzymes. Nucleic Acids Res 32:D438–D442. https://doi.org/10.1093/nar/gkh100

Kumar S, Chauhan PS, Agrawal L et al (2016) *Paenibacillus lentimorbus* inoculation enhances tobacco growth and extenuates the virulence of cucumber mosaic virus. PLoS One 11(3): e0149980. https://doi.org/10.1371/journal.pone.0149980

Kurtz S, Phillippy A, Delcher AL et al (2004) Versatile and open software for comparing large genomes. Genome Biol 5:1–9. https://doi.org/10.1186/gb-2004-5-2-r12

Langille MGI, Hsiao WWL, Brinkman FSL (2008) Evaluation of genomic island predictors using a comparative genomics approach. BMC Bioinform 9:329. https://doi.org/10.1186/1471-2105-9-329

Lee I, Chalita M, Ha S et al (2017) ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol 67(6): 2053–2057. https://doi.org/10.1099/ijsem.0.001872

Liu M, Li X, Xie Y (2019) ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. Nucleic Acids Res 47:D660–D665. https://doi.org/10.1093/nar/gky1123

Lobb B, Tremblay BJM, Moreno-Hagelsieb G et al (2020) An assessment of genome annotation coverage across the bacterial tree of life. Microbe Genomics 6:e000341. https://doi.org/10.1099/mgen.0.000341

Margulies M, Egholm M, Altman WE et al (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. https://doi.org/10.1038/nature03959

Markowitz VM, Chen IMA, Palaniappan K (2012) IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res 40:D115–D122. https://doi.org/10.1093/nar/gkr1044

McArthur AG, Tsang KK (2017) Antimicrobial resistance surveillance in the genomic age. Ann N Y Acad Sci 1388:78–91. https://doi.org/10.1111/nyas.13289

Merriman B, R&D Team IT, Rothberg JM (2012) Progress in ion torrent semiconductor chip based sequencing. Electrophoresis 33:3397–3417. https://doi.org/10.1002/elps.201200424

Muller D, Medigue C, Koechler S et al (2007) A tale of two oxidation states: bacterial colonization of arsenic-rich environments. PLoS Genet 3:53. https://doi.org/10.1371/journal.pgen.0030053

Myers EW, Sutton GG, Delcher AL et al (2000) A whole-genome assembly of Drosophila. Science 287:2196–2204. https://doi.org/10.1126/science.287.5461.2196

Parks DH, Imelfort M, Skennerton CT et al (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25(7): 1043–1055. https://doi.org/10.1101/gr.186072.114

Pattnaik S, Mohapatra B, Kumar U et al (2019) Microbe-mediated plant growth promotion: a mechanistic overview on cultivable plant growth-promoting members. In: Giri B et al (eds) Biofertilizers for sustainable agriculture and environment, soil biology 55. Springer Nature, Switzerland, pp 435–463. https://doi.org/10.1007/978-3-030-18933-4_20

Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground, molecular plant-rhizobacteria interactions. Plant Cell Environ 26:189–199. https://doi.org/10.1046/j.1365-3040.2003.00956.x

Phale PS, Shah BA, Malhotra H (2019) Variability in assembly of degradation operons for naphthalene and its derivative, Carbaryl, suggests mobilization through horizontal gene transfer. Genes (Basel) 10(8):569. https://doi.org/10.3390/genes10080569

Prjibelski A, Antipov D, Meleshko D et al (2020) Using SPAdes de novo assembler. Curr Protocol Bioinform 70:e102. https://doi.org/10.1002/cpbi.102

Sanger F, Air GM, Barrell BG et al (1977) Nucleotide sequence of bacteriophage phi X174 DNA. Nature 265:687–695

Santoyo G, Urtis-Flores CA, Loeza-Lara PD et al (2021) Rhizosphere colonization determinants by plant growth promoting rhizobacteria (PGPR). Biology (Basel) 10(6):475. https://doi.org/10.3390/biology10060475

Sar P, Mohapatra B, Ghosh S et al (2017) Geomicrobiology of arsenic contaminated groundwater of Bengal Delta Plain. In: Das S, Dash HR (eds) Handbook of metal-microbe interactions and bioremediation. CRC Press, pp 221–242. https://doi.org/10.1201/9781315153353-20

Sayers EW, Agarwala R, Bolton EE et al (2019) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 47:D23–D28

Shapir N, Mongodin EF, Sadowsky MJ et al (2007) Evolution of catabolic pathways: genomic insights into microbial *s*-triazine metabolism. J Bacteriol 189:674–682. https://doi.org/10.1128/JB.01257-06

Sharma P, Kumari H, Kumar M et al (2008) From bacterial genomics to metagenomics: concept, tools and recent advances. Indian J Microbiol 48:173–194

Shokralla S, Spall JL, Gibson JF et al (2012) Next-generation sequencing technologies for environmental DNA research. MolEcol 21:1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Sutton GG, White O, Adams MD et al (1995) TIGR Assembler: a new tool for assembling large shotgun sequencing projects. Genom Sci Technol 1:9–19

Tan KZ, Radziah O, Halimi MS et al (2014) Isolation and characterization of Rhizobia and Plant Growth-Promoting Rhizobacteria (PGPR) and their effects on growth of rice seedlings. Am J Agric Biol Sci 9(93):342–360. https://doi.org/10.3844/ajabssp.2014.342.360

Vallenet D, Engelen S, Mornico D et al (2009) MicroScope: a platform for microbial genome annotation and comparative genomics. Database (Oxford), 2009:bap021. https://doi.org/10.1093/database/bap021

van der Meij A, Worsley SF, Hutchings MI et al (2017) Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev 41:392–416. https://doi.org/10.1093/femsre/fux005

Vanlnsberghe D, Arevalo P, Chien D et al (2020) How can microbial population genomics inform community ecology? Philosophic Transact Royal Soc B 375:20190253. https://doi.org/10.1098/rstb.2019.0253

Vilchez-Vargas R, Junca H, Pieper DH (2010) Metabolic networks, microbial ecology and "omics" technologies: towards understanding in situ biodegradation processes. Environ Microbiol 12:3089–3104. https://doi.org/10.1111/j.1462-2920.2010.02340.x

Wang J, Wong GK, Ni P et al (2002) RePS: a sequence assembler that masks exact repeats identified from the shotgun data. Genome Res 12:824–831. https://doi.org/10.1101/gr.165102

Wattam AR, Abraham D, Dalay O (2014) PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:D581–D591. https://doi.org/10.1093/nar/gkt1099

Weiss RA (2002) Virulence and pathogenesis. Trends Microbiol 10:314–317. https://doi.org/10.1016/s0966-842x(02)02391-0

Winsor GL, Khaira B, Van Rossum T et al (2008) The *Burkholderia* genome database: facilitating flexible queries and comparative analyses. Bioinformatics 24:2803–2804. https://doi.org/10.1093/bioinformatics/btn524

Winsor GL, Lam DKW, Fleming L (2011) *Pseudomonas* genome database: improved comparative analysis and population genomics capability for *Pseudomonas* genomes. Nucleic Acids Res 39: D596–D600. https://doi.org/10.1093/nar/gkq869

Wozniak RA, Waldor MK (2010) Integrative and Conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat Rev Microbiol 8:552–563. https://doi.org/10.1038/nrmicro2382

Zerbino DR (2010) Using the velvet de novo assembler for short-read sequencing technologies. Curr Protocol Bioinform 31:11–15. https://doi.org/10.1002/0471250953.bi1105s31

Ziemert N, Alanjary M, Weber T (2016) The evolution of genome mining in microbes-a review. Nat Prod Rep 33(8):988–1005. https://doi.org/10.1039/c6np00025h

Bioinformatics: A Tool for Sustainable Agriculture

13

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Abstract

Bioinformatics is a discipline of research that uses computational power for extraction of useful information from biological data. Bioinformatics utilizes computer technology for storage, analysis, and retrieval of genetic information which is achieved as a result of various scientific processes. In line with the research of biological bodies, keeping information of 100,000 genes of a human being without the availability of computational power would be beyond imagination. Biotechnology has become more efficient with the availability of highly reliable computer-assisted predictions. With the need for high amounts of agricultural products for ever-growing population, pesticides and chemical fertilizers were once successful in catering to the demands. After decades of practices of such products for agriculture, it was revealed that chemical fertilizers and pesticides leave a negative impact on the atmosphere which in turn is harmful for sustainable environment. Recent progress in computational software and computing tools along with modern bioinformatics analysis and genome analysis are proving to be successful in diminishing the effects of harmful agriculture and creating a better and sustainable agroatmosphere.

Keywords

Bioinformatics · Web tools · Sustainable agriculture · Genomics · Metabolomics · Interactomics

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_13

13.1 Introduction

Bioinformatics utilizes computational power and techniques for extraction of knowledge from biological data stored in digital forms. These methods comprise collection, storage, retrieval, manipulation, and data modeling which can otherwise be utilized to develop computer-based algorithms and solutions involving bioinformatics. Bioinformatics, along with Human Genome Product (HGP), combines life science, computer science, and statistical methods for the development of creative application utilizing computational methods and statistical techniques. Bioinformatics can also be perceived as the use of computer techniques for accumulation, storage, analysis, and comparison of biological information with improved speed and accuracy. For example, if we want to study 15,000 gene structures of *Arabidopsis* plant with another plant, it would take years to complete without computers. If we look forward to keep track of 100,000 genes of a human being without computers, it will be practically inconceivable. Computers make the comparison process automated and store information real time, thereby simplifying the immense task of comparison and analysis. This stored information can be used for the construction of models for simplifying the experimentation and analysis process.

