Rhizosphere Biology

Jayachandra S. Yaradoddi
Merja Hannele Kontro
Sharanabasava V. Ganachari *Editors*

Actinobacteria

Ecology, Diversity, Classification and Extensive Applications

Springer

Rhizosphere Biology

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Editors
Jayachandra S. Yaradoddi
Biomaterials Laboratory, Center of Material Science
KLE Technological University
Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program
University of Helsinki
Lahti, Finland

Sharanabasava V. Ganachari
Center for Material Science
Advanced Research in Nanoscience & Nanotechnology, KLE Technological University
Hubballi, Karnataka, India

Merja Hannele Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme
University of Helsinki
Lahti, Finland

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Preface

Microbial populations are likely to play a crucial role in contributing to most human diseases and treatment. These microbes can carry different kinds of metabolites within their genome. Moreover, they are the most abundantly available sources among all occurring living organisms. Microbes are a vital sources in balancing the environment, ecological functions, human and livestock health, sustainable agriculture (through their effective crop productivity and safety), and forestry. Further, they can be harnessed to produce many bioactive compounds such as enzymes, pigments, vitamins, fats, oils, antibiotics, and other essential chemicals. They are the significant constituents of soil biodiversity and perform a broad range of functions through their huge functional gene pool and metabolic potentials. In recent years due to massive industrialization and chemical utilization in the agriculture sector led to heavy air, water and soil pollutions and created unhealthy environment, this nasty situation demand the biological processes to take part in restoration activities through natural ecological activities management. The biodegradation especially by microbial means are the key factors in conversion of complex plants and animal residues, and thus they are involved in the biodegradation of soil organic and inorganic pollutants. At the same time, they act as a plant growth promoter by their symbiotic association with the agriculture crop plants. Several interactive actions occurs in plant roots rhizospheres, they are generally led by accumulation of root exudates by the microbial communities will routinely support the growth, development, and resistance upon the risks from the various biotic and abiotic stresses. By considering the significance insights and numerous beneficial effects of these microbes, they can be identified, characterized, and explored to produce different bioactive molecules, which is practiced from the past few decades. They can be utilized and implemented effectively at the industrial level. Among all recognized microbes, the actinobacteria are dominant groups of organisms that find value in biotechnological, biomedical, pharmaceutical, and environmental applications, especially in bioremediation, agricultural, and plant disease management.

Actinomycetes or actinobacteria are the diverse groups of aerobic, spore-forming filamentous bacterial communities; they comprise the highest guanine-cytosine ratio, i.e., between 57 and 75% within their genome, and they mainly belong to the order Actinomycetales. These actinomycetes are ubiquitously distributed in our ecosystem and are predominantly occur in dry, alkaline soil habitats. They carry unique properties like recycling organic waste present in the soil. Actinomycetes are also well versed in producing different kinds of primary and secondary metabolites. The significance of the accumulation of these novel secondary metabolites by actinomycetes is to sustain their lives in extreme environmental stress conditions. The secondary metabolites produced by the actinomycetes are of medicinal importance and play a crucial role in the production of antibiotics, anticancer, antiviral, antifungal, antiprotozoal, anticholesterol, antihelminth, and immunosuppressive agents. Their part is not only limited to the medical sector; they also act as a significant factor in agriculture industries. They promote the yield of crop plants by inhibiting the growth of plant pathogenic organisms. Actinomycetes can grow in diverse environmental conditions, in temperature of 5–7 °C and 45–80 °C has been phenomenal, because industrial processes usually occur in extremely harsh conditions. Actinomycetes are also an important source of many industrial enzymes, and these enzymes carry commercial properties similar to chemical catalysts. Economical production of enzymes may lead to the green revolution and foster biotechnological applications with all adaptability potentials of the actinomycetes. These can also be utilized for ecological and medical applications. Several robust techniques applied in carefully understanding the metabolic and micromolecular phenomenon encircled by these groups of organisms can dictate the advanced innovation in various aspects of these versatile organisms. This book emphasizes the occurrence, diversity, isolation, production, and characterization of the vast array of actinobacteria and rare actinobacteria. This book also offers insights on their availability, inhabitance, and usefulness, such as biotechnological, agriculture, bioremediation, biopharmaceutical, and nanotechnological implications of these organisms. The edited book disseminates the best knowledge on actinomycetes to a wide range of readers including students, researchers, and other scientific communities in understanding these groups of microbes.

Hubballi, Karnataka, India/Lahti, Finland — Jayachandra S. Yaradoddi
Lahti, Finland — Merja H. Kontro
Hubballi, Karnataka, India — Sharanabasava V. Ganachari

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Contents

Editors and Contributors

About the Editors

Jayachandra S. Yaradoddi is a Research Scientist in Biomaterials Laboratory, Dept. of Center for Materials Science, KLE Technological University, Hubballi, Karnataka, India. He obtained his Masters and Doctor of Philosophy of Biotechnology from the Gulbarga University, Kalburagi. He has executed research work on "diversity and molecular characterization of halophilic bacteria from Karnataka" during his Ph.D. He was served as a project fellow (JRF and SRF) under UGC major research project entitled "Siderophore as a biocontrol agent on phytopathogens of regional crops." Recently, he has completed post-doctoral studies on "Recent trends in actinobacterial diversity of environment and their potential bioactive molecules" from the University of Helsinki, Finland. He has published more than 40 publications in various national and international journals of repute and filed two Indian patents. He has completed the Govt. funded project under the IDEA2PoC scheme by Bangalore Bioinnovation Center and KBITS, Govt. of Karnataka. His major thrust area of research is microbial taxonomy, biomaterials, industrial biotechnology, and bio-nanotechnology.

Merja H. Kontro (nee Suutari) is an associate professor of Environmental Biotechnology at the University of Helsinki, Finland. She achieved M.Sc. (chemeng.; microbiology, biochemistry, biotechnology, food technology), and further Ph.D. from Aalto University (Finland) on microbial lipids in temperature adaptation. After a post-doc in the United States on molecular biochemistry, she worked as a senior researcher in the Environmental Microbiology Laboratory of the National Public Health Institute (Finland) on topics related to sick building syndrome, especially Actinobacteria in water-damaged buildings; and on drinking water quality until moved to Helsinki University. Current research topics include pesticide contamination in groundwater and related remediation, microbial safety in waste treatment, and circular economy related transformation of waste carbon to polyhydroxyalkanoates, a plastic raw material. Further, the survey of antibacterial compounds from waste against hospital bacteria biofilms is also underway. She has 60 peer-review publications, 7 book chapters, 2 patent publications, and about 32 peer-review proceeding publications.

Sharanabasava V. Ganachari was born in 1983. He got his Ph.D. in Materials Science from Gulbarga University in 2013. During his Ph.D. He was awarded Project Fellowship from University Grants Commission, the one most prestigious government funding organization. He is currently working as a Research Scientist & Assistant Professor at the Centre for Material Science, KLE Technological University, Hubballi, Karnataka, INDIA since November 2014. He has more than 50 publications, including journal articles, conference articles, book chapters and an inventor of several patents. He was honored Dr. A. P. J. Abdul Kalam, Best Young Scientist in 2015–16, Fellow member, Senior and life member of several national and international scientific societies. Editorial board member and reviewer for several prestigious journals &. His research area of interest is in green nanotechnology, materials chemistry, bio-nano-composites, green synthesis of nanoparticles, conducting polymers and multifunctional applications as biological, sensors, water purification, energy and agriculture.

Contributors

Anil Kumar S Katti Department of Biotechnology, Walchand College of Arts and Science, Solapur, Maharashtra, India

Kaveri Badiger Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

Nagaraj R. Banapurmath Biomaterials Laboratory, Center for Materials Science, KLE Technological University, Hubballi, Karnataka, India

Savitha S. Desai Independent Researcher, Hubballi, Karnataka, India

Mahesh Divatar Viral Research and Diagnostic Laboratory, Gulbarga Institute of Medical Sciences, Gulbarga, Karnataka, India

Sharanabasava V. Ganachari Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

Shankar Hallad Biomaterials Laboratory, Centre for Material Science, KLE Technological University, Hubballi, Karnataka, India

Samay Honnangi Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

Basavaraj S. Hungund Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

Somappa Jaggal Department of Crop improvement and Biotechnology, College of Horticulture, Munirabad, Karnataka, India
University of Horticultural Sciences, Bagalkot, Karnataka, India

Kartik C. Kamath Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

Chandrakanth Kelmani Department of Biotechnology, Gulbarga University, Gulbarga, Karnataka, India

Zareen Kousar Kazi Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

Merja H. Kontro Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland

H. M. Navya Department of Biotechnology, Davangere University, Davangere, Karnataka, India

Ajaykumar Oli SDM Research Institute for Biomedical Sciences, a Constituent Unit, Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

A. B. Parameshwar Faculty Biotechnology Skill Enhancement Program (BiSEP), Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India

Seema J. Patel Department of Biotechnology, Davangere University, Davangere, Karnataka, India

Prabhurajeshwar Department of Biotechnology, Davangere University, Davangere, Karnataka, India

N. Rajeshwari Spices, Plantation, Medicinal and Aromatic Crops, College of Horticulture, Munirabad, Karnataka, India
University of Horticulture Sciences, Bagalakot, Karnataka, India

Venkatesh Ramaswamy Biomaterials Laboratory, Centre for Material Science, KLE Technological University (formerly known as B.V. Bhoomaraddi College of Engineering and Technology), Hubballi, Karnataka, India

Shilpa AK Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India

Nagaveni Shivshetty Department of Microbiology and Food Science and Technology, GITAM Institute of Science, GITAM (Deemed to be University), Gandhi Nagar, Rushikonda, Visakhapatnam, Andhra Pradesh, India

H. Shoba Agricultural Engineering, College of Horticulture, Munirabad, Karnataka, India
University of Horticulture Sciences, Bagalakot, Karnataka, India

Manzoore Elahi M. Soudagar Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur, Malaysia

Sulochana B Mudgulkar Department of PG Studies and Research in Biotechnology, Gulbarga University, Kalburagi, Karnataka, India

Gururaj B. Tennalli Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

M. K. Umesh Department of Microbiology, Gulbarga University, Kalaburagi, Karnataka, India

Jayshree Uppin Department of Biotechnology, Gulbarga University, Gulbarga, Karnataka, India

Jayachandra S. Yaradoddi Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India
Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

H. Yogeeshappa Soil Science, College of Horticulture, Munirabad, Karnataka, India
University of Horticulture Sciences, Bagalakot, Karnataka, India

Chapter 1
Microbial Ecology

Merja H. Kontro and Jayachandra S. Yaradoddi

Abstract Microbes usually occur in vast communities. Many reports state that the species richness can be enormous even in a small quantity of soil or water, indicating the complex interactions among different microbial habitats in communities. Moreover, differences in physicochemical conditions led to the establishment of unique communities in each environment, such as in water, sediment, soil, plants, fungi, protozoa, and animals. Since many centuries of coexistence of these diverse communities in different habitats have led to the essential adaptation and speciation. One of the extremely important bacterial phyla in these communities is *Actinobacteria*. Understanding the microbial ecology, i.e., importance of *Actinobacteria* as part of the activities of these microbial communities, is among the utmost important, challenging, and fascinating areas of science. This pattern of colonizing microbial community distribution can trigger various complex interactions such as mutualism, symbiotic association, and antagonistic or pathogenic and parasitic relationships. Many scientists have indicated that the bioactive compounds or secondary metabolites recovered from the microorganisms are involved in these microbial interactions, often in low quantities. One of the major challenges of the researchers is to produce medically important drugs, which can be achieved through complex microbial interactions. These microbial interactions take part as pivotal role in the development and preservation of colonization with beneficial and harmful properties, including infective microorganisms. In addition to this, activated host defense mechanisms can able to produce antimicrobial agents against the harmful organisms in the environment. Microbial interactions or communications are also affecting gene regulation within microbial communities and in host organisms in response to the infections caused by the pathogenic microorganisms.

M. H. Kontro (✉)
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

J. S. Yaradoddi
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

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1.1 Introduction

Microbial interactions are an utmost critical challenge in the effective establishment and sustenance of microbial communities. These complicated interactions can be realized upon the environmental constrains tailed by the transfer of genetic and molecular information, including the numerous signaling mechanisms and variety of molecules involved. The molecular mechanisms permit microbes to flourish in a population truly based on the complex interactions possessed by the organisms with higher diversity. Sophisticated interactions often result in beneficial or pathogenic effects on host organisms. In human being, for instance, the microbial population plays a key function in protecting the host against the diseases, which are caused by pathogenic microbes or due to physiological issues (Frey-Klett et al. 2011).

The communities of soil microorganisms are important for the protection of plant species from various diseases and also from abiotic stress or inability of nutrient uptake. Organisms in microbial communities are hardly exposed as a sole species among populations under certain environmental conditions; meanwhile, the investigations perceived in a small proportion suggest that the microbial interactions are intrinsic factors for the inhabitants of communities in the environment that involves sediments, soils, water, air, plants, animals, protozoa, and fungal cells. Several years of evolution of various taxa guide toward variation and specialism that outcome as a huge range of associations offering cohabitation progression, including antagonistic, competitive, and parasitism associations, as well as mutualistic and symbiotic interdependences (Faust and Raes 2012).

Numerous secondary metabolites have been produced during complex microbial communication. These secondary metabolites are typically bioactive molecules that elicit a variety of ecological interactions (Sulochana et al. 2014a, b; Yaradoddi et al. 2020a, b; Yaradoddi et al. 2019). One of the majorly investigated mechanisms of microbial communication is the quorum sensing system, which comprises a stimuli-response structure that is concerning to the cellular concentration by regulating genes. Furthermore, the synthesis of signaling compounds permits cells to be headed for communication and gives a response toward the external surroundings in a synchronized path (Phelan et al. 2012). Throughout the interaction process that is defined by host cells, the microbial-associated molecular pattern was preserved in various microbial species permitting to enhance the stability during the interaction by animal or plant cells (Stuart et al. 2013), and that indeed regulates the microbial communications among different hosts.

Considerable focus has been provided to the microbial communication, which is the key for the effective establishment and sustenance of colonization and infections. In addition, antimicrobials produced by host resistance and environmental conditions play a vital role. Cell communications empower the microbial population to

jointly regulate the gene expression in reply to the host as well as environmental stresses, which can be produced by similar or different kinds of species (Jayachandra et al. 2012a, b; Mohan Reddy et al. 2015a, b; Yaradoddi et al. 2018). This outcomes as a coordinated response among the population, attaining a successful pathogenic product that is not usually provided by individual cells (Jimenez et al. 2012; Peters et al. 2012; Brickman and Armstrong 2009). Accordingly, understanding the mechanisms possessed by microbes during interactions could be an ideal way of evolving precise agents that may disturb or avoid the defensive or offensive abilities of pathogenic organisms.

The examination of molecular mechanisms can significantly contribute to the understanding of microbial pathogenesis in order to develop novel antimicrobial agents (Peleg et al. 2010). Additionally, the microbial interactions taking place in the human host could also be beneficial; several diseases are frequently concerning disparities within the healthy microbial communities. Consequently, the investigations of beneficial microbial populations in host organisms are important to uncover the mechanisms of disease progression and to develop suitable treatments (Clemente et al. 2012; Knights et al. 2011; Virgin and Todd 2011). The interactions between microorganisms likewise necessitate the attention of researchers from the natural product discovery area. A group of bioactive molecules that are quite under laboratory conditions in development can stimulate via activating the establishment of the natural environment for microorganisms. Previous reports have revealed that the coculturing method along with related microbes from identical habitats can enhance the activation process of silent biosynthetic pathways that lead to synthesis and also the recognition of novel natural compounds (Brakhage and Schroeckh 2011; Oh et al. 2007; Schroeckh et al. 2009; Marmann et al. 2014; Netzker et al. 2015). Besides this, the acquired knowledge can be useful in the genetic manipulation (genetic engineering) of phytopathogens or parasites, keeping the target as an enhanced biocontrol activity (Chamoun et al. 2015).

1.2 Ecological Species Concepts

The most significant part of the ecological system relies on the concept of species: the population's ecology tallies the individuals among the species, while macroecology and community ecology tally the total number of the species or taxa. Organisms up to species level, generally described by the biological species perception, were introduced by the Mayr (1957). It is a genomic description that visualizes a taxonomic group of multiplying individuals recovered from the different groups through the recombination blockage. If a genetic exchange of information among the species is appropriately widespread, and between species it is adequately lower, taxon could be comparatively homogeneous within themselves and habitually divergent from another taxon.

Unfortunately, the prokaryotic organisms are asexual reproducers, and through disregarding with the recombination expectation, they do not develop into taxon in

this genetic path. In substitution, the environmental species concept describes taxon as the typical individual which measures to be similar in every applicable ecological properties. Cohan (2002) has contended that bacteria are ecological species. He has hypothesized that the bacteria live in distinct habitats, along with recurrent assortment, leading to the removal of genetic variation in each individual habitat, i.e., they are deprived of stopping discrepancy among the inhabitants of dissimilar habitats. Thus, the ecological and genetic variation of species occurs, providing there is very little recombination, and the ecological principles also assume that such taxon must be applied for bacteria, which also forecast that the molecular assortment must be connected in a straight line to ecological multiplicity. Cohan's ecotypes are based upon distinct habitats; nevertheless, speciation will be an added issue to predict once the pertinent environmental shifting is constant. Bacterial species concept within these conditions can be explored using adaptive dynamics principles (Metz et al. 1996; Dieckmann and Doebeli 1999), but again, which is not leading to easy mapping among the ecological niche of the molecular market.

It is critical to designate the speciation and environmental taxon description; there is a massive requirement of bacterial gene transfer techniques. However, it is also an unpredictable process, which may transfer a small portion of the whole genome. It has provided possible machinery in maintaining the biological species as per Mayr' report (Dykhuizen 1998), due to a received gene that can substitute a homologous gene copy in the genome simultaneously maintaining the inherited genetic arrangement of the species. Apart from the above, these mechanisms can also outcome in a horizontal gene transfer without complementing the host sustained upon plasmid or associated with nonhomologous exchange of genetic material. Besides this, homologous exchange of genetic material and also lateral gene transfer differs extensively in significance between thoroughly studied bacterial taxon and conceivably still more within the uncultured organisms in the environment. Since the heterogeneous nature, the researchers are yet far away from the concurrent upon the landscape of bacterial taxon as per the recent discovery of Royal Society meeting and discussion (Spratt et al. 2006).

Consequences of gene transmission due to bacterial genome comprise two diverse units, accessory genome and core genome (Young et al. 2006). The core genome contains genes required for the environmental effects and could originate from the Mayrian species which maintain rationality by homologous recombination processes. The accessory genome contains a unique environmental adaptation among the genes immediately acquired or lost. Microbial cultures belonging to the strains of a species, based on the core genome, may vary in the composition of numerous accessory genes and, thus, may have different ecological abilities. Regarding present prospects, ecotypes presented by Cohan are transitory lineage through specific patterns of accessory genes, and the environmental habitat cannot be explained by the superficial organization of taxon that is distinct through the core genes (Wertz et al. 2003).

Analysis of 16S rRNA gene sequences has revealed enormous diversity of the bacterial population. Simultaneously there is the most fascinating environmental adaptability assured via observing accessory genome, and factual ecological

multiplicity occurs within rich divergence of catabolic plasmids, including pathogenic markers of resistance transposons. They could be collective between unique bacterial species within an environment that favors them, however, then again not present among similar bacterial taxon growing away. Tools available in evolutionary biology were adapted in understanding the mechanism of interaction within these accessory genes and concerning to their host bacterium. Bergstrom et al. (2000) described the various parameters considered for maintaining the plasmid. In the latest theoretical investigation accomplished, at which evolutionary artillery race within bacteria, the bacteriophages lead to speciation among host (Weitz et al. 2005). These studies offer a vital assay for those investigating prokaryotic organisms and also working on community ecology. The present resolution can be utilized in description using taxonomic units; these researchers are far away from a comprehensible body of concept of a theory, which is associated with the fluidic properties of a bacterial cell in the ecology of bacterial populations.

Regarding the mutable activities of microbes, primary molecular investigations did not distinguish among active and inactive microbes; however, a considerable portion of the cells within a specific environment was inactive at a specific point of time (Chaparro et al. 2013). Numerous bacterial genera turn to resistant spores; however, many non-sporulating bacteria could also convert to inactive vegetative or dormant forms which are generally tolerant toward the adverse environmental conditions (Peters et al. 1986). Inactivity among the microbial populations will interfere with many characteristic features among ecology that include population dynamics and biodiversity, obscuring the advantageous ecological concept that generally concentrates on free-living and bioactive individuals. Prevailing to this concept, the function of the seed banks within the plant biology can be appropriate. This seed bank is very imperative, as germination can cause sequential disparity among the observed plant diversity. The dormant seeds have a beneficial role in the ecological succession of plant species; however, the variety of the seed bank (prospective diversity) and that of the traditional vegetation (comprehended diversity) is frequently changing at a considerable amount (Akiyama et al. 2005). Cells and seeds of inactive organisms usually do not directly promote ecological processes; however, they help to understand the flexibility of a population to agitation and could become crucial as soon as the environmental conditions change. The addition of inactive members of the population thus necessitates the contemplation of the type of the study.

1.2.1 Organisms Involved

Microbes can rarely be seen as a single species in a population; hence, they are encountered among various environments or hosts. Therefore, a huge number of interactions is associated with microorganisms within different populations. Interactions between bacteria, fungi, plants, and animals include mutualistic and parasitic relationships, involving several pronounced cellular mechanisms that permit toward

the progression of strategies to engineer these interactions, which can lead to increased host abilities or the appearance of novel metabolites within their genome. As per the reports of van Elsas et al. (2012), the invasion of a novel taxon into any environment is based on the features and activities of the local microbial population. Usually, the ecological systems with species diversity loss have remarkably reduced potential to defense against the invader; meanwhile, the further existing native species could take advantage to occupy the existing niche. The invasive organism must interact with the local species in a specific environment during the occupation process of the niche. The molecular mechanisms of interactions between organisms are not always entirely understood, although bacterial-fungal interactions have been vastly studied.

A wide range of different organisms are involved in interactions in all types of environments, such as bacteria, fungi, insects, plants, humans, and higher animals. Understanding these interactions can be utilized in substantial number of different biotechnological applications among food industry, medicine, and environmental research related to biocontrol, bioremediation, and material recycling. Apart from this, the bacterial and fungal associations tend to be a metabolically and physically dependent entity that defines the discrete properties that are of biotechnological values, specifically concerning to the exploration of natural products and areas of synthetic biology (Frey-Klett et al. 2011; Tarkka et al. 2009). Plenty of the host-microbial interdependences are utmost interesting due to pathogenic or beneficial relationships in animals and plant systems. Among other things, the organization of microorganisms in biofilms and planktons can lead to a variety of unique physiological, molecular, and genetic regulatory mechanisms. The interactions presented above will be described in the current chapter.

1.2.2 Soil and Plant

As we know, all living organisms are associated with microorganisms that include bacteria, archaea, viruses, and fungi. These groups of microbes are particularly important for the healthy development of the host (Berg et al. 2016). Microorganisms connected to the plant species can be considered as a second genome. Interactions with microbes form the basis for plant growth, health, and fitness and subsequently the production (Lakshmanan et al. 2014). At the same time, the microbes of different plant parts, including the rhizosphere, phyllosphere, and endosphere, are designated for a certain variety of microbes with specific functions. Culture-independent approaches have indicated that the plant microbiome may attain densities higher than the host cell frequency, and simultaneously microbial genes may be more actively expressed than those in a plant. In this context, it is important to recall that the metagenomic approach through sequencing has indicated that not more than about 5% of the bacterial population can be cultured in laboratory conditions using the present techniques, revealing that microbial counts and their relative functions have so far been insufficiently defined (Mendes et al. 2013). One

of the important approaches in the interaction of microbes and plants is the identification of plant secretions by soil microorganisms. There is an evolved concept in nature that plants can possess microbes through exudates, ideally consisting of carbohydrates and amino acids, as well as organic acid metabolites that vary in composition between plants and depending on environmental conditions.

Various plants choose a particular microbial population, as revealed by Berg et al. (2016). When comparing the rhizosphere colonization of two critical medicinal plants grown under the same conditions, they possessed microbial communities of different composition (16S rDNA sequencing) and function (nitrogen fixation *nifH* gene). The plants were nightshade (*Solanum distichum*) and chamomile (*Matricaria chamomilla*). Furthermore, plant exudates differ rendering to plant growth and development stages pertaining to specific microbial populations (Haldar and Sengupta 2015). Several plant exudate secretion compounds accountable for particular type of interactions have been described by researchers, such as the presence of flavonoids in rhizobia-legume (Chaparro et al. 2013) and strigo-lactone as a key fungal signaling molecule in arbuscular mycorrhiza (Peters et al. 1986). A model has been designed for microbial colonization in plants (Reinhold-Hurek et al. 2015). Bulk soil contains vast groups of microbial communities with a significant proportion of diversity. The communities are predisposed only through the type of soil and other environmental factors. Recognition of microbial communities in the vicinity of the plant root rhizosphere exudates revealed a small quantity of species and an added specialized population. Limited amounts of the taxon passed through the plant cell wall and developed within the host species.

Once microbes enter into the plant, their populations differ among various organs, such as in roots, stems, flowers, fruits, and leaves in different parts of the plant (Tarkka et al. 2009). Symbiotic microbial species could protect plants from pathogens by secreting antibiotics or through stimulating plant tolerance. The plant-induced systemic resistance (ISR) produces the highest response toward the pathogens. Although harmful organisms are commonly present in the soil, diseases rarely occur. The machinery related to the disease suppression can have plenty of scopes. In this direction, Mendes and colleagues (2011) have determined the microbiome in a soil that is suppressive toward fungal pathogens, such as *Rhizoctonia solani*, which results in dumping off in some crops. By the use of 16S rRNA, oligonucleotide microarray method enabled to recognize and classify about 33,000 archaeal and bacterial species within the sugar beet seedlings rhizosphere developed in soil. The researchers reported that diverse bacterial taxa, such as *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, including *Pseudomonadaceae*, continued to exist as a rich microbial community in the soil under disease suppressive conditions in contrast to the conducive soil. Through the use of random transposon mutation methods in a *Pseudomonas* sp., the genes responsible for the antifungal agent biosynthesis controlling fungal infection were recognized to encode chlorinated lipopeptide with nine amino acids.

1.3 Secondary Metabolism

Secondary metabolites are a famous molecular group produced by microorganisms. They are not essential for producers in the functions like cellular development, growth, and reproduction (Keller et al. 2005). Nonetheless, these secondary metabolites can be habitually bioactive compounds, and they give the host organism a significant competitive advantage in factors such as resistance, ecological interactions, and signaling (Demain and Fang 2000). In order to be active in the microbial interaction networks, the microbes generally communicate through the exchange of metabolites, leading to the complicated responses involved in the regulation of secondary metabolite biosynthesis. The types of signaling activities could be related to the parasitic, antagonistic, or competitive relationships. Imaging mass spectroscopy (IMS) and metabolomics are techniques that have been used to examine the structures and functions of bioactive compounds (Tata et al. 2015). As examples of secondary metabolites, the iron-chelating siderophores concern beneficial and competitive properties to bacteria by solubilizing iron, and, in addition, they can perform supplementary functions, such as antibiotic activity or cell signaling (Johnstone and Nolan 2015). Hopanoids perform key actions in bacterial interaction, deliberating resistance, and improvising the adaptability potentials of bacteria among dissimilar environments (Lopez-Lara et al. 2003). Concerning fungi, the secondary metabolites can be differentially controlled in an interaction, and they are always bioactive compounds, such as trichothecenes, atranones, polyketides, and diketopiperazines. Plenty of knowledge is provided on understanding of existing mechanisms and the function of many differentially expressed bioactive genes throughout the microbial interaction processes. Against this background, some fascinating examples from previous studies concerning secondary metabolites and microbial interactions are given below.

1.3.1 Microbes and Community Interactions

Actinobacteria are excellent sources of numerous natural products due to their wide variety of bioactivities. An investigation on *Streptomyces coelicolor* interaction with another actinobacterium indicated that the greatest part of the compounds synthesized during every relations are exclusive, reporting a differential response among each case. Numerous unknown compounds and a family of acyl-desferrioxamine-type siderophores, which were never previously designated in *S. coelicolor*, were identified. About 227 types of compounds were determined, which are differentially synthesized during the interactions: however, half of them were described as metabolites associated with actinorhodins, prodiginines, acyl-desferrioxamines, and coelichelins. Therefore, the interactions between actinobacterial species appears to be highly complex and at the same time accurate (Traxler et al. 2013). Interactions between bacteria and fungi have been shown to include specific biosynthesis of

Fig. 1.1 Colony morphology of *Streptomyces* sp. grown on tryptone-soy agar (TSA)

particular fungal molecules, and communication has not been based solely on diffusible factors. Nevertheless, physical interactions also play a role. An investigation (Schroeckh et al. 2009) also confirmed that a close physical interaction among the actinobacterium *Streptomyces* sp. (Fig. 1.1) and *Aspergillus nidulans* can trigger silent fungal genes and activate encoding for metabolites concerning the biosynthesis of polyketides. The polyketide synthase (PKS) gene was recognized to be essential for the biosynthesis of the biomolecules F-9775A and F-9775B (cathepsin K inhibitors), archetypal aromatic polyketide orsellinic acid, and lecanoric acid, a typical lichen metabolite. Actinomycetes have further been reported to trigger changes in fungal histone acetylation, mediated by the Saga/Ada complex, leading to PKS cluster triggering instead of silent genes. These results indicate that the bacteria can stimulate the variation of histone acetylation in fungal species (Nutzmann et al. 2011).

1.3.1.1 The Siderophores

The synthesis and accumulation of siderophores within microorganisms bring about vital machinery to gain iron from the soil. Several microbes can produce siderophores under different environmental conditions, so that the cell surface receptors recognize and ensure controlled supply of iron carrying compounds and

transfer into the microbial cells from the surrounding (Faraldo-Gomez and Sansom 2003). Therefore, they have been associated with mutual and competitive microbial interactions. Apart from this, siderophores can perform many other cellular functions. They can act as antibiotics, signaling molecules, and agents in the management of oxidative stress and in sequestering the wide variety of metals, including toxic heavy metals (Johnstone and Nolan 2015). Several species of *Pseudomonas* produce siderophore compounds known as pyoverdines. They may be indispensable in regulating growth during infection and biofilm formation (Sulochana et al. 2014a, b). The pyoverdines have been revealed to function as signaling compounds in stimulating a cascade leading to the biosynthesis of some factors related to the virulence, like PrpL endoprotease, other pyoverdines, and exotoxin A (Lamont et al. 2002). Extracellular siderophores affect their biosynthesis and iron uptake mechanisms in other microbial species, i.e., they act as signaling molecules that regulate, for instance, the growth of marine bacteria in iron scarcity. For example, *Vibrio* sp. produces *N*,*N*′-bis(2,3-dihydroxybenzoyl)-*O*-serylserine siderophore and outer membrane proteins under the control of iron only in the presence of extracellular siderophores produced by other bacteria (Guan et al. 2001).

1.3.1.2 Symbiotic Interactions

The symbiotic or mutualistic relationships are essential for many organisms. However, the complicated symbiotic interactions, such as plant-pathogen or fungal-bacterial connections, are yet inadequately uncovered concerning the metabolites and regulatory machineries used during the host-invader channeling and interactions. The unique, complex symbiotic relationship between kingdoms has been reported to occur between the endosymbiotic bacteria of the genus *Burkholderia* and the phytopathogenic fungus of the genus *Rhizopus*, both of which are required for the production of fungal rhizoxin phytotoxin to cause seedling blight disease in rice (Partida-Martinez and Hertweck 2005). Further, in the absence of *Burkholderia* spp. as an endosymbiont, *Rhizopus* sp. could not produce spores, which shows that the fungus is dependent on factors synthesized through the symbionts to fulfill its life cycle (Partida-Martinez et al. 2007). The exopolysaccharide (EPS) structure of *Burkholderia rhizoxinica* was elucidated, as EPSs were expected to act key roles in the interactions with *Rhizopus microsporus*. EPSs are generally involved in bacterial interactions with organisms. Nevertheless, no endosymbiotic interactions by EPS were observed in *R. microsporus* after the development of knockout mutant (Uzum et al. 2015). In contrast, *Burkholderia gladioli* synthesized the polyketide antibiotic enacyloxins in symbiotic coculturing with *R. microsporus*. The fungus stimulated the growth and development of *B. gladioli*, while the bacterium produced bongkrekic acid in the stationary growth phase and prevented fungal growth. Antifungal and antibacterial compounds, such as toxoflavin and complex polyketides, were produced during bacterial-fungal cultivation (Ross et al. 2014).

1.3.1.3 Parasitic Interaction

As an example of parasitic interactions, the investigations about the mycoparasitic associations among *Rhizoctonia solani* and *Stachybotrys elegans* have revealed that the gene expression and biosynthesis of several bioactive molecules are altered during the parasitic interactions. Based on the gene expression and metabolomic studies on the secondary metabolite profiles, the association may cause *S. elegans* to produce cell wall degrading enzymes and compounds supporting parasitic interaction, including trichothecene and atranone mycotoxins. Trichothecenes are inhibitory components in eukaryotic protein synthesis and causal agents of oxidative stress (Morissette et al. 2008; Chamoun and Jabaji 2011). As a response in *R. solani*, significant changes were observed in metabolism. The synthesis of various antimicrobials was reduced, only diketopiperazine and pyridoxal reductase biosynthesis were activated (Chamoun and Jabaji 2011; Martins and Carvalho 2007). Diketopiperazines have shown many biological activities including antifungal properties, while pyridoxal reductases are scavengers of reactive oxygen species (Martins and Carvalho 2007; McCormick et al. 2011). The hypothesis was that *R. solani* induced the trichothecene biosynthesis in *S. elegans*, followed by changes in the overall metabolism of *R. solani*.

1.3.1.4 Plant and Endophyte-Pathogen Association

The bioactive molecules and related regulatory mechanisms between phytopathogens, endophytes, and host plants during their interactions are yet undistinguishable, and several secondary metabolites are assumed to be involved. The endophytic fungi have been reported to synthesize vast varieties of bioactive molecules (Suryanarayanan and Shaanker 2015). The molecules have been associated with complex interactions of endophytes with the host plant and phytopathogens, acting as growth factors in plant development and in defense against phytopathogens, and they can also participate in important ecological functions (Strobel 2003).