By the evolution of such computational methods researchers are now aided with tools that can produce technically reliable predictions of test results of genetic modifications. This has on the other side made biotechnology more efficient (Hoffmann and Valencia 2004). Scientists are able to use fairly reliable computer-assisted predictions of test results on genetic modifications. This indeed helps in predicting the result prior to in vivo application. This complements the time-consuming process involved in growing out every modified plant in the laboratory or greenhouse to test for the desired modification.

13.2 Role of Bioinformatics

Bioinformatics evolved as a major discipline in biology in its success in locating the genes and in phylogenetic comparisons. These tools range from image processing techniques that read out the data to visualization tools that provide a first-sight hint to the biologists, and from preprocessing techniques (Durbin et al. 2002) that remove the systematic noise in the data to clustering methods (Eisen et al. 1998; Sheng et al. 2003), and reveal genes that behave similarly under different experimental conditions. Proteomics, another field of bioinformatics, can be utilized for protein structure analysis and discovery of sequence sites in the locations of protein-protein interaction. Stuart et al. (2003) emphasized the need of bioinformatics for revealing the biology at the systems level. Further, metabolomics—study of *metabolome*, which is the study of cell dynamics can be used to simulate cellular interactions. Bioinformatics provides analytical tools for microarray data (Brady and Provart 2009; Chellappan and Jin 2009), just to name a few. Microarray technology has opened genomics and bioinformatics has aided in genome sequencing, and has shown new dimensions.

13.2.1 Web Tools and Resources

The World Wide Web (www) and tools like Web services and Web 2.0 have provisioned numerous tools for the purpose of data sharing within research groups. Publishing research and innovation findings over the Web has been simpler and faster than before. Data accession simplicity has improved collaborations and data dissemination among institutions which has tremendous impact on the sharing of large-size data sets. In addition to this, numerous security improvements in terms of access security, cryptography, and network security have a crucial role in securing the research finding within intended research groups or proving access-based control to parties or groups over shared information or data sets (Berners-Lee et al. 2001).

These developments and collaborations have made some tools de facto standards among major institutions. We can find that BLAST (Altschul et al. 1997) family of applications has evolved as a Web application which provides dynamic access to genome and protein data sets over Web apart from the ability to search *homologs* of DNA input sequences and protein libraries. Numerous laboratories provide online *BLAST* interface to their DNA or protein sequence databases which allows scientists to identify homologs of provided input sequences. This capability empowers the researchers with the ability to compare new sequences with previously known sequences and have their findings validated by other members of the research community.

There are numerous Web lists of bioinformatics resources, with many aimed at the biologist looking for software. Some of these, such as Bioinformatics.net, include discussion forums on the use of biology software. These are useful for biologists, as well as bioinformatics engineers looking for tools related to their work, or to be used at service centers. Many of these share a similar organization by functional categories, with many of the same links often with different functionalities. As a result of these types of collaborations, some tools become de facto standards in the communities as they are shared among a large number of institutions. For instance, consider the BLAST (Altschul et al. 1997) family of applications, which allow biologists to find homologs of an input sequence in DNA and protein sequence libraries. BLAST is an example application that has been enhanced as a Web source, which provides dynamic access to large data sets. Many genomics laboratories provide a Web-based BLAST interface (http://blast.wustl.edu/) to their sequence databases that allow scientists to easily identify homologs of an input sequence of interest. This capability enhances the genomics research environment by allowing scientists to compare new sequences with every known sequence and to have their work validated by other members of the community. The addition of new sequences at an increasingly frequent rate (NIAS DNA Bank, http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.htm) further increases the value of this capability. General resources such as Google, Amazon's Alexa, and Open Directory Project at Mozilla.org include biology and bioinformatics categories in their directories. These directories are populated by robots or from submissions; they tend to lack the comprehensiveness of biologist-maintained lists (Hucka et al. 2003).

Bioinformatics.ca is a Web-based tool that provisions a categorized and curated list of links with sections including human genome and model organizations, sequences, gene expressions, education, and computer-related resources. The Genome Web at MRC, UK, presents a similar list of links with editorial abstracts. Bioinformatics.ca uses RSS which is an XML technique for sharing bioinformatics resources. RSS feeds can be utilized to notify users about any modifications to the user catalogue.

The "Bionetwork" project at "Pasteur Institute" provides an example of resource lists that can be explored by providing different types of bioinformatics criteria: biological domain, resource type, and organism (Blais and Dynlacht 2005). This is a search engine which is dedicated to the retrieval of resources that can be relevant for research in the biology and bioinformatics domain. For update of search databases, this search engine has a provision of link maintenance by using semiautomatic scanning of news and resources over the Internet.

BioHunt is another such tool that uses Internet robot technology to search and update molecular biology resources. *BioHunt* maintains its entries with update times review months for several searches, which makes it easier to find new or updated tools. *Bioinformatics.net* is a catalogue of online biology resources, specializing in bioinformatics tools. Its focus is towards the needs of molecular biologists and life science professionals, more than for bioinformaticians, and includes discussion and help forums on the use of software and bioscience topics. Jonathan Rees, who developed this resource, also curates biology lists in the Open Directory project. This service is supported in part by advertising, as are others reviewed here, one of the limited options available to maintain such services. Bioinformatik.de offers a similar directory-style collection of curated bioinformatics and biology resource links. The CMS molecular biology resource is an extensive catalogue of biology resources, including software tools. The Southwest Biotechnology Center also maintains a useful catalogue covering a broad range of biology resources. Bioinformatics.org and SourceForge.net are resources that support software developers and bioinformatics engineers, but are also useful to biologists looking for tools. Open-source software development in bioinformatics and other fields is being invigorated through agencies such as these. The number of active, widely used, and valuable bioinformatics projects at these services is growing, including Generic Model Organism Database, Gene Ontology, GeneX Gene Expression Database, and Staden Package for sequence analysis (Blazejczyk et al. 2007). These agencies allow for software archiving, but the primary attractions to software developers are infrastructure and tools that enable collaborative software development (Harris et al. 2004). A historical archive or catalogue service of bioinformatics software is limited, and maintenance of software releases is left to developers using this service.

13.3 Agriculture and India: A Brief History

The agricultural history of India can be related to the times of Indus Valley Civilization. In terms of farm output India is regarded as the country having second rank. As per statistical records of 2018, the agriculture sector provides employment opportunities for more than 50% of the total unstructured workforce and contributes to roughly 18% of the country's gross domestic product (Sundar 2018). It has also been reported that agriculture provides source of income for 58% of Indian households (IBEF 2019).

Agriculture sector along with animal husbandry, forestry, and fisheries accumulated for 15.4% of the total gross domestic product (CIA Factbook 2021; IBEF 2021; FAO 2010). India holds the first position in the world having the highest net cropped area followed by the USA and China. However, the contribution of agriculture towards Indian GDP growth is at a state of declination with the broad-based economy. But agriculture is arguably one sector that can have a pivotal role in weaving the fabric of economy for a country like India. The agricultural exports market in 2013 of $38 billion dollars put India at seventh place in the global context (Flake 2014). Indian agricultural/horticultural products and processed foods are exported to more than 120 countries, primarily to Japan, Southeast Asia, SAARC countries, the European Union, and the USA (Flake 2014).

13.3.1 Postindependence Agricultural Scenario in India

The *Green Revolution* in India started in 1965 which was an initiative of M.S. Swaminathan. The 1943 famine of West Bengal, erstwhile known as Bengal state under the British rule, was one of the most severe famines that accounted for the death of over three million people. With little help from the industrial sector and minimal appreciation of importance of agriculture in the British era, the agriculture sector was in a disastrous state in the country. Very soon after independence, India realized that the country needs to be self-sufficient in forms of agriculture which led to the birth of the *Green Revolution*. It began with the decision to adopt superior yielding disease-resistant wheat varieties in combination with better farming knowledge to improve productivity. The major stakeholders in executing the plan comprised the Government of India, multilateral and bilateral donors, International Agricultural Research Institution/s, farmers, and peasants.

The *Green Revolution* marks the implementation of technological advancements to the traditional farming and agricultural methods. The traditional methods of farming include improved irrigation systems, practice of harvesting mixed crops, and more importance to harvesting local crops/plant species. Advancements in the form of unsustainable technological approaches have resulted in the reduction of groundwater table, soil erosion, and loss of biodiversity. The Green Revolution that was focused on the growth of high-yielding crop varieties of plants and grains quickly found its application in Indian states like Punjab, Haryana, Uttar Pradesh, Tamil Nadu, and Kerala. The paradigm shift and the change in methods have been

continuously making changes to the environment and ecology which are irreversible in nature. Furthermore, it is difficult to revert to organic farming on a land which has been through a series of mass production methods as such methods tend to deteriorate the soil quality and other nutrients.

Punjab was the first state in India to steer the Green Revolution and became to be known as the country's bread basket. The early stage of growth in agricultural production was initiated mostly by states of Punjab, Haryana, and western Uttar Pradesh, and most importantly with aided support from government officials, production witnessed a significant increase. Farms in India produced wheat at an average rate of 0.8 tonnes in 1948 and the same was 4.7 tonnes in 1975 per hectare. The average rate of production of wheat in 2000 was 6 tonnes per hectare from the same fields. This data has been received as per the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) data (FAOSTAT 2014).

There have been several discrepancies regarding agricultural production since the inception of the Green Revolution. Proponents of the Green Revolution say that it solved the problem of malnutrition but adversaries say that it made it worse. One of the reasons is because monoculture and chemical fertilizers have taken the nutrition out of the food and the soil. There have been many myths regarding soil, high-yielding varieties (HYV), and industrial breeding in comparison to organic breeding of plants. The myths regarding HYVs are that they produce a high partial yield and low total system yield, and indigenous varieties get a better yield than HYVs. Industrial breeding focuses on quantity rather than nutrition per acre and partial yield rather than multiple crops; this type of breeding and planting also removes the focus from local varieties of plants and shifts it to plants that are traded worldwide (Agriculture Marketing 2008).

The postindependence agricultural scenario has been quite different from what it used to be under the British rule. Along with the growth in population, the food grain production has been on a substantial rise. This has also improved the per capita food grain availability to Indian citizens. Prior to mid-1960, India relied on agricultural imports and aids of food from foreign nations and organizations for its domestic requirements. The draughts of 1965 and 1966 forced India to restructure its agricultural policy. It was also perceived that India's dependence on foreign aid and imports for ensuring food security may not be considered as a viable option for a longer time and this perception propelled the country's need for becoming self-sufficient in forms of agriculture and food grains which eventually gave birth to the Green Revolution.

After receiving overwhelming success in the production of wheat, the technology of Green Revolution was passed on to the production of rice. At that time with limited amount of irrigation, several methods for harvesting of groundwater were innovated. This knowledge of water harvesting was passed on to eastern Indian states like Bihar, West Bengal, and Odisha. The seed quality was improved and the total agricultural yield from the irrigated areas accounted for around one-third of the total crop harvest of the country. In 1980s the policy changed to "production in line with the demand" and also generated focus on several other agricultural crops like oilseeds, fruits, and vegetables. Other areas like dairy, fisheries, and livestock also

found infusion of new methods and techniques to meet the requirement of an ever-growing population. The history of Indian agriculture has a significant impression in the world history from the eighteenth century to the nineteenth century (Vertovik 1995).

13.4 The Need for Sustainable Agriculture

Agricultural sustainability nurtures the mankind and the land resource. With the advent of industrialization, agrochemicals were the propelling force behind the development of agriculture in India and they became a major necessity for rapid crop growth and prevention of crops from destruction which was much needed for the demand of growing population in the postindependence era. After decades of such practices in agriculture, issues like new diseases, disturbances in organic cycles, and other such parameters which are necessary for sustainable environment and ecological balance were witnessed.

These circumstances mandate the need for necessary measures to reverse such adverse effects on an urgent basis (Nayak et al. 2018). The best practices for sustainable agriculture call for the need of healthy quality food without adversely impacting the ability of future generations. In order to achieve this kind of environment-friendly cultivation methods, we need to maintain a right equilibrium between production of crops and preservation of the ecosystem.

13.4.1 Positive Impacts of Sustainable Agriculture

13.4.1.1 Towards a Healthier Ecosystem by Employing Natural Resources

The practices for sustainable agriculture ensure optimized utilization of natural resources, thereby making it utilizable for future generations. A case in point is the use of efficient irrigation techniques like sprinkle and drip irrigation techniques used by companies like Jain Farm Fresh Limited. The utilization of these evolved techniques has resulted in as much as a 60% reduction in water usage. Sustainable agricultural practices promote healthy ecosystems, as everything and everybody flourish in a balanced environment (Mishra et al. 2021).

13.4.1.2 Reduces Pollution and Adverse Effects on the Land Resources

Sustainable farming techniques work towards adopting good agricultural practices so that there is minimum wastage of crop and resource. Farmers are trained to use pesticides and fertilizers most optimally, which results in two positives. One, there are zero pesticide residues in the crop. Two, the land around the farms, which is made redundant by the overload of chemicals in the soil, can be salvaged.

13.5 Bioinformatics as a Tool for Sustainable Agriculture

The issue of sustainable agricultural production has emerged as a global issue in response to climate change and population increase. The need and the research for biofuels have made biofuel-yielding crops as new profitable crops. We can achieve solutions to this problem in a way if we can have modified plant genetic functions which can in turn provide tolerance to environmental factors and yield more growth. Plant genomics research helps us discover and regulate important plant genes which can provide better yields and more tolerance to environmental factors.

13.5.1 Genomics, Metabolomics, and Interactomics for Sustainable Agricultural Development

In addition to being a major occupation for people in a few countries, agriculture can also be associated with having its impact on the lifestyle and cultural diversities of populations. Cereals and sugarcane have always been considered as important food by majority of populations worldwide. From ancestral times, people are using their own indigenous techniques for cultivation of such crops. The Green Revolution that happened during 1960–1970 resulted in improving of productivity and side by side preserving of the taste and nutritional values of the crop yield.

However, with the side effects of Green Revolution, we are no longer capable to survive by a few numbers of "high-yield" varieties. This creates the need for advanced methods in agronomy to maintain the supply to the growing population and preserve nutritional values of food grains. This also aims to cater to the demand for future generations with less availability of land and energy and climatic changes.

The last few years have witnessed a new area of agriculture assisted by bioinformatics and computational biology. Computer science along with plant biology has made rapid progress in genomic sequencing and also made this a cost-effective technique (Esposito et al. 2016).

Inclusion of modern methods of biotechnology will have significant boost to sectors like bioenergy and agroindustries along with scopes for improved plants and better environmental management. Modern genome sequencing methods provide scopes for the study of genetics of various plant species and trace the difference in scores of such species within and outside the population (Xue et al. 2008). It also looks for genetic mutations which can in turn be essential in the development of a plant species with desired traits (Fig. 13.1).

Plant genomics can be helpful for the following activities:

- De novo assembling and genomic sequencing of new plant varieties
- Listing of genes with ontology and functional annotation
- Discovery of a great quantity of single-nucleotide polymorphism (SNP)/insertion-deletion length polymorphism (InDeL) markers for selection of improved crop breed

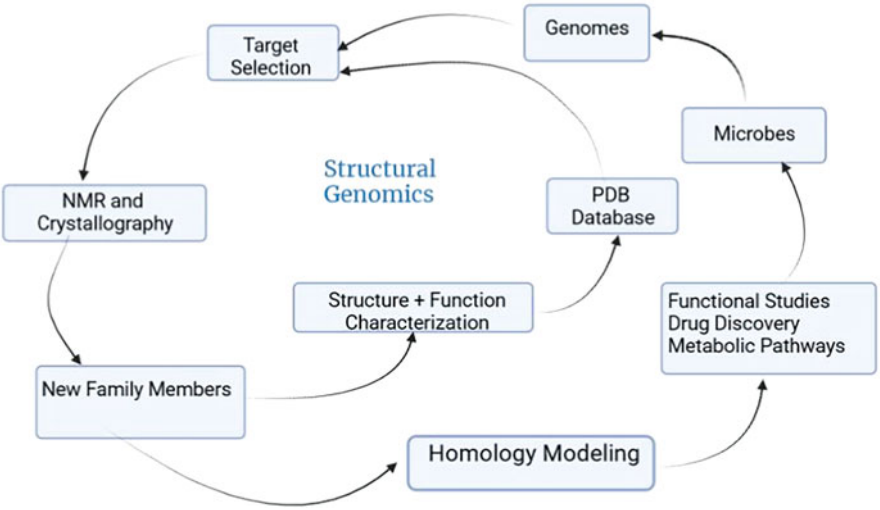

Fig. 13.1 Schematic diagram of structural genomics

- Candidate gene/allele identification with desired qualities after differentiation of necessary *quantitative trait loci* (QTLs) using various mapping techniques
- Creating of "MarkerChip Panel" for the purpose of genotyping and selection

With these capabilities metabolomics can be termed as one of the first emerging techniques in the field of *omics* and can be used to scan all metabolites present in the sample using LC-MS, NMR-MS, and GC-MS instruments (Choi and Pavelka 2012). Figure 13.2 represents the process of metabolomics.

13.5.2 Impact of Genome Sequencing in Agriculture

The term genome can be applied particularly to the whole genetic material of an organism including the full set of nuclear DNA (i.e., nuclear genome) and also to the genetic information stored within organelles, which have their own DNA—the "mitochondrial genome" or the "chloroplast genome" (Alonso et al. 2015; Ansorge 2009).

There are some organisms who have multiple copies of chromosomes which are termed as *diploid, triploid,* and *tetraploid*; typically in eukaryotes the gamete has half of the number of chromosomes of the somatic cell and the genome is a complete set of chromosomes in a gamete (Heinrich and Schuster 2012). In addition to this, genomes can contain genetic components like viruses, plasmids, and other transposable elements. There are some biological elements which are often more complex than viruses and tend to possess extra genetic materials along with their own chromosomes. Hence, we can say that "genome" describes all of the genes and related information on the noncoding DNA which may have potential chances of

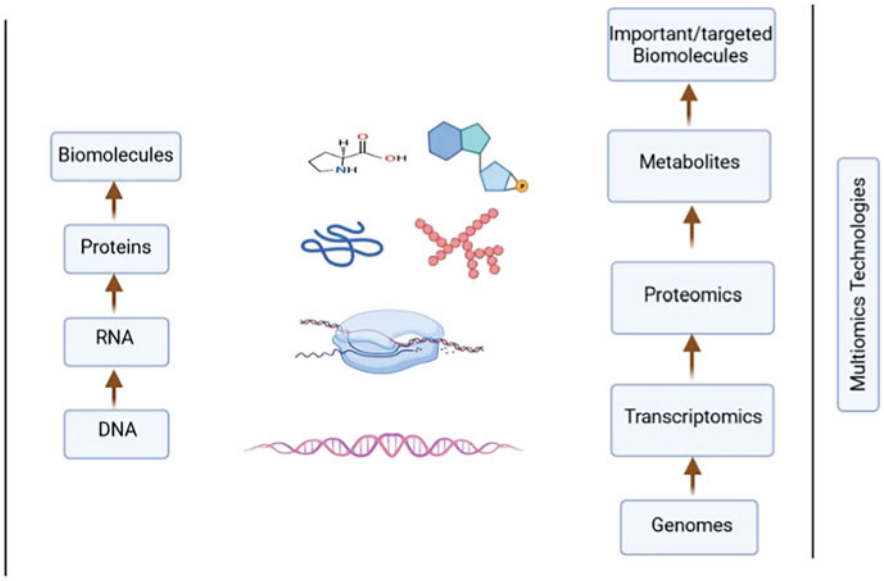

Fig. 13.2 Multiomics technology

presence (Chellappan and Jin 2009). But in plants and animals, "genome" is typically associated with only the information on chromosomal DNA. The genetic information contained by DNA within organelles, i.e., chloroplast and/or mitochondria, is not considered to be a part of the genome. Practically, mitochondria are sometimes mentioned to carry their own genome often called "mitochondrial genome." The DNA established in the chloroplast may be called "plastome."