The endophytic fungus of the cocoa trees, *Trichoderma harzianuman*, has been used in biocontrol, and it is considered as an antagonist of the phytopathogenic fungus *Moniliophthora roreri*. The two fungi were cultivated separately and in coculture to examine the association between the plant pathogen and the endophyte. As a result, four different bioactive molecules could be identified from the coculture, harzianolide, T39-butenolide, sorbicillinol, and an unidentified compound, while none of them were detected in pure cultures of the strains. T39-butenolide and harzianolide have been shown to be antifungal, whereas sorbicillinol belongs to the metabolic intermediates of secondary metabolites from the family bisorbicillinoids (Tata et al. 2015). *Trichoderma atroviride* is another endophytic fungi used in biocontrol. It has promoted *Arabidopsis thaliana* growth in the rhizosphere by producing indoles. As inoculated in the roots, it appeared to protect plant leaves from hemibiotrophic (*Pseudomonas syringae*) and nectrotrophic

(*Botrytis cinerea*) phytopathogens by activating genes involved in the biosynthesis of salicylic acid, jasmonic acid, ethylene, camalexin, and reactive oxygen species scavenging enzymes (Salas-Marina et al. 2011).

Bacteria, such as *Methylobacterium* and *Curtobacterium* strains, have also been found to be involved as endophytes in plant-endophyte-pathogen interactions. Species of both bacteria have been isolated from healthy and asymptomatic citrus plants, while *Xylella fastidiosa* is the causative agent of citrus-variegated chlorosis disease. The interaction studies in vitro showed that endophytic bacterium *Methylobacterium extorquens* stimulated and endophytic bacteria *Methylobacterium mesophilicum* and *Curtobacterium flaccumfaciens* inhibited the growth of phytopathogenic *X. fastidiosa* (Lacava et al. 2004; Dourado et al. 2015). The transcriptional RNA profile of *X. fastidiosa* revealed that the genes related to growth were downregulated, and those related to stress, motility, transport, and energy production were upregulated as an adaptive response to the occurrence of *M. mesophilicum* during in vitro cocultivation (Dourado et al. 2015).

The interrelationship between orchid leaf necrosis causing *Burkholderia gladioli* and suppressing endophytic bacterium *Burkholderia seminalis* was studied using the sequencing of whole genome and transposon insertion. The mutant *B. seminalis* strain deficient of leaf necrosis suppression had alternations in the biosynthesis of bacterial capsule polysaccharides, which are known factors in host-bacterial interactions (Araujo et al. 2016; Sim et al. 2010). Additionally, the gene clusters presumed to be interrelated to indole acetic acid biosynthesis and ethylene precursor (aminocyclopropane-1-carboxylic acid) deaminase were recognized, suggesting that the endophytic *B. seminalis* interact by affecting the metabolism of plant hormones (Araujo et al. 2016).

The hopanoids are a subclass of triterpenoids contained in the cell membrane of several bacteria, performing a similar function to eukaryotic cholesterol. They have the ability to stabilize the cell membrane and regulate its permeability and fluidity (Bradley et al. 2010; Welander et al. 2009). Hopanoid deficiency did not affect the normal growth and development of *Rhodopseudomonas palustris* or *Streptomyces scabies* based on mutation experiments with biosynthetic genes like squalene-hopene cyclase gene *hnp*F (Welander et al. 2009; Seipke and Loria 2009). Hopanoids were not essential for osmotic or oxidative stress, ethanol, low pH, or high temperature tolerance in *S. scabies*. However, a morphological defect was observed in hopanoid-deficient *R. palustris* under acidic or basic growth conditions (Welander et al. 2009; Seipke and Loria 2009), and in acidophile *Acidithiobacillus* sp., hopanoids were also associated with pH homeostasis (Jones et al. 2012).

Hopanoid metabolism was also altered in *Methylobacterium extorquens* during the degradation of toxic dichloromethane (Muller et al. 2011), and hopanoid depletion damaged energy-dependent multidrug transportation (Sáenz et al. 2015). Antibiotic and low pH sensitivity increased in *Burkholderia cenocepacia* upon deletion of hopanoid biosynthetic genes, and motility defects were observed (Schmerk et al. 2011). Overall, hopanoids are involved in boosting the bacterial tolerance to harsh environmental conditions.

Table 1.1 Potential antiviral and antiparasitic agents produced by the actinobacteria

Antiviral species	Compounds
Streptomyces hygroscopicus	Hygromycin
Streptomyces antibioticus	9-β-D-Arabinofuranosyladenine
Streptomyces spp.	Panosialins
Antiparasitic species	
Streptomyces coelicolor	Prodiginine
Streptomyces avermitilis	Avermectins
Streptomyces bottropensis	Trioxacarcin

Hopanoids are utmost interesting factors in plant-bacterial interactions, as they reinforce resistance to stress conditions that include extreme temperature, pH, and exposure to antibiotics and detergents. They are assumed to be involved in protecting nitrogenase from oxygen diffusion in *Frankia* species, which has been connected to the adaptation to saprophytic and symbiotic interactions with plants, and nitrogen metabolism (Sáenz et al. 2015; Nalin et al. 2000). Another nitrogen-fixing bacterium, *Bradyrhizobium diazoefficiens*, has synthesized methylated hopanoids under free growth state under microaerobic and acidic conditions in the tropical legume host *Aeschynomene afraspera*. In contrast, C35 hopanoids were produced under stress conditions, such as high temperature, low pH, oxidative stress, osmotic pressure, and in the presence of bile acids or antimicrobial peptides. The host plant produced antimicrobial peptides that apparently induced the production of C35 hopanoids, as they were not essential in symbiosis with soybean. The synthesis of C35 hopanoids was associated with avoiding plant defense and simultaneous utilization of host photosynthetic products and nitrogen fixation (Kulkarni et al. 2015).

Microorganisms, especially actinomycetes species, are a versatile group among microbes; they are potential sources for various bioactive compounds such as antitumor drug, antiviral, antiparasitic, and antifungal activities (Tables 1.1 and 1.2); one of the main advantages of actinobacteria possessing unique sporulation ability (Fig. 1.2) is their capability to provide immense biotechnologically important compounds.

1.4 Conclusion

The Gram-positive bacteria from the phylum *Actinobacteria*, occurring in habitats including terrestrial, aquatic, plant, animal, and human connections, are of remarkable importance due to their unique contribution to different ecosystems. They are significant producers of multiple natural compounds with a broad range of bioactivity, including metabolites and degrading enzymes. Many of currently commercially utilized actinobacterial bioactive compounds are produced by single species, while studies of their interactions with plants, animals, endophytes, and pathogens, as well

Table 1.2 Antitumor drugs produced by the actinobacteria

Potential antitumor producing actinobacteria	List of bioactive agents/drugs
Nocardia asteroides	Asterobactine
Micromonospora sp.	Diazepinomicin
M. echinospora	LL-E33288 complex
Thermoactinomyces spp.	Mechercharmycin
M. lomaivitiensis	Lomaiviticins
Actinomadura spp.	IB-00208
Streptomyces peucetius	Doxorubicin (adriamycin)
Salinispora tropica	Salinosporamide
Micromonospora spp.	Tetrocarcin
Micromonospora spp.	Lupinacidins
Actinomadura sp.	1,6-phenazinediol
Streptomyces sp.	3,6-Disubstituted indoles
Actinomycete sp.	Arcyriaflavin A
Marinispora sp.	Lynamycins

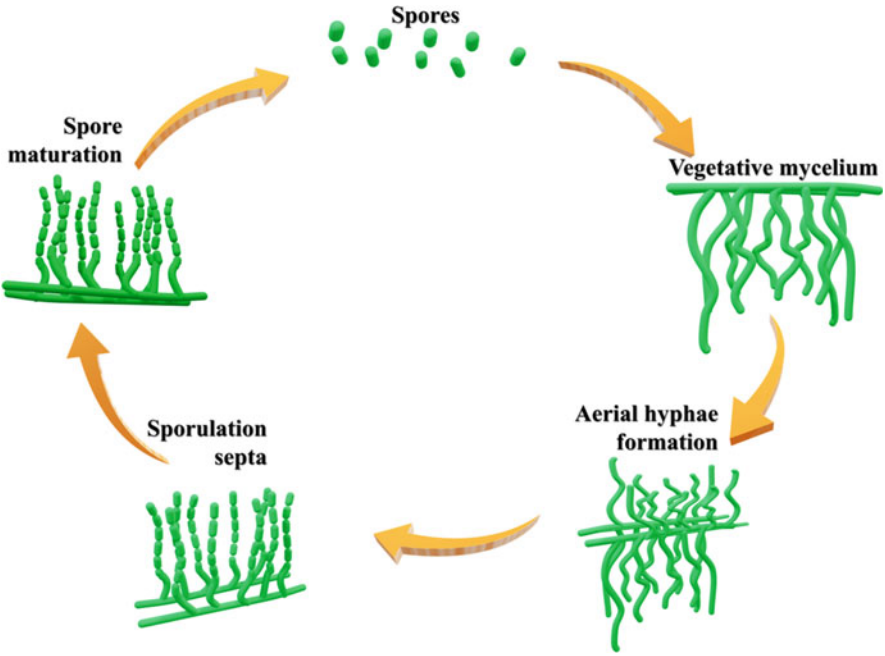

Fig. 1.2 The mechanism of sporulation among actinobacteria

as with the bacteria are not yet revealed, their elucidation would enable the development of second-generation products and product mixtures with multiple associated bioactivities. Exploring such possibilities is still largely at an early stage.

The above-presented examples of symbiotic, parasitic, and plant-endophyte-pathogen relationships, as well as simultaneously produced metabolites, including siderophores and hopanoids, showed that organisms can have an enormous yet poorly known capacity to interact in a variety of ways while maintaining the diversity of both beneficial and harmful microbes. In particular, the investigation of the cocultivation of *S. coelicolor* with other actinobacteria indicated that out of 227 differentially synthesized compounds, half were already identified metabolites, containing actinorhodins, prodiginines, coelichelins, and acyl-desferrioxamines differing in acyl chain length. The molecules in the second half were exceptional, demonstrating the variety of responses in separate cases. *S. coelicolor* genome has been found to include many gene clusters for uncharacterized metabolites, and some of them could account for the biosynthesis of detected unknown compounds (Traxler et al. 2013). Using *A. nidulans* and *Streptomyces* sp., it has been established that the interaction resulted in the synthesis of biologically active molecules by otherwise silent biosynthetic genes, and the phenomenon was not due to the diffusion of signal molecules. The close physical interaction of fungi and *Streptomyces* sp. led to the biosynthesis of bioactive metabolites (Nutzmann et al. 2011). In conclusion, by examining the ecology of actinobacteria in microbial communities, it may be possible to find numerous new forms of interactions and bioactive molecules for wide variety of applications that can be utilized in, for example, food technology and medicine.

References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Araújo WL, Creason AL, Mano ET, Camargo-Neves AA, Minami SN, Chang JH, Loper JE (2016) Genome sequencing and transposon mutagenesis of *Burkholderia seminalis* TC3.4.2R3 identify genes contributing to suppression of orchid necrosis caused by *B. gladioli*. Mol Plant-Microbe Interact 29:435–446

Berg G, Rybakova D, Grube M, Koberl M (2016) The plant microbiome explored: implications for experimental botany. J Exp Bot 67:995–1002

Bergstrom CT, Lipsitch M, Levin BR (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. Genetics 155:1505–1519

Bradley AS, Pearson A, Sáenz JP, Marx CJ (2010) Adenosylhopane: the first intermediate in hopanoid side chain biosynthesis. Org Geochem 41:1075–1081

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites—strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22

Brickman T, Armstrong S (2009) Temporal signaling and differential expression of *Bordetella* iron transport systems: the role of ferrimones and positive regulators. Biometals 22:33–41

Chamoun R, Jabaji S (2011) Expression of genes of Rhizoctonia solani and the biocontrol Stachybotrys elegans during mycoparasitism of hyphae and sclerotia. Mycologia 103:483–493

Chamoun R, Aliferis KA, Jabaji S (2015) Identification of signatory secondary metabolites during mycoparasitism of *Rhizoctoni asolani* by *Stachybotrys elegans*. Front Microbiol 6:353

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8:e55731

Clemente JC, Ursell LK, Parfrey LW, Knight R (2012) The impact of the gut microbiota on human health: an integrative view. Cell 148:1258–1270

Cohan FM (2002) What are bacterial species? Annu Rev Microbiol 56:457–487

Demain AL, Fang A (2000) The natural functions of secondary metabolites. Adv Biochem Eng Biotechnol 69:1–39

Dieckmann U, Doebeli M (1999) On the origin of species by sympatric speciation. Nature 400:354–357

Dourado MN, Santos DS, Nunes LR, da Costa de Oliveira RLB, de Oliveira MV, Araújo WL (2015) Differential gene expression in *Xylella fastidiosa* 9a5c during co-cultivation with the endophytic bacterium *Methylobacterium mesophilicum* SR1.6/6. J Basic Microbiol 55:1357–1366

Dykhuizen DE (1998) Santa Rosalia revisited: why are there so many species of bacteria? Antonie Van Leeuwenhoek 73:25–33

Faraldo-Gómez JD, Sansom MSP (2003) Acquisition of siderophores in Gram-negative bacteria. Nat Rev Mol Cell Biol 4:105–116

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10: 538–550

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial–fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75:583–609

Guan LL, Kanoh K, Kamino K (2001) Effect of exogenous siderophores on iron uptake activity of marine bacteria under iron-limited conditions. Appl Environ Microbiol 67:1710–1717

Haldar S, Sengupta S (2015) Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. Open Microbiol J 9:1–7

Jayachandra SY, Parameshwar AB, Mohan Reddy K, Sulochana MB (2012a) Characterization of extracellular hydrolytic enzymes producing extremely halophilic bacterium *Virgibacillus* sp. JS5. World J Sci Technol 2(2):23–26

Jayachandra SY, Anil Kumar S, Merley DP, Sulochana MB (2012b) Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. Recent Res Sci Technol 4(4):46–49

Jimenez PN, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ (2012) The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. Microbiol Mol Biol Rev 76:46–65

Johnstone TC, Nolan EM (2015) Beyond iron: non-classical biological functions of bacterial siderophores. Dalton Trans 44:6320–6339

Jones DS, Albrecht HL, Dawson KS, Schaperdoth I, Freeman KH, Pi Y, Pearson A, Macalady JL (2012) Community genomic analysis of an extremely acidophilic sulfur-oxidizing biofilm. ISME J 6:158–170

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3:937–947

Knights D, Costello EK, Knight R (2011) Supervised classification of human microbiota. FEMS Microbiol Rev 35:343–359

Kulkarni G, Busset N, Molinaro A, Gargani D, Chaintreuil C, Silipo A, Giraud E, Newman DK (2015) Specific hopanoid classes differentially affect free-living and symbiotic states of *Bradyrhizobium diazoefficiens*. MBio 6:e01251–e01315

Lacava PT, Araújo WL, Marcon J, Maccheroni W Jr, Azevedo JL (2004) Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. Lett Appl Microbiol 39:55–59

Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166:689–700

Lamont IL, Beare PA, Ochsner U, Vasil AI, Vasil ML (2002) Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 99:7072–7077

López-Lara IM, Sohlenkamp C, Geiger O (2003) Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Mol Plant-Microbe Interact 16:567–579

Marmann A, Aly AH, Lin W, Wang B, Proksch P (2014) Co-cultivation—a powerful emerging tool for enhancing the chemical diversity of microorganisms. Mar Drugs 12:1043–1065

Martins MB, Carvalho I (2007) Diketopiperazines: biological activity and synthesis. Tetrahedron 63:9923–9932

Mayr E (1957) In: Mayr E (ed) The species problem. American Association for the Advancement of Science, Washington, DC, pp 1–22

McCormick SP, Stanley AM, Stover NA, Alexander NJ (2011) Trichothecenes: from simple to complex mycotoxins. Toxins 3:802–814

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37: 634–663

Metz JAJ, Geritz SAH, Meszéna G, Jacobs FJA, van Heerwaarden JS (1996) In: van Strien S, Verduyn Lunel Sjoerd M, Verduyn Lunel SM (eds) Stochastic and spatial structures of dynamical systems. North Holland, Amsterdam, pp 183–231

Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867

Morissette DC, Dauch A, Beech R, Masson L, Brousseau R, Jabaji-Hare S (2008) Isolation of mycoparasitic-related transcripts by SSH during interaction of the mycoparasite *Stachybotrys elegans* with its host *Rhizoctonia solani*. Curr Genet 53:67–80

Muller EEL, Hourcade E, Louhichi-Jelail Y, Hammann P, Vuilleumier S, Bringel F (2011) Functional genomics of dichloromethane utilization in *Methylobacterium extorquens* DM4. Environ Microbiol 13:2518–2535

Nalin R, Putra SR, Domenach A-M, Rohmer M, Gourbiere F, Berry AM (2000) High hopanoid/ total lipids ratio in *Frankia* mycelia is not related to the nitrogen status. Microbiology 146: 3013–3019

Netzker T, Fischer J, Weber J, Mattern DJ, König CC, Valiante V, Schroeckh V, Brakhage AA (2015) Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6:299

Nützmann H-W, Reyes-Dominguez Y, Scherlach K, Schroeckh V, Horn F, Gacek A, Schümann J, Hertweck C, Strauss J, Brakhage AA (2011) Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation. Proc Natl Acad Sci U S A 108:14282–14287

Oh D-C, Kauffman CA, Jensen PR, Fenical W (2007) Induced production of emericell amides A and B from the marine-derived fungus *Emericella* sp. in competing co-culture. J Nat Prod 70: 515–520

Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884–888

Partida-Martinez LP, Monajembashi S, Greulich K-O, Hertweck C (2007) Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. Curr Biol 17:773–777

Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial–fungal interactions. Nat Rev Microbiol 8:340–349

Peters NK, Frost JW, Long SR (1986) A plant flavone, luteolin, induces expression of rhizobium-meliloti nodulation genes. Science 233:977–980

Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME (2012) Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev 25:193–213

Phelan VV, Liu W-T, Pogliano K, Dorrestein PC (2012) Microbial metabolic exchange—the chemotype-to-phenotype link. Nat Chem Biol 8:26–35

Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol 53:403–424

Ross C, Opel V, Scherlach K, Hertweck C (2014) Biosynthesis of antifungal and antibacterial polyketides by *Burkholderia gladioli* in coculture with *Rhizopus microsporus*. Mycoses 57:48–55

Sáenz JP, Grosser D, Bradley AS, Lagny TJ, Lavrynenko O, Broda M, Simons K (2015) Hopanoids as functional analogues of cholesterol in bacterial membranes. Proc Natl Acad Sci U S A 112:11971–11976

Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. Eur J Plant Pathol 131:15–26

Schmerk CL, Bernards MA, Valvano MA (2011) Hopanoid production is required for low-pH tolerance, antimicrobial resistance, and motility in *Burkholderia cenocepacia*. J Bacteriol 193:6712–6723

Schroeckh V, Scherlach K, Nützmann H-W, Shelest E, Schmidt-Heck W, Shuemann J, Martin K, Hertweck C, Brakhage AA (2009) Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. Proc Natl Acad Sci U S A 106:14558–14563

Seipke RF, Loria R (2009) Hopanoids are not essential for growth of *Streptomyces scabies* 87-22. J Bacteriol 191:5216–5223

Sim BMQ, Chantratita N, Ooi WF, Nandi T, Tewhey R, Wuthiekaun V, Thaipadungpanit J, Tumapa S, Ariyaratne P, Sung W-K, Sem XH, Chua HH, Ramnarayanan K, Lin CH, Liu Y, Feil EJ, Glass MB, Tan G, Peacock SJ, Tan P (2010) Genomic acquisition of a capsular polysaccharide virulence cluster by non-pathogenic *Burkholderia* isolates. Genome Biol 11:R89

Spratt BG, Staley JT, Fisher MC (2006) Introduction: species and speciation in microorganisms. Phil Trans R Soc B 365:1897–1898

Strobel GA (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Stuart LM, Paquette N, Boyer L (2013) Effector-triggered versus pattern-triggered immunity: how animals sense pathogens. Nat Rev Immunol 13:199–206

Sulochana MB, Jayachandra SY, Anil Kumar S, Parameshwar AB, Mohan Reddy K, Dayanand A (2014a) Siderophore as a potential plant growth-promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5):418–424. https://doi.org/10.1002/jobm.201200770

Suryanarayanan TS, Shaanker RU (2015) Fungal endophytes—biology and bioprospecting preface. Curr Sci India 109:37–38

Tarkka MT, Sarniguet A, Frey-Klett P (2009) Inter-kingdom encounters: recent advances in molecular bacterium–fungus interactions. Curr Genet 55:233–243

Tata A, Perez C, Campos ML, Bayfield MA, Eberlin MN, Ifa DR (2015) Imprint desorption electrospray ionization mass spectrometry imaging for monitoring secondary metabolites production during antagonistic interaction of fungi. Anal Chem 87:12298–12304

Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R (2013) Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome. MBio 4:e00459–e00413

Uzum Z, Silipo A, Lackner G, De Felice A, Molinaro A, Hertweck C (2015) Structure, genetics and function of an exopolysaccharide produced by a bacterium living within fungal hyphae. ChemBioChem 16:387–392

van Elsas JD, Chiurazzi M, Mallon CA, Elhottovã D, Krištůfek V, Salles JF (2012) Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc Natl Acad Sci U S A 109: 1159–1164

Virgin HW, Todd JA (2011) Metagenomics and personalized medicine. Cell 147:44–56

Weitz JS, Hartman H, Levin SA (2005) Coevolutionary arms races between bacteria and bacteriophage. Proc Natl Acad Sci U S A 102:9535–9540

Welander PV, Hunter RC, Zhang L, Sessions AL, Summons RE, Newman DK (2009) Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas palustris* TIE-1. J Bacteriol 191:6145–6156

Wertz JE, Goldstone C, Gordon DM, Riley MA (2003) A molecular phylogeny of enteric bacteria and implications for a bacterial species concept. J Evol Biol 16:1236–1248

Yaradoddi JS, Shoba H, Banapurmath NR, Hunashyal AM, Sulochana MB, Shettar AS, Ganachari SV (2018) Alternative and renewable bio-based and biodegradable plastics. In: Martínez LMT, Kharissova OV (eds) Handbook of ecomaterials. Springer International Publishing AG, Cham. https://doi.org/10.1007/978-3-319-68255-6_150

Yaradoddi J, Kontro M, Ganachari S, Sulochana M, Agsar D, Tapaskar R, Shettar A (2019) Protein nanotechnology (Dr. Kharisov BI (Editor-in-Chief) Handbook of ecomaterials). Springer, pp 3573–3585. https://doi.org/10.1007/978-3-319-682556_192

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV, Soudagar MEM, Mubarak NM, Hallad S, Hugar S, Fayaz H (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Young JPW, Crossman LC, Johnston AWB, Thomson NR, Ghazoui ZF, Hull KH, Wexler M, Curson ARJ, Todd JD, Poole PS, Mauchline TH, East AK, Quail MA, Churcher C, Arrowsimth C, Cherevach I, Chillingworth T, Clarke K, Cronin A, Davis P, Fraser A, Hance Z, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabbinowictsch E, Sanders M, Simmonds M, Whitehead S, Parkhill J (2006) The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. Genome Biol 7:R34

Chapter 2
Actinobacteria in Marine Environments

Jayachandra S. Yaradoddi, Merja H. Kontro, Sharanabasava V. Ganachari, Nagaraj R. Banapurmath, Ajaykumar Oli, Anilkumar S. Katti, and M. B. Sulochana

Abstract The marine environment is one of the significant habitats for exploring novel compounds from diverse microorganisms; among these organisms, marine actinobacteria are considered to be a leading contributor. Recently, imperative advancements have been made in the field of marine microbial ecology with particular emphasis on molecular studies, including 16S rRNA analysis and metagenomics libraries, which have indicated the predominance of actinobacterial diversity in the soil sample. Both culture-dependent and culture-independent approaches have revealed the importance of marine actinobacterial diversity in biomedical science and bioengineering applications.

J. S. Yaradoddi (✉)
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

M. H. Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

N. R. Banapurmath
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

A. Oli
SDM Research Institute for Biomedical Sciences, a Constituent Unit, Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

A. S. Katti
Department of Biotechnology, Walchand College of Arts and Science, Solapur, Maharashtra, India

M. B. Sulochana
Department of PG Studies and Research in Biotechnology, Gulbarga University, Kalburagi, Karnataka, India

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The major habitats for marine actinobacteria are the seashore, sea snow, hydrocarbon seeps, saturated brines, cold seeps, and hydrothermal vents. Many reports have shown the presence of epibiont and symbiotic actinobacteria in the marine micro as well as macrofauna. Actinobacteria are unevenly distributed in the marine environment in small but substantial quantities along with the significant levels of biodiversity. The presence of viruses within the marine actinobacteria forms the marine actinophages that have been recognized for their ubiquitous nature. However, the extent of marine actinobacterial biodiversity, distribution, and abundance is still undistinguishable due to fewer reports, intermittent research work, and inappropriate identification methods.

Keywords Actinobacteria · Marine environment · Metagenomics · Biodiversity · Habitats · Epibiont · Symbiotic association

2.1 Introduction

Gram-positive, aerobic, and nonmotile *Actinobacteria* can have a high DNA guanosine-cytosine (GC) base content of 70–80%. According to the 16S rRNA gene phylogeny, they are evolutionarily much more bacterial-like than fungal; although partly due to the filamentous morphology, they were originally considered to be intermediates between bacteria and fungi. Accordingly, members of the phylum *Actinobacteria* are classified as prokaryotes and belong to the order *Actinomycetales*, which have substrate hyphae and form aerial spores and mycelium. The aerial hyphae of actinomycetes tend to produce sporophores, and their structure varies widely. The spore-forming hyphae with aerial mycelium possess enormous lengths compared to substrate mycelium. An additional interesting characteristic feature of the spores is their resistance toward desiccation, and the spores can be viable for long periods. The life process gives resistance to harsh environmental circumstances, such as reduced water availability and nutrient deficiency. Such microorganisms are phenotypically and genetically unique and can be seen in most environments.

2.1.1 Basis and Distribution of Marine Actinomycetes

Actinobacteria or actinomycetes are typically discovered from oceanic sediments, and they occur abundantly within the soils. Their diversity and distribution in the aquatic system has mostly been unrevealed for several years. Many of the researchers have questioned the nativity of the marine actinomycetes due to resistant spores that may have migrated from the terrestrial environments to sea and other aquatic systems.

2.1.2 Actinobacteria in the Marine Environments

Marine microbiology is emerging globally with a discrete focus on secondary metabolite production. Blunt et al. (2007) reported that between 1965 and 2014,

more than 25,000 new compounds were discovered in distinct marine organisms in 22 oceanic regions worldwide, including the Indian Ocean and islands; Atlantic Europe and Baltic Sea; South, North, and Central America; Australia; some African countries; and Arctic and Antarctica. Inspired by this, marine actinomycetes have also been explored for the possible ability to produce unique secondary metabolites, and, as the previous reports have revealed, they are the abundant sources for bioactive molecules. These microorganisms hold an exceptional position as significant targets for major screening processes, as their diversity provides support to anticipate that they also have the capability to synthesize various pharmaceutically important molecules and novel secondary metabolites (Ellaiah et al. 2004).

Following the discovery of actinomycin (Lechevalier 1982), bioactive molecules were screened from actinomycetes to produce antitumor agents, commercial bioactive molecules, and desired industrial enzymes (Tanaka and Omura 1990). As much as about two-thirds of the recovered natural metabolites have been derived from these microbes (Takaizawa et al. 1993), among which most of the bioactive compounds have been discovered from *Streptomyces* spp. (Goodfellow and O'Donnell 1993). The produced bioactive molecules have been found to be of major structural interest and essential in promoting the development of novel antibiotic derivatives from their molecular backbone (Sivakumar et al. 2007).

Although the microbial assortment in the terrestrial conditions is intrinsically remarkable, utmost diversity can also be seen in the oceanic environments (Donia and Hamann 2003). It is well-known that about 70% of the Earth exterior is the ocean, from which life was originated. Several research investigations have revealed that in the marine environments, like coral reefs and deep seafloor, the biological diversity is quite high compared to, for example, tropical rainforests (Haefner 2003). This is due to marine ecological circumstances, which are very unusual and different from the terrestrial environment; it can be inferred that actinobacteria from the oceans have possessed in evolution toward different characteristic features than the terrestrial ones (Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020). Consequently, they could have the potential to produce diverse classes of secondary metabolites. The adaptability of marine actinobacteria toward the extreme and harsh living conditions has resulted from the vast evolutionary range of extreme environments, covering high seabed pressure (upper limit about 1100 atmosphere), anaerobicity, sometimes extreme acidic conditions (pH low, about 2.8), and temperatures close to 0 °C or in the other extreme about 100 °C near the hydrothermal vents on ridges in the middle of the ocean.

The unique conditions are undoubtedly reflected in the metabolic and genetic multiplicity of the marine actinobacteria, which continues immensely to be unknown. Indeed, the marine conditions are almost untapped sources of novel types of actinobacterial diversity and, consequently, the novel metabolites (Stach et al. 2003a; Jensen et al. 2005a; Fiedler et al. 2005; Magarvey et al. 2004). The diversity and distribution of actinomycetes inside the sea have been hugely ignored, and many original marine actinobacteria remain uncharacterized. This gap is created because of limited research work conducted toward exploring marine actinobacteria, whereas the terrestrial actinobacteria are much more utilized for the investigation

and production of novel bioactive molecules. Various computational approaches are promoting in understanding the actinobacteria at the gene level to explore novel natural products (Fig. 2.1).

2.2 Origin of Marine Actinobacteria

Marine actinobacteria have remained dormant for several years; actually, these bacteria have been estimated to be migrated as leached dormant spores from soil that are able to survive but not grow (Goodfellow and Williams 1983). Nowadays it is unambiguous that the explicit communities of marine inhabited actinomycetes not only occur in the marine environmental conditions but also contribute by adding diversity within a wide array of actinobacterial taxa (Mincer et al. 2002; Stach et al. 2004). Reports have also indicated that actinomycetes can be recovered from the coastal environments, deep-sea sediments, and mangrove swamps (Sivakumar 2001; Tae et al. 2005), despite the selective techniques used in the cultivation of actinobacteria aimed only at mycelium-producing strains, thus excluding the interesting marine populations such as mycolate actinobacteria (Colquhoun et al. 1998). It has been realized that the marine actinobacteria comprise of novel phenotypes and are undoubtedly different from those recognized to occur in soil. While the biological properties of marine and aquatic actinobacteria continue to be undefined, there is a scope in understanding their ecological roles as terrestrial ones. The terrestrial actinobacteria are involved in degradation of recalcitrant organic compounds, mainly chitin, a biopolymeric material abundant in the ocean. As long as actinobacteria are living inside the sea, which undergoes a significantly diverse ecological circumstances when compared with terrestrial populations, the occurrence of speciation in marine actinobacteria with several exceptional taxa is not surprising. Besides being a wide range of marine actinobacterial multiplicity, it has yet to be described. Researchers must understand the mechanisms of adaptation of the organisms in the ocean that lead to the production of bioactive molecules; there is a need for these interactions to be established.

Marine ecosystems have a substantial actinobacterial diversity, allowing for the extraction of new metabolites and their genes, thereby increasing global awareness to microorganisms in the oceans and their bioactive molecule products. Based on the potential associated with marine actinobacteria, several new molecules in previously unknown configurations have been uncovered (Subramani and Aalbersberg 2012). The intertidal or littoral zone regular changes between exposure to air during low tide and high tide flooding are a unique part of the sea shores and estuary. The zone is also a habitat for actinobacteria, though their communities, biological activity, and genetic capacity have been infinitely little studied, and the niche most likely is arousing curiosity for the discovery of novel genes of biological origin and potential antimicrobial producing strains. However, the biodiversity and bioactive molecule biosynthesis in intertidal sediments have been assessed using cultivation-based methods. The results using genomic fingerprints demonstrated the occurrence of

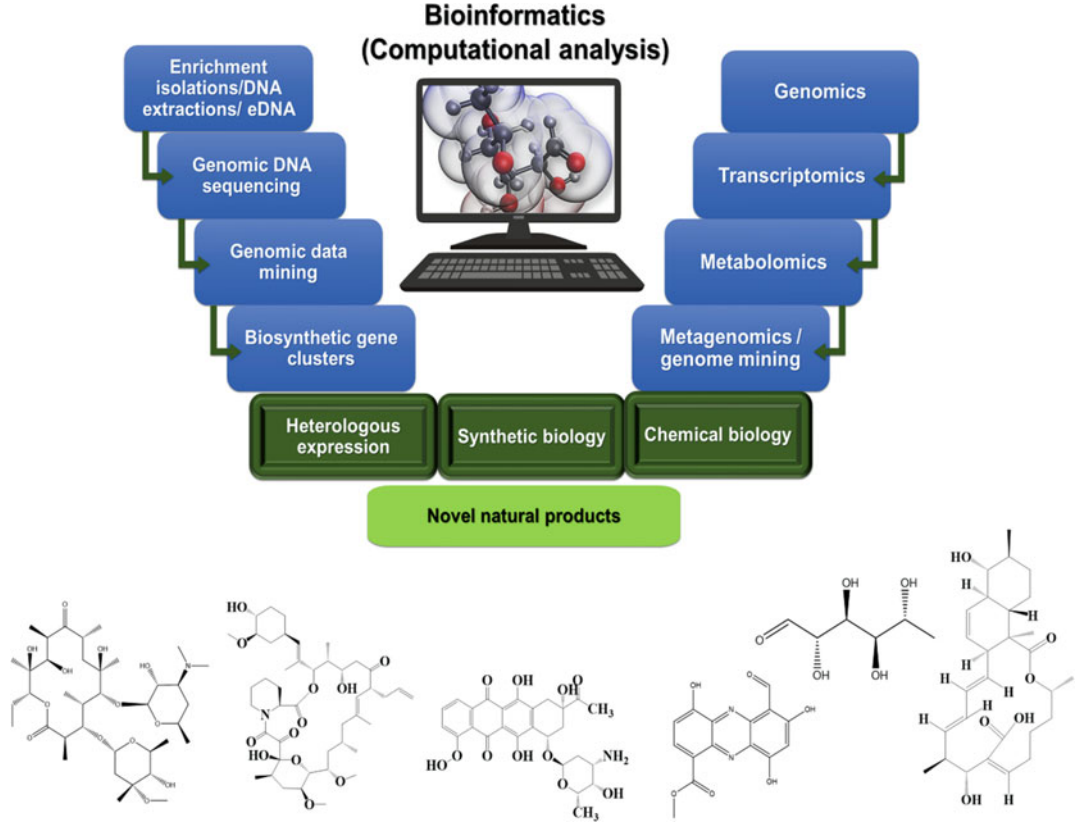

Fig. 2.1 Advanced computational and molecular approaches existing in the identification of novel natural products

high diversity and potential for multiple biosynthetic pathways (Jose and Jha 2017). Furthermore, the 39 km^2 Diu Island (20.71°N 70.98°E) near Saurashtra Peninsula (Gujrat, India) in the Arabian Sea is an area where unique, diverse microorganisms grow, and the diversity and biological capacity of actinobacteria have not been examined. These few examples reveal well that there are many areas in the marine environment that are poorly studied in terms of actinobacteria.