13.5.3 Applications of Agricultural Bioinformatics

The facilities for storing and collecting plant genetic resources can be utilized to develop stronger, disease- and insect-resistant crops and improve the quality of agro-yield. Collection and storage of plant genetic resource can be used to manufacture stronger, disease- and insect-resistant crops and improve the quality of livestock making them healthier, more resistant to diseases, and more productive. The process of comparative genetics of various model and non-model plant species can be helpful in gene organization which is used for information transfer from model crop species to other crop species. The examples of existing full plant genomes are *Arabidopsis thaliana* (watercress) and *Oryza sativa* (rice) which can be taken for consideration (Caicedo et al. 2007; He et al. 2010).

Plant-based biomass can be useful in the conversion of biomass to biofuels which could be utilized as fuels in vehicles and higher machineries. For this purpose, crops which facilitate the production of biomass like maize and lignocellulosic species like

bagasse and straw can be cultivated in a rapid scale along with the use of genomics and bioinformatics. This method of production will solve a two-dimensional purpose. The first one will cater the demand of the growing population and the second one will be helpful in catering to the demand for nonconventional sources of energy.

In this context, *Bacillus thuringiensis*—a bacterium that lives in the soil and can control a number of serious pests—has been successfully transferred to plants like potatoes, cotton, and maize and this new feature of the plants to resist insect outbreaks will significantly reduce the use of conventional insecticides and would improve the nutritional value of the crop.

Developing certain cereal varieties that possess greater tolerance to soil alkalinity and other toxic materials like iron and free aluminum can also be regarded as one of the major achievements in sustainable agriculture. These varieties possess the capabilities like more plant growth in areas having poor soil quality which will in turn add to the amount of cultivated areas. The purpose of plant genomics in this regard can be related to the understanding of genetic and molecular basis of biological processes of plants that correspond to the species. This will allow explicit exploitation of plant species as biological resources in the evolution of new plant species with better quality and lesser cost on the environment.

13.5.4 Molecular Plant Breeding

Genetic maps in important crops have the tendency to expand. Moreover, the molecular basis for certain characteristics or physiological responses can be further clarified with the tendency for expansion. This will enable researchers to associate potential candidate genes that are found in model species with relevant loci in crop plants. With available relevant data it will be possible to develop gene sequences or genetic map positions. With implementation of new tools that can search for possible candidate genes, there will be less difference between breeding and molecular genetics. Computer models can be utilized to formulate predictive hypotheses to establish new phenotypes from complex allele combinations and then make those combinations by scoring major populations for a lot of numbers of genetic markers (Parray et al. 2019).

The resources utilized in this technology over years will be linked to basic plant biology and will be helpful in clarifying gene functions in model organisms. For example, characteristics that are badly determined at the biochemical level but well established as a visible phenotype can be related to high-resolution mapping with candidate genes. The particular phenotypes of commercial interest which are expected to be spectacularly improved by this progress include both the improvement of factors which frequently limit agronomic performance (input traits) and the change of the amount and type of materials that crops produce (output traits). Examples include:

- Abiotic stress tolerance (cold, drought, immersion, salt)
- Biotic stress tolerance (fungal, bacterial, viral)

- Nutrient-use efficiency
- Management of plant architecture and progress (size, shape, number, position, and timing of evolution, senescence)
- Metabolite division (redirecting of carbon flow through existing pathways, or moving into new pathways)

13.6 Conclusion

The era of computational biology, information management, and retrieval techniques has been quite prevalent in recent times and more such dimensions are expected to appear with more research in this particular field. Plants play diversified roles in the growth of society and maintenance of major ecological chains and cycles. Crop growth improvement is a surety owing to the development in agricultural techniques and other enhanced breeding techniques. Using genomics, a large amount of information can be utilized to study and develop new plant phenotypes. Genomic analysis is possible by gathering sequence data from various databases. These research mechanisms will together be helpful in a multitude of actions like providing food and nutrition to the growing population, preserving nutritional values of crops, saving the crops from pests, and enhancing fertile lands for growing population. In addition to this, new horizons like research for plants for biofuels will bring new dimensions to the requirement for clean and renewable energy sources. By combining all such methods, we can be able to minimize the loss that has so far occurred to the environment by the use of chemical fertilizers and pesticides that have been in practice for decades and it will also make the future generations ready for living in a sustainable atmosphere with balanced cycles and organic chains.

References

FAO (2010) The state of world fisheries and aquaculture. United Nations

Agriculture Marketing (2008) https://india.gov.in. Accessed Feb 2008

Alonso R, Salavert F, Garcia-Garcia F et al (2015) Babelomics 5.0:functional interpretation for new generations of genomic data. Nucleic Acids Res 43(W1):W117–W121. https://doi.org/10.1093/nar/gkv384

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402. https://doi.org/10.1093/nar/25.17.3389

Ansorge WJ (2009) Next-generation DNA sequencing techniques. New Biotechnol 25(4):195–203. https://doi.org/10.1016/j.nbt.2008.12.009

Berners-Lee T, Hendler J, Lassila O (2001) The semantic web. Sci Am 284(5):34–43

Blais A, Dynlacht BD (2005) Constructing transcriptional regulatory networks. Genes Dev 19(13):1499–1511. https://doi.org/10.1101/gad.1325605

Blazejczyk M, Miron M, Nadon R (2007) FlexArray: a statistical data analysis software for gene expression microarrays. Genome Quebec, Montreal, Canada. http://genomequebec.mcgill.ca/FlexArra

Brady SM, Provart NJ (2009) Web-queryable large-scale data sets for hypothesis generation in plant biology. Plant Cell 21(4):1034–1051. https://doi.org/10.1105/tpc.109.066050

Caicedo AL, Williamson SH, Hernandez RD et al (2007) Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS Genet 3(9):1745–1756. https://doi.org/10.1371/journal.pgen.0030163

Chellappan P, Jin H (2009) Discovery of plant microRNAs and short-interfering RNAs by deep parallel sequencing. Methods Mol Biol 495:121–132. https://doi.org/10.1007/978-1-59745-477-3_11

Choi H, Pavelka N (2012) When one and one gives more than two: challenges and opportunities of integrative omics. Front Genet 2:105. https://doi.org/10.3389/fgene.2011.00105

CIA Factbook (2021) India-Economy. https://www.cia.gov/the-world-factbook/countries/india/. Accessed 20 July 2021

Durbin BP, Hardin JS, Hawkins DM et al (2002) A variance-stabilizing transformation for gene-expression microarray data. Bioinformatics 18(suppl_1):S105–S110. https://doi.org/10.1093/bioinformatics/18.suppl_1.S105

Eisen MB, Spellman PT, Brown PO et al (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci 95(25):14863–14868

Esposito A, Colantuono C, Ruggieri V et al (2016) Bioinformatics for agriculture in the next-generation sequencing era. Chem Biol Technol Agric 3:9. https://doi.org/10.1186/s40538-016-0054-8

FAOSTAT (2014) Data. Faostat.fao.org. Accessed 17 Sept 2011

Harris MA, Clark J, Ireland A et al (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32:D258–D261. https://doi.org/10.1093/nar/gkh036

He G, Zhu X, Elling AA et al (2010) Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. Plant Cell 22(1):17–33. https://doi.org/10.1105/tpc.109.072041

Heinrich R, Schuster S (2012) The regulation of cellular systems. Springer US. https://doi.org/10.1007/978-1-4613-1161-4

Hoffmann R, Valencia A (2004) A gene network for navigating the literature. Nat Genet 36(7):664. https://doi.org/10.1038/ng0704-664

Hucka M, Finney A, Sauro HM et al (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19(4):524–531. https://doi.org/10.1093/bioinformatics/btg015

IBEF (India Brand Equity Forum) (2021) Agriculture and food industry and exports. https://www.ibef.org/industry/agriculture-and-food-industry-india.aspx. Accessed June 2021

IBEF (India Brand Equity Forum) (2019) Agriculture in India: information about Indian Agriculture & Its Importance. https://www.ibef.org/industry/agriculture-india.aspx

Mishra BB, Nayak SK, Mohapatra S et al (eds) (2021) Environmental and agricultural microbiology: applications for sustainability. Wiley-Scrivener publisher, Beverly

Nayak SK, Dash B, Baliyarsingh B (2018) Microbial remediation of persistent agro-chemicals by soil bacteria: an overview. In: Patra J, Das G, Shin HS (eds) Microbial biotechnology. Springer, Singapore, pp 275–301. https://doi.org/10.1007/978-981-10-7140-9_13

Flake L (2014) India's Agricultural Exports Climb to Record High. International Agricultural Trade Report, US Department of Agriculture. https://www.fas.usda.gov/sites/default/files/2015-02/india_iatr_august_2014

Parray JA, Mir MY, Shameem N (2019) Advancement in sustainable agriculture: computational and bioinformatics tools. In: Sustainable agriculture: biotechniques in plant biology. Springer, Singapore, pp 465–547. https://doi.org/10.1007/978-981-13-8840-8_10

Sheng Q, Moreau Y, De Moor B (2003) Biclustering microarray data by Gibbs sampling. Bioinformatics 19(suppl_2):ii196–ii205. https://doi.org/10.1093/bioinformatics/btg1078

Vertovik S (1995) Indian indentured migration to the Caribbean. In: Cohen R (ed) The Cambridge survey of world migration. Cambridge University Press, Cambridge, New York, pp 57–68

Stuart JM, Segal E, Koller D et al (2003) A gene-coexpression network for global discovery of conserved genetic modules. Science 302(5643):249–255. https://doi.org/10.1126/science.1087447

Sundar S (2018) India economic survey 2018: farmers gain as agriculture mechanisation speeds up, but more R & D needed. In: The Financial Express. https://www.financialexpress.com/budget/india-economic-survey-2018-for-farmers-agriculture-gdp-msp/1034266/. Accessed 29 Jan 2018

Xue J, Zhao S, Liang Y et al (2008) Bioinformatics and its applications in agriculture. In: Li D (ed) IFIP international federation for information processing, Volume 259; Computer and computing technologies in agriculture, vol 2. Springer, Boston, pp 977–982

Recent Advances in Deep Learning CNN Models for Plant Disease Detection

14

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Abstract

Machine learning and deep learning techniques are being used frequently in recent days for plant disease detection. The deep CNN models have been used in different fields and have gained immense result. With the growing population in the world, the importance of plant protection that produces food is also tremendously increasing. Various recent works have applied deep CNN models in the agricultural field and contributed a lot to specially w.r.t. various disease detection. It not only gives high prediction accuracies but also improves the other parameters, i.e., sensitivity, specificity, and F1 score of the model, which signifies better model for plant disease detection. Here, a survey of papers has been presented showing the use of different pre-trained CNN models in the field of plant disease detection. The summarized findings clearly indicate that CNN models are enriched with techniques that give promising performance with better precision and accuracy.

Keywords

Deep learning · AlexNet · ResNet · CNN model · Plant disease

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S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_14

14.1 Introduction

Every farmer should go through smart farming in order to increase productivity, face the adverse environment, and more importantly ensure food security (Gebbers and Adamchuk 2010). Due to the hike in global population (Kitzes et al. 2008), food production should also increase in the same proportion to meet the balance. There are various reasons behind plant diseases; it can be caused by viruses, bacteria, fungi, pest, or other agents. The symptom of disease can be found in root, stem, leaf, and fruit (Riley et al. 2002). The crop yield decreases due to plant diseases resulting in food crisis. The aim must be not only to produce high-quality nutritious food but also to maintain the farming ecosystem (Carvalho 2006). For these, there is the requirement to understand the complex agricultural ecosystem. This can be achieved by continuously measuring various complex phenomena. Disease detection is getting more challenging with the introduction of various crop varieties. The method of disease detection is very tedious and costly, so there is the requirement of evolvement of new techniques (Sharma et al. 2020). With the introduction of computer vision, new techniques are getting evolved for the quick and accurate detection of plant diseases with visible symptoms. Difficulties of identifying different features of diseases have been reduced with the introduction of deep learning models. Various studies in recent years have proved the capabilities of deep learning models in the identification of diseases (Kurniawati et al. 2009; Mohanty et al. 2016; Wang et al. 2017). The main challenge with different models is the huge difference between the training and the testing accuracies. This in technical terms is known as the model overfitting or the model underfitting. Different methodologies like simplifying or enhancing the complexities of deep models have been followed to overcome the challenges. Also, the volume of data set significantly impacts the achievement of better accuracy. Convolutional neural network (CNN) has been used in deep models for feature extraction by identifying the patterns. But it needs huge data set known as training and testing set containing images (Lee et al. 2015).

This chapter presents the survey on various deep CNN models like AlexNet, VGGNet, and ResNet used for plant disease detection with their output and accuracy. It contains seven sections. It highlights the knowledge about various plant diseases and describes about different CNN models in detail. It also focuses on various data sets available, highlights the deep models used in various papers, and makes comparison in terms of various parameters. At last, a brief summarization of results, further research scope, and conclusion is made.

14.2 Various Plant Diseases

Plant diseases can be biotic or abiotic. Biotic diseases are caused by the living organisms, and abiotic diseases are due to bad environmental effect. The latter is less dangerous and can be avoided (Sankaran et al. 2010). But biotic diseases are much dangerous and cause severe damage to food production. There are three major players.

14.2.1 Caused by Fungus

More than 80% of plant diseases are caused by fungus. Wide varieties of vegetables are affected by fungus. Due to the damage of cell by fungal infection, the plant stress increases. The source of infection can be contaminated soil, water, animals, etc. They enter through natural stomatal opening or through wounds caused by harvesting, insects, animals, etc. Table 14.1 shows fungal diseases with the crops they affect and conducive factors that help them to grow.

14.2.2 Caused by Bacteria

There are approximately 200 types of bacteria that cause plant diseases. With conducive environment, bacteria get active and harm the plant because they multiply themselves in favorable conditions like high humidity, poor soil health, and irregular watering. Bacteria of different strains harm different types of vegetable crops. Some bacterial diseases with their conducive environment and the plants they affect with the symptom are given in Table 14.2.

Table 14.1 Various fungal diseases (Dean et al. 2012)

Fungal disease	Factors conducive to spread	Crops affected	Symptoms
White rust	Within 3–4 h (6–24 °C)	Brassicas	White blisters and swellings on the leaves
Downy mildews	High humidity and leaf wetness	Onion, peas, and spinach	Yellow spot on leaves turns brown later
Powdery mildews	Moderate temperature (20–25 °C)	Potato, tomato, cabbage, and peas	Small white patches on the underside of leaves
Clubroot	Warm weather and acidic soil	Brassicas	Plant becomes yellow with clubroots
Pythium species	Cold and wet soil	Brassicas and cucurbits	Seedlings are affected and will die
Sclerotinia rots	Moist and warm condition	Beans, beets, carrots, and potatoes	Yellowish growth surrounds the disease area
Botrytis rots	Cool and wet weather	Cucumber, brassicas, and tomato	Sunken spot appears on leaves
Anthracnose	Wet and cool atmosphere	Tomato, potato, and capsicum	Yellowish growth surrounds the disease area
Tuber disease		Potato and sweet potato	Infection in potato tuber
Black root rot	Cool and moisture soil	Beans and cucurbits	Blackening of root

Table 14.2 Various bacterial diseases (Mansfield et al. 2012)

Bacterial disease	Factors conducive to spread	Crops affected	Symptoms
Black rot	Wet and warm condition	Brassicas	V-shaped yellow leaves
Bacterial canker	High humidity	Capsicum and tomato	Yellow leaves and tissue discolor
Bacterial leaf	Wind and overirrigation	Capsicum, tomato, and cucurbits	Black outer leaves and stems get greasy spot
Bacterial blight	Windy and wet condition	Peas	Dark brown leaves
Bacterial brown spot	Cool and windy condition	Beans	Reddish-brown leaves

Table 14.3 Various viral diseases (Scholthof et al. 2011)

Viral disease	Type of virus	Crop affected	Symptoms
Tobacco mosaic virus	Single-stranded RNA virus	*N. tabacum*	Mosaic patches on tobacco
Tomato spotted wilt virus	RNA virus	Tomato plant	Necrotic or chlorotic rings on leaves
Tomate yellow leaf curl virus	Single-stranded DNA	Tomato plant	Yellow leaf tomato
Cucumber mosaic virus	RNA virus	Cucumber plant	Light or dark green mosaic pattern
Potato virus	Single-stranded RNA	Potato plant	Brown and black line pattern
Cauliflower mosaic virus	DNA virus	Cauliflower	Mosaic marbling effect on leaf

14.2.3 Caused by Virus

Plant diseases caused by viruses are the rarest. If any plant gets affected, then the solution is to remove all infected ones, as it cannot be stopped by chemical treatment. Table 14.3 gives some of the plant diseases caused by viruses.

14.3 Different Deep CNN Models

Deep CNN models are the group of neural network models which have taken part in different computer vision competitions and have shown outstanding performances. They are giving exciting results in some of the applications like segmentation, classification, object detection, and natural language processing. For the automatic feature extraction in a deep model, we need huge data set because deep model is a complex model with huge parameters to set, so if the data set size is small, then there

might be the chance of overfitting. Layer by layer, the feature extraction and weight optimization are given in Eq. (14.1):

$$X^1 -> W^1 -> X^2 -> \ldots\ldots -> X^{l-} -> W^{l-1} -> X^l -> W^l -> \quad (14.1)$$

X^1 is the input layer and W^1 is the weight vector associated with the neurons. The output layer is X^l, which gives the resultant feature matric. With each training, the error generated leads to the upgradation of the weight by backpropagating. A loss function known as least square error is given below in Eq. (14.2):

$$\text{Loss} = \frac{1}{2}\left\|t - x^l\right\|^2 \quad (14.2)$$

The loss is needed in the neural network for the learning or the upgradation of the parameter. Weight upgradation takes place by the following different ways like stochastic gradient descent (SGD). SGD is a way of optimization given in Eq. (14.3):

$$w^i < -w^i - \eta \frac{\partial \text{loss}}{\partial w^i} \quad (14.3)$$

η is the learning rate.