2.2.1 Different Niches of Marine Bacteria

Actinobacteria can be discovered in several free-swimming marine invertebrates and vertebrates, as well as in immobile organisms. These distinguished bacteria are found in marine living beings that produce bioactive molecules. For instance, the puffer fish was recognized as a producer for the potential neurotoxin, tetrodotoxin. However, several current marine/aquatic organisms are also found to be important producers of tetrodotoxin. The ability has often been associated with different prokaryotic bacteria, such as actinomycetes. Puffer fish usually has high levels of toxins in the liver and ovaries, where it also possesses tetrodotoxin-producing actinobacteria. The identified bacteria have been strongly associated with *Nocardiopsis dassonvillei*, which has also been recovered through puffer fish ovaries (Wu et al. 2005).

2.2.2 Actinobacteria in Marine Snow

Marine snow is mostly organic detritus that falls into deeper layers of the water column. Previous investigations (DeLong et al. 1993; Simon et al. 2002) revealed that actinomycetes have not been successfully detected in marine snow through molecular techniques, although cultivation-based methods have been flourishing (Grossart et al. 2004). A total of 10% fraction of actinobacteria was recovered from marine macroaggregates called marine snow. The actinobacteria in the aggregates were connected with competitive interactions, as 80% of the actinobacterial cultures indicated antagonistic effects on the growth of other bacterial cultures utilizing aggregates. The contradictory results of these investigations regarding the existence or lack of actinobacteria in the marine snow were emphasized to be based on the geographical conditions and environmental heterogeneity of marine snow.

2.2.3 Actinobacteria in Sediments

Since, as per the literature, actinomycetes are among the key phyla in marine sediments; several research advancements have been developed in isolation,

identification, and classification of actinobacteria as part of the indigenous microbial flora. During the latest decades, various strategies have been successfully developed for isolation and screening for secondary metabolite production, with particular focus on marine actinomycetes (Ma et al. 2009). As a result, novel and unambiguously marine actinomycete genera, *Salinispora* and *Marinophilus*, were successfully defined, which later on led to a significant improvement in culture-dependent discovery of drugs (Jensen et al. 2005b; Newman 2016). The isolation of actinobacteria from samples collected from different sea areas, covering mud, (subtidal) sediments, sponge, ascidian, and different depth series has expanded the identified diversity. Detailed information is available on actinobacterial quantification and classification in different geographical locations and on their biological activity. This process has led to an increase in the number of isolated and classified actinobacteria and discovery of their new bioactive products (Blunt et al. 2011, 2016; Newman and Cragg 2012; Claverías et al. 2015; Stach et al. 2003b; Magarvey et al. 2004). As an example, hypersaline Hamelin Pool stromatolites in Shark Bay (Western Australia) are a structure of organo sedimentary material composed due to microbial metabolic activity. The morphology of living stromatolites is analogous to that of the fossil ones, which can be as old as 3.5 billion years. Examination of microorganisms in these exceptional mat communities showed significant differences between stromatolite structural types, with a cyanobacterial portion of about 5% being lower than expected, while an actinobacterial abundance was approximately 14% with the average sequence identity of 95.5% to the closest relatives in databases. Actinomycetes appeared to be ubiquitous in stromatolites under marine environmental conditions (Papineau et al. 2005).

2.2.4 Association with Fauna

Bioactive molecules from the sea can also be derived from fauna, sponges, and marine invertebrates, that is, from sessile organisms. Site-bound organisms require an effective mixture of chemical defense molecules. In particular, the sponges are considered to be abundant sources of new metabolites (Hill 2004). They are associated with sophisticated bacterial communities within tissues. Bioactive secondary metabolite-producing actinobacteria are widespread among these microbial communities, which comprise a wide variety of sponge-specific lineages that include actinobacteria from the genera *Theonella*, *Rhopaloeides*, *Aplysina*, *Xestospongia*, *Gordonia*, *Micrococcus*, *Brachybacterium*, *Salinospora*, *Micromonospora*, *Actinoplanes*, *Streptomyces*, and many inadequately characterized and uncultured bacterial clones (Hentschel et al. 2002; Montalvo et al. 2005; Kim et al. 2005).

2.2.5 Deep Subfloor Biosphere

The fascinating ecology of the seafloor and its sediments and the evolution of microbial communities reveal the abundance of bacteria, archaea, fungi, and viruses in extreme environments at sea depths down to 5500 m and even below (Orsi 2018; Walsh et al. 2016). The compiled data from 65 studies showed that bacteria and archaea in the subseafloor have almost the same abundances. Both microbial groups decreased with increasing depth, bacteria more than archaea (Lloyd et al. 2013). The species richness and genetic diversity of actinobacteria also varied, as sediment depth increased below the seafloor at 3814 m so that diversity shifted toward dominance, while each sediment section had distinct characteristic phylogeny, that is, the actinobacterial genetic relatedness in sediment sections collected 5–46 cm below the seafloor was different. Actinobacteria were most closely related to *Corynebacterineae*, *Frankineae*, and *Streptomycineae*, though only 9% of the operational taxonomic unit groups (OTUs) showed 99–100% homology to cultivated actinobacteria; the rest had 94–98% homology (Stach et al. 2003b). In the vertical microbial diversity profile from sea surface down to subseafloor sediments, the quantities of *Actinobacteria*, *Planctomycetes*, and *Firmicutes* OTUs were among the most abundant in water columns. OTUs, which were abundant in deep subseafloor sediments, were often common in shallow sediments and were also observed at low concentrations in the water column, suggesting that they are ultimately seeded from the water column (Walsh et al. 2016). Since only 7 actinobacterial strains could be isolated from subseafloor sediments out of 194 cultivated (southwestern Sea of Okhotsk) (Inagaki et al. 2003), while 16S rDNA sequencing has revealed a much higher diversity and abundance (Stach et al. 2003b), it can be concluded that the greatest part of the metabolic divergence and bioactivity of subfloor biosphere actinobacteria is yet to be discovered.

2.2.6 Methane-Hydrate-Associated Sediments

Actinomycetes contributed as much as 40% of all sequences present in methane-hydrate-associated sediment clones in Nankai Trough, indicating that actinobacteria may cover a remarkable portion of biodiversity in particular geographical extreme sites (Reed et al. 2002). Actinomycetes have spread widely to the marine ecosystem in a little but important portions of genetic multiplicity. Apart from actinomycetes, the oceans are also occupied by different groups of viruses (Suttle 2005), and the ubiquitous occurrence of the actinomycetes has also appeared in the existence of actinophages in the marine environment (Kurtböke 2005). The profusion and degree of actinomycete diversity in various biogeographical locations remains unclear; this is due to lower sampling rates. Further, the identification of actinomycetes by fragmented biased methods has not been clearly described (Suttle 2005; Kurtböke 2005).

2.3 Marine Actinobacteria in Phytopathogen Control

In recent decades, the major focus in the agricultural sector has been on pollution, which is usually released through widespread use of highly hazardous agrochemicals, mainly pesticides (Rai et al. 2011; Prévost et al. 2006). Meanwhile, in the 1970s, in addition to the hazardous effects on the public health conditions, over a period of time-continuous exposure toward the pesticides has led to progress in phytopathogen tolerance (Aktar et al. 2009). The occurrence of pathogenic infections in agricultural crops in the global economy position is relentless; both academies and industry have improved their studies in search of solutions to the present issue.

Bacterial cells of both beneficial and also pathogenic strains were identified as social populations, which are capable to control their gene expression in the density-based pathway, the mechanism called as quorum sensing (QS) (Helman and Chernin 2015). Quorum sensing controls the biological mechanisms associated with metabolism, growth, and virulence among bacterial cells by synthesizing signaling molecules, which intensify the concentration with respect to an increase in cell numbers (Grandclément et al. 2016). When the amount of the molecules attains a particular threshold, unlikely signal transduction cascades are stimulated as a result of changes in gene expression, which includes a pathogenic effect. The QS dictates the expression of various virulence characters, and several plant pathogens are dependent on this type of system to induce disease in its host plant (Andersen et al. 2010; Barnard et al. 2007). For instance, it is a well-known fact that quorum sensing system regulates toxoflavin biosynthesis in several members of *Burkholderia* species (specifically among the *Burkholderia glumae*) and, thus, phytotoxin can be recognized as a critical pathogenic factor in wilt disease affecting the plant vascular system and in rice rot disease causing black lesions (Kim et al. 2004). To control this problem, there are several antagonistic compounds, mainly antibiotics, which can be obtained from microorganisms. Thus, microbes are mainly recognized as a chief source of antimicrobial compounds that can be used against phytopathogens of agricultural crops.

To date, the most powerful source of such antibiotic-producing microorganisms has been the terrestrial environment (Sulochana et al. 2014a, b). However, microorganisms from seas have also been documented to be a vital basis for bioactive compounds in the future due to their ability to control these phytopathogens (Ma et al. 2009; Blunt et al. 2016). Furthermore, marine bacteria belonging to phylum *Actinobacteria* have been identified as one of the most imperative species cluster with immense biotechnological applications (Blunt et al. 2016; Shellikeri et al. 2018; Yaradoddi et al. 2020b), accordingly contributing by increasing the supply of novel bioactive compounds (Newman 2016). The metabolites from the marine origin have become a model for the advancements in putative antimicrobial and insecticidal compounds and, thus, they have turned to be an excellent candidate in agrochemical production (Blunt et al. 2011; Newman and Cragg 2012). For example, concerning to kasugamycin hydrochloride, it is a general antifungal

agent used against the *Magnaporthe grisea* and a potential antibacterial agent against *Burkholderia glumae* (Yoshii et al. 2012). These secondary metabolites were initially recovered from the terrestrial actinobacterium *Streptomyces kasugaensis*, and afterward it was also extracted from the marine strain *Streptomyces rutgersensis* subsp. *gulangyunensis* (Betancur et al. 2017).

An approach of the therapeutic value of antibiotics can be ascribed toward in vivo bacterial growth inhibition once antibiotic concentrations surpass the minimum inhibitory concentration (MIC). Besides, although the concentration is lower than the MIC, it can still be able to reduce the growth activity and also the expression of different bacterial virulence factors, diminishing possible effects of the pathogenic organisms on causing the disease. The specific action of antibiotics known as sub-MIC effects, further compounds that are used in quorum quenching activities are called as quorum quenching compounds (QQC) (Helman and Chernin 2015).

The QQC have been applied for the inhibition of the expression of virulence factors of the phytopathogens. There are different mechanisms directed to the biosynthesis of enzymes, which lead to the interference with virulence factor signaling. Inhibiting enzymes can interfere with the signaling molecule biosynthesis at the transcriptional level, or the enzymes may inhibit receptor activation by producing quenching compounds (Helman and Chernin 2015). Numerous existing research outcomes demonstrate the ability of bacterial strains to inhibit QS systems of phytopathogenic strains. For example, several species from the genus *Streptomyces* encompass potential of inhibition against the various QS-controlled virulence factor expression in *Pectobacterium carotovorum*. The inhibition occurs by synthesizing several bioactive compounds that have been recognized as containing piericidin A and glucopiericidin A, indicating that the compounds have potential for biocontrol of plant pathogens (Kang et al. 2016). The molecules extracted from marine territory microorganisms could be valuable, when appropriately used as bioactive agents in quorum quenching to prevent pathogenic bacterial communication and to lower the injury to the host (Kalia 2013). Furthermore, *N*-amido–αproline and the linear dipeptide (proline, glycine) produced by actinobacterium in aquatic sponge presented preventing actions upon quorum sensing and facilitated the adverse influences of *Pseudomonas aeruginosa* (Naik et al. 2013).

2.4 Marine Bacterial Cultures

Several conventionally used cultivation media and their derivatives are available for cultivating actinobacteria from terrestrial environments, such as starch-casein-KNO_3 agar, actinomycete-isolation agar, glycerol-arginine agar, tryptone-yeast extract-glucose agar, tryptone-soy agar, glucose-yeast extract agar, and humic acid-vitamins agar (Suutari et al. 2002; Maldonado et al. 2005). Marine bacteria have typically been cultivated on marine agar (ZoBell 1946). As these media contain quite high concentrations of organic substrates and select microorganisms that grow rapidly to

high densities under rich nutritional conditions, later especially in marine environments, the growth media development has focused more on low-nutrient substrates.

Media with low nutrient concentrations represent the composition of the marine environment. Among the first cultivation techniques developed were dilution cultures combined with flow cytometry, in which marine bacteria were diluted and then cultivated in seawater based-media (Button et al. 1993). The most probable number (MPN) cultivation on mineral media with different compositions was used to quantify Mediterranean sapropel bacteria (Süß et al. 2004). Further, high-throughput cultivation in small quantities (extinction culturing) under low substrate conditions on microtiter plates was developed to improve screening efficiency by mimicking nutrient concentrations in situ (Connon and Giovannoni 2002). End point dilution using microtiter plates and dilute growth medium, such as diluted nutrient broth, combined with automated cell array and imaging were used successfully to isolate novel marine bacteria (Janssen et al. 2002; Keller and Zengler 2004; Mincer et al. 2002; Rappe et al. 2002). Gel microcapsules were developed to encapsulate and cultivate individual cells under low nutrient conditions, and growth was monitored by flow cytometry until the microcolonies could be sorted individually into dishes with selective growth medium (Zengler et al. 2002, 2005; Toledo et al. 2006). Moreover, diffusion chambers were designed to simulate marine environmental conditions for bacterial cultivation (Kaeberlein et al. 2002). Incubation times were extended up to 6 weeks and even longer to allow growth of slow-growing microorganisms (Keller and Zengler 2004; Mincer et al. 2002; Toledo et al. 2006; Gontang et al. 2007).

Alongside media development, selective microbial isolation methods were improved. The practices include, for example, use of antibiotics with various carbon sources; $K_2Cr_2O_7$ to inhibit fungal growth; nalidixic acid to prevent the growth of fast-growing Gram-negative bacteria; and cAMP and acyl homoserine lactone supplements. Sample pretreatment was also developed, such as heat shock enrichment for spore-forming bacteria (Maldonado et al. 2005; Mincer et al. 2002; Gontang et al. 2007; Zhang et al. 2006; Bruns et al. 2002). Finally, the drying wet intertidal sediment overnight, followed by stamping onto various agar media, resulted in the isolation of 65.6% of actinobacterial strains, with the remainder of the isolates belonging to the class *Bacilli* (Gontang et al. 2007). The various approaches outlined above have significantly improved the cultivability of previously uncultivated marine bacteria.

2.4.1 Antimicrobial Actions of the Extracts

Various in vitro screening methods are available to examine antimicrobial susceptibility. Among the most commonly used bioassays are diffusion methods, including agar disk diffusion, agar well diffusion, and agar plug diffusion methods, as well as antimicrobial gradient, cross streak, and poisoned food methods. The agar disk diffusion method is simple to perform and allows large series of antimicrobials

and microorganisms to be examined, and the results are easily interpreted. Thus by testing the antibiogram one can measure the susceptibility results to classify microorganisms to resitant, intermediate, and susceptible. However, the method does not distinguish microbicidal or microbiostatic effects and cannot really be used to evaluate the minimum inhibitory concentration (MIC). An antimicrobial gradient method is required for MIC determination (Balouiri et al. 2016). A modification of these methods, the direct confrontation assay, has been successfully used to evaluate the antibacterial activity of marine actinobacteria strains against *Burkholderia* species. The in vitro antagonism assay originally developed to test for fungal growth inhibition by soil actinomycetes has also been used successfully to measure the antifungal activity of marine bacteria (Betancur et al. 2017; Crawford et al. 1993).

Organic extracts of marine bacterial strains were used to survey their antibacterial activity against *Burkholderia* pathogens by the diffusion method. After cultivating the marine bacteria in 100 mL of tryptone-soy broth, the liquid phase was separated by centrifugation and sterile filtration (0.22μm), followed by liquid extraction with ethyl acetate. The antibacterial activity of the concentrated extract was determined using a diffusion test on a microtiter plate. *Burkholderia* sp. was cultivated in King B medium, followed by the dilution of 30μL in 200μL of the same medium using microtiter plate. The organic extract (500μg) and 5% DMSO (30μL) were added, and the plate was incubated for 24 h. In the absence of *Burkholderia* sp. growth, the extract was evaluated to be positive for antibacterial agents. *Burkholderia* sp. was not inoculated into the negative control, and gentamicin (0.2μg/mL) was added to the positive control as per the report (Balouiri et al. 2016; Betancur et al. 2017). Besides being potential producers of antibiotics, actinomycetes are susceptible to a few important antibiotics as listed (Table 2.1).

The activity of marine bacterial extracts against fungi was also examined. Fungal cultures on potato-dextrose agar plates were collected in 0.85% aqueous NaCl solution, and the suspension was inoculated into PDA (2 mL) in a well. The bacterial extract (500μg) dissolved in 5% DMSO (30μL) was added, and after 96-h cultivation, fungal growth was evaluated. Positive controls contained clotrimazole (5μL of 1% solution), and fungi was not inoculated to the negative controls (Betancur et al. 2017).

Table 2.1 Effective concentration of antibiotics on actinobacteria

Name of the antibiotics	Concentration per mL
Erythromycin	15 and 30μg
Aureomycin	30μg
Gentamicin sulfate	10μg
Kanamycin	15μg
Amikacin	30μg
Chloramphenicol	30μg
Novobiocin	5 and 30μg
Ciprofloxaci	10μg
Penicillin G	10 U
Tetracycline	10 and 30μg
Vancomycin	10μg

2.4.2 *Marine Strain Quorum Quenching actions*

A plate assay for disc diffusion to screen for antagonists of quorum sensing (QS) signals has been developed and proven to be suitable for marine bacteria as well (Betancur et al. 2017; McLean et al. 2004; Tello et al. 2012). The biosensor indicator *Chromobacterium violaceum* ATCC31532 synthesizing acylated homoserine lactones (AHLs) was inoculated on agar plates, where discs (diameter 5 mm) with the marine bacterial extract (300μg) were placed. The QS inhibition affects AHL-related signaling. After 24-h incubation, quenching molecules were assessed for the lack of production of purple violacein pigment in the discs surrounding, while the occurrence of growth inhibition was judged to be due to antibacterials. In positive controls, 4-hydroxybenzaldehyde (200μg) was added to the disc, and in negative controls, 300μL of DMSO (5%) was added.

2.5 Future Prospective

Microbes inherently possess unique biotechnologically important secondary metabolites (Jayachandra et al. 2013a, b; Anil Kumar et al. 2010). Extensive investigations have been carried out in screening the terrestrial ecosystem, and a large number of actinobacteria have already been explored for the production of interesting bioactive compounds (Mohan et al. 2015a, b). However, much research is still required to dig up coastal regions, slat pan, sponge, salt marshes, and other marine environments as sources of novel marine microorganisms, mainly marine actinobacteria. Marine actinobacteria have the ability to thrive well at a high concentration of salinity, pressure, and pH and thus improve the possibilities of using such microbes in industrial applications, as these industrial processes usually operate at relatively high temperatures, pH, and pressures. Most excitingly, these actinobacteria from marine origin have gained enormous potential for sustenance at adverse environments. Marine organisms could have a remarkable hidden genome with efficient novel genes that would only be expressed if specific substrates were provided.

References

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1–12. https://doi.org/10.2478/v10102-009-0001-7. PMID: 21217

Andersen A, Joergensen B, Bjarnsholt T, Johansen H, Karlsmark T, Givskov M et al (2010) Quorum-sensing-regulated virulence factors in Pseudomonas aeruginosa are toxic to Lucilia sericata maggots. Microbiology 156(2):400–407

Anil Kumar S, Arunashri R, Jayachandra SY, Sulochana MB (2010) Screening of extracellular hydrolytic enzymes from *Marinobacter hydrocarbonoclasticas* strain AK5. Int J Bioscan 5(1): 97–99

Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 6(2):71–79

Barnard AM, Bowden SD, Burr T, Coulthurst SJ, Monson RE, Salmond GP (2007) Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. Philos Trans R Soc B Biol Sci 362(1483):1165–1183

Betancur LA, Naranjo-Gaybor SJ, Vinchira-Villarraga DM, Moreno-Sarmiento NC, Maldonado LA, Suarez-Moreno ZR, Acosta-González A, Padilla-Gonzalez GF, Puyana M, Castellanos L, Ramos FA (2017) Marine actinobacteria as a source of compounds for phytopathogen control: an integrative metabolic-profiling/bioactivity and taxonomical approach. PLoS One 12: e0170148

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR (2007) Marine natural products. Nat Prod Rep 24:31–86

Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR (2011) Marine natural products. Nat Prod Rep 28(2):196–268. https://doi.org/10.1039/c005001f. PMID: 21152619

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR (2016) Marine natural products. Nat Prod Rep 33:382–431

Bruns A, Cypionka H, Overmann J (2002) Cyclic AMP and acyl homoserine lactones increase the cultivation efficiency of heterotrophic bacteria from the central Baltic Sea. Appl Environ Microbiol 68:3978–3987

Button DK, Schut F, Quang P, Martin R, Robertson BR (1993) Viability and isolation of marine bacteria by dilution culture: theory, procedures and initial results. Appl Environ Microbiol 59: 881–891

Claverías FP, Undabarrena A, González M, Seeger M, Cámara B (2015) Cultuable diversity and antimicrobial activity of actinobacteria from marine sediments in Valparaíso bay, Chile. Front Microbiol 6:737. https://doi.org/10.3389/fmicb.2015.00737

Colquhoun JA, Mexson J, Goodfellow M, Ward AC, Horikoshi K, Bull AT (1998) Novel rhodococci and other mycolata actinomycetes from the deep sea. Antonie Van Leeuwenhoek 74:27–40

Connon SA, Giovannoni SJ (2002) High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl Environ Microbiol 68:3878–3885

Crawford DL, Lynch JM, Whipps JM, Ousley MA (1993) Isolation and characterization of actinomycete anatgonists of a fungal root pathogen. Appl Environ Microbiol 59:3899–3905

DeLong EF, Franks EG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached- vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Donia M, Hamann MT (2003) Marine natural products and their potential applications as anti-infective agents. Lancet Infect Dis 3:338–348

Ellaiah P, Ramana T, Bapi Raju KVVSN, Sujatha P, Uma Sankar A (2004) Investigation on marine actinomycetes from Bay of Bengal near Kakinada coast of Andhra Pradesh. Asian J Microbiol Biotechnol Environ Sci 6:53–56

Fiedler HP, Bruntner C, Bull AT, Ward AC, Goodfellow M, Potterat O, Puder C, Mihm G (2005) Marine actinomycetes as a source of novel secondary metabolites. Antonie Van Leeuwenhoek 87:37–42

Gontang EA, Fenical W, Jensen PR (2007) Phylogenetic diversity of Gram-positive bacteria culutured from marine sediments. Appl Environ Microbiol 73:3272–3282

Goodfellow M, O'Donnell AG (1993) Roots of bacterial systematic. In: Goodfellow M, O'Donnell AG (eds) Handbook of new bacterial systematics. Academic Press, London, pp 3–54

Goodfellow M, Williams ST (1983) Ecology of actinomycetes. Annu Rev Microbiol 37:189–216

Grandclément C, Tannières M, Moréra S, Dessaux Y, Faure DD (2016) Quorum quenching: role in nature and applied developments. FEMS Microbiol Rev 40(1):86–116

Grossart HP, Schlingoff A, Bernhard M, Simon M, Brinkhoff T (2004) Antagonistic activity of bacteria isolated from organic aggregates of the German Wadden Sea. FEMS Microbiol Ecol 47: 387–396

Haefner B (2003) Drugs from the deep: marine natural products as drug candidates. Drug Discov Today 8:536–544

Helman Y, Chernin L (2015) Silencing the mob: disrupting quorum sensing as a means to fight plant disease. Mol Plant Pathol 16(3):316–329. https://doi.org/10.1111/mpp.12180. PMID: 25113857

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Hill RT (2004) Microbes from marine sponges: a treasure trove of biodiversity for natural products discovery. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM Press, Washington, DC, pp 225–231

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. Appl Environ Microbiol 69:7224–7235

Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M (2002) Improved culturability of soil bacteria and isolation in pure culture of novel members of the dividions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. Appl Environ Microbiol 68:2391–2396

Jayachandra SY, Anil Kumar S, Shouche YS, Sulochana MB (2013a) Culturable diversity of extremely halotolerant bacteria from Arabian Sea Karnataka, India. Int J Biol Pharm Allied Sci 2(2):391–405

Jayachandra SY, Mohan Reddy K, Paramesh B, Shouche YS, Sulochana MB (2013b) Screening of halophilic bacteria extracellular enzymes production from west coast of Karnataka, India. Int J Univ Pharm Biosci 3(1):31–45

Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W (2005a) Culturable marine actinomycete diversity from tropical Pacific Ocean sediments. Environ Microbiol 7:1039–1048

Jensen PR, Mincer TJ, Williams PG, Fenical W (2005b) Marine actinomycete diversity and natural product discovery. Antonie Van Leeuwenhoek 87:43–48

Jose PA, Jha B (2017) Intertidal marine sediment harbours actinobacteria with promising bioactive and biosynthetic potential. Sci Rep 7:1–15

Kaeberlein T, Lewis K, Epstein SS (2002) Microorganisms in pure culture in a simulated natural environment. Science 296:1127–1129

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31(2):224–245. https://doi.org/10.1016/j.biotechadv.2012.10.004. PMID: 23142623

Kang JE, Han JW, Jeon BJ, Kim BS (2016) Efficacies of quorum sensing inhibitors, piericidin A and glucopiericidin A, produced by Streptomyces xanthocidicus KPP01532 for the control of potato soft rot caused by Erwinia carotovora subsp. atroseptica. Microbiol Res 184:32–41. https://doi.org/10.1016/j.micres.2015.12.005. PMID: 26856451

Keller M, Zengler K (2004) Tapping into microbial diversity. Nat Rev Microbiol 2:141–150

Kim J, Kim J-G, Kang Y, Jang JY, Jog GJ, Lim JY, Kim S, Suga H, Nagamatsu T, Hwang I (2004) Quorum sensing and the LysR-type transcriptional activator ToxR regulate toxoflavin biosynthesis and transport in Burkholderia glumae. Mol Microbiol 54:921–934

Kim TK, Garson MJ, Fuerst JA (2005) Marine actinomycetes related to the '*Salinospora*' group from the great barrier reef sponge *Pseudoceratina clavata*. Environ Microbiol 7:509–518

Kurtböke DI (2005) Actinophages as indicators of actinomycete taxa in marine environments. Antonie Van Leeuwenhoek 87:19–28

Lechevalier H (1982) The development of applied microbiology at Rutgers. The State University of New Jersey, New Brunswick, p 3

Lloyd JG, May MK, Kevorkian RT, Steen AD (2013) Meta-analysis of quantification methods shows that archaea and bacteria have similar abundances in the subseafloor. Appl Environ Microbiol 79:7790–7799

Ma GBZ, Xia Z, Wu S (2009) Inhibiting effect of seven marine actinomycete strains against vegetable pathogenic microorganisms. Crops 5:3–9

Magarvey NA, Keller JM, Bernan V, Dworkin M, Sherman DH (2004) Isolation and characterization of novel marine-derived actinomycete taxa rich in bioactive metabolites. Appl Environ Microbiol 70:7520–7529

Maldonado LA, Stach JEM, Pathom-aree W, Ward AC, Bull AT, Goodfellow M (2005) Diversity of cultivable actinobacteria in geographically widespread marine sediments. Antonie Van Leeuwenhoek 87:11–18

McLean RJC, Pierson LS, Fuqua C (2004) A simple screening protocol for the identification of quorum signal antagonists. J Microbiol Methods 58(3):351–360. https://doi.org/10.1016/j.mimet.2004.04.016. PMID: 15279939

Mincer TJ, Jensen PR, Kauffman CA, Fenical W (2002) Widespread and persistent populations of a major new marine actinomycetes taxon in ocean sediments. Appl Environ Microbiol 68:5005–5011

Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867

Montalvo NF, Mohamed NM, Enticknap JJ, Hill RT (2005) Novel actinobacteria from marine sponges. Antonie Van Leeuwenhoek 87:29–36

Naik D, Wahidullah S, Meena R (2013) Attenuation of *Pseudomonas aeruginosa* virulence by marine invertebrate-derived *Streptomyces* sp. Lett Appl Microbiol 56(3):197–207. https://doi.org/10.1111/lam.12034. PMID: 23210926

Newman DJ (2016) Developing natural product drugs: supply problems and how they have been overcome. Pharmacol Ther 162:1–9

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75(3):311–335. https://doi.org/10.1021/np200906s. PMID: 22316239

Orsi WD (2018) Ecology and evolution of seafloor and subseafloor microbial communities. Nat Rev Microbiol 16:671–683. https://doi.org/10.1038/s41579-018-0046-8

Papineau D, Walker JJ, Mojzsis SJ, Pace NR (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl Environ Microbiol 71:4822–4832

Prévost K, Couture G, Shipley B, Brzezinski R, Beaulieu C (2006) Effect of chitosan and a biocontrol streptomyceteon field and potato tuber bacterial communities. BioControl 51(4):533–546

Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan A (2011) Developing stress tolerant plants through in vitro selection—an overview of the recent progress. Environ Exp Bot 71(1):89–98

Rappe MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633

Reed DW, Fujita Y, Delwiche ME, Blackwelder DB, Sheridan PP, Uchida T, Colwell FS (2002) Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. Appl Environ Microbiol 68:3759–3770

Shellikeri A, Kaulgud V, Yaradoddi J, Ganachari S, Banapurmath N, Shettar A (2018). Development of neem-based bioplastic for food packaging application. IOP Conf Ser Mater Sci Eng 376:012052. https://doi.org/10.1088/1757899X/376/1/012052

Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates. Aquat Microbiol Ecol 26:175–211

Sivakumar K (2001) Actinomycetes of an Indian mangrove (Pichavaram) environment: an inventory. PhD thesis, Annamalai University, India, p 91

Sivakumar K, Sahu MK, Thangaradjou T, Kannan L (2007) Research on marine actinobacteria in India. Indian J Microbiol 47:186–196

Stach JEM, Maldonado LA, Ward AC, Goodfellow M, Bull AT (2003a) New primers for the class actinobacteria: application to marine and terrestrial environments. Environ Microbiol 5:828–841

Stach JEM, Maldonado LA, Masson DG, Ward AC, Goodfellow M, Bull AT (2003b) Statistical approaches to estimating bacterial diversity in marine sediments. Appl Environ Microbiol 69: 6189–6200

Stach JEM, Maldonado LA, Ward AC, Bull AT, Goodfellow M (2004) *William siamaris* sp. nov., a novel actinomycete isolated from the Sea of Japan. Int J Syst Evol Microbiol 54:191–194

Subramani R, Aalbersberg W (2012) Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res 167:571–580

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Süß J, Engelen B, Cypionka H, Sass H (2004) Quantiative analysis of bacterial communities from Mediterranean sapropels based on cultivation-dependent methods. FEMS Microbiol Ecol 51: 109–121

Suttle CA (2005) Viruses in the sea. Nature 437:356–361

Suutari M, Lignell U, Hyvärinen A, Nevalainen A (2002) Media for cultivation of indoor streptomycetes. J Microbiol Methods 51:411–416

Tae KK, Garson MJ, Fuerst JA (2005) A marine actinomycetes related to the 'Salinospora' group from the Great Barrier Reef sponge Pseudoceratina clavata. Environ Microbiol 7(4):509–518

Takaizawa M, Colwell W, Hill RT (1993) Isolation and diversity of actinomycetes in the Chesapeake Bay. Appl Environ Microbiol 59:997–1002

Tanaka Y, Omura O (1990) Metabolisms and products of actinomycetes: an introduction. Actinomycetologica 4:13–14

Tello E, Castellanos L, AreÂvalo-Ferro C, Duque C (2012) Disruption in quorum-sensing systems and bacterial biofilm inhibition by cembranoid diterpenes isolated from the octocoral Eunicea knighti. J Nat Prod 75(9):1637–1642. https://doi.org/10.1021/np300313k. PMID: 22905751

Toledo G, Green W, Gonzalez RA, Christoffersen L, Podar M, Chang HW, Hemscheidt T, Trapido-Rosenthal HG, Short JM, Bidigare RR, Mathur EJ (2006) High throughput cultivation for isolation of novel marine microorganisms. Oceanography 19:120–125

Walsh EA, Kirkpatrick JB, Rutherford SD, Smith DC, Sogin M, D'Hondt S (2016) Bacterial diversity and community composition from seasurface to subseaflor. ISME J 10:979–989

Wu Z, Xie L, Xia G, Zhang J, Nie Y, Hu J, Wang S, Zhang R (2005) A new tetrodotoxin-producing actinomycete, *Nocardiopsis dassonvillei*, isolated from the ovaries of puffer fish Fugu rubripes. Toxicon 45:851–859

Yaradoddi JS, Sulochana M (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Yoshii A, Moriyama H, Fukuhara T (2012) The novel kasugamycin 20-N-acetyltransferase gene aac (20)-Ila, carried by the IncP island, confers kasugamycin resistance to rice-pathogenic bacteria. Appl Environ Microbiol 78:5555–5564. https://doi.org/10.1128/AEM.01155-12

Zengler K, Toledo G, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. Proc Natl Acad Sci U S A 99:15681–15686

Zengler K, Walcher M, Clar G, Haller I, Toledo G (2005) High-throughput cultivation of microorganisms using microcapsules. Methods Enzymol 397:124–130

Zhang H, Lee YK, Zhang W, Lee HK (2006) Culturable actinobacteria from the marine sponge Hymeniacidon perleve: isolation and phylogenetic diversity by 16S rRNA gene-RFLP analysis. Antonie Van Leeuwenhoek 90:159–169

ZoBell C (1946) Marine microbiology, a monograph on hydrobacteriology. Chronica Botanic Company, Waltham, MA, 240 pp

Chapter 3
Terrestrial Ecology of *Actinobacteria*

Basavaraj S. Hungund, Savitha S. Desai, Kartik C. Kamath, and Gururaj B. Tennalli

Abstract Terrestrial environments, generally soil, encompass diverse groups of microbial communities which are able to produce various bioactive molecules, including antibiotics, industrial enzymes, dyes, and pigments. *Actinobacteria*, particularly the genus of *Streptomyces*, are generally saprophytic in nature and form semi-dormant spores in their life cycle. This adaptation is mainly because of the lack of sufficient nutrients. Owing to their high level of adaptability, they thrive in the harshest of ecological and environmental conditions. Indeed, they are ubiquitous in nature, present in soil, freshwater, saline water, deep sea, volcanic caves, saline soil, and air. *Actinobacteria* are dominated among the soil microbial communities, especially in forest soil. Many scientists have recovered actinobacteria from different terrestrial habitats and explored their potential to produce novel drug candidates. Despite the current rigorous research, only a fraction of the cultivable actinobacterial diversity has been reported. Many underexplored areas, such as biodiversity hotspots, have unique types of environments that may have affected the progression of secondary metabolite synthesis. Thus, the variety of tropical and temperate forests and wetlands found in the northeastern region of India may have a rich and varied population of *Actinobacteria* to offer. These actinobacteria colonize with host plants and produce plant growth-promoting substances. They are involved in carbon cycling; plant growth promotion by fixing nitrogen, chelating iron, solubilizing phosphorous, and producing enzymes like ACC deaminase for stress alleviation or hydrolytic enzymes for biomass degradation; and hormone production. They also produce biologically active metabolites. The diverse functions of actinobacteria make it a unique group of organisms. This book chapter deals with the potential role of actinobacteria in various ecological processes.