Convolution means the inter-twinning of two functions as given in Eq. (14.4), where $f(x, y)$ and $h(m, n)$ represent the image and kernel, respectively:

$$(x, y) = \sum_{m=\frac{-M}{2}}^{\frac{M}{2}} \sum_{n=\frac{-N}{2}}^{\frac{N}{2}} h(m, n) f(x - m, y - n) \quad (14.4)$$

Then activation function is acted upon to add nonlinearity. Here, the deep CNN models that we will focus on will be the model that participated in ImageNet challenges like AlexNet, VGGNet, and ResNet.

14.3.1 AlexNet

AlexNet is a deep network model proposed by the group of members named A. Krizhevsky, G. Hinton, and I. Sutskever. It has bagged the first position of the ImageNet challenge in 2012. ImageNet is a database consisting of around 1.1 million images with 1000 classes. AlexNet has set up a strong base for the future of the CNN models. The top 5% error rate of AlexNet using the ImageNet database for classification was around 25% (Krizhevsky et al. 2012). Figure 14.1 gives the architecture of AlexNet, which contains five convolutional and three fully connected layers. The use of ReLU activation function here adds nonlinearity. Around 60 million parameters have been updated during the training through ImageNet data set. Convolution in deep network is the inter-twinning of image and the filter to generate

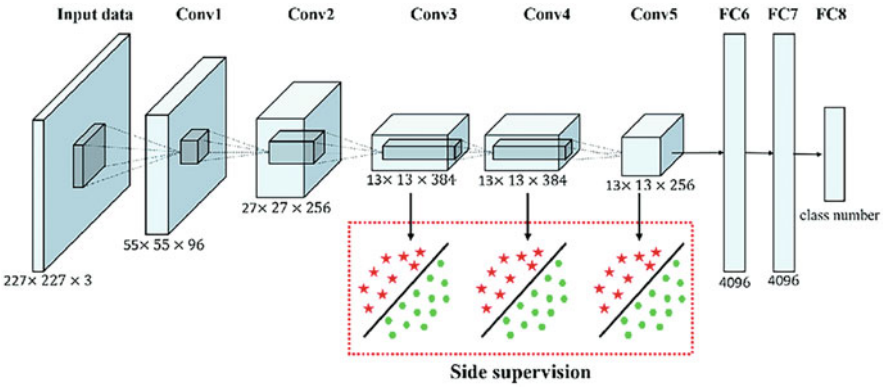


Fig. 14.1 AlexNet architecture (Krizhevsky et al. 2012)

Table 14.4 Detailed components of AlexNet architecture (Krizhevsky et al. 2012)

Layer	Input	Kernel	Output
Conv2D/4	227 × 227 × 3	11 × 11 × 64/4	55×55 × 64
Pool/2	55 × 55 × 64	3 × 3/2	27 × 27 × 64
Conv2D	27 × 27 × 64	5 × 5 × 192	27 × 27 × 192
Pool/2	27 × 27 × 192	3 × 3/2	13 × 13 × 192
Conv2D	13 × 13 × 192	3 × 3 × 384	13 × 13 × 384
Conv2D	13 × 13 × 384	3 × 3 × 384	13 × 13 × 384
Conv2D	13 × 13 × 384	3 × 3 × 256	13 × 13 × 256
Pool/2	13 × 13 × 256	3 × 3/2	6 × 6 × 256
FC1	6 × 6 × 256	5 × 5 × 4096	1 × 1 × 4096
FC2	1 × 1 × 4096	1 × 1 × 4096	1 × 1 × 4096
FC3	1 × 1 × 4096	1 × 1 × 1000	1 × 1 × 1000

a feature map. After one or many convolutions, the pooling layer has been used to extract the max or average feature using a window (Table 14.4).

Here, FC represents fully connected layer, pooling is done max value, and Conv2D represents 2D convolution. The drawback associated with AlexNet was the depth, which may lead to overfitting. This drawback has been challenged by Krizhevsky et al. (2012), by adapting the concept of Hughes et al. (2015), where they introduced the idea of neuron dropout. Neuron dropout is a technique for regularization. Also, the introduction of ReLU solves the problem of vanishing gradient. Here, the large-size filters like 11 × 11 and 5 × 5 have been used to restrict the length of deep model.

14.3.2 VGGNet

A research group of the University of Oxford has developed the deep network named Visual Geometry Group (VGG). It is also known as VGG-16 because it consists of 16 convolution layers. It has bagged the first runner-up position at ImageNet challenge 2014. It has been trained with ImageNet data set with 4 GPUs for 3 weeks. It is the most commonly used pre-training for classification. In VGGNet, around 138 million parameters have been trained. The architecture is explained below (Figs. 14.2 and 14.3).

Here, it has been considered that the reduced filter size can enhance the network performance. Here, instead of 11 × 11 and 5 × 5, 3 × 3 has been used. Also, it reduces the computational complexity. In VGG, padding has been done for maintaining spatial resolution. But here it is required to update approximately 138 million parameters, so it is computationally expensive.

14.4 ResNet

ResNet model was developed by Kaiming et al. (2015). It was the winner of ImageNet challenge 2015. It has introduced the skip connection concept. It has solved the problem of vanishing gradient. A total of 152 layers are there in ResNet. It reduced the top 5% error to 3.57%. The performance has enhanced much in object detection because of the residual network concept (Fig. 14.4).

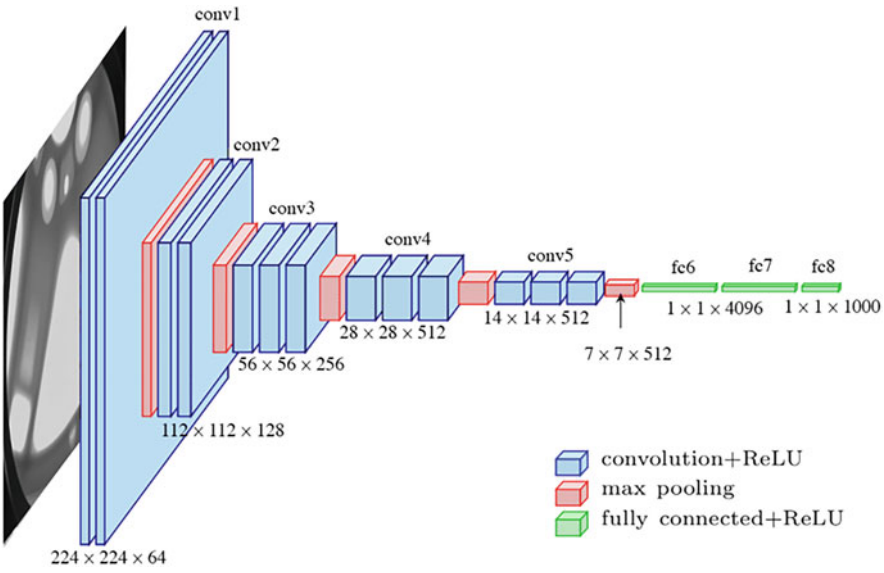


Fig. 14.2 VGGNet architecture (Simonyan and Zisserman 2014)

Layer (type)	Output shape	Param #
input_1 (InputLayer)	[(None, 224, 224, 3)]	0
block1_conv1 (Conv2D)	(None, 224, 224, 64)	1792
block1_conv2 (Conv2D)	(None, 224, 224, 64)	36928
block1_poo1 (MaxPooling2D)	(None, 112, 112, 64)	0
block2_conv1 (Conv2D)	(None, 112, 112, 128)	73856
block2_conv2 (Conv2D)	(None, 112, 112, 128)	147584
block2_pool (MaxPooling2D)	(None, 56, 56, 128)	0
block3_conv1 (Conv2D)	(None, 56, 56, 256)	295168
block3_conv2 (Conv2D)	(None, 56, 56, 256)	590080
block3_conv3 (Conv2D)	(None, 56, 56, 256)	590080
block3_pool (MaxPooling2D)	(None, 28, 28, 256)	0
block4_conv1 (Conv2D)	(None, 28, 28, 512)	1180160
block4_conv2 (Conv2D)	(None, 28, 28, 512)	2359808
block4_conv3 (Conv2D)	(None, 28, 28, 512)	2359808
block4_pool (MaxPooling2D)	(None, 14, 14, 512)	0
block5_conv1 (Conv2D)	(None, 14, 14, 512)	2359808
block5_conv2 (Conv2D)	(None, 14, 14, 512)	2359808
block5_conv3 (Conv2D)	(None, 14, 14, 512)	2359808
block5_pool (MaxPooling2D)	(None, 7, 7, 512)	0
flatten (Flatten)	(None, 25088)	0
fc1 (Dense)	(None, 4096)	102764544
fc2 (Dense)	(None, 4096)	16781312
predictions (Dense)	(None, 1000)	4097000

Fig. 14.3 VGGNet layer details

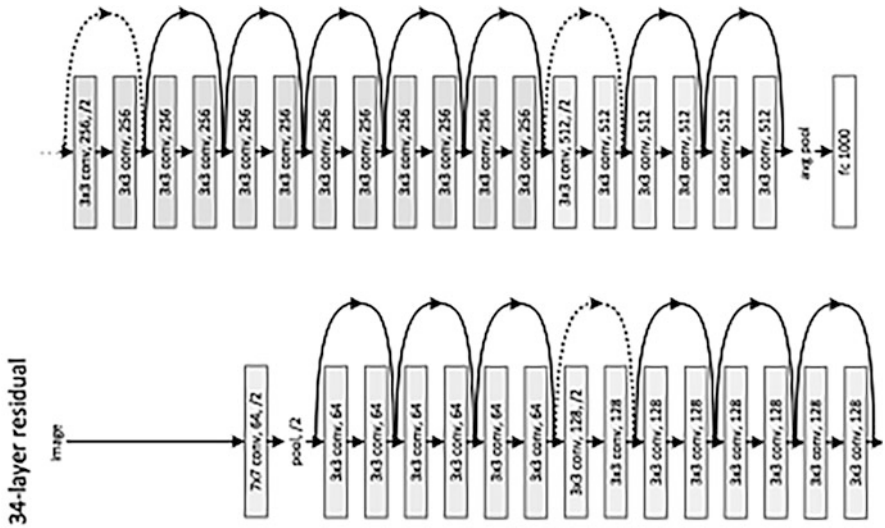


Fig. 14.4 ResNet50 architecture (Kaiming et al. 2015)

It has introduced the idea of residual network in deep CNN. The depth of ResNet is around 10–20 times than VGG and AlexNet. ResNet shows good performance over image localization and recognition. Different ResNet models are ResNet50 or 101 or 152 depending on their depth.

14.5 Various Materials Available Related to Plant Diseases

For different plant diseases, different image data are available. To train and test the huge deep CNN model, a large volume of data is needed. Also, different pre-trained models like AlexNet, VGGNet, and ResNet can be used via transfer learning to utilize the optimized weight. Hughes et al. (2015) have described very few number of data sets. It contains 58 classes with corresponding diseases and also some healthy plants (Table 14.5).

Sibiya and Sumbwanyambe (2019), presented in Table 14.3, have captured the possible maize plant diseases using their smart phone. Here, they have considered images for the diseases like leaf blight, leaf spot, leaf rust, and normal plant with 100 images each. They have used these data for the classification of different diseases and achieved an average of 92.85% accuracy. Zhang et al. (2018) used data set for different tomato disease detection using some predefined neural network like ResNet, GoogleNet, and AlexNet. Here, they have considered the diseases like early blight, Corynespora leaf spot, late blight, leaf mold, Septoria leaf spot, spider mite, virus diseases, and yellow leaf. The data set is divided into 80:20 as training and testing data. Then, the training data again undergoes augmentation process to generate large data set by doing horizontal, vertical, and diagonal flipping and

Table 14.5 Data set details

Author	Data set	Plant	Classes of disease	No. of images/ class
Sibiya and Sumbwanyambe (2019)	PlantVillage	Maize	Leaf blight, leaf spot, rust, normal images	100 each
Zhang et al. (2018)	PlantVillage	Tomato	Early blight	405
			Corynespora	547
			Late blight	726
			Leaf mold	480
			Septoria leaf	734
			Spider mite	720
			Virus disease	481
			Yellow leaf	814
			Normal leaf	643
Amara et al. (2017)	PlantVillage	Banana	Black sigatoka	240
			Black speckle	1817
			Normal	1643
Ferentinos (2018)	PlantVillage in-field image	Apple	Apple scab	630
			Apple rust	276
			Black rot	712
		Cabbage	Black rot	64
		Cassava	Brown leaf spot	43
		Celery	Early blight	1204
		Cherry	Powdery mildew	1052
		Corn	Cercospora leaf spot	1457
			Common rust	1614
		Cucumber	Downy mildew	1318
		Gourd	Downy mildew	114
		Grape	Black rot	1180
			Black measles	1384
			Leaf blight	1074
		Orange	Huanglongbing	5507
		Peach	Bacterial spot	2297
		Pepper	Bacterial spot	997
		Potato	Late blight	1000
			Early blight	3167
		Pumpkin	Cucumber mosaic	2387
		Soybean	Downey mildew	851
			Frogeye leaf spot	2023
		Strawberry	Leaf scorch	3396
Türkoğlu and Hanbay (2019)	Real-field data set	Walnut	Walnut leaf mite	69

(continued)

Table 14.5 (continued)

Author	Data set	Plant	Classes of disease	No. of images/ class
		Apricot	Apricot monilia laxa	85
		Rice	Xanthomonas	143
			Arboricola	
Lu et al. (2017)	Real-field data	Rice	Rice brown spot	500

contrast changing. The best accuracy of 96.8% is achieved with ResNet50. Amara et al. (2017) used the data set for different diseases in banana like black sigatoka and black speckle. The images are of different sizes, poses, orientation, and illuminations. Ferentinos (2018) has used the open database with 87,848 images, including 58 classes. Among those, 70,300 and 17,548 are used as training and testing images, respectively. It got an accuracy of 99.53% with VGGNet. Türkoğlu and Hanbay (2019)) have obtained images for different plant diseases as described in Table 14.3 using Nikon camera. Each color (RGB) image is with a resolution of 4000×6000. It is observed that AlexNet with SVM classifier achieved an accuracy of 95.5% over the combination of classifiers like Extreme Learning Machine (ELM) and K-nearest neighbor (KNN) with deep models like AlexNet, VGG16, and VGG19. Lu et al. (2017) have used the data set for rice diseases of ten kinds and achieved an accuracy of 95.48% with a deep model with stochastic pooling in comparison to mean and max pooling.

14.6 Related Work

14.6.1 Application

Jadhav et al. (2020) have used AlexNet for the classification of disease plant and normal soybean plant. Three types of soybean plant diseases have been classified here named as bacterial blight, brown spot, and frogeye spot. The final fully connected layer of AlexNet has been changed with a layer of four neurons. Using 649 images as training and 80 images as testing, the model has achieved 98.75% accuracy in 20 epochs. Zhang et al. (2018) have used fine-tunned ResNet50 by unfreezing the last three layers to classify the tomato diseases. The eight categories of tomato diseases like early blight, yellow leaf, virus disease, spotted spider, leaf mold, late blight, leaf spot, Corynespora, and one healthy leaf have been classified with 97.19% accuracy. Here, ResNet50 has been fine-tuned with last three layers and trained and tested with 4440 and 1110 images, respectively. Brahimi et al. (2017) have used a large data set containing around 14,828 images. The visualization method has been used here to analyze the model. Using AlexNet, they have achieved 99% accuracy than the classification models like SVM or Random Forest. Liu et al. (2018) have used AlexNet for the four-class classification of apple diseases where the deep model is used not only for the retrieval of feature but also to learn layered

features. The deep model achieved 91.19% accuracy. Durmuş et al. (2017) have used the AlexNet and SqueezeNet for the classification of diseases in tomato plants. PlantVillage data set has been used here with 80:20 as training and testing data. By keeping the batch size of 20 and using stochastic gradient descent optimizer, AlexNet has achieved 97.22% accuracy. Hu et al. (2020) proposed a deep network with IoT technology for multiple crop disease recognition. Here, they found that ResNet gives good result in comparison with others. The proposed system is the combination of video cameras and deep networks. The system achieved an average accuracy of 93.96% over VGGNet and AlexNet. Saleem et al. (2020) have made a comparative analysis of classification of 26 classes using various pre-trained deep networks available, but with ResNet, they achieved 95.66% accuracy. Srivastava et al. (2020) came up with a technique for sugarcane disease detection. Here, they have used different pre-trained models like VGG-16, VGG-19, and Inception V3 model in combination with different classifiers like SVM, KNN, and naive Bayes and found that VGG-16 with SVM classifier gives AUC as 90.2%. Models named VGGNet and ResNet have been used by Aversano et al. (2020) with around 1600 images to classify them into 10 classes. VGGNet gives an accuracy of 97% with a good precision. Qiu et al. (2021) have used the VGGNet as a feature extractor and linear discriminant technique for classification using 10 classes, where 9 are leaf with diseases and 1 is healthy leaf. By using augmentation, 5000 images are generated out of 1000 images where each class gets balanced with 500 images each. After tenfold cross-validation, the model got an accuracy of 97.08% with average precision and recall of 94.83% and 83.75%, respectively. Jiang et al. (2020) have done the identification of plant diseases by using ResNet. Here, they have frozen the layers to use the weights of ResNet optimized by training with ImageNet data set.

14.6.2 Comparison Accuracies with Training Samples

Figure 14.5 shows accuracies of different deep models like AlexNet, VGGNet, and ResNet w.r.t. a number of training samples as per Table 14.6. It is seen that AlexNet with less number of training sample is showing better performance over others. But as we know, with less number of training samples, there is the possibility of overfitting. Then, also with more number of training samples, AlexNet shows good performance in comparison with others.

Figure 14.6 shows the amount of training samples used for different deep models like AlexNet, VGGNet, and ResNet to show more or less same accuracy as per Table 14.6. So, for different cases, different pre-trained deep CNN models are chosen. It can be done by transfer learning or fine-tuning. In transfer learning, they can be directly used as feature extractor, and in fine-tuning, we can go for changing some of the layers or some hyperparameters.