B. S. Hungund (✉) · K. C. Kamath · G. B. Tennalli
Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

S. S. Desai
Independent Researcher, Hubballi, Karnataka, India

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3.1 Introduction

Actinobacteria constitute a phylum of Gram-positive bacteria with high G + C content whose origins date back to around 2700 million years ago. They are found in many habitats ranging from marine, aquatic, terrigenous, tidal flat ecosystems to aerial, extreme environments. They are also found to have symbiotic associations with a variety of macroorganisms such as marine tunicates and sponges, as well as with ants and termites. The members of this phylum range from anaerobic unicellular organisms to multicellular, filamentous, and spore-forming lineages (Lewin et al. 2016). The diversity of actinobacteria remained unclear until the late twentieth century since most of the strains were uncultivable or the sampling methods used were destructive. With the advent of various molecular techniques in the 1990s, metagenomic studies helped researchers better understand the ubiquity of these bacteria (Kurtböke 2017). Since then, various extreme biospheres have been explored, and a vast variety of actinobacteria has thus been identified and isolated (Fig. 3.1). Be it the *Actinobacteria* in the cold extremes of the Antarctic (Shing et al. 2013), the hot extremes of the deserts (Idris et al. 2017), or the microbiologically unexplored forests of Northeast India (Das et al. 2018), there are numerous species and strains of actinobacteria that are waiting to be discovered and have a whole lot to offer to the world today. *Actinobacteria* form a special area of interest as they produce a variety of biotechnologically relevant compounds like immune modifiers, antibiotics, enzyme inhibitors, plant growth-promoting substances, natural dyes, enzymes like L-glutaminase and L-asparaginase (Desai and Hungund 2018; Desai et al. 2016), and a host of other compounds. As a source of over 5000 bioactive compounds, 90% of the antibiotics used for commercial and research purposes are derived from them (Das et al. 2018). Apart from this, actinobacteria play a vital role from an ecological perspective: they help to provide a variety of hosts with nutrition and defense from other organisms, and they help recycle elements in nature. This chapter is aimed at shedding light on the multitude of roles *Actinobacteria* play in nature and the research methodologies followed to study the various populations that have been discovered.

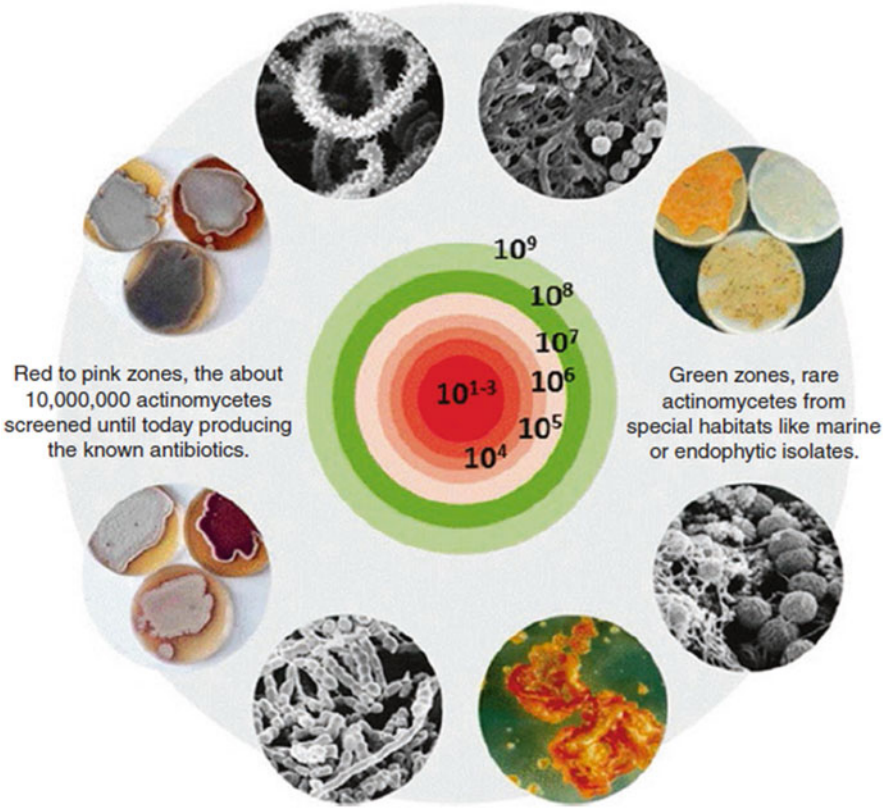

Fig. 3.1 Pictorial presentation for the isolation of novel *Actinobacteria* from unexplored ecological locations. Novel bioactive compounds can be obtained from rare actinomycetes

3.2 Isolation, Identification, and Purification of *Actinobacteria*

Since actinobacteria are predominantly found in soil, several researchers have isolated various actinobacteria from a variety of soils. The numbers and type of actinomycetes present in a particular soil are greatly influenced by geographical location, soil temperature, soil type, soil pH, organic matter content, cultivation, aeration, and moisture content. Actinomycete populations are usually small in waterlogged soils with low oxygen tension. Forest soils usually have a relatively low pH and contain a predominance of *Streptomyces* that are tolerant of acid conditions (Davies and Williams 1970; Williams et al. 1971), while arid soils of alkaline pH tend to contain fewer *Streptomyces* and more of the "rare" genera such as *Actinoplanes* and *Streptosporangium*.

As an example of a generic isolation methodology, a study by Shing et al. (2013) was considered wherein they isolated 95 strains of actinobacteria from 8 different

Antarctic soil samples. The soil samples were appropriately diluted and cultured, and colonies were separated based on morphology. The DNA of the putative actinobacteria is extracted, and the 16S rDNA (ribosomal DNA) is amplified using the DNA as a template with appropriate restriction endonucleases (RENs). The amplified rDNA is the subject to amplified ribosomal DNA restriction analysis (ARDRA), and a phylogenetic tree was constructed. Similar methods for isolation are employed in all studies with a few variations. Some physicochemical treatments may be included to specifically isolate certain strains of actinomycetes like air drying which eliminates most of the unwanted Gram-negative bacteria that produce mucoid. Other methods include alternate drying and wetting (Labeda 1990), rehydration, and centrifugation which select motile actinomycetes (Hayakawa et al. 2000). Wet-heating for 15 min at 70 °C and phenol treatment (Seong et al. 2001), pretreatment by mixing with $CaCO_3$ incubated for 10 days at 28 °C (Rizk et al. 2007), and use of antibiotics to eliminate certain bacteria have also been used for isolation of actinobacteria**.** Hirsch and Christensen (1983) used a cellulose ester membrane filter on the media to selectively isolate the actinomycetes by penetration of mycelia into the media. Use of polyol gel Lutrol FC127 for selective isolation of *Thermoactinomyces* sp. (Wood 1985), use of peptone (6%) and lauryl sulfate (0.05%) at 50 °C for 10 min (You and Park 1996), use of high-frequency (EHFs) radiation to eliminate most of bacteria, and resulting in selective isolation of rare actinomycetes genera (Li 2002) were reported by various researchers. The dilution may be carried out in Ringer's solution or physiological saline. Solutions of soil samples may be subject to heating to screen thermophilic strains. Further studies may be carried out where their antimicrobial activity, for example, is tested against various test strains.

Studies show that actinomycetes as a whole are favored by soils rich in organic content, are relatively dry, and are neutral or alkaline in nature (Davies and Williams 1970). Temperature and moisture tension were also found to be contributing factors to actinobacterial activity in soil. Studies conducted in the pre-metagenomic era showed that, in grasslands, numbers of family *Nocardiae* were most common in winter and that of *Streptomycetes* were higher in summer. *Streptomyces malachiticus* and *Nocardia otitidiscaviarum* were confined to subtropical and tropical soils. The radial growth of *Streptomycetes* was lowered at moisture tension of pF 1.0 and the evolution of micromonosporae was promoted. *Actinobacteria* usually form dormant arthrospores and chlamydospores and germinate under conditions of the occasional presence of exogenous nutrients. Other nonspore-forming genera such as *Arthrobacter* were found to exist as resting cocci for long periods (Kurtböke 2017). However, as more and more actinobacteria are discovered, the likeliness of them producing unknown bioactive compounds is reducing. Thus, various extreme biospheres are being explored under the hope that those extreme conditions might have affected adaptations in actinobacteria that lead to the production of novel bioactive compounds. Kirby et al. (2011) list some of the actinobacteria that have been isolated from soil ecosystems in Antarctica, the Arctic Circle, and the Alpine Tundra region (Table 3.1; Kirby et al. 2011). Figure 3.2 depicts the phenotypic

Table 3.1 A list of actinobacteria isolated from Antarctica, the Arctic Circle, and Alpine Tundra regions

Species	Source	References
Brevibacillus levickii	Geothermal soils, Mt. Melbourne	Allan et al. (2005)
Cryobacterium psychrophilum	Antarctic soil	Suzuki et al. (1997)
Friedmanniella antarctica	Sandstone, McMurdo Dry Valleys	Schumann et al. (1997)
Micromonospora endolithica	Sandstone, McMurdo Dry Valleys	Hirsch et al. (2004)
Modestobacter multiseptatus	Soil Asgard Range, Transantarctic Mountains	Mevs et al. (2000)
Pseudonocardia antarctica	Cyanobacterial mat, McMurdo Valley	Prabahar et al. (2004)
Streptomyces hypolithicus	Hypolithic community, Miers Valley	Le Roes-Hill et al. (2009)
Actinoalloteichus spitiensis	Cold desert, Himalayas	Singla et al. (2005)
Agrococcus lahaulensis	Cold desert, Himalayas	Mayilraj et al. (2006a, b)
Arthrobacteral pinus	Alpine soil, Austria	Zhang et al. (2010)
Arthrobacter psychrophenolicus	Austrian alpine ice cave	Margesin et al. (2004)
Rhodococcus kroppenstedtii	Cold desert, Himalayas	Mayilraj et al. (2006a, b)

Fig. 3.2 Colony morphologies of various actinobacteria on starch casein agar medium otained during isolation process

characters (colony morphology), whereas Fig. 3.3 depicts the microscopic view of actinobacteria obtained during isolation process.

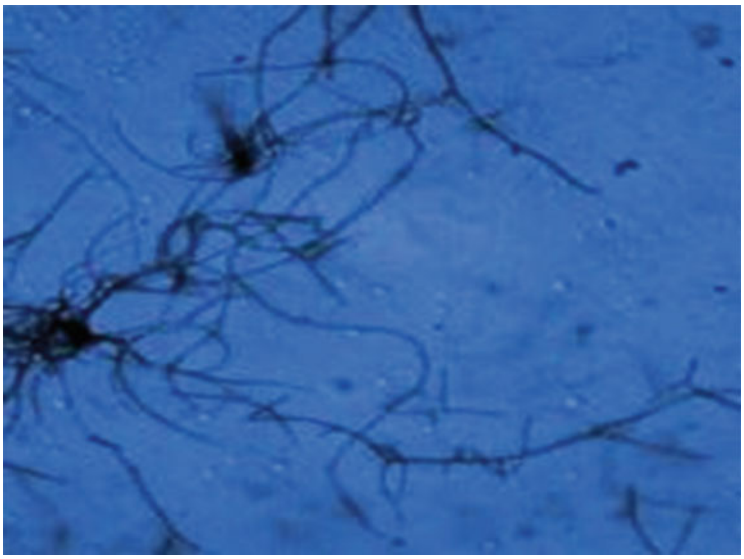

Fig. 3.3 Microscopic view of an isolated actinobacterium under light microscopy (visualized by Gram's staining technique)

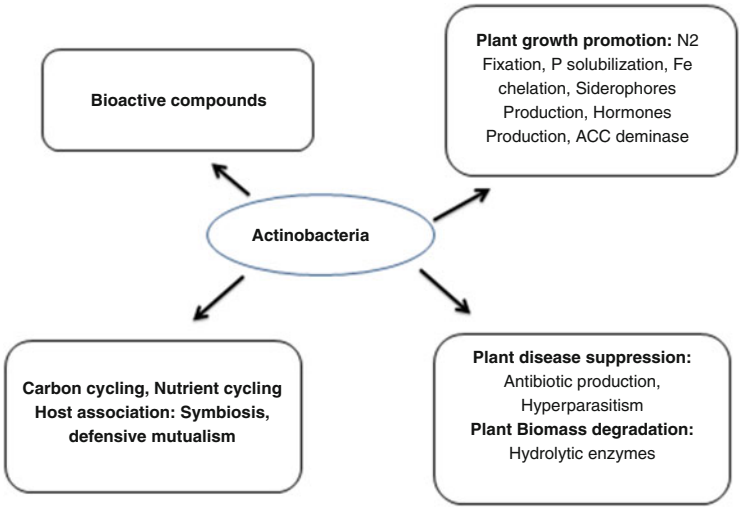

Fig. 3.4 Ecological implications of *Actinobacteria*

3.3 *Actinobacteria* and Their Ecological Interactions

Apart from the vital influence actinobacteria have on human health, they play a number of ecological roles and facilitate several biogeochemical cycles of the planet (Fig. 3.4). Waksman was among the first to establish the role of actinobacteria in the decomposition of plant biomass in soil. Unlike other phyla of bacteria, *Actinobacteria* are equipped with a full suite of enzymes to degrade the complex and recalcitrant plant polymers such as celluloses, hemicelluloses, lignin, and chitin. This allows them to grow on plant biomass, and its constituents are decomposing them in conjunction with fungi. For example (Gittel et al. 2014), the RNA transcripts for most cellulase and hemicellulase enzymes in the Arctic peat soil were assigned to *Actinobacteria* (Tveit et al. 2014).

Similarly, many genera of *Actinobacteria*, including *Cellulomonas*, *Nocardia*, and *Micromonospora* have been implicated in plant biomass degradation based on their ubiquity in soil and their capability to grow on plant biomass-derived substrates. In many composting systems, certain genera of *Actinobacteria* are particularly abundant during the thermophilic stage, viz., *Thermobifida*, *Saccharomonospora*, and *Thermobispora* (Lewin et al. 2016). *Actinobacteria* thus play an instrumental part in carbon cycling. They have also been found to be responsible for their plant and animal hosts' nutrition in many cases (Lewin et al. 2016). They also act as defensive mutualists with their plant and animal hosts.

Actinobacteria colonize the rhizosphere and can influence plant health and growth in direct or indirect ways. Their direct influences involve antagonizing deleterious effects of other microorganisms by producing bioactive secondary metabolites and antibiotics. For example, *Streptomyces griseorubens* possesses compounds called siderophores that chelate iron to fight of the *Fusarium* wilt in banana caused by *Fusarium oxysporum* f. sp. *cubenese* (Getha et al. 2005). Several actinobacteria including *S. antibioticus*, *S. aureofaciens*, *S. lividans*, *S. plicatus*, *S. halsteii* AJ-7, and *S. lydicus* WYEC108 have been found to produce chitinolytic enzymes (Barka et al. 2016). However, some *Actinobacteria* can also be pathogenic to plants especially potatoes, beet, carrot, turnip, and parsnip. Phytopathogenic *Actinobacteria* such as *S. scabiei*, *S. acidiscabies*, and *S. turgidiscabies* are most commonly found to induce devastating scab diseases on a broad spectrum of plants. *S. turgidiscabies* and *S. acidiscabies* are emergent pathogens that were first described in Japan and the Northeastern United States, respectively, and *S. scabiei*, the most ancient of these pathogens, is found worldwide (Barka et al. 2016). Millard and Taylor (1927), however, have shown that inoculating the soil with saprophytic nonpathogenic *Actinobacteria* could significantly reduce both disease (such as potato scab) and pathogen populations, thereby reducing plant disease.

Actinobacteria also play an important role in nutrient recycling. For example, *Rhodococcus rhodnii* provides B vitamins to the insect vector of Chagas disease, *Rhodnius prolixus*, and *Coriobacteriaceae* strains provide B vitamins to Pyrrhocoridae (Hemiptera), like firebugs. In the mammalian gut, the digestion of complex carbohydrates, including plant-derived oligosaccharides and gastric mucin,

is facilitated by *Bifidobacteria*. The association between nitrogen-fixing *Frankia* strains and actinorhizal plants, which enable their early colonization during primary succession, most clearly establishes the nutritional association between plants and *Actinobacteria*. Similarly, *Pseudonocadiae* coevolves with a tribe of fungi-growing ants called Attini. The exoskeleton provides nutrients to the bacteria, while the bacteria protect its food source (a fungal cultivar) from specific pathogens by way of certain antimicrobial compounds. Similar defensive mutualism exists between solitary digger wasps (Tribe Philanthini) and *Streptomyces* (Lewin et al. 2016).

3.3.1 Actinobacteria as Plant Growth-Promoting Agents

Actinobacteria increase plant growth by stimulating hormones and help plants by increasing the availability of iron, phosphate solubilization, nitrogen fixation, and stress alleviation. Hormones such as indole acetic acid (IAA) and cytokinin are involved in the growth of the plants. Actinomycetes produce IAA near the rhizosphere, which results in lateral root hair development, thereby increasing the shoot and root growth. Some actinomycetes produce only IAA, whereas some actinomycetes like *S. rhimosis* and *S. rochei* produce all the three hormones like auxins, cytokinin-like substances, and gibberellins which increase the plant growth. There are few reports on the actinomycete-producing cytokinin.

Manulis et al. (1994) observed that different *Streptomyces* spp. are capable of producing plant hormone indole-3-acetic acid (IAA). They also described the pathway of its synthesis. *Streptomyces* species like *Streptomyces rimosus*, *Streptomyces rochei*, and *Streptomyces olivaceoviridis* have the ability to produce exogenous auxins (IAA), gibberellins, and cytokinins. There are reports on use of *Streptomyces griseus* in treating seeds of barley, oat, wheat, and carrot to increase their growth. It is observed that culture filtrate of *Streptomyces* spp. increases the shoot length, whereas *S. olivaceoviridis* increases shoot mass of wheat plants. *S. rochei* were found to produce significant amounts of growth regulators influencing the growth of the plant (Aldesuquy et al. 1998). Very little information exists on the use of *Streptomyces* as plant growth promoters; also, its involvement in the mechanism of plant promotion is limited. It seems that like most rhizobacteria, *Streptomyces* are capable of enhancing growth of plant.

Actinomycetes are involved in increasing the availability of iron by plants in two ways. In soil, iron is available in the form of ferric (insoluble form Fe^{3+}). Plants take up iron in the form of ferrous (soluble form Fe^{2+}) compared to ferric form. Certain actinomycetes like actinobacteria can directly convert insoluble form to soluble form. Other way in which actinomycetes can help uptake of iron is by producing siderophores. They help in chelating iron in ferric form and help in the assimilation of iron by plants. It has been observed that along with iron assimilation, when plants were inoculated with siderophore-producing actinomycete like *Streptomyces tendae*, they assimilate iron along with nitrogen, phosphorus, and magnesium. It has been observed that these actinomycetes also help in phytoremediation of the cadmium.

Phosphorus always forms a complex with metals such as iron, aluminum, and silicon. Some genera of actinomycetes like *Streptomyces*, *Micromonospora*, *Micrococcus*, etc. are capable of solubilizing phosphate making it available to plants resulting in increase in plant growth. These actinomycetes can also inhibit the growth of organism which causes damping off. Thus, these have dual advantages and serve as PGPR. PGPR affects plant growth in two ways: (a) direct way wherein plant is supplemented with a compound or PGPR facilitates nutrient uptake (Glick 1995) or (b) indirect way by prevention of harmful effect of microorganism which is through the production of antibiotics and other secondary metabolite (Fenton et al. 1992). Actinomycetes can solubilize phosphorous (P) by production of organic acids near the rhizosphere thereby decreasing the pH which converts the insoluble form of P to soluble form.

Actinomycetes also help in the fixation of atmospheric nitrogen called nitrogen fixation. *Frankia*, an actinomycete, have the capacity of fixing nitrogen when inoculated with dicot plants. Other actinomycete genera influence root nodule formation. It is seen that in plants under abiotic stress condition, ethylene is produced, which affects plant growth. Certain actinomycetes produce enzyme ACC (1-aminocyclopropane-1-carboxylic acid) deaminase, which converts ACC which is precursor of ethylene to ketoglutarate and ammonia, in turn reducing the levels of ethylene and hence promoting plant growth (Glick 2005). Under biotic stress, actinomycetes compete with other organisms or pathogens by producing antibiotics, hydrolytic enzymes, or hyperparasitism. Several species of *Streptomyces* are able to produce antibiotics like nigericin and azalomycin which can inhibit the growth of pathogens like *Fusarium* and *Phytophthora*. S*treptomyces* spp. are used to control bacterial wilt, blights, and canker in tomato. Some actinomycetes also produce volatile antibiotics that inhibit the growth of fungi like *Aspergillus*, *Fusarium*, *Sclerotia*, etc.

Certain actinomycetes produce extracellular hydrolytic enzymes, which can degrade the cell wall of fungi. The enzymes include chitinases which act on the chitin component and 1–3-glucanases, which act on fungal cell wall. Few actinomycetes have been evaluated as biocontrol agents against phytopathogens (Khebizi et al. 2018; Suarez Moreno et al. 2019).

It is well known that *Streptomyces* is used as biocontrol agent since they increase plant growth (Aldesuquy et al. 1998). Though *Streptomyces* population is abundant in soil, they are effective in colonizing plant root system, and they can withstand adverse conditions by forming spores; it is poorly investigated for its potential as PGPR (Alexander Alex 1977). For some particular crops, the effect of strains of PGPR is beneficial (Glick 1995). Urauchimycin, a member of antimycin class, is an antifungal agent. Urauchimycins A and B isolated from *Streptomyces* sp. repressed the morphological differentiation of *Candida albicans* (ImamUra et al. 1993), whereas urauchimycins C and D did not show any activity against *C. albicans*, *Mucor miehei*, and bacteria (Yao et al. 2006). Polyenes are antifungal agents with many conjugated double bonds. They are produced by the members of the genus *Streptomyces*. Fungal infections are effectively treated using polyenes.

3.4 Actinobacteria as a Source of Bioactive Compounds

Nobel Laureate Selman Waksman and his colleagues discovered the first antibiotics in *Actinobacteria*. These included actinomycin from a culture of *Streptomyces antibioticus* in 1940, streptothricin from *Streptomyces lavendulae* in 1942, and streptomycin from *Streptomyces griseus* in 1944 (Barka et al. 2016). Some of the major antibiotics that are derived from *Actinobacteria* are listed in Table 3.2.

In the aforementioned study conducted by Shing et al. (2013), 95 strains of actinomyces were isolated, 53 of which were clustered as representative of *Streptococcus beijiangensis*, from the soils of Antarctica. This is also among the first studies to show the presence of non-ribosomal peptide synthetase (NRPS) genes while demonstrating their antimicrobial activities (Shing et al. 2013). Another study isolated 24 strains of actinomyces from the Pani Dihing Wildlife Sanctuary (PWS) and Nameri National Park (NNP) of Assam, India. They extensively studied their antimicrobial activity against *Candida albicans*, *Staphylococcus aureus*, *E. coli*, and MRSA (Das et al. 2018). Actinobacteria have also been sources of insecticides and herbicides (Goodfellow and Williams 1983). Various species of *Streptomyces* have been found to produce a class of compounds called macrotetrolides that are active against mites and helminths and also show immunosuppressive effects. *Streptomyces aureus* is found to produce a mixture of tetranactin (the most active compound in the class; is effective against carmine mites of fruits and tea), dinactin, and trinactin that have proven to be commercially successful. Similarly, *S. avermitilis* is the producer of the world's first endectocide, ivermectin, in the 1970s (Barka et al. 2016). Umezawa and coworkers (1965) observed that metabolite kasugamycin produced by *Streptomyces kasugaensis* is a bactericidal and fungicidal. It is used to control rice blast and bacterial diseases in several crops. Isono et al. (1965) observed a new class of natural fungicides called polyoxin B and D, which were produced by *Streptomyces cacaoi* var. *asoensis*. Polyoxin D is used to control rice sheath blight caused by *Rhizoctonia solani* Kùhn. Validamycin, an antifungal metabolite produced by *Streptomyces*, is active against rice sheath blight, whereas

Table 3.2 A list of antibacterial agents produced by *Actinobacteria*

Antibacterial agents	Producing actinobacteria	References
Neomycin	*Streptomyces fradiae*	Lo Grasso et al. (2016)
Kanamycin	*Streptomyces kanamyceticus*	
Streptomycin	*Streptomyces griseus*	
Erythromycin	*Saccharopolyspora erythraea*	McGuire et al. (1952)
Gentamicin	*Micromonospora purpurea*	Weinstein et al. (1963)
Auricin	*Streptomyces aureofacians*	Kormanec et al. (2014)
Landomycin	*Streptomyces* sp.	Henkel et al. (1990)
Moromycin	*Streptomyces* sp.	Kharel et al. (2012)
Rifamycin	*Streptomyces mediterranei*	Floss and Yu (2005)
Geldanamycin	*S. hygroscopicus*	Kang et al. (2012)
Vancomycin	*Amycolatopsis orientalis*	Brigham and Pittenger (1956)

Table 3.3 Bioactive agents derived from *Actinobacteria*

Bioactive agents	Producer organism	References
Antifungal		
Actinomycins	*Streptomyces anulatus*	Bister et al. (2004)
Amphotericin B	*S. nodosus*	Linke et al. (1974)
Transvalencin	*Nocardia transvalensis*	Hoshino et al. (2004)
Blasticidin	*S. griseochromogenes*	Takeuchi et al. (1958)
Candicidin	*S. griseus*	Acker and Lechevalier (1954)
Bioherbicide/biopesticide		
2,4-Dihydro-4-(β-D-ribofuranosyl)-1,2,4(3*H*)-triazol-3-one (herbicide)	*Actinomadura* spp.	Schmitzer et al. (2000)
Herbimycin	*S. hygroscopicus*	Mura et al. (1979)
Ivermectin	*S. avermitilis*	Õmura and Crump (2004)
Prasinons	*S. prasinus*	Box et al. (1973)
Spinosad (neurotoxic insecticides)	*Saccharopolyspora spinosa*	Waldron et al. (2001)
Antiparasitic agents		
Avermectins	*S. avermitilis*	Burg et al. (1979)
Prodiginine	*S. coelicolor*	Cerdeño et al. (2001)
Trioxacarcin	*S. bottropensis*	Tomita et al. (1981)
Antitumour agents		
Anthraquinones	*Micromonospora* spp.	Igarashi et al. (2007)
Asterobactine	*Nocardia asteroides*	Nemoto et al. (2002)
IB-00208	*Actinomadura* spp.	Malet-Cascón et al. (2003)
Mechercharmycin	*Thermoactinomyces* spp.	Kanoh et al. (2005)
Marinomycin	*Marinospora* spp.	Kwon et al. (2006)
Salinosporamide	*Salinispora tropica*	Barka et al. (2016)

mildiomycin is active against powdery mildews on various plants (Iwasa et al. 1978; Harada and Kishi 1978; Feduchi et al. 1985). Some such insecticides, herbicides, and antifungals are listed in Table 3.3.

3.5 Conclusion

From the presented data, it is clear that *Actinobacteria* are an important part of the ecosystem. They live in symbiosis and mutualism with a multitude of organisms, providing them with much needed nutrition and defense. They play a vital role in soil health and can be used as biocontrol agents for a number of crops and aid in composting organic matter, thus cycling and recycling elements within the ecosystem. They have also proven to be invaluable from a biotechnological perspective as a source of innumerable bioactive agents ranging from antibiotics, antifungal agents, insecticides, herbicides, and much more. Indeed, researchers cannot help but seek to understand and utilize the wealth and knowledge this phylum of bacteria has to offer to the world.

References

Acker RF, Lechevalier H (1954) Some nutritional requirements of *Streptomyces griseus* 3570 for growth and candicidin production. Appl Microbiol 2(3):152–157. https://doi.org/10.1128/aem.2.3.152-157.1954

Aldesuquy HS, Mansour FA, Abo-Hamed SA (1998) Effect of the culture filtrates of Streptomyces on growth and productivity of wheat plants. Folia Microbiol 43(5):465–470

Alexander Alex M (1977) Introduction to soil microbiology, 2nd edn. Krieger Publishing Company, Malabar. 467

Allan RN, Lebbe L, Heyrman J, De Vos P, Buchanan CJ, Logan NA (2005) *Brevibacillus levickii* sp. nov. and *Aneurinibacillus terranovensis* sp. nov., two novel thermoacidophiles isolated from geothermal soils of northern Victoria Land, Antarctica. Int J Syst Evol Microbiol 55(3):1039–1050. https://doi.org/10.1099/ijs.0.63397-0

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, Clément C, Ouhdouch Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol Biol Rev 80(1):1–43. https://doi.org/10.1128/MMBR.00019-15

Bister B, Bischoff D, Ströbele M, Riedlinger J, Reicke A, Wolter F, Bull AT, Zähner H, Fiedler HP, Süssmuth RD (2004) Abyssomicin C-A polycyclic antibiotic from a marine Verrucosispora strain as an inhibitor of the p-aminobenzoic acid/tetrahydrofolate biosynthesis pathway. Angew Chem Int Ed 43(19):2574–2576. https://doi.org/10.1002/anie.200353160

Box SJ, Cole M, Yeoman GH (1973) Prasinons A and B: potent insecticides from *Streptomyces prasinus*. Appl Microbiol 26(5):699–704. https://doi.org/10.1128/aem.26.5.699-704.1973

Brigham RB, Pittenger RC (1956) *Streptomyces orientalis*, n. sp., the source of vancomycin. Antibiot Chemother (Northfield) 6(11):642–647

Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, Hartman R, Kong YL, Monaghan RL, Olson G, Putter I, Tunac JB (1979) Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob Agents Chemother 15(3):361–367. https://doi.org/10.1128/AAC.15.3.361

Cerdeño AM, Bibb MJ, Challis GL (2001) Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): new mechanisms for chain initiation and termination in modular multienzymes. Chem Biol 8(8):817–829. https://doi.org/10.1016/S1074-5521(01)00054-0

Das R, Romi W, Das R, Sharma HK, Thakur D (2018) Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of northeast India. BMC Microbiol 18(1):1–16. https://doi.org/10.1186/s12866-018-1215-7

Davies FL, Williams ST (1970) Studies on the ecology of actinomycetes in soil. I. The occurrence and distribution of actinomycetes in a pine forest soil. Soil Biol Biochem 2(4):227–238. https://doi.org/10.1016/0038-0717(70)90029-5

Desai SS, Hungund BS (2018) Submerged fermentation, purification and characterization of L-asparaginase from *Streptomyces* isolated from soil. J Appl Biol Biotechnol 6(5):17–23

Desai SS, Chopra SJ, Hungund BS (2016) Production, purification and characterization of L-glutaminase from *Streptomyces* sp. isolated from soil. J Appl Pharm Sci 6(7):100–105

Feduchi E, Cosin M, Carrasco L (1985) Mildiomycin: a nucleoside antibiotic that inhibits protein synthesis. J Antibiot 38:415–419

Fenton AM, Stephens PM, Crowley J, O'callaghan M, O'gara F (1992) Exploitation of gene (s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a Pseudomonas strain. Appl Environ Microbiol *58*(12):3873–3878

Floss HG, Yu TW (2005) Rifamycin—mode of action, resistance, and biosynthesis. Chem Rev 105: 621–632. https://doi.org/10.1021/cr030112j

Getha K, Vikineswary S, Wong WH, Seki T, Ward A, Goodfellow M (2005) Evaluation of *Streptomyces* sp. strain G-10 for suppression of *Fusarium* wilt and rhizosphere colonization in pot-grown banana plantlets. J Ind Microbiol Biotechnol 32(1):24–32. https://doi.org/10.1007/s10295-004-0199-5

Gittel A, Bárta J, Kohoutová I, Mikutta R, Owens S, Gilbert J, Urich T (2014) Distinct microbial communities associated with buried soils in the Siberian tundra. ISME J 8(4):841–853. https://doi.org/10.1038/ismej.2013.219

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick (2005) The modulation of plant ethylene levels by the bacterial enzymes ACC deaminase. FEMS Microbiol Lett 251:1–7

Goodfellow M, Williams ST (1983) Ecology of actinomycetes. Annu Rev Microbiol 37(1): 189–216

Harada S, Kishi T (1978) Isolation and characterization of mildiomycin, a new nucleoside antibiotic. J Antibiot 31:519

Hayakawa M, Otoguro M, Takeuchi T, Yamazaki T, Iimura Y (2000) Application of a method incorporating differential centrifugation for selective isolation of motile actinomycetes in soil and plant litter. Antonie Van Leeuwenhoek 78:171–185

Henkel T, Rohr J, Beale JM, Schwenen L (1990) Landomycins, new angucycline antibiotics from *Streptomyces* sp. I. Structural studies on landomycins A-D. J Antibiot 43(5):492–503. https://doi.org/10.7164/antibiotics.43.492

Hirsch CF, Christensen DL (1983) Novel method for selective isolation of actinomycetes. Appl Environ Microbiol 46(4):925–929

Hirsch P, Mevs U, Kroppenstedt RM, Schumann P, Stackebrandt E (2004) Cryptoendolithic actinomycetes from Antarctic sandstone rock samples: *Micromonospora endolithica* sp. nov. and two isolates related to Micromonospora coerulea Jensen 1932. Syst Appl Microbiol 27:166–174. https://doi.org/10.1078/072320204322881781

Hoshino Y, Mukai A, Yazawa K, Uno J, Ando A, Mikami Y, Yamaguchi K (2004) Transvalencin A, a thiazolidine zinc complex antibiotic produced by a clinical isolate of *Nocardia transvalensis*: II. Structure elucidation. J Antibiot 57(12):797–802. https://doi.org/10.7164/antibiotics.57.803

Idris H, Goodfellow M, Sanderson R, Asenjo JA, Bull AT (2017) Actinobacterial rare biospheres and dark matter revealed in habitats of the Chilean Atacama Desert. Sci Rep 7(1):1–11. https://doi.org/10.1038/s41598-017-08937-4

Igarashi Y, Trujillo ME, Martínez-Molina E, Yanase S, Miyanaga S, Obata, Furumai T (2007) Antitumor anthraquinones from an endophytic actinomycete *Micromonospora lupini* sp. nov. Bioorg Med Chem Lett 17(13):3702–3705. https://doi.org/10.1016/j.bmcl.2007.04.039

ImamUra N, Nishijima M, Adachi K, Sano H (1993) Novel antimycin antibiotics, urauchimycins A and B, produced by marine actinomycete. J Antibiot 46(2):241–246

Isono K, Nagatsu J, Kawashima Y, Suzuki S (1965) Studies on polyoxins, an-tifungal antibiotics. Part I. Isolation and characterization of polyoxins A and B. Agric Biol Chem 29:848–854

Iwasa T, Suetomi K, Kusuka T (1978) Taxonomic study and fermentation of producing organism and antimicrobial activity of mildiomycin. J Antibiot 31:511–518

Kang Q, Shen Y, Bai L (2012) Biosynthesis of 3,5-AHBA-derived natural products. Nat Prod Rep 29:243–263. https://doi.org/10.1039/c2np00019a

Kanoh K, Matsuo Y, Adachi K, Imagawa H, Nishizawa M, Shizuri Y (2005) Mechercharmycins A and B, cytotoxic substances from marine-derived *Thermoactinomyces* sp. YM3-251. J Antibiot 58:289–292. https://doi.org/10.1038/ja.2005.36

Kharel MK, Pahari P, Shepherd MD, Tibrewal N, Nybo SE, Shaaban KA, Rohr J (2012) Angucyclines: biosynthesis, mode-of-action, new natural products, and synthesis. Nat Prod Rep 29:264–325. https://doi.org/10.1039/c1np00068c

Khebizi N, Boudjella H, Bijani C, Bouras N, Klenk HP, Pont F, Mathieu F, Sabaou N (2018) Oligomycins A and E, major bioactive secondary metabolites produced by *Streptomyces* sp. strain HG29 isolated from a Saharan soil. J Mycol Med 28(1):150–160

Kirby BM, Barnard D, Tuffin IM, Cowan DA (2011) Ecological distribution of microorganisms in terrestrial, psychrophilic habitats. In: Horikoshi K (ed) Extremophiles handbook. Springer, Berlin, pp 839–863. https://doi.org/10.1007/978-4-431-53,898-1

Kormanec J, Novakova R, Mingyar E, Feckova L (2014) Intriguing properties of the angucycline antibiotic auricin and complex regulation of its biosynthesis. Appl Microbiol Biotechnol 98:45–60. https://doi.org/10.1007/s00253-013-5373-0

Kurtböke Dİ (2017) Ecology and habitat distribution of actinobacteria. In: Biology and biotechnology of actinobacteria. Springer, Berlin, pp 123–149. https://doi.org/10.1007/978-3-319-60,339-1

Kwon HC, Kauffman CA, Jensen PR, Fenical W (2006) Marinomycins A-D, antitumor-antibiotics of a new structure class from a marine actinomycete of the recently discovered genus "*Marinispora*". J Am Chem Soc 128:1622–1632. https://doi.org/10.1021/ja0558948

Labeda DP (1990) Isolation of biotechnological organisms from nature. McGraw-Hill, New York

Le Roes-Hill M, Rohland J, Meyers PR, Cowan DA, Burton SG (2009) *Streptomyces hypolithicus* sp. nov., isolated from an Antarctic hypolith community. Int J Syst Evol Microbiol 59:2033–2036. https://doi.org/10.1099/ijs.0.007971-0

Lewin GR, Carlos C, Chevrette MG, Horn HA, McDonald BR, Stankey RJ, Currie CR (2016) Evolution and ecology of actinobacteria and their bioenergy applications. Annu Rev Microbiol 70(1):235–254. https://doi.org/10.1146/annurev-micro-102215-095748

Li YV (2002) Isolation of actinomycetes from soil using extremely high frequency radiation. Microbiology 71(1):119–122

Linke HAB, Mechlinski W, Schaffner CP (1974) Production of amphotericin B-14C by *Streptomyces nodosus* fermentation, and preparation of the amphotericin B-14C-methyl-ester. J Antibiot 27:155–160. https://doi.org/10.7164/antibiotics.27.155

Lo Grasso L, Chillura-Martino D, Alduina R (2016) Production of antibacterial compounds from actinomycetes. In: Actinobacteria—basics and biotechnological applications. IntechOpen, London. https://doi.org/10.5772/61525

Malet-Cascón L, Romero F, Espliego-Vázquez F, Grávalos D, Fernández-Puentes JL (2003) IB-00208, a new cytotoxic polycyclic xanthone produced by a marine-derived actinomadura. I. Isolation of the strain, taxonomy and biological activities. J Antibiot 56:219–225. https://doi.org/10.7164/antibiotics.56.219

Manulis S, Shafrir H, Epstein E, Lichter A, Barash I (1994) Biosynthesis of indole-3-acetic acid via the indole-3-acetamide pathway in *Streptomyces* spp. Microbiology 140(5):1045–1050

Margesin R, Schumann P, Spröer C, Gounot AM (2004) Arthrobacter psychrophenolicus sp. nov., isolated from an alpine ice cave. Int J Syst Evol Microbiol 54:2067–2072. https://doi.org/10.1099/ijs.0.63124-0

Mayilraj S, Krishnamurthi S, Saha P, Saini HS (2006a) Rhodococcus kroppenstedtii sp. nov., a novel actinobacterium isolated from a cold desert of the Himalayas, India. Int J Syst Evol Microbiol 56:979–982. https://doi.org/10.1099/ijs.0.63831-0

Mayilraj S, Suresh K, Schumann P, Kroppenstedt RM, Saini HS (2006b) Agrococcus lahaulensis sp. nov., isolated from a cold desert of the Indian Himalayas. Int J Syst Evol Microbiol 56(Pt 8): 1807–1810. https://doi.org/10.1099/ijs.0.64247-0

McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell HM, Smith JW (1952) Ilotycin, a new antibiotic. Antibiot Chemother 2(6):281–283

Mevs U, Stackebrandt E, Schumann P, Gallikowski CA, Hirsch P (2000) Modestobacter multiseptatus gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). Int J Syst Evol Microbiol 50(Pt 1):337–346. https://doi.org/10.1099/00207713-50-1-337

Millard WA, Taylor CB (1927) Antagonism of micro-organisms as the controlling factor in the inhibition of scab by green-manuring. Ann Appl Biol 14(2):202–216. https://doi.org/10.1111/j.1744-7348.1927.tb07076.x

Mura S, Iwai Y, Takahashi Y, Sadakane N, Nakagawa A, Oiwa H, Ikai T (1979) Herbimycin, a new antibiotic produced by a strain of streptomyces. J Antibiot 32(4):255–261. https://doi.org/10.7164/antibiotics.32.255

Nemoto A, Hoshino Y, Yazawa K, Ando A, Mikami Y, Komaki H, Gräfe U (2002) Asterobactin, a new siderophore group antibiotic from Nocardia asteroides. J Antibiot 55(6):593–597. https://doi.org/10.7164/antibiotics.55.593

Õmura S, Crump A (2004) The life and times of ivermectin—a success story. Nat Rev Microbiol 2(12):984–989. https://doi.org/10.1038/nrmicro1048

Prabahar V, Dube S, Reddy GSN, Shivaji S (2004) Pseudonocardia antarctica sp. nov. an actinomycetes from McMurdo Dry Valleys, Antarctica. Syst Appl Microbiol 27(1):66–71. https://doi.org/10.1078/0723-2020-00249

Rizk M, Tahany Rehman A, Metwally H (2007) Screening of antagonistic activity in different *Streptomyces* species against some pathogenic microorganism. J Biol Sci 7(8):1418–1423

Schmitzer PR, Graupner PR, Chapin EL, Fields SC, Gilbert JR, Gray JA, Gerwick BC (2000) Ribofuranosyl triazolone: a natural product herbicide with activity on adenylosuccinate synthetase following phosphorylation. J Nat Prod 63(6):777–781. https://doi.org/10.1021/np990590i

Schumann P, Prauser H, Rainey FA, Stackebrandt E, Hirsch P (1997) Friedmanniella antarctica gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. Int J Syst Bacteriol 47(2):278–283. https://doi.org/10.1099/00207713-47-2-278

Seong CN, Choi KH, Baik (2001) An improved selective isolation of rare actinomycetes from forest soil. J Microbiol 39(1):17–23

Shing YP, Geok YAT, Peter C, David AP, Irene KPT (2013) Diversity and bioactivity of actinomycetes from Signy Island terrestrial soils, maritime Antarctic. Adv Polar Sci 24(4): 208. https://doi.org/10.3724/sp.j.1085.2013.00208

Singla AK, Mayilraj S, Kudo T, Krishnamurthi S, Prasad GS, Vohra RM (2005) *Actinoalloteichus spitiensis* sp. nov., a novel actinobacterium isolated from a cold desert of the Indian Himalayas. Int J Syst Evol Microbiol 55(Pt 6):2561–2564. https://doi.org/10.1099/ijs.0.63720-0

Suarez Moreno ZR, Vinchira-Villarraga DM, Vergara-Morales DI, Castellanos L, Ramos FA, Guarnaccia C, Degrassi G, Venturi V, Moreno-Sarmiento N (2019) Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. Front Microbiol 10:290

Suzuki KI, Sasaki J, Uramoto M, Nakase T, Komagata K (1997) C*ryobacterium psychrophilum* gen. nov., sp. nov., nom. rev., comb. nov., an obligately psychrophilic actinomycete to accommodate 'Curtobacterium psychrophilum' Inoue and Komagata 1976. Int J Syst Bacteriol 47(2): 474–478. https://doi.org/10.1099/00207713-47-2-474

Takeuchi S, Hirayama K, Ueda K, Sakai H, Yonehara H (1958) Blasticidin S, a new antibiotic. J Antibiot 11(1):1–5

Tomita F, Tamaoki T, Morimoto M, Fujimoto K (1981) Trioxacarcins, novel antitumor antibiotics I. Producing organism, fermentation and biological activities. J Antibiot 34(12):1519–1524. https://doi.org/10.7164/antibiotics.34.1519

Tveit AT, Urich T, Svenning MM (2014) Metatranscriptomic analysis of arctic peat soil microbiota. Appl Environ Microbiol 80:5761–5772. https://doi.org/10.1128/AEM.01030-14

Waldron C, Matsushima P, Rosteck PR, Broughton MC, Turner J, Madduri K, Baltz RH (2001) Cloning and analysis of the spinosad biosynthetic gene cluster of *Saccharopolyspora spinosa*. Chem Biol 8(5):487–499. https://doi.org/10.1016/S1074-5521(01)00029-1

Weinstein MJ, Luedemann GM, Oden EM, Wagman GH, Rosselet JP, Marquez JA, Coniglio CT, Charney W, Herzog HL, Black J (1963) Gentamicin, a new antibiotic complex from *Micromonospora*. J Med Chem 6:463–464. https://doi.org/10.1021/jm00340a034

Williams ST, Davies FL, Mayfield CI, Khan MR (1971) Studies on the ecology of actinomycetes in soil II. The pH requirements of *Streptomycetes* from two acid soils. Soil Biol Biochem 3(3): 187–195

Wood S (1985) A method for the examination of the substrate mycelium of actinomycetes by scanning electron microscopy. Microbiology 131(9):2493–2495

Yao CF, Schiebel M, Helmke E, Anke H, Laatsch H (2006) Prefluostatin and new urauchimycin derivatives produced by *Streptomycete* isolates. Zeitschrift für Naturforschung B 61(3):320–325

You KM, Park YK (1996) A new method for the selective isolation of actinomycetes from soil. Biotechnol Techn 10(7):541–546

Zhang DC, Schumann P, Liu HC, Xin YH, Zhou YG, Schinner F, Margesin R (2010) *Arthrobacter alpinus* sp. nov., a psychrophilic bacterium isolated from alpine soil. Int J Syst Evol Microbiol 60(9):2149–2153

Chapter 4
Extremophilic Actinobacteria

Jayachandra S. Yaradoddi, Merja H. Kontro, Nagaraj R. Banapurmath, Sharanabasava V. Ganachari, M. B. Sulochana, Basavaraj S. Hungund, Zareen Kousar Kazi, S. K. Anilkumar, and Ajaykumar Oli

Abstract In nature, we can see many hostile or extreme environments, as these environments have made life more difficult to survive. Harsh environments can be designated as any considerably high change in the extent of chemical (pH, water content, organic solvents, and salt concentration) or physical variations (osmotic pressure, temperature, pressure, and radiation). Extremophilic organisms are rare

J. S. Yaradoddi (✉)
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

M. H. Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

N. R. Banapurmath
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

M. B. Sulochana
Department of PG Studies and Research in Biotechnology, Gulbarga University, Kalburagi, Karnataka, India

B. S. Hungund · Z. K. Kazi
Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

S. K. Anilkumar
Department of Biotechnology, Walchand College of Arts and Science, Solapur, Karnataka, India

A. Oli
SDM Research Institute for Biomedical Sciences, a Constituent Unit, Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

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organisms that can thrive well in these adverse physicochemical conditions. The discovery of novel actinobacterial species may lead to the recovery of new secondary metabolites. In another sense, the metabolites from the extremophilic actinobacteria have received immense value in harsh industrial applications. Extremophilic actinobacteria can be classified into thermophilic, psychrophilic, barophilic, acidophilic, alkaliphilic, halophilic, osmophilic, saprophytic, and xerophilic based upon their inherent properties. Apart from these extremophilic actinobacteria, there is a particular category of extreme tolerant actinobacteria in various environments. However, lots of research work needs to be carried out in the exploration of these groups of actinobacteria—both extremophilic and extreme tolerant actinobacterial communities' genomes inherently have novel potential bioactive compounds. However, the only fraction of the diversity of the extremophilic or extreme tolerant actinobacteria is known, but they have got enormous potential.

Keywords Extremophilic actinobacteria · Temperature · Pressure · Radiation · Acidophilic · Alkaliphilic actinobacteria

4.1 Introduction

4.1.1 Major Sources of Extremophilic Actinobacteria

Numerous environments could be known as extreme, moreover concerning chemical (salinity, pH, water content) or physical constraints (pressure, temperature, radiation) (Bull 2011). Organisms living in such an environment are known as extremophiles; these extremophilic organisms prefer to grow in the wide ranges of these physicochemical parameters (Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020). Despite these unique characteristic features, numerous microorganisms, denoted as extremotrophs, can able to grow; however, not basically adjusted despite the extreme environmental conditions such sas nutrient-depleted situations, those organisms can be considered as oligotrophs instead oligophile (Bull 2011). A number of *Actinobacteria* are isolated from a total range of extreme conditions.

The existence of alkaliphilic, acid-tolerant, thermotolerant, psychrotolerant, alkali-tolerant, halotolerant, xerophilous, and halo alkali-tolerant *Actinobacteria* has been described (Lubsanova et al. 2014). The novel chemodiversity is extra likely to be in rarely cultured strains. Consequently, the multiplicity among the extreme biosphere could help to address the challenges in rediscovering earlier known secondary metabolites to a significant period of time, because of this motive, exploration of the flourishing *Actinobacteria* in harsh conditions in recovering novel strains with immense industrial value. Though several widespread types of research were employed in the exploration of bacterial diversity, especially in the arid ecosystems, the multiplicity of *Actinobacteria* by such environmental conditions was not wholly investigated (Okoro et al. 2009).

4.2 Arid Niche and Subsistence of Biogeographical Barriers

Arid habitats encompass huge continental environment (which covers about 30% among the Earth area; within that, 7% is hyperarid) that is water proscribed. These arid or dry regions known as biomes by means of a ratio of average yearly rainfall denoted annual disappearance lower than 0.05 and slightly below 0.002 for hyperarid regions (Bull 2011). The extreme dried and aridity sorroundings in higher arid deserts is often acquired by higher temperatures, nutrient depletion, lower a_w (water activity), and prevailing radiation, whereas in some other ecosystems, lower temperature, pH, high salinity, higher metal concentration such as sulfate or nitrate and inorganic oxidant anions can be seen under arid regions (Koeberl et al. 2011).

Among all, the inaccessibility of aquatic resources and nutrients is the prime restraining factor for biological activity under arid and semiarid habitats (Saul-Tcherkas et al. 2013). Bacteria present in low water activity ecosystems inevitability of distribution higher energy to collect at a definite quantity of water and also the important robust bacteria typically arise, a condition of hydrobiosis as soon as a_w (water activity) is concentrated about 0.88 a_w, wherein the cells detained just before metabolize, though, persist workable (Connon et al. 2007; Yaradoddi et al. 2020b). Bacteria that thrive well in arid ecosystems can adjust to drought condition yet water is essential for their physiological requirements. Utmost occurs adjoining near mineral soils mainly halites, gypsum, or quartz; by spreading, a little water surrounds within mineral soils adequate for the bacterial growth and activity (Azua-Bustos et al. 2012).

Arid or dry zones are the interface alongside of the vegetated semi-arid regions, that also contains biologically infertile hyper or extreme arid desert ecosystems (Neilson et al. 2012). These regions harbor various untapped thermophilic, xerophilic, alkaliphilic, and halophilic *Actinobacteria* producing novel bioactive compounds. Adapting potential new techniques can lead toward the detection of culturable bacterial communities in deserts that were hypothetical to be infertile (Koeberl et al. 2011). The desert ecosystems are unique environments to tap the novel extremotrophs or extremophilic strains of *Actinomycetes,* they can be explored to yield new metabolites, *Actinobacteria* have possessed tolerance to desiccation, and solute stress among bacteria and these organisms were reported from the various antagonistic environment such as arid or hyper arids desert, which are supposed to be similar to habitats on Mars. However, high levels of propagation and that produce 0.5 a_w are described. *Actinobacteria* especially non-halophilic actinomycetes are generally improbable to be metabolically active beneath 0.8 a_w, but they might be ecologically active in water-suppressed microhabitats in soil that comprise water activity slightly higher than this value (Stevenson and Hallsworth 2014). In spite of the different geographical range of arid environments, a very minute is familiar in relation to the bacterial communities of these ecosystems with diverse metabolic activities. As for this concern, several reports are accessible in relation to the isolation, screening, and environmental diversity of rare actinomycetes inhabited in the desert habitat (Harwani 2013). In addition to this, habitats that alternate to the soils are besides deliberated as the novel basis in water-scarce conditions (Azua-Bustos et al. 2012).

4.3 Xerophiles Recovered Under Arid Environments

The actinomycetes recovered under extreme warm or acidic environments using hyper radiation or aridity situations (like a desert and new arid ecosystems) are inclined toward characteristically deepest genera of actinomycetes (Rubrobacteridae, Acidimicrobidae). The higher dry state of deserts has been one of the most dynamic environments for the progression of DNA repair mechanisms, which has produced tolerance toward the ionizing radiation (Gamma and UV), distinguished by numerous desert-based *Actinobacteria* (Makarova et al. 2001). The most resilient genera from such environments are strains of *Geodermatophilus* and *Deinococcus* that can resist up to 30 Gy of radiation. Members of this genus are not so far isolated from the non-arid soil, even employing radiation treatments. The xerophilic *Actinobacteria*, *G. siccatus*, and *Geodermatophilus arenarius* were recovered from Sahara deserts in Chad (Montero-Calasanz et al. 2013). Another important member of the genera *Geodermatophilus* has been reported from Negev Desert soil, and *Actinoplanes* and *Streptomyces* strains were recovered from Mojave Desert soil and California-Nevada border, through selective chemoattractants (Kurapova et al. 2012). The *Geodermatophilaceae* comprises two other genera such as *Modestobacter* and *Blastococcus*, which thrive well in water and nutrient limiting conditions; *Geodermatophilus* chooses dry soils as usual environments among 15 species designated in this genus; at least nine species are recovered from the desert's region (Euzeby 2015). In contrast, *Modestobacter* and *Blastococcus* are occupied in rock surfaces. Apart from this actinobacterium which was discovered from the desert ecosystem in Egypt, *Citricoccus alkalitolerans* was designated as alkali tolerant, and maximum growth can be seen at pH 8.0–9.0 (Li et al. 2005a). New strains of the nonsporulating actinomycetes *Mycetocola manganoxydans* which have capability to bring oxidation of manganese ions were recovered within Takalime Desert (Luo et al. 2012). Associated with the *Terra bacteria* genera are also categorized by its adaptation to the radiation, high salinity, and desiccation. Concerning the members of the genera *Streptomyces*, mainly *Streptomyces deserti* initially reported under hyperarid Atacama Desert can be seen in arid habitats (Santhanam et al. 2013); *Streptomyces bullii* was from hyperarid Atacama Desert, and the moderate thermophilic *Streptomyces* sp. 315 are xerotolerant in Mongolia Desert soil (Kurapova et al. 2012).

Apart from the *Streptomyces*, strains belonging to *Saccharothrix*, *Strepto sporangium*, *Cellulomonas*, and *Micromonospora* were isolated from the Qinghai-Tibet Plateau (Ding et al. 2013a), whereas *Actinomadura*, *Nocardiopsis*, and *Micromonospora* were recovered from soda saline soils of ephemeral salt lakes in Buryatiya (Lubsanova et al. 2014). Thermophilic and thermotolerant actinomycetes can be seen much abundantly, sometime beyond that of the neutrophilic forms in Mongolia Desert soil. Other members of the *Actinomadura*, *Streptomyces*, *Streptosporangium*, and also *Micromonospora* are utmost extensively spread thermoresistant species in deserts soils. Numerous members of *Streptomyces*, which belonged to actinobacterial genera *Nocardia*, *Micromonospora*,

Saccharopolyspora, *Nonomuraea*, and *Nocardiopsis*, were also reported from the Arabian Sea, solar salterns of the Bay of Bengal, and inland surrounding the Sambhar Salt Lake (Jose and Jebakumar 2012). However, surprisingly it has been revealed that *Actinobacteria* in desert soil land dominated 20.7%, whereas agricultural soil comprises 4.6% relatively in poorer quantity in farmland when compared with desert ecosystem (Ding et al. 2013b). Especially concerning to the genera *Rhodococcus*, an *Actinobacteria* has dominated in desert soil. More specifically, tolerant to the salinity (*Actinobacteria* obtained using saline soil of the infertile territories), higher temperature, alkaline situation, and drought have been practically proven. It was understood that all the halotolerant strains (strains can able to grow up to 5% NaCl), unlike non-halophilic isolates, have the potential to grow in medium with pH 10, whereas non-halophilic strains do not have such potentials. In this prospect, a moderate thermophilic strain of *Streptomyces sp.* which was recovered from desert soil was practically demonstrated as a xerotolerant. The halotolerant and alkaliphile *Streptomyces aburaviensis* reported from the salt arid region of Kutch in India have an antagonistic effect against Gram-positive bacteria. The strain was able to grow slowly at 15% NaCl and in neutral pH, whereas the maximum growth was observed in 5–10% NaCl and at pH 9 (Thumar et al. 2010). The mesophilic actinobacteria from the Mongolian desert soil habitat belonged to the genera *Streptomyces*, while thermotolerant organisms belonged to the genera *Actinomadura*, *Micromonospora*, and *Streptosporangium*. Plant associated with *Actinobacteria* from desert origin also exists. Concerning to drought-tolerant endophytic *Actinobacteria*, *S. olivaceus* DE10, *S. geysiriensis* DE27, and *Streptomyces coelicolor* DE07 were isolated from plants of arid and drought-affected areas. These strains demonstrated plant growth promotion (PGP) activity similar to other bacterial (Sulochana et al. 2014a, b) and inherent tolerance to water stress (−0.05 to −0.73 MPa) (Yandigeri et al. 2012). Roughly extremophile bacteria, mainly *Deinococcus-Thermus*, *Rubellimicrobium*, and *Acidimicrobium* intensely have below stated agricultural use.

In contrast to this, original desert bacteria can enhance plant health in desert agro-based ecosystems. *Actinobacteria* in lower water activity regions of Antarctica (comparable condition in desert habitat) were pronounced. The bacterial multiplicity of Lake Hodgson and the Antarctic Peninsula comprises 11.6% *Chloroflexi*, 20.2% *Plantomycetes*, 21% *Proteobacteria*, and 23% *Actinobacteria* (Pearce et al. 2013). Although from Dry Valley soil of Antarctic, the *Actinobacteria* (26%), *Acidobacteria* (16%), and *Cyanobacteria* (13%) belonged to the majority of the recognized as resident bacteria (Smith et al. 2006). The culture-independent evaluation of different domain bacterial variety in the cold desert of the McKelvey Valley established which is very specific communities to be colonized in discrete lithic habitats can be seen concurrently among this ecosystem. In spite of relatively barren soil, the maximum part of variety was found in chasmoliths and endoliths of sand stone. The complete phylum level structures of numerous arid regions are indicated to be dominated by the *Actinobacteria*. They were also disclosed to be most abundant phyla about 72–88% from areas of Atacama Desert (Crits-Christoph et al. 2013), whereas in other dry area, they are among the three predominant

phyla (generally along with the *Proteobacteria* and *Firmicutes*) in the deserted soil of the Aridic Calcisols in Kazakhstan (Kutovaya et al. 2015), alkaline-saline (Keshri et al. 2013), deserts comprising shrub root zone (Steven et al. 2012), and elevated deserts (Lynch et al. 2014). The dominant genera of *Actinobacteria* are not yet described as per metagenomic studies in concern, except the studies focused on haloalkaline semiarid regions in India, wherein two-thirds of the *Actinobacteria* clones were identified among order *Rubrobacterales* (Keshri et al. 2013).

4.4 Mixed Extreme Environments

Among other extreme environments, brief structures of two ecosystems detailed here are water polluted sites and inland. The inland waters comprised of salt and soda lakes could be unseemly on primary encounter of freshwater lakes, nevertheless the circumstantial attention on freshwater lakes turns around their high radiation exposure and their oligotrophy. Recently, there have been distinctive freshwater bacteria identified (Zwart et al. 2002); mostly, the predominant category belonged to *Actinobacteria* (70%), and they have been considered as ultrasmall microorganisms (Hahn et al. 2003). Warnecke et al. in the year 2005 ensured that the bacteria of planktonic origin dominating in the high altitude and in ultraviolet (UV) transparent lakes were native actinobacteria; however, it has been cautioned that the adjustment to ultraviolet stress was relatively, not essentially, causal. At present, no such pure strains have recovered from these original actinobacteria concerning to the UV tolerance. The cocultures and phylotypes of these freshwater organisms are often associated with the representatives of *Micrococcineae*, and more recently, Hahn has identified the potential novel monophyletic and recently has described a novel monophyletic group among family *Microbacteriaceae* (Hahn 2009). Seven new species were recognized but again lonely as candidate species because pure and isolated cultures have not been accomplished; the helper bacteria mostly related proteobacteria are required to form quite close interaction to allow the development of the actinomycetes. The mechanism about this interaction remains unknown. Aside from freshwater, inland waters such as soda and salt lakes are also abundant sources of new actinobacteria; soda lake-derived organisms consist of *Nitriliruptor* (Sorokin et al. 2009), *Yonghaparkia* (Yoon et al. 2006), and *Microcella* (Tiago et al. 2005). However, the *Nutriliruptor alkaliphilus* is probably the exciting organism because it is the first identified member of a novel, extremely branched order within the *Actinobacteria*, and it is moderately halophilic, obligatory alkaliphilic and can able to grow in a range of nitriles. Also, thermophilic actinobacteria have been recovered from hot springs (*Rubrobacter* (Chen et al. 2004)), whereas the first culture-independent methods have revealed the foremost diversity of actinobacteria most commonly seen within the environments of higher temperature (81 °C) (Song et al. 2009); the significant phylotypes and associated members include the *Rubrobacteriales* and the actinobacteria suborder *Frankineae*. The salt lake is embedded with the presence of *Haloactinospora* (Tang et al. 2008), *Haloglycomyces*

Table 4.1 Bioactivity of compounds extracted from various actinomycetes

Compounds	Actinomycetes	Action
Sclerothricin	*Streptomyces sp.*	Antifungal activity
Lomofungin	*Streptomyces lomondensis*	Antifungal
Spoxamicin	*Streptosporangium oxazonolinicum*	Antitrypanosomal
Antimycin	*Streptomyces* sp.	Antifungal
Avermectin	*Streptomyces avermitilis*	Antiparasitic
Rosamicin	*Micromonospora rosaria*	Antibacterial
Roseoflavin	*Streptomyces dawavensis*	Antibacterial
Validamycin	*Streptomyces* sp.	Antifungal
Rifamycin	*Micromonospora rifamycinica*	Antibacterial

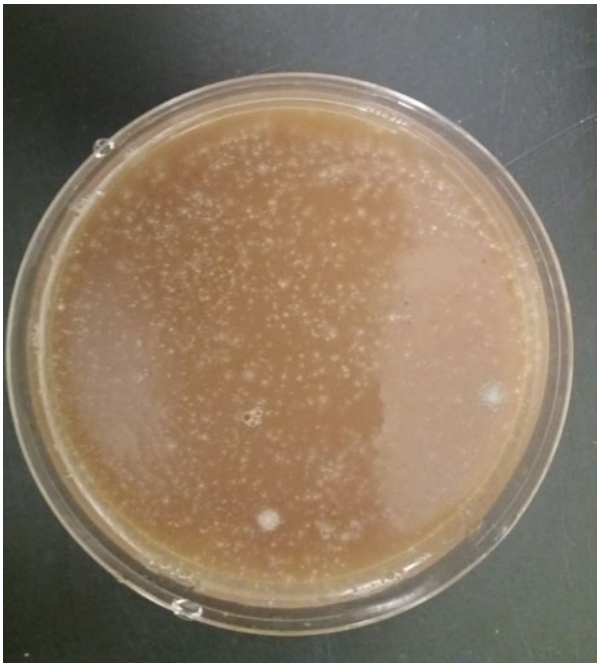

Fig. 4.1 Indicating potential strain of thermostable actinobacteria from compost sample

(Guan et al. 2009), and *Streptimonospora* (Cui et al. 2001). However, the culture-based methods can be more beneficial in isolation and maintenance of the potential microbial consortia for various industrial applications (Anil Kumar et al. 2010; Jayachandra et al. 2013a; Mohan Reddy et al. 2015a, b; Jayachandra et al. 2013b). There are numerous actinomycetes reported for their antimicrobial activities such as antifungal, antibacterial, antitrypanosomal, antiparasitic, etc. (Table 4.1). Recently we could be able to recover potential lignocellulose degrading actinobacteria from the compost samples in Finland (Fig. 4.1).

4.5 Actinobacteria in Alkaline Soils

In traditional terms, actinobacteria which are the organisms that are tolerant to the environmental conditions recognized as mycelia prokaryotes occurring under alkaline conditions have been investigated. The actinobacteria isolate grown well on alkaline media were initially described by Baldacci (1944). The alkaliphilic actinomycetes were recovered from a variety of soils by Taber (1960). These actinobacteria are reportedly recovered from soda lakes and saline soils. Mycelial-forming bacteria of the *Geodermatophilus* genera employ specific life cycle amount to the multiple part of the microbial consortium in desert ecosystems, plants of the Kyzylkum Desert, salt crust, desert, and solonchaks in the southern coastal regions of the Aral and Dead seas (Dobrovol'skaya 2002). On the other hand, these actinobacteria were not studies from the perspective of their resistance capacity to higher pH and a higher concentration of salinity. Before, a considerable amount of information on the isolation of alkalophilic actinobacteria from the soils and concerned substrates has been utilized. During one of the study, the amino acid composition of the cell wall of certain alkaliphilic actinobacteria (Mikami et al. 1982; Yoshida et al. 1979) reported the occurrence of a mesoisomers of DAPa (diaminopimelic acid) in some of them. The alkaliphilic types of bacteria not only were restricted to the *Streptomyces* genus but also common among other genera such as *Streptoverticillium*, *Elytrosporangium*, *Microellobosporia*, *Nocardioides*, *Chainia*, *Sporichthia*, *Saccharothrix*, *Micromonospora*, and *Nocardiopsis* (Prabahar 2004; Prauser 1976a, b). Thereafter, they were grouped under alkaliphilic actinobacteria to few of the abovementioned genera confirmed by using 16S rRNA gene sequence analysis (Antony-Babu et al. 2003). Previous reports also revealed these alkaliphilic actinobacteria could be resulted in the description of new taxa (Kroppenstedt and Evtushenko 2004; Nakajima et al. 1999) and biologically active substances synthesized by novel species, alkaline proteases, and new antibiotics (Sato et al. 1983; Song et al. 2001).

Several attempts able to divide the alkaliphilic isolates according to their requirement for acidity were mentioned in the literature. Few authors (Jiang and Xu 1993) distinguish the extremes as alkaliphilic actinobacteria with an optimum growth rate at pH 10–11 and not viable at pH 7.0; moderate alkaliphilic can be classified based on the pH 10 but weakly grows at pH ranging between 6.0 and 11.0. As per the report of Jiang et al., the alkaliphilic type can be classified into 2 groups: alkaliphilic with the optimum growth at pH ranges between 9.0 and 9.5 and growth stops at 8.0–11.5 and alkali tolerant with the optimum growth occurs at pH 7.0 and growth stops at pH 11.5. The range of the pH value optimum toward the growth of the isolates was analyzed, measuring the intensity of incorporated adenine into the cell wall. For alkaliphilic actinobacteria, this intensity is maximum at pH between 9.0 and 9.5. Till now, a great amount of information is available on the recovery of actinobacteria in unusual requirements for the acidity of the environment has accumulated. However, there is a lack of data on the regular distribution of population, and ecological persistence of the alkaliphilic actinobacteria is not yet

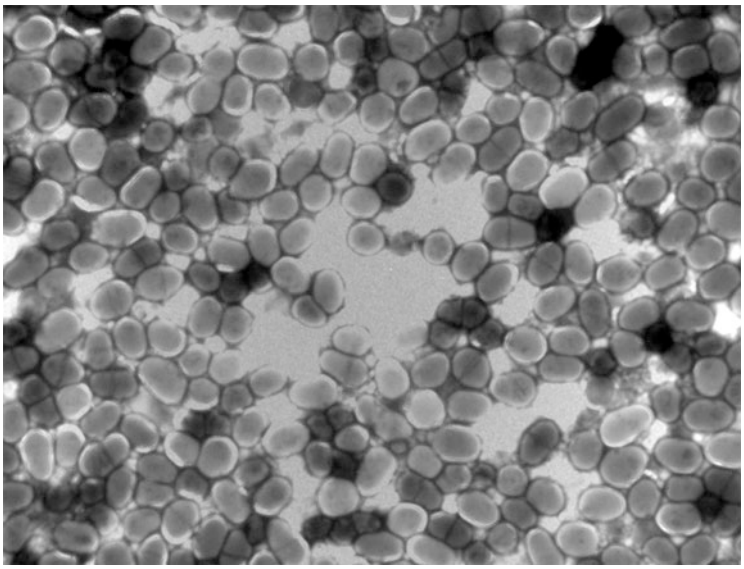

Fig. 4.2 TEM micrograph of actinobacteria: *Cellulomonas* sp.

widespread. There is also scarcity of detailed descriptions of taxa belonging to mycelial prokaryotes by unique pH requirements. As per the literature, the issues are associated with the specific property of secondary metabolism in alkaliphilic actinobacteria for the production of antibiotics (Mikami et al. 1986), and thus the synthesis of alkaline-stimulated enzymes (Sato et al. 1983) is most commonly considered, or novel taxa among the alkaliphilic and acidophilic actinobacteria were reported (Tsuchiya et al. 1997; Li et al. 2005b; Wael et al. 2004; Wang and Ruan 1994; Wang et al. 2001, 2004). The likely occurrence of the mycelia bacteria under alkaline medium is of no doubt. Applied methods like TEM can be used in understanding the structural morphology and behaviors of actinobacteria (Fig. 4.2).