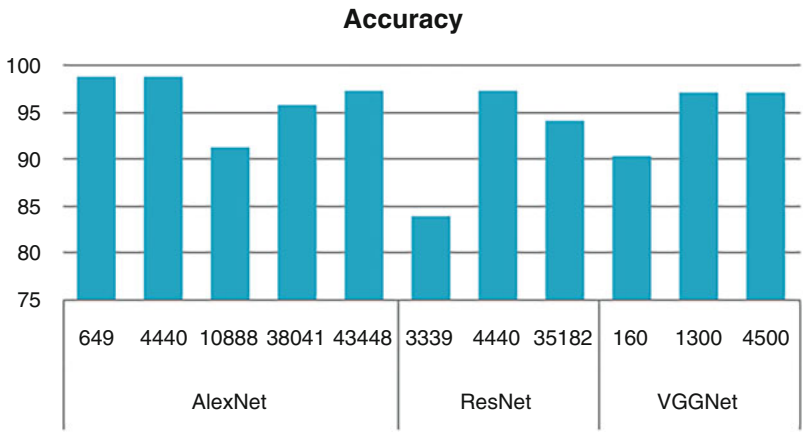


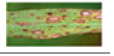
Fig. 14.5 Accuracies of different deep models w.r.t. training samples

14.7 Conclusion

In different types of computer vision-related analyses, CNN has always shown its supremacy. Various experiments have been done to improve the performance of CNN. The main parameters to build a better CNN model involve activation function, loss function, regularization, optimization, learning rate, etc. Here, we came across different CNN models like AlexNet, VGGNet, and ResNet with their architectural design, parameters, accuracies, etc. Various papers that include these models have been discussed with their advantages and challenges.

In recent years, the structural modifications have been experimented to study the efficiency of deep models. Different pre-trained CNN models have also proved their capabilities. Their architectures come with different modules and make the entire phase clear to understand. We have shown the architecture of three pre-trained models named AlexNet, VGGNet, and ResNet with the data they get trained and accuracies. Our takeaway from here is that type of convolution, pooling, skip connection, connectivity of layers, and kernel size have improved the performances of different deep CNN models. We believe that it will help the researchers in future to carry out their research in the field of plant disease detection for sustainable agriculture.

Table 14.6 Classification of crop diseases with deep network

Author	Data	No. of classes	Models used	Trainable parameter	Train	Test	Accuracy (%)	Epoch	Precision (%)	Recall	Leaf image
Jadhav et al. (2020)	Soybean	4	AlexNet	0	649	80	98.75	20	98.75	100	
Brahimi et al. (2017)	Tomato	9	AlexNet	0	4440	1110	98.66	4992	98	97.85	
Liu et al. (2018)	Apple	4	AlexNet (SGD)	56,884,612	10,888	2801	91.19	20	97.81	99.67	
Durmuş et al. (2017)	Tomato	10	AlexNet (SGD)	0	43,448	10,861	97.22	–	–	–	
Saleem et al. (2020)	P.Village	26	AlexNet	60 M	38,041	5430	95.66	54	95.63	95.70	
Srivastava et al. (2020)	Sugarcane	6	VGGNet	0	160	80	90.2	–	–	–	
Aversano et al. (2020)	Tomato	10	VGGNet	0	1300	300	97	30	–	–	
Qiu et al. (2021)	Rice	10	VGGNet	0	4500	500	97.08	–	94.83	96.88	
Hu et al. (2020)	P.Village	10	ResNet	0	35,182	4540	93.96	–	82.79	82.33	

Jiang et al. (2020)	P.Village	4	ResNet	0	3339	835	83.75	4000	–	–	
Zhang et al. (2018)	Tomato	9	ResNet50	4092	4440	1110	97.19	4992	–	–	

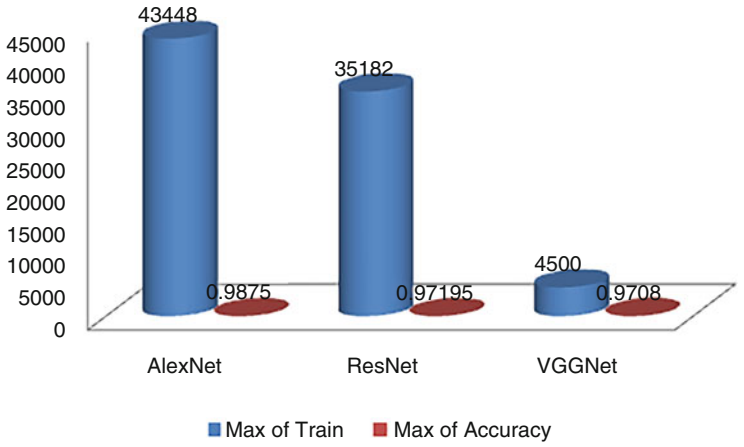


Fig. 14.6 Models showing max accuracy with max training samples

References

Amara J, Bouaziz B, Algergawy A (2017) A deep learning-based approach for banana leaf diseases classification. Datenbanksysteme für Business, Technologie und Web (BTW 2017)-Workshopband

Aversano L, Bernardi ML, Cimitile M et al (2020) Tomato diseases classification based on VGG and transfer learning. In: 2020 IEEE international workshop on metrology for agriculture and forestry (MetroAgriFor). IEEE, pp. 129–133

Brahimi M, Boukhalfa K, Moussaoui A (2017) Deep learning for tomato diseases: classification and symptoms visualization. Appl Artif Intel 31(4):299–315

Carvalho FP (2006) Agriculture, pesticides, food security and food safety. Environ Sci Pol 9(7–8): 685–692

Dean R, Van Kan JA, Pretorius ZA et al (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13(4):414–430

Durmuş H, Güneş EO, Kırcı M (2017) Disease detection on the leaves of the tomato plants by using deep learning. In: 2017 6th international conference on agro-geoinformatics. IEEE, pp. 1–5

Ferentinos KP (2018) Deep learning models for plant disease detection and diagnosis. Comput Electron Agric 145:311–318

Gebbers R, Adamchuk VI (2010) Precision agriculture and food security. Science 327(5967): 828–831

Hu WJ, Fan J, Du YX et al (2020) MDFC–ResNet: an agricultural IoT system to accurately recognize crop diseases. IEEE Access 8:115287–115298

Hughes D, Salathé M et al (2015) An open access repository of images on plant health to enable the development of mobile disease diagnostics. arXiv preprint arXiv:151108060

Jadhav SB, Udupi VR, Patil SB (2020) Identification of plant diseases using convolutional neural networks. Int J Inform Technol 13(6):2461–2470

Jiang H, Xue ZP, Guo Y (2020) Research on plant leaf disease identification based on transfer learning algorithm. J Phys Conf Ser 1576:012023. IOP Publishing

Kaiming H, Xiangyu Z, Rea S (2015) Deep residual learning for image recognition. ResNet model. arXiv preprint arXiv:151203385

Kitzes J, Wackernagel M, Loh J et al (2008) Shrink and share: humanity's present and future ecological footprint. Philos Trans R Soc B 363(1491):467–475

Krizhevsky A, Sutskever I, Hinton GE (2012) Advances in neural information processing systems
Kurniawati NN, Abdullah SNHS, Abdullah S et al (2009) Investigation on image processing techniques for diagnosing paddy diseases. In: 2009 international conference of soft computing and pattern recognition. IEEE, pp. 272–277
Lee SH, Chan CS, Wilkin P, Remagnino P (2015) Deep-plant: plant identification with convolutional neural networks. In: 2015 IEEE international conference on image processing (ICIP). IEEE, pp. 452–456
Liu B, Zhang Y, He D et al (2018) Identification of apple leaf diseases based on deep convolutional neural networks. Symmetry 10(1):11
Lu Y, Yi S, Zeng N et al (2017) Identification of rice diseases using deep convolutional neural networks. Neurocomputing 267:378–384
Mansfield J, Genin S, Magori S et al (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13(6):614–629
Mohanty SP, Hughes DP, Salathé M (2016) Using deep learning for image-based plant disease detection. Front Plant Sci 7:1419
Qiu J, Lu X, Wang X et al (2021) Research on rice disease identification model based on migration learning in VGG network. In: IOP conference series: earth and environmental science, vol 680. IOP Publishing, p. 012087
Riley MB, Williamson MR, Maloy O (2002) Plant disease diagnosis. Plant Health Instr:10
Saleem MH, Potgieter J, Arif KM (2020) Plant disease classification: a comparative evaluation of convolutional neural networks and deep learning optimizers. Plan Theory 9(10):1319
Sankaran S, Mishra A, Ehsani R et al (2010) A review of advanced techniques for detecting plant diseases. Comput Electron Agric 72(1):1–13
Scholthof KBG, Adkins S, Czosnek H et al (2011) Top 10 plant viruses in molecular plant pathology. Mol Plant Pathol 12(9):938–954
Sharma P, Berwal YPS, Ghai W (2020) Performance analysis of deep learning CNN models for disease detection in plants using image segmentation. Inf Process Agric 7(4):566–574
Sibiya M, Sumbwanyambe M (2019) A computational procedure for the recognition and classification of maize leaf diseases out of healthy leaves using convolutional neural networks. Agri Eng 1(1):119–131
Simonyan K, Zisserman A (2014) Very deep convolutional networks for large-scale image recognition. arXiv preprint arXiv:14091556
Srivastava S, Kumar P, Mohd N et al (2020) A novel deep learning framework approach for sugarcane disease detection. SN Comput Sci 1(2):1–7
Türkoğlu M, Hanbay D (2019) Plant disease and pest detection using deep learning-based features. Turk J Elec Eng Comput Sci 27(3):1636–1651
Wang G, Sun Y, Wang J (2017) Automatic image-based plant disease severity estimation using deep learning. Comput Intel Neurosci 2017:1–8
Zhang K, Wu Q, Liu A et al (2018) Can deep learning identify tomato leaf disease? Adv Multimed 2018:1–10