4.6 Prospective

Several properties of these isolates have been investigated (Hozzein et al. 2004); however, the ecology of alkaliphilic actinobacteria is poorly understood. There are huge opportunities in exploration of the actinobacteria complexes in a broad range of soils and artificial substrates and the identification of the taxonomic structure and ecological specificities of alkaliphilic actinobacteria, corresponding to the places of these mycelia bacteria under the microbial consortia of alkaline and saline soils and which could significantly contribute toward the understanding of biological diversity. The soils by means of their pH values (saline chestnut, saline alluvial meadow, meadow solonchaks, semidesert brown, and crusty) were studied. With the high

alkaline (pH >8) soils, numerous actinobacterial species were recovered by cultivating on medium with pH 9 which was quite high compared to neutral and, specifically, on the acidified media (Selyanin et al. 2005). This excess can be seen interestingly in the solonchak by about pH 9.5 produced within the underneath of the dried salt lake in Buryatia. On the substrate, numerous actinobacterial species can grow under alkaline medium conditions that surpass the density that was isolated under acidic pH. Reportedly in every soil that was investigated at above pH 7, numerous actinomycetes were recovered under the alkaline medium, which was relatively higher as compared to that cultivated under neutral conditions.

References

Anil Kumar S, Arunashri R, Jayachandra SY, Sulochana MB (2010) Screening of extracellular hydrolytic enzymes from *Marinobacter hydrocarbonoclasticas* strain AK5. Int J Bioscan 5(1): 97–99

Antony-Babu S, Pathom-aree, Nam S, et al (2003) Alkali-tolerant streptomycete diversity in littoral and sand dune systems. In: The 13th international symposium on the biology of actinomycetes, Melbourne, Australia, 2003, p 23

Azua-Bustos A, Urrejola C, Vicuña R (2012) Life at the dry edge: microorganisms of the Atacama Desert. FEBS Lett 586:2939–2945. https://doi.org/10.1016/j.febslet.2012.07.025

Baldacci E (1944) Contributo alla systematica degli actenomyceti: X–XVI *Actinomyces madurae*; *Proactinomyces ruber*; *Proactinomyces pseudomadurae*; *Proactinomyces polychromogenus*; *Proactinomyces violaceus*; *Actinomyces coeruleus*; con un elencio alfabetico delle specie e delle varieta finora studiate. Atti Ist Dot Univ Pavia Ser 3:139–193

Bull AT (2011) *Actinobacteria* of the extremobiosphere. In: Horikoshi K (ed) Extremophiles handbook. Springer, Berlin, pp 1203–1240

Chen MY, Wu SH, Lin GH, Lu CP, Lin YT, Chang WC, Tsay SS (2004) *Rubrobacter taiwanensis* sp. nov., a novel thermophilic, radiation-resistant species isolated from hot springs. Int J Syst Evol Microbiol 54:1849–1855

Connon SA, Lester ED, Shafaat HS, Obenhuber DC, Ponce A (2007) Bacterial diversity in hyperarid Atacama Desert soils. J Geophys Res 112:G4. https://doi.org/10.1029/2006JG000311

Crits-Christoph A, Robinson CK, Barnum T, Fricke WF, Davila AF, Jedynak B et al (2013) Colonization patterns of soil microbial communities in the Atacama Desert. Microbiome 1:28. https://doi.org/10.1186/2049-2618-1-28

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptimonospora salina* gen. nov., sp nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 51: 357–363

Ding D, Chen G, Wang B, Wang Q, Liu D, Peng M et al (2013a) Culturable actinomycetes from desert ecosystem in northeast of Qinghai-Tibet Plateau. Ann Microbiol 63:259–266. https://doi.org/10.1007/s13213-012-0469

Ding GC, Piceno YM, Heuer H, Weinert N, Dohrmann AB, Carrillo A et al (2013b) Changes of soil bacterial diversity as a consequence of agricultural land use in a semi-arid ecosystem. PLoS One 8:e59497. https://doi.org/10.1371/journal.pone.0059497

Dobrovol'skaya TG (2002) Structure of soil bacterial communities. Akademkniga, Moscow. [in Russian]

Euzeby JP (2015) List of prokaryotic names with standing in nomenclature. http://www.bacterio.cict.fr

Guan T-W, Tang S-K, Wu J-Y, Zhi X-Y, Xu L-H, Zhang L-L, Li W-J (2009) *Haloglycomyces albus* gen. nov., sp. nov., a halophilic, filamentous actinomycete of the family *Glycomycetaceae*. Int J Syst Evol Microbiol 59:1297–1301

Hahn MW (2009) Description of seven candidate species affiliated with the phylum *actinobacteria*, representing planktonic freshwater bacteria. Int J Syst Evol Microbiol 59:112–117

Hahn MW, Lunsdorf H, Wu QL, Schauer M, Hofle MG, Boenigk J, Stadler P (2003) Isolation of novel ultramicrobacteria classified as *actinobacteria* from five freshwater habitats in Europe and Asia. Environ Microbiol 69:1442–1451

Harwani D (2013) Biodiversity of rare thermophilic actinomycetes in the great Indian Thar desert: an overview. Indo Am J Pharm Res 3:934–939

Hozzein WN, Li WJ, Ali MIA et al (2004) *Nocardiopsis alcaliphila* sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. Int J Syst Evol Microbiol 54:247–252

Jayachandra SY, Mohan Reddy K, Paramesh B, Shouche YS, Sulochana MB (2013a) Screening of halophilic bacteria extracellular enzymes production from west coast of Karnataka, India. Int J Univ Pharm Biosci 3(1):31–45

Jayachandra SY, Anil Kumar S, Shouche YS, Sulochana MB (2013b) Culturable diversity of extremely halotolerant bacteria from Arabian Sea Karnataka, India. Int J Biol Pharm Allied Sci 2(2):391–405

Jiang C, Xu L (1993) Actinomycetes diversity in unusual habitats. Actinomycetes 4:47–57

Jose PA, Jebakumar SRD (2012) Phylogenetic diversity of actinomycetes cultured from coastal multipond solar saltern in Tuticorin, India. Aquat Biosyst 8:23. https://doi.org/10.1186/2046-9063-8-23

Keshri J, Mishra A, Jha B (2013) Microbial population index and community structure in saline–alkaline soil using gene targeted metagenomics. Microbiol Res 168:165–173. https://doi.org/10.1016/j.micres.2012.09.005

Koeberl M, Müller H, Ramadan EM, Berg G (2011) Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. PLoS One 6:e24452. https://doi.org/10.1371/journal.pone.0024452

Kroppenstedt RM, Evtushenko LI (2004) In: Dvorkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. Springer, New York

Kurapova I, Zenova GM, Sudnitsyn II, Kizilova AK, Manucharova NA, Norovsuren ZH et al (2012) Thermotolerant and thermophilic actinomycetes from soils of Mongolia Desert Steppe Zone. Microbiology 81:98–108. https://doi.org/10.1134/S0026261712010092

Kutovaya OV, Lebedeva MP, Tkhakakhova AK, Ivanova EA, Andronov EE (2015) Metagenomic characterization of biodiversity in the extremely arid desert soils of Kazakhstan. Eurasian Soil Sci 48:493–500. https://doi.org/10.1134/S106422931505004X

Li WJ, Chen HH, Zhang YQ, Kim CJ, Park DJ, Lee JC et al (2005a) *Citricoccus alkalitolerans* sp. nov., a novel actinobacterium isolated from a desert soil in Egypt. Int J Syst Microbiol 55:87–90. https://doi.org/10.1099/ijs.0.63237-0

Li WJ, Zhang YG, Zhang YQ et al (2005b) *Streptomycetes sodiiphilus* sp. nov., a novel alkaliphilic actinomycete. Int J Syst Evol Microbiol 55:1329–1333

Lubsanova DA, Zenova GM, Kozhevin PA, Manucharova NA, Shvarov AP (2014) Filamentous *actinobacteria* of the saline soils of arid territories. Moscow Univ Soil Sci Bull 69:88–92. https://doi.org/10.3103/S0147687414020057

Luo X, Wang J, Zeng XC, Wang Y, Zhou L, Nie Y et al (2012) *Mycetocola manganoxydans* sp. nov., an actinobacterium isolated from the Taklamakan desert. Int J Syst Microbiol 62 (Pt 12):2967–2970. https://doi.org/10.1099/ijs.0.038877-0

Lynch RC, Darcy JL, Kane NC, Nemergut DR, Schmidt SK (2014) Metagenomic evidence for metabolism of trace atmospheric gases by high elevation desert actinobacteria. Front Microbiol 5:698. https://doi.org/10.3389/fmicb.2014.00698

Makarova KS, Aravind L, Wolf Y, Tatusov RL, Minton KW, Koonin EV et al (2001) Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the

perspective of comparative genomics. Microbiol Mol Biol Rev 65:44–79. https://doi.org/10.1128/MMBR.65.1.44-79.2001
Mikami Y, Miyashita K, Arai T (1982) Diaminopimelic acid profiles of alkalophilic and alkaline—resistant actinomycetes. J Gen Microbiol 128:1709–1712
Mikami Y, Miyashita K, Arai T (1986) Alkaliphilic actinomycetes. Actinomycetes 19:176–191
Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363
Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867
Montero-Calasanz MC, Göker M, Pötter G, Rohde M, Spröer C, Schumann P et al (2013) *Geodermatophilus siccatus* sp. nov., isolated from arid sand of the Saharan desert in Chad. Antonie Van Leeuwenhoek 103:449–456. https://doi.org/10.1007/s10482-012-9824-x
Nakajima Y, Kitpreechevanich V, Suzuki K, Kudo T (1999) *Microbispora coralline* sp. nov., a new species of genus Microbispora isolated from Thai soil. Int J Syst Evol Microbiol 49:1761–1767
Neilson JW, Quade J, Ortiz M, Nelson WM, Legatzki A, Tian F et al (2012) Life at the hyperarid margin: novel bacterial diversity in arid soils of the Atacama Desert, Chile. Extremophiles 16:553–566. https://doi.org/10.1007/s00792-012-0454-z
Okoro CK, Brown R, Jones AL, Andrews BA, Asenjo JA, Goodfellow M et al (2009) Diversity of culturable actinomycetes in hyper arid soils of the Atacama Desert, Chile. Antonie Van Leeuwenhoek 95:121–133. https://doi.org/10.1007/s10482-008-9295-2
Pearce DA, Hodgson DA, Thorne MAS, Burns G, Cockell CS (2013) Preliminary analysis of life within a former Subglacial Lake sediment in Antarctica. Diversity 5:680–702. https://doi.org/10.3390/d5030680
Prabahar V (2004) *Pseudonocardia antarctica* sp. nov., an actinomycete from McMurdo Dry Valleys, Antarctica. Syst Appl Microbiol 27:66–71
Prauser H (1976a) In: Arai T (ed) Actinomycetes: the boundary microorganisms. Toppan, Tokyo, pp 193–207
Prauser H (1976b) *Nocardioides,* a new genus of the order Actinomycetales. Int J Syst Bacteriol 26:58–65
Santhanam R, Rong X, Huang Y, Andrews BA, Asenjo JA, Goodfellow M (2013) Streptomyces bullii sp. nov., isolated from a hyper-arid Atacama Desert soil. Antonie Van Leeuwenhoek 103:367–373. https://doi.org/10.1007/s10482-012-9816-x
Sato M, Beppu T, Arima K (1983) Studies on antibiotics produced at high alkaline pH. Agric Biol Chem 47:2019–2027
Saul-Tcherkas V, Unc A, Steinberger Y (2013) Soil microbial diversity in the vicinity of desert shrubs. Microbial Ecol 65:689–699. https://doi.org/10.1007/s00248-012-0141-8
Selyanin VV, Oborotov GV, Zenova GM, Zvyagintsev DG (2005) Soil alkalophilic actinomycetes. Mikrobiologiya 74(6):838–844
Smith JJ, Tow LA, Stafford W, Cary C, Cowan DA (2006) Bacterial diversity in three different Antarctic cold desert mineral soils. Microbial Ecol 51:413–421. https://doi.org/10.1007/s00248-006-9022-3
Song J, Weon HV, Yoon SH et al (2001) Polygenetic diversity of thermophilic actinomycetes and thermoactinomyces spp. isolated from mushroom composites in Korea based on 16 S rRNA gene sequence analysis. FEMS Microbiol Lett 202(1):97–102
Song ZQ, Zhi XY, Li WJ, Jiang HC, Zhang CL, Dong HL (2009) Actinobacterial diversity in hot Springs in Tengchong (China), Kamchatka (Russia), and Nevada (USA). Geomicrobiol J 26:256–263
Sorokin DY, van Pelt S, Tourova TP, Evtushenko LI (2009) *Nitriliruptor alkaliphilus* gen. nov., sp nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of *Nitriliruptoraceae* fam. nov and *Nitriliruptorales* ord. nov. Int J Syst Evol Microbiol 59:248–253

Steven B, Gallegos-Graves LV, Starkenburg SR, Patrick S, Chain PC, Kuske CR (2012) Targeted and shotgun metagenomic approaches provide different descriptions of dryland soil microbial communities in a manipulated field study. Environ Microbiol Rep 4:248–256. https://doi.org/10.1111/j.1758-2229.2012.00328.x

Stevenson A, Hallsworth JE (2014) Water and temperature relations of soil actinobacteria. Environ Microbiol Rep 6:744–755. https://doi.org/10.1111/1758-2229.12199

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424

Taber WA (1960) Evidence for the existence of acid-sensitive actinomycetes in soil. Can J Microbiol 6:534–544

Tang SK, Tian XP, Zhi XY, Cai M, Wu JY, Yang LL, Xu LH, Li WJ (2008) *Haloactinospora alba* gen. nov., sp. nov., a halophilic filamentous actinomycete of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 58:2075–2080

Thumar JT, Dhulia K, Singh SP (2010) Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. World J Microbiol Biotechnol 26:2081–2087. https://doi.org/10.1007/s11274-010-0394-7

Tiago I, Pires C, Mendes V, Morais PV, da Costa M, Verissimo A (2005) *Microcella putealis* gen. nov., a Gram-positive alkaliphilic bacterium isolated from a nonsaline alkaline groundwater. Syst Appl Microbiol 28:479–487

Tsuchiya K, Ikeda I, Tsuchiya T, Kimura T (1997) Cloning and expression of an intracellular alkaline protease gene from alkalophilic thermoactinomyces sp. HS682. Biosci Biotechnol Biochem 61:298–303

Wael N, Hozzein WN, Li WJ et al (2004) *Nocardiopsis alkaliphila* sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. Int J Syst Evol Microbiol 54:247–252

Wang L, Ruan JS (1994) Classification of actinomycetes isolated from saline–alkaline soils. In: ISBA 94, international symposium on the biology of actinomycetes, Moscow, Russia, 1994, p 238

Wang YM, Zhang YS, Xu XL et al (2001) *Actinopolymorpha singaporensis* gen. nov., sp., nov., a novel actinomycete from the tropical rainforest of Singapore. Int J Syst Evol Microbiol 51:467–473

Wang YM, Cao GM, Jiang WB, Zhang YS (2004) Study on actinomycetes population of alpine meadow soil in Qinghai. Wei Sheng Wu Xue Bao 44(6):733–736

Yandigeri MS, Meena KK, Singh D, Malviya N, Singh DP, Solanki MK et al (2012) Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. Plant Growth Regul 68:411–420. https://doi.org/10.1007/s10725-012-9730-2

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Yoon J-H, Kang S-J, Schumann P, Oh T-K (2006) *Yonghaparkia alkaliphila* gen. nov., sp. nov., a novel member of the family *Microbacteriaceae* isolated from an alkaline soil. Int J Syst Evol Microbiol 56:2415–2420

Yoshida K, Iguchi A, Mukaisako M, et al (1979) JP patent 151,196

Zwart G, Crump BC, Agterveld MPKV, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141–155

Chapter 5
Actinobacteria: Basic Adaptation to Harsh Environments

Jayachandra S. Yaradoddi and Merja H. Kontro

Abstract Adverse physicochemical environments are significant sources to recover the extreme actinobacterial communities. In these harsh environmental conditions, most mesophilic microbes perish because of the lack of adaptability. However, despite the harsh environments, few types of microbes can still be able to survive and even reproduce at different physicochemically limited conditions, and they are popularly known as extremophiles. The main reason behind this adaptation is due to their abundance in amino acid composition, which influences the protein folding capabilities of these microbes. The current chapter has revealed extreme actinobacteria communities in different hostile environments such as high or low temperature, high salinity, acidic or alkaline conditions, high pressure, and dry-desiccation conditions. One of the distinguishing features of the extremophilic actinobacteria is concerns about their propagation in a broader range of environments. Advancements have been made in developing L-strategy for categorizing the microbes under unfavorable conditions as either highly tolerant or resistant to environmental stress conditions.

Keywords Adaptation of actinobacteria · High salinity · High or low temperature · Acidophiles · Alkaliphiles · Extremotolerant organisms

J. S. Yaradoddi
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

M. H. Kontro (✉)
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

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5.1 Introduction

Actinobacteria are vital sources for pharmaceutical substances, especially antimicrobials. They contribute about two-thirds of antibiotics synthesized among the microbial origin (Challis and Hopwood 2003), which accounts for about 70% of bioactive molecules recommended for treating human diseases (Bérdy 2005). The rise in drug tolerance exhibited by several bacterial pathogens and the contemporary progression among numerous fungal infections have resulted in renewed attention in discovering new reservoirs of secondary metabolites (Zerikly and Challis 2009). The discovery of novel bioactive compounds (Table 5.1) is a basic requirement for developing new pharmaceutical products. This inevitability is directed towards searching novel biologically active compounds among the different underexplored and unexplored ecological niches (Bérdy 2012; Sulochana et al. 2014a, b; Yaradoddi et al. 2020a, b; Yaradoddi and Sulochana 2020). Research on actinomycetes has concentrated on special habitat that consists of extreme environment and existing tissues (Qin et al. 2009). The researcher Bull (2011) also emphasized the significance of actinomycetes, which are recovered within the extreme biosphere.

Tengchong County in Yunnan province, southwest China, has a large number of geothermal springs. Some investigation has been carried out to evaluate the microbial ecology even at these hot springs (Song et al. 2009). Song and his colleagues have revealed the actinobacterial biodiversity at Tengchong hot spring mixtures and two related hot springs situated in Nevada (USA) and Kamchatka (Russia) through a culture-independent approach. The clones recovered in hot springs at temperatures up to 81 °C revealed the occurrence of scarce actinobacteria of the suborder *Frankineae* as well as other uncultured actinobacteria. Song et al. (2010) further reported that temperature may control over pH the biodiversity of *Crenarchaeota* inhabitation in hot springs. Hedlund et al. (2012) revealed the existence of chief thermophilic bacteria under Rehai geothermal regions from Tengchong concerning the phylum *Deinococcus-Thermus*. The majority of bacteria reported in the above region were capable of producing thermostable polymer denaturing enzymes. In another investigation, the diverse candidates in order *Aquificales* were revealed under geothermal springs (Hedlund et al. 2015).

Table 5.1 Bioactivity of compounds extracted from various Actinomycetes

Compounds	Actinomycetes	Action
Sclerothricin	*Streptomyces sclerogranulatus*	Antifungal activity
Lomofungin	*Streptomyces lomondensis*	Antifungal
Spoxamicin	*Streptosporangium oxazonolinicum*	Antitrypanosomal
Antimycin	*Streptomyces* sp.	Antifungal
Avermectin	*Streptomyces avermitilis*	Antiparasitic
Rosamicin	*Micromonospora rosaria*	Antibacterial
Roseoflavin	*Streptomyces dawavensis*	Antibacterial
Validamycin	*Streptomyces* sp.	Antifungal
Rifamycin	*Micromonospora rifamycinica*	Antibacterial

The reports on cultivable actinobacteria among these geothermal regions are rare. Consequently, it is essential to study the cultivable actinomycetes among these hot springs. It is frequently realized that a wide variety of actinomycetes recovered from unique habitats comprising medicinal plants, marine sponges, fish guts, limestone habitats, or samples from the gulf are well connected with antimicrobial abilities (Table 5.1) (Gottardi et al. 2011). One of the essential antibacterial molecules abyssomicin synthesized by the marine organism *Verrucosisporamaris* AB-18-032 has an intricated structure and biosynthetic pathway (Riedlinger et al. 2004). Abyssomicin inhibits the biosynthesis of aromatic amino acids, i.e. it targets the biosynthetic pathway present in plants, parasites and microorganisms, but is absent from vertebrates. The antimicrobial potentials are mostly connected with the expression of biosynthetic genes. It is consequently important to determine the ability to synthesize the bioactive molecules in association with the different varieties of actinomycetes that occur in the habitat. Till today, the greatest part of the screening techniques against biosynthetic potential depend on amplification of specific domains of the gene cluster using degenerated primers (Donadio et al. 2007). Primers were designed or premeditated based on the conserved properties of the PKS (polyketide synthases) and NRPS (non-ribosomal peptide synthetases), particularly the KS (keto synthase) domain, and adenylation domain of NRPS, there through enabling to determine variabilities under gene clusters (Liu et al. 2016). The present chapter also reveals a culturable group of actinomycetes present in the hot springs from various parts around the globe and their biosynthetic gene profiles.

The entire free-living bacterial communities are usually challenged within the environment; bacterial communities exist based upon their abilities to adapt to rapid exposure towards the harsh environmental conditions. For example, soil bacteria could encounter rapid osmotic stress caused due to flooding, rain, and desiccation (Sleator and Hill 2002; Poolman et al. 2004; Kysela et al. 2016; Claessen et al. 2008; Höltje 1998). The bacterial cells characteristically give a response towards the osmotic fluctuations through changing the osmotic potentials with their cells, either through importing or exporting the compatible solutes and ions (Jayachandra et al. 2012a). Whereas these quick responses classically occur instantly after cells have been challenged towards the altered environments, they also have the potential to modulate the expression of metabolic activities of pathways consisting of vital enzymes (Jayachandra et al. 2012b). How can osmotic fluctuations affect cellular morphology has not been completely established. The cell shape is hugely sheltered by the presence of a cell wall, which is an extremely dynamic structure that acts as the key barrier and provides the necessary osmotic protection (Mohan et al. 2015a).

The biosynthesis of their major constituents, PG (peptidoglycan), encompasses the activity of the huge part of a protein complex which supportively builds and integrates new PG precursors exclusively in developing glycan strands over cell surface (Mohan et al. 2015b). These strands are later cross-linked to shape as a single, massive structure that encircles cells (Jayachandra et al. 2018). The position in the insertion of a new PG is a foremost different between the planktonic bacteria, which grow through the extension of the lateral cell wall, and actinomycetes, which can grow by apical extension and thus inserting the new PG structure at the cell poles

(Flärdh and Buttner 2009; Claessen et al. 2014). Actinomycetes possess an extensive diversity of morphologies, which include bacteria from the genera *Rhodococcus* (cocci), *Corynebacterium,* and *Mycobacterium* (rods), *Kitasatospora* and *Streptomyces* (mycelia) or even *Arthrobacter* (multiple shapes) (Barka et al. 2016). Taxa belonging to these genera are capable of modifying their morphology to adapt to harsh situations. For instance, *Rhodococcus* sp. generally originated under arid conditions; they have capacity to adapt in aridity conditions e.g. by changing their lipid composition, and also by arranging into small fragmented cells (Alvarez et al. 2004). The *Arthrobacter* species display excellent tolerance to cold stress and desiccation conditions. After exposure to hyperosmotic stress, these cells can change the production of osmoprotectants and shift among rod-shaped and myeloid cells (Höltje 1998).

Although the cell wall can be a vital component for almost all types of bacteria, the greatest part of species can be engineered within laboratory conditions to synthesize L-forms, which are forms that allow to proliferate without their wall (Studer et al. 2016). Characteristically, L-forms are produced by exposing walled bacteria to higher levels of lysozyme and antibiotics, which target cell wall biosynthesis in media comprising increased levels of osmolytes (Studer et al. 2016; Innes and Allan 2001). The formation of cell wall-dedicient L-form bacteria, which proliferate indefinitely without a the cell wall requires two mutations that fall in different classes. The primary class of mutations in wall precursor biosynthesis may lead to intensification of membrane synthesis, either straight away by enhancing fatty acid biosynthesis or indirectly through lowering the cell wall synthesis (Mercier et al. 2013; Errington et al., 2016). The secondary type of mutations involve oxidative damage through the reactive oxygen substances that is disadvantageous to the propagation of L-forms (Kawai et al. 2015). Especially, the propagation of L-forms is autonomous of the FtsZ-dependent division mechanism (Mercier et al. 2014). Alternatively, their propagation can also be deliberated exclusively via biophysical processes. A disparity among the cell surface area to volume ratio can lead to unpremeditated blebbing and the generation of progeny cells. The clean biophysical phenomenon of L-form propagation or proliferation is not strain-specific. The present thought has led to the hypothesis that initial life forms proliferated in the same way well before the cell wall evolution (Errington 2013). Whether the L-forms have functional significance under modern bacteria, though, is unclear. At this point, reports show that the filamentous actinomycetes have a natural potential to express the CWD (cell wall deficient) cells once exposed to higher concentrations of osmolytes (Ramijan et al 2018). These types of novel identified cells, which are known as S-cells, synthesize PG precursors and are capable of altering the canonical mycelial mode of growth—strangely, after prolonged exposure to a higher osmotic stress situation. The S-cells can attain mutations that can enable them to propagate within the CWD state as L-forms. These observations conclude that the removal of S-cells and their changeover in propagation to the L-forms is an innate adaptation approach in filamentous actinomycetes produced during continued exposure towards osmotic stress.

Organisms that are resistant to a substantial amount of environmental stress produced via altered situation are called as poikilophilic or poikilotrophic

according to Gorbushina and Krumbein (1999). Such environments are predominant in harsh circumstances for life. They contain extremely little water, low nutrients, extreme temperatures, and elevated levels of toxic compounds; nevertheless the conditions can be irregularly, intermittently changed, and also converted suitable for microorganism's growth and development. For instance, the poikilotrophic organisms are known to be rock-dwelling fungi and prokaryotes. Microbes present under desert ecosystems or arid-based soils by extreme lower nutrients, highly variable temperature, as well as precipitation may also be viewed as poikilotrophic.

The added perception of the extreme environments is mainly dependent on the factual conditions, in particular, on chemical and physical boundaries during cellular processes. These confining factors are connected to the characteristic features of biological molecules and biochemical reactions, besides the limitations of cellular sustainability. These extreme environmental conditions, as per one perception, can be described as those close to the confines for cellular integrity and functioning, which is mainly a limitation for enzymatic activities or detrimental to the biomolecules (Rothschild and Mancinelli 2001; Marion et al. 2003). One of the finest examples of the physical confines of life is the absence of liquid water. As we all know that without water, there is no life; the main reason is that this solvent is basic essential for every biochemical reaction to occur. Other similar limitations are extreme or harsh conditions, distinctive ends of gradients, higher or lower pHs and temperatures, higher doses of radiation and higher salinity, nutritional depletion conditions, and higher concentrations of toxic substances. In spite of these harsh physiochemical constraints for biochemical processes and the strength of biological molecules, growing microbes at extremes were evolved adaptive in their lifecycle and processes. Common serial dilution methods can be used in isolating actinobacteria (Fig. 5.1).

The microorganisms that sustain their lives under such harsh conditions can be divided into various categories rendering towards the nature of adaptability. They are thermophilic (resistance to higher temperatures), psychrophiles (lower temperature), halophiles (higher salts), alkaliphiles (higher alkalinity), and xerophiles (dried and desiccation). The corresponding suffix -phile is mainly used for those organisms requiring extreme state for their growth, and troph or tolerant refers to those organisms tolerant towards the relative extreme conditions. These organisms are

Fig. 5.1 Serial dilution adapted in isolation of Actinobacterial species

further categorized by considering the characteristic features of xerophile and psychrophilic nature.

The extremophilic organisms are adapted to and confined by a narrow range of environmental circumstances, and some may thrive well or must require extreme conditions. Extremotolerant or extremotrophic microbes can sustain lives and also propagate in a wide range of environmental situations. Usually, they can tolerate extreme environments but generally grow best in moderate conditions. Under the ecological perspective, the major growth approach of each organism is frequently defined as R and K strategy (Panikov 1999). The microorganisms, along with the main K-strategy, could live close to the fetching ability of the environment. They have comparatively lower growth rates yet require this through modest advantages such as lower maintenance energy, high affinity for substrate molecules, accumulation of storage polymers, and high affinity for the substrate molecules. Contradictorily, R-selected living organisms possess the ability of fastidious propagation and quick response to profuse and readily presented substrates. A particular life strategy, described as L-strategy, is generally used to characterize organisms that are screened under adverse conditions and are extremely resistant or tolerant to stress. This also comprises microbes by specific adjustment to artificial stress conditions. Therefore, soils can be characterized as extreme, which support the microorganisms that can tolerate anthropogenic disruption and harsh conditions caused by higher concentrations of contaminants and other toxic substances.

5.2 Physicochemical Parameters Limiting to Life

5.2.1 Water

Soil water occurs as free water within pores or adsorbed upon the surface or films within the soil particles. The soil water's position is defined through the water potential, which measures the energy per volume in relation to the pure reference water, and influences the passage of water below the ground. It is associated with the a_w (water activity) modification of free life among the soil water. Significant soil water likely has matric potential (reserved in pores or the energy by which soil water binds to the solid surface) and osmotic potential. Since these substances diminish the free energy of the water, the water potential is negative. This shows free energy in organisms essentially used to remove water from the soil. With reduced water potential, the water turns into limited access, and also the level of stress in organisms increases. The higher level of soil water stress can be seen occasionally in the greatest portion of the soils, in climates or environments by sufficient precipitation, wherever there is water accessibility based on soil composition, plant cover, and precipitation drainage. The prokaryotes in distinct environments, such as soil, water bodies, nearby particles, or within water-filled pores are sensitive to water diminution.

Soil fungal communities are generally more resistant to water stress than bacterial communities. They have better resistance to adapt to moisture fluctuations because they possess hyphal growth, allowing them to cover dried pores and, on the other hand, receive water using small pores, where the water remains for extended periods (Killham 1994). The microbial cells have a turgor pressure by attracting solute molecules inside the cell's presence slightly higher than outside. The difference in water action within the atmosphere is rapidly trailed by a water flux across the semipermeable membrane from higher to lower water potential. As a result, there is a risk of the denaturation and swelling of the cell inside hypotonic solutions, or dehydrating inside a hypertonic solution (Kempf and Bremer 1998). Henceforth, the cell must preserve an intracellular water level. Consequently, the cell should sustain an intracellular water potential corresponding to that of the exterior of the cells.

5.2.2 Soil Microbes at Extreme Environments

To sustain the cell unity at mesophilic temperatures (11–40 °C) generally necessitates soil water potential higher than −4 MPa (0.95 a_w) for maximum bacterial populations and slightly above −22 MPa (0.86 a_w) for actinobacteria and most of the fungi. It is usually measured that the lower limit of water potential for a living being is −70 MPa (0.60 a_w) (Zvyagintsev et al. 2005), but in recent years it has been confirmed that spore elongation and germination of several actinobacteria could happen at a water potential of −96 MPa (0.50 a_w; described (Doroshenko et al. 2005). Few organisms, for instance, lichens, can even persist on water vapor.

The xerophile or xerotolerant organisms can resist salinity and water stress since they can counterpoise a lower water potential in the atmosphere by acquiring extremely soluble small molecules inside the cytoplasm (Kempf and Bremer 1998). These solute molecules could be inorganic or organic compounds (polyols, carbohydrates, amino acids, ammonium compounds). As a result of the regulation of solutes, the intracellular water potential decreases or increases. These compounds can then stimulate and control particular enzyme activities, however, without inhibiting the overall metabolism of the cells, and so they are labeled as osmoprotectants or compatible solutes. Few osmoprotectants can be constitutively synthesized, while the others are induced. The energy consuming process of osmoregulation is an ubiquitous mechanism of soil microbes to maintain intracellular enzyme activities for long duration adaptation under stress circumstances as unicellular, in cell aggregates or biofilms, or in association with plants (Bonatelli et al. 2021).

The rest of the approaches which protect bacterial communities from dehydration include the biosynthesis of exopolysaccharides and pigments that will help to preserve water molecules (Bonatelli et al. 2021; Gorbushina and Broughton, 2009; Wright et al. 2005). The construction of cell microaggregates and biofilms to maintain sufficiently high a_w (water activity) is perceived further to safeguard

microbes from drying out. Actinobacteria are more specifically osmoregulated, as the cell membranes of these microbes restrict permeabilities and retain organic solutes as well as salt ions inside the cells. Just like fungal strains, they can segregate into dormant cells and they are even more resistant to being dried (Chen et al. 2017; Dose et al. 2001). Several halophilic actinobacteria were reported from different saline habitats.

5.2.3 Saline Environments

The stresses caused by osmotic pressure or salts, and water scarcity are interconnected, as the solutes affect the a_w (water availability). However, the water-caused stress on microorganisms occurs recurrently in almost all terrestrial habitats, while high salt concentration is naturally evident in only very small fraction of the environment. The earth layers with high salinity have a very uneven water distribution over time, location and altitude. Such alterations can cause particular stress for the microorganisms and increase the risk of death due to which there is a huge requirement of instantaneous response towards the desiccation conditions to adjust at higher salt concentrations. Saline terrestrial counterparts are distinctive for naturally arid areas employing high evaporation rates. They can also be discharge pollution caused by mining, chemical or metallurgical industries. Microorganisms in such saline soils are usually salt-tolerant (halotolerant) or halophilic. On the other side the extreme halophilic organisms can resist very limited water potential and grow well even at −40 Mpa (0.75 a_w, with respect to saturated NaCl). The maximum limit is generally assessed by the solubility of salt in preference to the normal functioning of the cells (Brown 1976). Corresponding to xerotolerant and xerophilic organisms, the halotolerant and halophilic microbes acquire a wide variety of compounds in their cytoplasm (compatible solutes or osmolytes) in response to the external osmotic stress (Roberts 2005). Commonly, bacteria synthesize and accumulate compatible solutes, which are mainly nonionic or zwitterions, such as carbohydrates, amino acids, polyols, neutral peptides, glycine betaine, methylamines, proline, and other derivatives. The osmoprotectants of archaea are frequently inorganic cations usually absorbed by passive diffusion or through selective ion transportation systems. Most of the archaea bacteria have evolved proteins rich in negatively charged acidic amino acids, which require cation counterions, like K^+ ions, for appropriate protein folding and activity. The organic osmoprotectants accumulated inside the archaea resemble bacterial counterparts in structure; however, most of them are anionic because of negatively charged functional groups, such as phosphate, sulfate, or carboxylate (Roeßler and Müller 2001; Martin et al. 1999). Furthermore, concerning their functions as osmotic gated active compounds, the compatible solutes may also function as protein stabilizers and activity regulators.

5.2.4 Temperature

Optimum temperature plays a vital role in the sustenance of living beings similarly to microbes. The temperature range appropriate for life is typically associated with the freezing and boiling points of water. Yet, many microorganisms have established mechanisms to extend their lives to even broader ranges of temperatures that exceed the freezing and boiling values of water at atmospheric pressure. Currently, the temperature restrictions for microbial life and activities are realized as ranging from about −40 °C to +130 °C (Shivlata and Satyanarayana 2015; Kashefi and Lovley 2003). Microorganisms adapted to very cold Arctic climatic conditions have been revealed to be active at temperatures as low as −10 to −20 °C (Panikov and Dedysh 2000). Even below the freezing temperatures, there may still be water as a liquid layer adsorbed on the surface of the soil particles, hygroscopic water (Steven et al. 2006). Substantial cell membrane modifications are required for the microbial growth at low temperatures to maintain the fluidity vital in cellular functions and nutrient transportation across the cell membrane. As the temperature lowers, unsaturation, cyclicity, chain length, branching, and the quantity of membrane fatty acids can change (Suutari and Laakso, 1992, 1993, 1994). Life at low temperatures is much enabled by accumulating within the cytoplasm the antifreeze components, which may be at least partially the same as compatible solutes in the osmoprotection.

5.2.5 Microorganisms at Extreme Soils

Several strategies have been developed by the microbial communities for acclimation to soil even under extreme conditions. For example, adaptation to lower temperatures by bacteria involves modifications among cell membrane constituents along with cold-active proteins and ribosomes comprised in basic cell activities or functions (Suutari and Laakso, 1994; Cavicchioli et al. 2000). The adaptation to extreme weather conditions in soil, for example, may imply such substantial changes in the molecular constituents of a cell, that an organism can no longer live outside the extreme. Cold-growing psychrophiles often have an optimal temperature of 15 °C and below, and can withstand their life even at 20 °C, while cellular components can become labile a few degrees above the optimum. Another interesting feature of the psychrotropic organisms is that they can even grow well at below 0 °C, while their optimal temperature is about 15 °C, and their maximum temperature is even 30 to 40 °C (Suutari et al. 1990; Margesin and Miteva, 2011). Price and Sowers (2004) have investigated temperature-based metabolic activities in various environments, including permafrost; clouds; subsurface aquifer, freshwater, and marine sediments; lakes, oceans, ice and snow. Based on metabolic rates, these researchers classified microbial communities into three categories: first one, rates adequate for growth; second, transitional rates required for maintaining the cellular

functions, however too low nutrient levels for growth; third category, the minimum rate required for survival of the largely dormant cells and to repair the damaged molecules. The rate of metabolism was tremendously low at -40 °C, while microbes from different environments had surprisingly similar growth rates at the same temperature (Price and Sowers 2004).

In contrast, soil bacteria called thermotrophs and thermophiles have adapted to high temperatures. The minimum growth temperature for thermotrophic microorganisms is 20–30 °C, and for thermophilic over 50 °C. At the upper-temperature limit, one or more key molecules of cellular metabolism become labile or denatured, such as a protein or nucleic acid, and consequently, the ability of these molecules to do their functions is lost. The temperature of both natural and manmade soils can exceed 50 °C. The thermophilic organisms have been recovered from a wide variety of soils such as tropical deserts, volcanic eruption and geothermal soils, and compost heaps. Microbes possess essential mechanisms for safeguarding their membranes, nucleic acids and proteins from damage caused by high temperature. SEM (scanning electron microscopy) can be used to know about the cellular membrane morphology of actinobacteria (Fig. 5.2). The biological molecules of thermophilic microbes are thermally stable and maintain their active structure at temperatures that usually

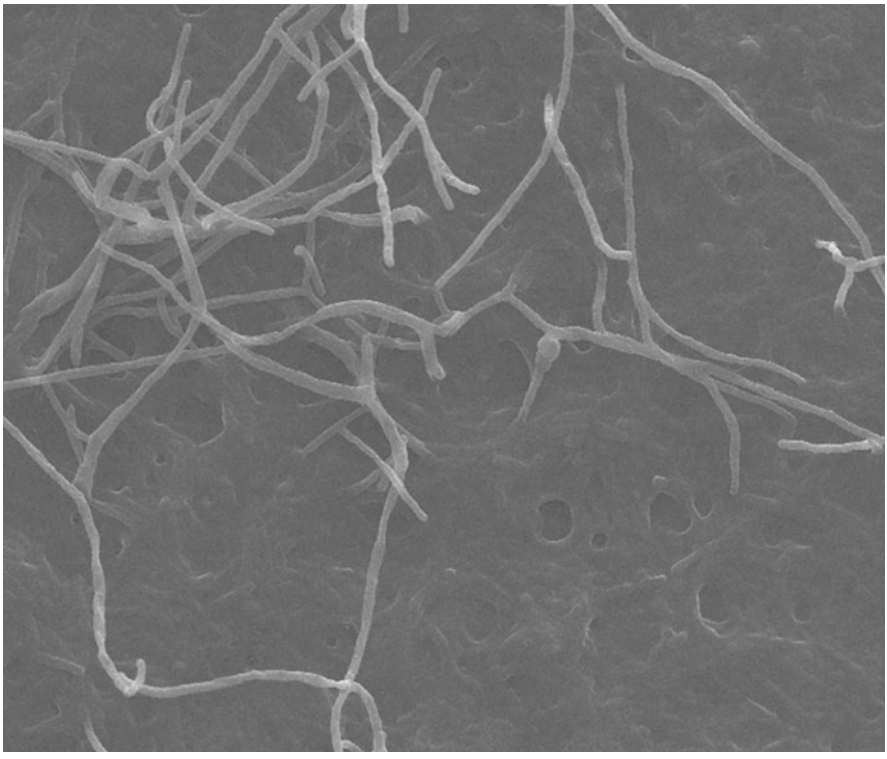

Fig. 5.2 A typical scanning electron micrograph of *Streptomyces* sp

inactivate the proteins, nucleic acids, and lipids in mesophiles (Rothschild and Mancinelli 2001). Protein stabilization is attained by changes in amino acid residues toward charged and hydrophobic residues; and suitable for stabilization by hydrogen and disulfide bonds; as well as salt bridge-forming ones (Xu et al. 2020). The nucleic acid molecules can be thermostabilized, for example, by increasing GC content in sequences, nucleotide arrangement and methylation, and by means of histone-like proteins (Trivedi et al. 2005). At elevated temperatures, the cell membrane containing fatty acids can increase chain length and saturation, and alter branching and cyclization along with the changes in lipid quantity. Such variations within the membrane bilayer structure reduce membrane fluidity and improve thermal stability (Suutari and Laakso 1994).

5.2.6 *Effect of pH*

The pH values for habitats in which bacteria, archaea, fungi and algae have been found to grow range from pH 0 to 13, although most of the prokaryotic bacteria grow at a comparatively narrow pH range of about 5.5-9.0, that is close to neutral. It appears that regular adaptation to the extremely high or low pH of bacterial habitats is to control the pH within the cell to preserve it close to neutral. For example, the acidophiles typically grow at a pH ranging from 0 to 3. such as *Acidithiobacillus ferrooxidans* an acidophilic bacterium formerly known as *Thiobacillus ferrooxidans*, prefers to grow under acidic conditions near about pH 1. Despite the very low surrounding pH, the bacterium efficiently excludes protons through dynamic pH control mechanisms, and the cytoplasmic pH is kept at about 6.0 (Marion et al. 2003 Krulwich et al. 2011). The optimum pH for microbial enzymes is typically neutral to alkaline, still, the pH optimum for the enzymes of acidopiles and alkalophiles can deviate much from this (Thapa et al. 2019). Concerning archaea bacteria, they include few acidophiles that can grow in extremely acid environments, even at pH 0. For instance, iron oxidizing bacteria of the genus *Ferroplasma*, lacking cell wall, have been recovered at pH values of 0-2.5, with optimal growth at 1.2. The species from this genus have been isolated especially in mining waste waters worldwide. These groups of organisms can deploy sulfide ore metals and tolerate high quantities of hazardous heavy metals (Golyshina and Timmis 2005; Baker-Austin et al. 2005). The extreme thermoacidophilic archaea *Picrophilus oshimae* and *Picrophilus torridus* were recovered from solfataric habitats in Japan. They were growing at about pH 0 while their optimum growth pH was 0.7 and at 60 °C. The isolates did not growth below pH 3.5 and the cells were lysed at pH values above 5 (Schleper et al. 1995). Acid tolerant and acidophilic microbes have cyclic structures (e.g. cyclopropane or cyclopentane rings) and alkyl, especially methyl-branced side chains in membrane lipids. The basic adaptation of bacteria to acidic conditions is promoted through unique tetraether lipids within the cell membranes; caldarchaetidylglycerol tetraether lipids with isoprenoid core in *Picrophilus* spp. and *Ferroplasma* spp.; and high lipopolysaccharide concentration in the cell surface

of *A. ferrooxidans* (Golyshina and Timmis 2005; Arredono et al. 1994; Siliakus et al. 2017). The glyceroldialkyl-glycerol-tetraether lipids of acidophilic archaea form a monolayer membrane, which is a very impermeable to protons. Different types of proton pumps repels the cytoplasmic protons, including primary proteon pumps, secondary acitve transporters and proton-coupled ATPases (Siliakus et al. 2017; Krulwich et al. 2011). In the *P. torridus* genome, the quantity of genes for secondary transport systems was much higher than for ATP-consuming primary transporters, indicating that the high surrounding proton concentration is related to transportation processes (Futterer et al. 2004).

At the other end, the extreme alkaliphilic microbes have a pH optimum of about 9 to 11, while they do not grow at pH below 7.5. The cytoplasm pH is commonly about 2 units below the external pH, indicating that the cell membrane bears a barrier to OH^- ion flow, and an efficient internal proton transportation system, e.g., pH homeostasis associated with K^+/H^+ or Na^+/H^+ antiporters, and ATP synthase translocating protons (Krulwich et al. 2011). The pH of the soil usually ranges from 4.0 to 8.5 (Lynch 1979). The soil covered with organic matter is typically acidic in nature as the plant litter decay inclines to lower the pH. The redox reactions can also affect the pH of the soil. For instance, under suitable conditions, the oxidation of FeS_2 and NH_4^+ results in the synthesis of acids H_2SO_4 and HNO_3, respectively, which lowers the pH. Therefore, it seems that the prokaryotes can stimulate their nearby environment. Because of the metabolic activities, the pH within the organism's microhabitats can differ significantly from that in bulk soil (Marion et al. 2003; Siebers and Kruse 2019; Dennis et al. 2009; Frerichs et al. 2013). The highest levels are most common among soils comprising alkaline minerals (soda lakes), sewage plants, or can be seen temporally in circumscribed zones due to, for example, animal excretions. Related to the microbial growth pH ranges, Actinobacteria from the genus *Streptomyces* spp. grew over a wide pH range of 5.5-11.5 on media with high organic load, whereas in nutritionally scarce media, the strains grew only near neutral pH of 7-10. The growth pH ranges, and those for optimal growth and sporulation appreared to be nutrient-dependent rather than strain-specific properties (Kontro et al. 2005).

5.2.7 Exposure to Intensive Radiation

It is a well-known fact that the exposure of microbes to the UV (ultraviolet) light and ionizing radiation kills these organisms, even though very few of them are astonishingly resistant to higher doses of radiation. In general, the highest associations are detected among resistance against radiation, DNA damaging substances, and desiccation (Shukla et al. 2007). The strain *Deinococcus radiodurans* is among the greatest radiation-tolerant organisms identified, and capable of surviving high doses (nearly 1000 J/m^2) of ultraviolet light and higher than 20 kGy of γ-radiation (Rainey et al. 2005). The identified microbe is strangely well adapted to extreme radiation conditions, and it can also live in drought and nutrient-depleted situations

(Battista 1997). The red-pigmented spherical bacterium was identified in 1957 from meat, which was spoiled in spite of being treated with radiation. The bacteria from the genus are extensively distributed. They have been observed in a diversity of terrestrial environments as well as in the arid soils from the Antarctic Dry Valley (Niederberger et al. 2015; Matrosova et al. 2017). Desiccation and radiation can be the main reasons for DNA damage. Tolerance in *Deinococcus* species is described to be through a specific competent system for DNA repair mechanism. The dehydration or elevated radiation gives rise to a breakdown in the double-helical structure of DNA. *Deinococcus* spp. generally have 4–10 genome copies, and repairs the fragments through the intrachromosomal recombination process, which reconstructs the entire chromosome in about a few hours (Sale 2007). The prokaryotic and algal organisms in the environment have adaptive mechanisms, which permit them to evade, or in any case minimizing the damage by UV. For instance, biological molecules such as mycosporine-like amino acids, carotenoids, melanoids, scytonemin, and pigments protect against oxidative damage and extreme radiation (Fuentes-Tristan et al. 2019).

5.2.8 Low Nutrient Conditions

Environments are also categorized according to life-threatening physical and chemical conditions. They can be permafrost soil and desert ecosystems that are always limited in organic as well as inorganic nutrients. Several native microbes inhabited in such harsh environmental conditions generally belong to oligotrophs, which means that these organisms have adapted to lower nutrient sources (Steven et al. 2006; Mohammadipanah and Wink 2016). Yet, there are a plenty of oligotrophic prokaryotes that can be observed in soils at higher concentrations of organic matter. Under such low substrate conditions, the main organic materials found are humic substances, which are recalcitrant and not easily degradable or decomposable. Bacteria under the phyla actinobacteria includes oligotrophic members. These microbes are particularly profuse in soils with the scarcity of nutrient sources, and their diversity and density may decrease after modification by a readily accessible carbon source. The prokaryotes adapted to grow and establish in oligotrophic conditions are commonly K-selected, or K-strategists, i.e. populations vary near the carrying capacity of the environment. They possess slow growth rates, and competent substrate uptake metabolism with low, down to nM concentrations half-saturation factors for the uptake or absorption of nutrients. Microorganisms adapted to such physiology are frequently unable to grow under nutrient conditions (Bernard et al. 2007; Mohammadipanah and Wink 2016; Steven et al. 2006).

5.3 Microbial Diversity and Community Structure in Extreme Soils

The microbial diversity designates various features on variability and complexity within the microbial communities and their populations. This contains the genetic variations among species or taxa, differences in the community structure, complexity of interactions, number of associations, trophic stages, and the conclusive constraint in describing functional diversity. Biodiversity can be pronounced in numerous ways; by accounting for taxonomic clusters, or as individual species numbers (typically diversity indices), that depend mainly on the number of species or OTUs (operational taxonomic units). The diversity can also be characterized based on phylogenetic trees (evolutionary trees), or comprehended through analyzing the numerous functional associations. In modest and steady environments, the soil microorganisms' communities could grow into complex organizations with high functional and phylogenetic diversities. Hence, such microbial communities are among those that are difficult to characterize through phenetic and genetic techniques. Apart from this, the enormous divergence among the culturable and actual cell numbers in the specific environments has pushed towards the awareness of the great plate count anomaly (Ward et al. 1990). Following this perception, the biodiversity analysis dependent on the cultivable type of microorganism is being replaced by DNA metagenomics. The indicated technique possesses the construction of multifaceted community libraries that leads to the cloning of huge genomic DNA constituents (>40 kb) and then the environmental DNAs inserted into BAC (bacterial artificial chromosome) vectors or fosmid vectors are sequenced. One of the major challenges of this application is the ability to extract high molecular weight DNA in the highest purity. The metagenome technique can be utilized to produce a huge amount of information on the possible functioning of each distinct microbial taxon in environmental soils to investigate the extensive role of microbes within the specific ecosystem. The most widely used technique to assess microbial diversity is PCR (polymerase chain reactions), which is intended for the amplification of the 16S rRNA gene pertaining to the community (Rondon et al. 2000; Pace et al. 1986). The amplified products of the gene may be cloned, and these clones can be recognized through DNA sequencing methods, and used later for comparative patterning analysis. These amplification and gene sequencing methods are very time inefficient and also labor intensive for continuous analysis of large sample volumes. To address these problems, sequencing technologies have evolved towards high-throughput analysis of sequence data without cultivation and PCR amplification approaches. In particular, shotgun sequencing rapidly provides a huge amount of information about environmental microbial communities and their functions (Jo et al. 2020). Mere screening for any deviations in space and time, the microbial community fingerprinting method like DGGE (denaturing gradient gel electrophoresis), T-RFLP (terminal restriction fragment length polymorphism), ARISA (automated ribosomal intergenic spacer region analysis), and SSCP (single conformational polymorphism analysis) for the PCR amplified products of genes are often used.

All these techniques provide a vast amount of information concerning the numerically dominant members within the community, and can motivate to select one specific method as an alternative to another based on the equipment and expertise that are available. Besides, the evolutionary affiliation of the abundant organisms can be evaluated depending on successful sequence analysis, such as the DGGE-separated PCR products (Hewson et al. 2007; Nocker et al. 2007). The rDNA-dependent fingerprint analysis and amplification methods have led to the description of numerous prokaryotic species; among them, some are novel divisions. Several investigations have indicated that a significant proportion of the 16S rRNA sequences attained from a specific soil type reveal unique previously unknown microorganisms. Because of the heterogeneity of the soil communities, and the limitations of amplification and sequencing-dependent approaches, only a fraction of microbes in environmental soils have been revealed employing such methods, and researchers' knowledge and understanding of soil microbial diversity is yet very restricted. In-depth studies using advanced molecular approaches are really useful in the determination of microorganism's communities (Urich et al. 2008; Zhang et al. 2013; Zielinska et al. 2017).

5.4 Conclusion

Irrespective of the constraints presented, the culture-independent molecular approaches have prolonged the researcher's perception of extreme soils as niches for many microbes. Numerous studies have revealed the effects formed by human activities in harsh environments, and contamination among microbial communities. Microorganisms within soil with heavy metal-contaminated sludge were studied concerning the quantity and diversity of various bacterial genomes of the same size as the typical *E. coli* genome. The microbial multiplicity of the metal-contaminated soil was condensed based on the grade of pollution. The concentration of DNA recovered from the clean soil sample, and from the soil with lower and higher grade of metal pollution corresponded to a divergence of approx. 16000, 6400 and 2000 genomes, respectively (Sandaa et al. 1999). Therefore, it appears that genetic diversity could be a leading indicator in the analysis of environmental damage caused due to pollution.

Moreover, it has also been recognized that environmental stress stimulates variation among the community structures. The contaminations and disturbance may lead to a reduction in the functional availability and consistency of species, as dominant taxa may be replaced in the community. Based on this, it is concluded that microbial diversity can be inclined within extreme environmental stress situations. Their community structure may have been altered to the degree that the functioning of the community has changed.

References

Alvarez HM, Silva RA, Cesari AC, Zamit AL, Peressutti SR, Reichelt R, Keller U, Malkus U, Rasch C, Maskow T, Mayer F, Steinbüchel A (2004) Physiological and morphological responses of the soil bacterium *Rhodococcus opacus* strain PD630 to water stress. FEMS Microbiol Ecol 50:75–86

Arredono R, Garía A, Jerez CA (1994) Partial removal of lipopolysaccharide from *Thiobacillus ferrooxidans* affects its adhesion to solids. Appl Environ Microbiol 60:2846–2851

Baker-Austin C, Dopson M, Wexler M, Sawers RG, Bond PL (2005) Molecular insight into extreme copper resistance in the extremophilic archaeon '*Ferroplasma acidarmanus*' Fer1. Microbiology 151:2637–2646

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43

Battista JR (1997) Against all odds: the survival strategies of *Deinococcus radiodurans*. Annu Rev Microbiol 51:203–224

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Bérdy J (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot 65:385–395

Bernard L, Mougel C, Maron P-A, Nowak V, Lévêque J, Henault C, el Zahar Haichar F, Berge O, Marol C, Balesdent J, Gibiat F, Lemanceau P, Ranjard L (2007) Dynamics and identification of soil microbial populations actively assimilating carbon from 13C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. Environ Microbiol 9:752–764

Bonatelli ML, Lacerda-Júnior GV, dos Reis Junior FB, Fernandes-Júnior PI, Melo IS, Quecine MC (2021) Beneficial plant-associated microorganisms from semiarid regions and seasonally dry environments: a review. Front Microbiol 11:553223

Brown AD (1976) Microbial water stress. Bacteriol Rev 40:803–846

Bull AT (2011) Actinobacteria of the extremobiosphere. In: Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO (eds) Extremophiles handbook. Springer, Tokyo, pp 1203–1240

Cavicchioli R, Thomas T, Curmi PMG (2000) Cold stress response in archaea. Extremophiles 4: 321–331

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc Natl Acad Sci U S A 100(Suppl 2):14555–14561

Chen X, An L, Fan X, Ju F, Zhang B, Sun H, Ziao J, Hu W, Qu T, Guan L, Tang S, Chen T, Liu G, Dyson P (2017) A trehalose biosynthetic enzyme doubles as an osmotic stress sensor to regulate bacterial morphogenesis. PLoS Genet 13:e1007062

Claessen D, Emmins R, Hamoen LW, Daniel RA, Errington J, Edwards DH (2008) Control of the cell elongation-division cycle by shuttling of PBP1 protein in *Bacillus subtilis*. Mol Microbiol 68:1029–1046

Claessen D, Rozen DE, Kuipers OP, Søgaard-Andersen L, van Wezel GP (2014) Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. Nat Rev Microbiol 12:115–124

Dennis PG, Hirsch PR, Smith SJ, Taylor RG, Valsami-Jones E, Miller AJ (2009) Linking rhizoplane pH and bacterial density at the microhabitat scale. J Microbiol Methods 76:101–104

Donadio S, Monciardini P, Sosio M (2007) Polyketide synthases and nonribosomal peptide synthetases: the emerging view from bacterial genomics. Nat Prod Rep 24:1073–1109

Doroshenko EA, Zenova GM, Zvyagintsev DG, Sudnitsyn II (2005) Spore germination and mycelial growth of streptomycetes at different humidity levels. Microbiology 74:690–694

Dose K, Bieger-Dose A, Ernst B, Feister U, Gómez-Silva B, Klein A, Risi S, Stridde C (2001) Survival of microorganisms under the extreme conditions of the Atacama desert. Orig Life Evol Biosph 31:287–303

Errington J (2013) L-form bacteria, cell walls and the origins of life. Open Biol 3:120143

Errington J, Mickiewicz K, Kawai Y, Wu LJ (2016) L-form bacteria, chronic diseases and the origins of life. Philos Trans R Soc Lond B Biol Sci 371:20150494

Flärdh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol 7:36–49

Frerichs J, Oppermann BI, Gwosdz S, Möller I, Herrmann M, Krüger M (2013) Microbial community changes at a terrestrial volcanic CO vent induced by sol acidification and anaerobic microhabitats within the soil column. FEMS Microbiol Ecol 84:60–74

Fuentes-Tristan S, Parra-Saldivar R, Iqbal HMN, Carrillo-Nieves D (2019) Bioinspired biomolecules: mycosporine-like amino acids and scytonemin from Lyngbya sp. with UV-protection potentialities. J Photochem Photobiol B 201:111684

Futterer O, Angelov A, Liesegang H, Gottschalk G, Schleper C, Schepers B, Dock C, Antranikian G, Liebl W (2004) Genome sequence of *Picrophilus torridus* and its implications for life around pH 0. Proc Natl Acad Sci U S A 101:9091–9096

Golyshina OV, Timmis KN (2005) *Ferroplasma* and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments. Environ Microbiol 7:1277–1288

Gorbushina AA, Broughton WJ (2009) Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. Annu Rev Microbiol 63:431–450

Gorbushina AA, Krumbein WE (1999) The poikilotrophic micro-organism and its environment – microbial strategies of establishment, growth and survival. In: Seckbach J (ed) Enigmatic microorganisms and life in extreme environments. Kluwer Academic, Dordrecht, pp 175–185

Gottardi EM, Krawczyk JM, von Suchodoletz H, Schadt S, Mühlenweg A, Uguru GC, Plezer S, Fiedler H-P, Bibb MJ, Stach JEM, Süssmuth RD (2011) Abyssomicin biosynthesis: formation of an unusual polyketide, antibiotic-feeding studies and genetic analysis. ChemBioChem 12: 1401–1410

Hedlund BP, Cole JK, Williams AJ, Hou W, Zhou E, Li W, Dong H (2012) A review of the microbiology of the Rehai geothermal field in Tengchong, Yunnan Province, China. Geosci Front 3:273–288

Hedlund BR, Reysenbach A-L, Huang L, Ong JC, Liu Z, Dodsworth JA, Ahmed R, Williams AJ, Briggs BR, Liu Y, Hou W, Dong H (2015) Isolation of diverse members of the Aquificales from geothermal springs in Tengchong, China. Front Microbiol 6:157. https://doi.org/10.3389/fmicb.2015.00157

Hewson I, Jacobson/Meyers ME, Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ Microbiol 9:923–933

Höltje J-V (1998) Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. Microbiol Mol Biol Rev 62:181–203

Innes CMJ, Allan EJ (2001) Induction, growth and antibiotic production of *Streptomyces viridifaciens* L-form bacteria. J Appl Microbiol 90:301–308

Jayachandra SY, Parameshwar AB, Mohan Reddy K, Sulochana MB (2012a) Characterization of extracellular hydrolytic enzymes producing extremely halophilic bacterium *Virgibacillus* sp. JS5. World J Sci Technol 2(2):23–26

Jayachandra SY, Anil Kumar S, Merley DP, Sulochana MB (2012b) Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. Recent Res Sci Technol 4(4):46–49

Jayachandra SY, Shoba H, Banapurmath NR, Hunashyal AM, Sulochana MB, Shettar AS, Ganachari SV (2018) Alternative and renewable bio-based and biodegradable plastics. In: Martínez L, Kharissova O, Kharisov B (eds) Handbook of ecomaterials. Springer International AG, Cham. https://doi.org/10.1007/978-3-319-68255-6_150-1

Jo H, Oh J, Park C (2020) Microbial community analysis using high-throughput sequencing technology: a beginner's guide for microbiologists. J Microbiol 58:176–192

Kashefi K, Lovley DR (2003) Extending the upper temperature limit for life. Science 301:934

Kawai Y, Mercier R, Wu LJ, Domínguez-Cuevas P, Oshima T, Errington J (2015) Cell growth of wall-free L-form bacteria is limited by oxidative damage. Curr Biol 25:1613–1618

Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Arch Microbiol 170:319–330

Killham K (1994) Soil ecology. Cambridge University Press, Cambridge

Kontro M, Lignell U, Hirvonen M-R, Nevalainen A (2005) pH effects on 10 *Streptomyces* spp. growth and sporulation depend on nutrients. Lett Appl Microbiol 41:32–38

Krulwich TA, Sachs G, Padan E (2011) Molecular aspects of bacterial pH sensing and homeostasis. Nat Rev Microbiol 9:330–343

Kysela DT, Randich AM, Caccamo PD, Brun YV (2016) Diversity takes shape: understanding the mechanistic and adaptive basis of bacterial morphology. PLoS Biol 14:e1002565

Liu L, Salam N, Jiao J-Y, Jiang H-C, Zhou E-M, Yin Y-R, Ming H, Li W-J (2016) Diversity of culturable thermophilic actinobacteria in hot springs in Tengchng, China and studies of their biosynthetic gene profiles. Microb Ecol 72:150–162

Lynch JM (1979) The terrestrial environment. In: Lynch JM, Poole NJ (eds) Microbial ecology: a conceptual approach. Blackwell Scientific, Oxford, pp 67–91

Marion GM, Fritsen CH, Eicken H, Payne MC (2003) The search for life on Europa: limiting environmental factors, potential habitats, and earth analogues. Astrobiology 3:785–811

Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Res Microbiol 162:346–361

Martin DD, Ciulla RA, Roberts MF (1999) Osmoadaptation in archaea. Appl Environ Microbiol 65: 1815–1825

Matrosova VY, Gaidamakova EK, Makarova KS, Grichenko O, Klimenkova P, Volpe RP, Tkavc R, Ertem G, Conze IH, Brambilla E, Huntemann M, Clum A, Pillay M, Palaniappan K, Varghese N, Mikhailova N, Stamatis D, Reddy TBK, Daum C, Shapiro N, Ivanova N, Kyrpides N, Woyke T, Daligault H, Davenport K, Erkkila T, Goodwin LA, Gu W, Munk C, Teshima H, Xu Y, Chain P, Woolbert M, Gunde-Cimerman N, Wolf YI, Grebenc T, Gostinčar C, Daly MJ (2017) High-quality genome sequence of the radioresistant bacterium *Deinococcus ficus* KS 0460. Stand Genomic Sci 12:46

Mercier R, Kawai Y, Errington J (2013) Excess membrane synthesis drives a primitive mode of cell proliferation. Cell 152:997–1007

Mercier R, Kawai Y, Errington J (2014) General principles for the formation and proliferation of a wall-free (L-form) state in bacteria. Elife 3:e04629

Mohan RK, Siva DS, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan RK, Siva DS, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) *In silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol App Sci 4(11):858–867

Mohammadipanah F, Wink J (2016) Actinobacteria from arid and desert habitats: diversity and biological activity. Front Microbiol 6:1541

Niederberger TD, Sohm JA, Guderson TE, Parker AE, Tirindelli J, Capone DG, Carpenter EJ, Cary SC (2015) Microbial community composition of transiently wetted Antarctic dry valley soils. Front Microbiol 6:9

Nocker A, Burr M, Camper AK (2007) Genotypic microbial community profiling: a critical technical review. Microb Ecol 54:276–289

Pace NR, Stahl DA, Lane DJ, Olsen GJ (1986) The analysis of natural microbial populations by ribosomal-RNA sequences. Adv Microb Ecol 9:1–55

Panikov NS (1999) Understanding and prediction of soil microbial community dynamics under global change. Appl Soil Ecol 11:161–176

Panikov NS, Dedysh SN (2000) Cold season CH_4 and CO_2 emission from boreal peat bogs (West Siberia): winter fluxes and thaw activation dynamics. Glob Biogeochem Cycles 14:1071–1080

Poolman B, Spitzer JJ, Wood JM (2004) Bacterial osmosensing: roles of membrane structure and electrostatics in lipid–protein and protein–protein interactions. Biochim Biophys Acta 1666:88–104

Price PB, Sowers T (2004) Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc Natl Acad Sci U S A 101:4631–4636

Qin S, Li J, Chen H-H, Zhao G-Z, Zhu W-Y, Jiang C-L, Xu L-H, Li W-J (2009) Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176–6186

Rainey FA, Ray K, Ferreira M, Gatz BZ, Nobre F, Bagaley D, Rash BA, Park M-J, Earl AM, Shank NC, Small AM, Henk MC, Battista JR, Kämpfer P, da Costa MS (2005) Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample. Appl Environ Microbiol 71:5225–5235

Ramijan K, Ultee E, Willemse J, Zhang Z, Wondergem JAJ, van der Meij A, Heinrich D, Briegel A, van Wezel GP, Claessen D (2018) Stress-induced formation of cell wall-deficient cells in filamentous actinomycetes. Nat Commun 9:5164

Riedlinger J, Reicke A, Zähner H, Krismer B, Bull AT, Maldonado LA, Ward AC, Goodfellow M, Bister B, Bischoff D, Süssmuth RD, Fiedler H-P (2004) Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine *Verrucosispora* strain AB-18-032. J Antibiot 57:271–279

Roberts MF (2005) Organic compatible salutes of halotolerant and halophilic microorganisms. Sal Syst 1:5

Roeßler M, Müller V (2001) Osmoadaptation in bacteria and archaea: common principles and differences. Environ Microbiol 3:743–754

Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, Tiong CL, Gilman M, Osburne MS, Clady J, Handelsman J, Goodman RM (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66:2541–2547

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Sale JE (2007) Radiation resistance: resurrection by recombination. Curr Biol 17:R12–R14

Sandaa R-A, Torsvik V, Enger ∅, Daae FL, Castberg T, Hahn D (1999) Analysis of bacterial communities in heavy metalcontaminated soils at different levels of resolution. FEMS Microbiol Ecol 30:237–251

Schleper C, Puehler G, Holz I, Gambacorta A, Janekovic D, Santarius U, Klenk H-P, Zillig W (1995) *Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. J Bacteriol 177:7050–7059

Shivlata L, Satyanarayana T (2015) Thermophilic and alkaliphilic Actinobacteria: biology and potential applications. Front Microbiol 6:1014

Shukla M, Chaturvedi R, Tamhane D, Vyas P, Archana G, Apte S, Bandekar J, Desai A (2007) Multiple-stress tolerance of ionizing radiation-resistant bacterial isolates obtained from various habitats: correlation between stresses. Curr Microbiol 54:142–148

Siebers N, Kruse J (2019) Short-term impacts of forest clear-cut on soil structure and consequences for organic matter composition and nutrient speciation: a case study. PLoS One 14:e0220476

Siliakus MF, van der Oost J, Kenen SWM (2017) Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. Extremophiles 21:651–670

Sleator RD, Hill C (2002) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71

Song Z, Zhi X, Li W, Jiang H, Zhang C, Dong H (2009) Actinobacterial diversity in hot springs in Tengchong (China), Kamchatka (Russia), and Nevada (USA). Geomicrobiol J 26:256–263

Song Z-Q, Chen J-Q, Jiang H-C, Zhou E-M, Tang S-K, Zhi X-Y, Zhang L-X, Zhang C-LL, Li W-J (2010) Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China. Extremophiles 14:287–296

Steven B, Léveillé R, Pollard WH, Whyte LG (2006) Microbial ecology and biodiversity in permafrost. Extremophiles 10:259–267

Studer P, Staubli T, Wieser N, Wolf P, Schuppler M, Loessner MJ (2016) Proliferation of *Listeria monocytogenes* L-form cells by formation of internal and external vesicles. Nat Commun 7: 13631

Sulochana MB, Jayachandra SY, Anil Kumar S, Parameshwar AB, Mohan Reddy K, Dayanand A (2014a) Siderophore as a potential plant growth-promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Suutari M, Laakso S (1992) Changes in fatty acid branching and unsaturation of *Streptomyces griseus* and *Brevibacterium fermentans* as a response to growth temperature. Appl Environ Microbiol 58:2338–2340

Suutari M, Laakso S (1993) Effect of growth temperature on the fatty acid compostion of *Mycobacterium phlei*. Arch Microbiol 159:119–123

Suutari M, Laakso S (1994) Microbial fatty acids and thermal adaptation. Crit Rev Microbiol 20: 285–328

Suutari M, Liukkonen K, Laakso S (1990) Temperature adaptation in yeasts: the role of fatty acids. J Gen Microbiol 136:1469–1474

Thapa S, Li H, OHair J, Bhatti S, Chen F-C, Al Nasr K, Johnson T (2019) Biochemical characteristics of microbial enzymes and their significance from industrial perspectives. Mol Biotechnol 61:579–601

Trivedi S, Rao SR, Gehlot HS (2005) Nucleic acid stability in thermophilic prokaryotes: a review. J Cell Mol Biol 4:61–69

Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the metatranscriptome. PLoS One 3:e2527

Ward DM, Weller R, Bateson MM (1990) 16S rRNA sequences reveal numerous uncultured inhabitants in a natural community. Nature 345:63–65

Wright DJ, Smith SC, Joardar V, Scherer S, Jervis J, Warren A, Helm RF, Potts M (2005) UV irradiation and desiccation modulate the three-dimensional extracellular matrix of *Nostoc commune* (cyanobacteria). J Biol Chem 280(48):40271–40281

Xu Z, Cen Y-K, Zou S-P, Xue Y-P, Zheng Y-G (2020) Recent advances in the improvement of enzyme thermostability by structure modification. Crit Rev Biotechnol 40:83–98

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV, Soudagar MEM, Mubarak NM, Hallad S, Hugar S, Fayaz H (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Zerikly M, Challis GL (2009) Strategies for the discovery of new natural products by genome mining. ChemBioChem 10:625–633

Zhang Y, Cong J, Lu H, Yang C, Yang Y, Zhou J, Li D (2013) An integrated study to analyze soil microbial community structure and metabolic potential in two forest types. PLoS One 9:e93773

Zielińska S, Radkowski P, Blendowska A, Ludwig-Gałęzowska A, Łoś JM, Łoś M (2017) The choice of the DNA extraction method may influence the outcome of the soil microbial community structure analysis. MicrobiologyOpen 6:e453

Zvyagintsev DG, Zenova GM, Sudnizin II, Doroshenko EA (2005) The ability of soil actinomycetes to develop at an extremely low humidity. Dokl Biol Sci 405:461–463

Chapter 6
Diversity and Classification of *Streptomyces*

Basavaraj S. Hungund, Samay Honnangi, Savitha S. Desai, Kaveri Badiger, and Gururaj B. Tennalli

Abstract When compared to other bacteria, the genus *Streptomyces* involves a distinct assembly of Gram-positive bacteria that are universal in nature and display complex range in colony color, secretion of pigments, etc. The greatest role of *Streptomyces* is its capacity to generate biologically active specialized products, for instance, antivirals, antihypertensives, anticancer, fungicides, immunosuppressors, and most importantly antibiotics. Taxonomy or systematics of bacteria involves the study of a range of organisms that makes use of classification, nomenclature, and identification. *Streptomyces* taxonomy is established on limited characters such as structural coloration, bionomical necessities, and structural aspect of chains of fungal spore morphology. *Streptomyces* have been classified based on different approaches like the International *Streptomyces* Project (ISP) of 1966, genomic sequence (phylogenetic approach), molecular techniques (genotyping approach), chemical characteristics (chemotaxonomy), or numerical taxonomy. As far as the diversity is concerned, in the majority of the bacterial domain, the genus *Streptomyces* is highly diversified and is scattered around substantial terrestrial sections.

Keywords *Streptomyces* · Classification · Classification approaches · Taxonomy · Diversity · Antibiotics

B. S. Hungund (✉) · S. Honnangi · K. Badiger · G. B. Tennalli
Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

S. S. Desai
Independent Researcher, Hubballi, Karnataka, India

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6.1 Introduction

6.1.1 Streptomyces *Genus*

The *Streptomyces* genus encompasses a diverse assembly of Gram-positive, aerobic bacteria which are extensively scattered in nature (Stackebrandt et al. 1997). In nature, *Streptomyces* are universal, and when compared to other bacteria, they display a complex range in colony color, secretion of pigments, etc. (Lanoot et al. 2004). They are widely used in the production of many secondary products, especially antibiotics where half of them are produced by *Streptomyces* (van der Aart et al. 2018). The structural differentiation of *Streptomyces* includes the development of hyphal layer that can segregate to form a chain of spores. The greatest role of *Streptomyces* is its capacity to generate biologically active secondary products, for instance, antivirals, antihypertensives, anticancer, fungicides, immunosuppressors, and most importantly antibiotics (Procópio et al. 2012). Another characteristic feature of *Streptomyces* genus is its complicated cellular development, where the germinating spores develop into hyphae having multinucleated mycelium which forms septa at frequent intervals forming chain of spores (Ohnishi et al. 2008). When various conditions such as nutrient availability, humidity, and temperature are favorable for the spore, a tube is generated, and hyphae develop. Then the reproductive part (aerial hyphae) is developed, which in turn commences the association of several developments including the growth of the cell and its cycle. Species of the genus *Streptomyces* produce two types of mycelia (aerial and substrate) during growth (Pridham et al. 1958) which are distinguished by the morphological characteristics of their spores: three major types, viz., rectiflexibiles, retinaculiaperti, and spirales (Kampfer et al. 2014; Nguyen and Kim 2015). The surfaces of the spores of *Streptomyces* are categorized into five groups, which are spiny, hairy, rugose, smooth, and warty. They are categorized by examining under the scanning electron microscope (Dietz and Mathews 1971).

6.1.2 Ecology and Evolution of Streptomyces

The origin of *Streptomyces* mostly found in soil is thought to be commenced roughly 400 million years back, when green plants inhabited the land. Their significant role in the dissociation of cell wall or other components of fungi, insects, and plants (Chater et al. 2010) implies that the genus *Streptomyces* played a significant part in the creation of primitive soil by decomposition of the organic matter from the land plants. At the genomic level, over the evolutionary time, this sustained ecology of the *Streptomyces* is exhibited. *Streptomyces reticuli*, for example, has approximately 456 genes for the proteins encompassed in the degradation and binding of cellulase and other simple and complex carbohydrates. This situation of sustained ecology is prevalent among the genus *Streptomyces*, which has prompted to speculate that most of the isolates of the *Streptomyces* have progressed to live in varied communities.

Streptomyces role is not only limited to manufacturing of secondary products, but ecologically, they also play important part in recycling of rich cell walls of plants and fungi. Several of species from the *Streptomyces* genus have also developed close associations with plants or insects. Some of them have developed pathogenic properties (Chater 2016).

6.1.3 *Useful Applications of* Streptomyces

6.1.3.1 Antibiotic Production

Production of antibiotics is carried out by a broad collection of microorganisms such as bacteria and fungi. At lower concentrations, antibiotics restrain or destroy another microorganism (Marinelli 2009). It is remarkably known that *Streptomyces* are widely used in the production of many secondary products, especially antibiotics (Fig. 6.1). Some of the important antibiotic compounds produced by various *Streptomyces* species include anthracyclines used as an antitumor, caboxamycin used as antibacterial, chloramphenicol used as antibacterial, hygromycin used as antimicrobial, rapamycin used as immunosuppressant, and many more.

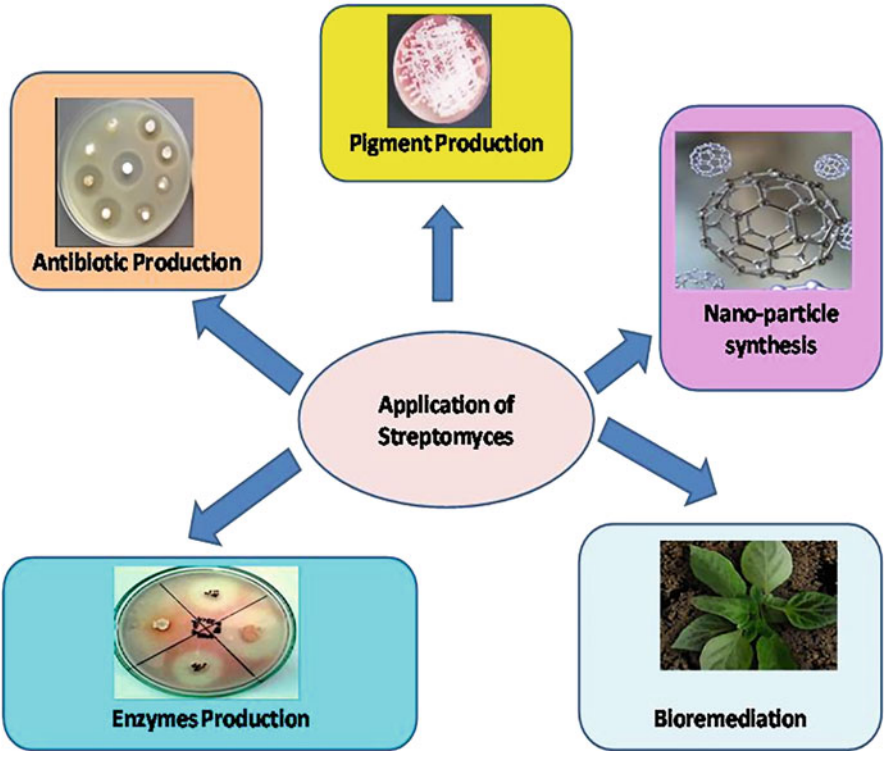

Fig. 6.1 Figure indicating various application of *Streptomyces* spp.

6.1.3.2 Enzyme Production

For the production of various enzymes such as xylanases, proteases, cellulases, chitinases, amylases, etc., several strains of *Streptomyces* have been used. For instance, *S. olivaceus* and *S. roseiscleroticus* produces aminoacylase used in the pharmaceutical industry. *S. erumpons* produces amylase used in many industries. *S. karnatakensis* and *S.halstedii* produce L-asparaginase used as a therapy for acute lymphoid leukemia. Lipase enzyme, used in many industries, is produced by various *Streptomyces* sp. like *S. griseus*, *S. coelicolor*, *S. fradiae*, etc.

6.1.3.3 Pigment Production

Actinobacteria were characterized based on a variety of pigment production on media like synthetic/natural media. Some *Streptomyces* species produce pigments like undecylprodigiosin, metacycloprodigiosin, granaticin, rhodomycin, etc.

6.1.3.4 Nanoparticle Synthesis

A large number of nanoparticles are produced by *Actinobacteria*, which exhibit various biological properties. Examples of nanoparticles produced by some *Streptomyces* species include silver (by *S. albidoflavus, S. hygroscopicus and S. rochci*) and gold (by *S. aureofaciens, S. glaucus, S. viridogens*).

6.1.3.5 Bioremediation

In technologically advanced countries, the increasing toxic waste such as solvents, petroleum residues, or herbicides from wastewater and soil has caused significant environmental pollution. In some processes such as the reprocessing of organic carbon and the degradation of complex polymers, the genus *Streptomyces* has played a significant role (Marchant and Banat 2012). Some of the species of *Actinobacteria* have the ability to survive in the environment, which are rich in oil. Such species of *Actinobacteria* can be used in the bioremediation process to diminish oil pollutants.

6.2 Classification

6.2.1 Taxonomy

The taxonomy of bacteria can be described as a study which involves scientific analysis of the diversity of organisms. The main goal of the bacterial taxonomy is

differentiating and assembling them in structured manner (Truper and Schleifer 1991). As the process of taxonomy became more used, the scope of bacterial taxonomy also changed, which included various processes such as identification, nomenclature, phylogeny, and classification (Goodfellow 2000; Vandamme et al. 1996). The approach of classification involved categorizing organisms into several taxonomic groups called taxons based on similarity. These taxonomic groups included family, species, genus, class, strain, and order. The extent of information provided, the stability, the predictability, and the objectives of such classification should be clear (Goodfellow 2000). Within the domain of the bacteria, *Actinobacteria* phylum is one of the major units of taxonomy among key ancestries (Ludwig et al. 2012). The phylum *Actinobacteria* displays vast range related to the structural, metabolic, and physiology competencies. There is a significant evolution in the taxonomy of the phylum *Actinobacteria* over time with a buildup of knowledge. The taxonomy of the genus *Streptomyces* has had an elongated and complicated background. The primary illustrations of *Streptomyces* were centered on limited characters such as structural coloration, bionomical necessities, and structural aspect of chains of fungal spore morphology. In 1943, a major breakthrough in the taxonomy of the genus *Streptomyces* was proposed by Henrici and Waksman. The advancement in the numerical taxonomic methods which involved observable characters or traits (phenotypic characteristics) played an important role in settling the intergeneric associations within the family *Streptomycetaceae*. As a result of this, six additional genera were reclassified into the genus *Streptomyces*: *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa*, and *Microellobosporia* (Lanoot 2004).

6.2.2 *Approaches Used for* Streptomyces *Classification*

There are different approaches for classification of *Streptomyces* (Fig. 6.2).

6.2.2.1 International *Streptomyces* Project (ISP)

Two committees were involved in the establishment of the International *Streptomyces* Project (ISP), which was founded in 1966. The two committees were bacteriological nomenclature subcommittee and taxonomical subcommittee of the American Society of Microbiology. A significant effect on the systematics of *Streptomyces* was visible when ISP was set up based on collaborative projects. The main aim of ISP was to deliver stable, new, and more complete description of prevailing and original strains of *Streptomyces* and *Streptoverticillium*. In ISP, many steps were followed: many varieties including the type and neotype of strains of more than 450 species were selected and were directed to three or more experts. These experts had to follow standardized procedures to describe the selected species on the basis of structural coloration and the utilization of the carbon source. By following standard protocols,

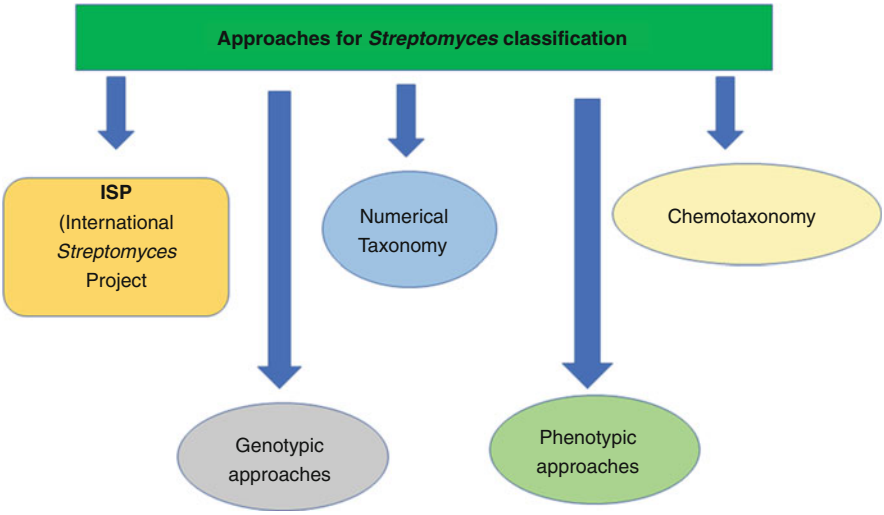

Fig. 6.2 Different approaches for classification of *Streptomyces*

the final and the updated description of the species was then published (Lanoot 2004). The type of stains were then submitted to culture collection center, e.g., NRRL. In the taxonomy of *Streptomyces*, the final results from International *Streptomyces* Project (ISP) proved to be a milestone as it laid down the foundation for the classification of the genus *Streptomyces* in the Bergey's Manual of Determinative Bacteriology-Edition 8.

I

6.2.2.2 Numerical Taxonomy

In numerical taxonomy, classification of the genus *Streptomyces* was done by numerical techniques of units of taxonomy. Classification was done based on the ability of their states of character. It aimed to develop methods that are objective, clear, and repeatable, both in assessment of taxonomic relationships and in the formation of taxa. In contrast to the ISP approach, computer-aided computation of similarity among greater number of units which are phenotypic was used for the classification in the numerical taxonomy approach. The phenotypic units comprise conventional features which are not used previously in taxonomy of *Streptomyces* such as structural studies, coloration, and data obtained from various tests, e.g., tests related to nutritive aspects, tests related to degradation, tolerance tests, and biochemical tests (Lanoot 2004).

The approach of numerical taxonomy was introduced to permit the constant assessment of considerable number of observable (phenotypic) characteristics. A study which was conducted by Williams et al. (1983a) involved ordered investigation of the numerical taxonomy of *Streptomyces* and other associated genera with

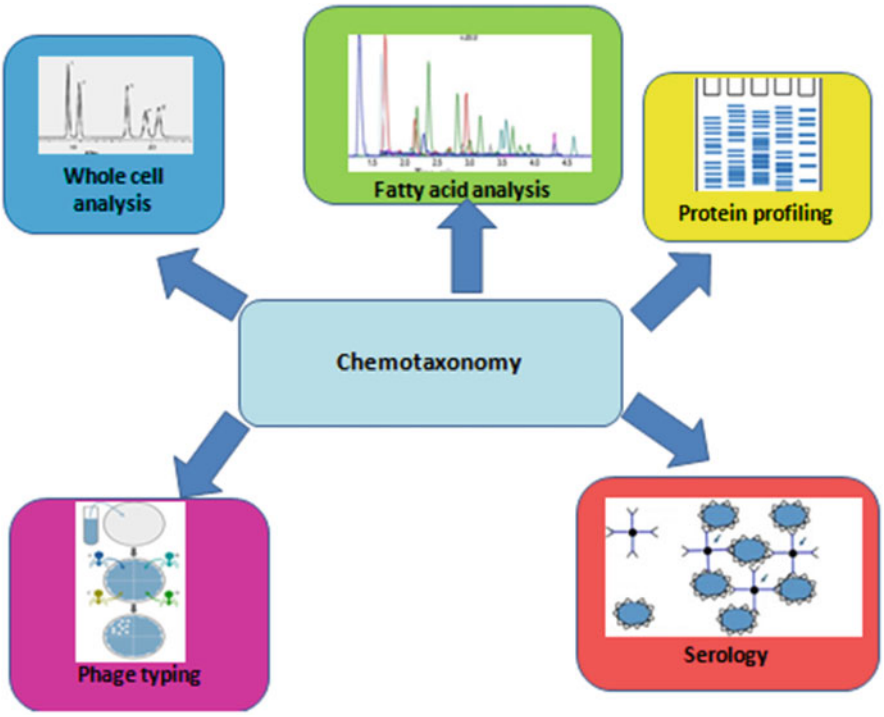

Fig. 6.3 Chemotaxonomical approach for *Streptomyces* classification

chemotype I type of cell wall (Lechevalier and Lechevalier 1970) with the help of using phenetic attributes. By making use of 139-unit attributes, 475 strains were characterized. The outcomes were examined with a universal similarity coefficient and the mean linkage calculation. The genus *Streptomyces* were further subdivided into groups of species by Williams et al. (1983a) (Anderson and Wellington 2001). According to the similarity results obtained from the phenetic tests, clustering of 394 type strains of *Streptomyces* was done (Fig. 6.3). These type strains were allocated to (1) 19 primary groups labeled as Category I, each of them involving 6–71 strains; (2) 40 secondary groups, labeled as Category II, each of them involving 2–5 strains; and (3) 18 individual groups, labeled as Category III. Category I was linked with groups of species, and both the Category II and Category III were linked to only species. In view of these outcomes, a proposal was made to reduce the number of species. The number of species was reduced from 463 species as it was in the Bergey's Manual-Edition 8–142. The results from the numerical taxonomy approach proved to be third milestone in taxonomy of *Streptomyces* as it laid down the foundation for the classification of the genus *Streptomyces* in the *Bergey's Manual of Determinative Bacteriology* 1989 Edition (Williams et al. 1989).

The matrix for classification, which was created by Williams et al. (1983b), was revised by Langham and team (Langham et al. 1989). The revision, which was done

by incorporating both the minor or primary groups and major or secondary groups, was done to enhance the resolution between the subgroups. The primary or the minor groups were characterized with the help of not more than two strains, rendering the groups to be statistically less reasonable when compared to the classification matrix created by Williams et al. (1983b). To characterize the secondary or the major groups, various tests were additionally used such as increased number of antibiotic resistances. With the help of the characterization matrix of Williams et al. (1983b), Huddleston et al. (1997) showed the inconsistency of the resistance of antibiotics across several taxonomic groups.

Using around 329 physiological tests, Kämpfer et al. (1991) reassessed a total of 821 *Streptomyces* and *Streptoverticillium* strains. Kämpfer et al. (1991) also reassessed the strains which were used by Williams et al. (1983a). Overall, 15 primary or major groups, 34 secondary or minor groups, and 40 individual groups were characterized with similarity index of 81.5%. Data from the taximetrics (phenetics) proved the existence of major phena which was obtained in the study done by Williams et al. (1983a). Kämpfer et al. later again determined the over-speciation of the genus *Streptomyces*. It was also determined that the reclassification of the genus *Streptoverticillium* should be done within the *Streptomyces* genus.

6.2.2.3 Chemotaxonomy

Classification of bacteria essentially depends on chemotaxonomy. As the name suggested, chemotaxonomy deals with study of chemical characteristics and chemical composition in microbial cell, and this study helps to classify and identify the bacteria including *Actinobacteria*. Different analytical techniques like electrophoretic technique, chromatographic technique, and spectroscopic technique were used for classification of *Streptomycetes* (Fig. 6.3). The polyphasic approach of species, genus, and higher taxa level can be recommended by chemotaxonomy; mainly classification of *Actinobacteria* depends on the presence of specific chemicals in cell envelope such as sugar (cell), amino acid (cell wall), polar lipids, menaquinones, mycolic acid, and fatty acid (plasma membranes) (Wang and Jiang 2016). Chemical techniques such as extraction, fractionation, and purification are applied to study these compositions. Protein profile, phage type, and serology are important studies for microorganisms (Wang and Jiang 2016).

Whole-Cell Analysis

The whole-cell analysis refers to analysis of the cell membrane composition and biomolecules present in the cell. The identification and classification of actinomycetes were done by Curie-point pyrolysis mass spectrometry (PyMS) (Sanglier et al. 1992). Difference between strains can be quantitatively identified by analyzing the pyrolysate with the help of mass spectroscopy in which pyrolysate was obtained by subjecting whole cells to high temperature and non-oxidative thermal degradation.

This ultimately provides fingerprint for the organism. Sanglier and team (Sanglier et al. 1992) used PyMS for analysis of strains from the largest *Streptomyces* species group; this species can be divided to *Streptomyces albidoflavus* and *Streptomyces anulatus*. Along with this, three out of six *Streptomyces halstedii* strains were grouped into a separate group, and the remaining strains clustered within the other two groups (Kämpfer et al. 1991). This study helped to recognize *Streptomyces albidoflavus* and *Streptomyces anulatus* belong to separate genomic species. Anderson A.S and Wellington E.M.H 2001 renamed the *Streptomyces anulatus* cluster by *Streptomyces griseous*.

Amino Acid of Cell Wall

The peptidoglycan polymer consists of alternative units of *N*-acetyl glucosamine and *N*-acetylmuramic acid linked by β,1–4 linkage. The polymer also contains amino acids. The carboxylic group of *N*-acetylmuramic acid is linked to peptide chain (consisting of four alternative D and L forms of amino acids) by peptide bond. Peptidoglycan chains are joined by cross-linking between the peptides. Chains of linked peptidoglycan subunits are joined by cross-links between the peptides. Often, the carboxyl group of the terminal D-alanine is connected directly or through a peptide inter-bridge to the amino group of diaminopimelic acid. *Actinobacteria* consists of cell wall about 20–80-nm-thick homogeneous peptidoglycan and 20% dry weight. Wall weight lies between 40% and 80%; along with this, it consists of macromolecules (lipids, teichoic acids, and acidic polysaccharides and proteins) covalently linked either directly to peptidoglycan or to one another (Wang and Jiang 2016).

Detection of Cell Wall Amino Acid

Precolumn derivatization and OPA (*o*-phthalaldehyde) were used to determine the amino acids in cell wall hydrolysates. Standard solutions (10 mL, 0.2 mM) of 10 amino acids were prepared. Also, purified cell wall (10 mL) was dissolved in 0.1 M (30 mL) borax buffer, and OPA (10 mL) was added to this mixture and kept at room temperature for 50 s, later analyzed by HPLC (high-performance liquid chromatography). The combination of HPLC and TLC can be used for better analysis (Wang and Jiang 2016).

Sugar of Whole-Cell Hydrolytes

The study of sugars from whole cell is essential for classification and identification of *Actinobacteria*; mainly five whole-cell sugar patterns are necessary for differentiating the meso-diaminopimelic acid containing actinomycetes. Those are (a) arabinose and galactose, (b) madurose, (c) no diagnostic sugars, (d) arabinose and xylose, and (e) rhamnose. The combination of characteristic diamino acid and some amino acids and cell wall sugars are used to describe eight wall

chemotypes to distinguish actinomycetes: (1) L-diaminopimelic acid, glycine; (2) *meso*-diaminopimelic acid, glycine; (3) *meso*-diaminopimelic acid; (4) *meso*-diaminopimelic acid, arabinose, galactose; (5) lysine and ornithine; (6) variable presence of aspartic acid and galactose; (7) diaminobutyric acid, glycine; (8) ornithine and meso-diaminopimelic acid, and L-diaminopimelic acid. HPLC can be applied to analyze the sugar of whole-cell hydrolytes (Wang and Jiang 2016).

Polar Lipids

The bacterial membranes are made up of polar lipids. The membranes also contain amphipathic polar lipids associated with specific membrane proteins; amphipathic polar lipids contain hydrophilic head groups that are linked to two hydrophobic fatty acid chains. Phospholipids are the most important polar lipids such as phophatidyglycerol, diphosphatidyglycerol, phasphatidycholine, phosphatidylethanolamine, phophatidylserine, phohatidyliositol, and other phosphatidyl glycolipids; in addition, glycolipids and acylated ornithine or lysine amides also fall into this category.

The actinomycetes are distinguished based on five types of phospholipids: (1) no nitrogenous phospholipids, (2) only one nitrogenous phospholipid phosphatidylethanolamine, (3) phosphatidylcholine and characteristic phospholipid, (4) glucosamine-containing phospholipids, and (5) phosphatidylglycerol and glucosamine-containing phospholipids. Polar lipids can be analyzed by one- or two-dimensional thin-layer chromatography (Wang and Jiang 2016).

Fatty Acid Analysis

Cytoplasmic membrane and lipopolysaccharides of the outer membrane of Gram-negative bacteria contain fatty acids; similarly, lipoteichoic acids possess 8 and 20 carbon atoms which can be found in the membrane of Gram-positive bacteria. Fatty acid composition varies from bacteria to bacteria, for example, variation in carbon chain length, type of methyl group present in fatty acids (iso-, anteiso-, and methylated within the molecule), saturated or unsaturated type of fatty acids, presence of cyclopropane fatty acid (cyclo 17:0, cyclo 19:0), and presence of OH-group at second or third position of the molecule in hydroxyl-fatty acid. Composition of fatty acids in bacteria depends on various parameters such as growth phase, temperature, and growth medium. The trypticase soy agar must be used as growth medium; if in extreme environment, then growth media must be optimized by tween and serum. Bacteria must be grown under standard conditions for analysis of fatty acids by biomass. Gas chromatography with Sherlock microbial identification software is required to identify the fatty acids at species level (Wang and Jiang 2016).

Fatty Acid Analysis (FAME) of Whole Cells

Streptomyces sp. consists of saturated straight chain and iso- as well as anteiso-branched chains, which are shown in Table 6.1 (Lanoot 2004).

Protein Profiling

Protein profiling is very important for distinguishing the actinomycetes. This profile is analyzed by polyacrylamide gel electrophoresis (PAGE) which may be one-dimensional or two-dimensional protein electrophoresis. The banding pattern of proteins from PAGE is necessary for identifying species and subspecies. Goodfellow and O'Donnell (1993) reported that many bacterial strains can be rapidly compared and reproduced by one-dimensional (1D) protein electrophoresis. The cellular protein profiles of 32 *Streptomyces* strains and *Streptoverticillium* strains were analyzed by SDS-PAGE (Manchester et al. 1990). Williams et al. (1983a) observed the taxonomic correlations between protein profiles and phenotypic groupings. Taxonomy of *Streptomyces* isolates from potato scab was elucidated by PAGE and DNA ± DNA hybridization technique by Paradis et al. (1994). The isolates were analyzed using PAGE, hybridization technique, and fatty acid analysis. Similarity factors were observed with PAGE and hybridization technique but not with fatty acid analysis. The growth conditions on the profiles influence the fatty acid analysis which may not correlate with SDS-PAGE and the DNA ± DNA hybridization grouping (Saddler et al. 1986, 1987). Higher resolution of total cellular protein can be obtained from 2D PAGE (two-dimensional) compared to 1D PAGE (O'Farrell 1975). This approach is suitable for analysis of bacterial ribosomal proteins which have lower evolutionary rate than other proteins; because it is very sensitive for proteins with high evolutionary rates, hence considering structural and functional constraints of bacteria, the ribosomal proteins were analyzed (Hori et al. 1987).

Mikulík et al. (1982) investigated the variations in ribosomal proteins for the taxonomy of streptomycete. Ochi (1989) used 2D PAGE to investigate the variability of the ribosomal proteins within *Streptomyces*. This study was further developed by the same scientist (Ochi 1992), mainly focusing on AT-L30 proteins which have genus-specific 2D profiles which can be used for classification and identification of *Streptomyces*. Later the phylogenetic groupings were done by sequencing the N terminal from the ribosomal AT-L30 protein of 81 streptomycetes from different

Table 6.1 Fatty acid composition in actinomycetes

Fatty acids	Relative amount (%)
15:0 anteiso	13–18
16:0 iso	19–26
15:0 iso	7–22
17:0 anteiso	7–17
16:0	4–14

taxonomic groups by Ochi (1995). Divergence between strains can be evaluated by extracting proteins and determining the specific protein sequence from streptomyces for taxonomic classification. SSI (*Streptomyces subtilis inhibitor*) proteins can be used to investigate the taxonomical statures of *Streptomyces coelicolor*. These proteins have molecular masses of 9 ± 12 kDa, which plays key role in physiological or morphological regulation of *Streptomyces coelicolor* strains. SSI from *Streptomyces lividans* 66, *Streptomyces coelicolor* Mu$ ller ISP5233T, and *Streptomyces coelicolor* A3(2) were compared to obtain taxonomic status. *Streptomyces coelicolor* A (3) was closely related to *Streptomyces lividans* 66 (cluster 21) than *Streptomyces coelicolor* Mu$ ller ISP5233T which were obtained by comparison of ribosomal sequences (Taguchi et al. 1996; Anderson and Wellington 2001).

SDS-PAGE of Whole-Cell Proteins

To identify whole-cell protein, it must be extracted as per the protocol of Manchester and team (Manchester et al. 1990). Pot et al. (1994) have given the protocol related to scanning and normalization of electrophoretic patterns of the whole-cell proteins by SDS-PAGE. Pearson product moment correlation coefficient with the software package Gel Compare version 4.2 (Applied Maths) was used to calculate the similarity between all traces from SDS-PAGE. Using the obtained similarity matrix, a dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Wang and Jiang 2016).

PAGE (Protein Profiling) of Whole-Cell Proteins

Williams et al. (1983a) studied the taxonomical consistency of the *S. cyaneus*, *S. albidoflavus*, and *S. anulatus* species by using SDS-PAGE for whole-cell proteins under denaturing conditions. Fatty acid, numerical phenetic, and DNA-DNA hybridization data revealed the scatter of *S. cyaneus* and *S. griseocarneus* over several groups, and the remaining *S. albidoflavus* and *S. anulatus* strains were recovered in discrete clusters. Protein profile concluded to differentiate the streptomyces at species level, and it can be used in identification purpose (Wang and Jiang 2016).

Bidirectional Polyacrylamide Gel Electrophoresis of Ribosomal Proteins

Ribosomal protein pattern revealed that *S. lavendulae* subsp. *lavendulae* and *S. lavendulae* subspecies *grasserius* showed a very similar pattern with that of *S. avidinii* (Ochi 1989, 1992). Bidirectional polyacrylamide gel electrophoresis of ribosomal protein approach is a time-consuming process for sample preparation, and it's difficult to standardize the highly complex protein patterns for interpretation (Wang and Jiang 2016).

N-Terminal Amino Acid Sequencing of ribosomal AT-L30 Proteins

The reclassification of genera *Kitasatospora* and *Streptoverticillium* in *Streptomyces* was done by identifying the similar terminal sequences to that of *S. exfoliates*, in addition to this, close relationship was observed with *S. lavendulae* by Ochi and Hiranuma, 1994. Ochi (1995) observed AT-L30 proteins from a large number of species (81) corresponding to Streptomyces. The results were similar to that of the corresponding numerical phenetic data of Williams et al. (1983a) and 16S rRNA sequence data (Wang and Jiang 2016).

Phage Typing

Host identification at the genus level and the species level can be done by examining the actinophages (Wellington and Williams 1981). *Streptomyces* phages can be divided either as polyvalent phage or species-specific phage. As the name suggests, polyvalent streptomycete phage has the ability to infect a wide number of members within the genus (Chater et al. 1986). The *Streptomyces hygroscopicus* phage SH10 was screened against 36 strains from different species groups in which 28 strains were susceptible (Klaus et al. 1981). Korn-Wendisch and Schneider (1992) observed that streptomycete phages are genus-specific although minor cross-reactivity was seen in some of genera such as *Nocardia*, *Streptosporangium*, and *Mycobacteria* (Bradley et al. 1961).

For instance, in a host range study involving 67 members of host species, these species were screened using three *Streptomyces coelicolor* Muller phages. It was observed that most of the streptomycetes tested were susceptible to the three phages. Also, the same study showed that 22 strains out of 40 *Streptomyces albus* strains were susceptible to the *Streptomyces albus* species-specific phage S3. This low number may be due to misclassification of strains or due to host phage resistance mechanisms (Chater et al. 1986; Korn-Wendisch and Schneider 1992). This resistance mechanism can be modified by restriction modification systems (Cox and Baltz 1984). In order to avoid problems that may occur with phage in fermentation plants, hence taxonomy of *Streptomyces* by phage has been avoided in industrial level. One of the examples of this is phi TG1, which was isolated from the thienamycin producer *Streptomyces catteleya* (Foor et al. 1985). Integrative vectors can be constructed using disabled phage which are lysogenic and which are unable to cause host-cell lysis. These vectors are used in antibiotic biosynthetic pathways of genetic engineering. Along with this, species-specific phages have been identified for *S. coelicolor* Müller, *S. griseus*, *S. griseinus*, *S. scabiei*, *S. venezulae*, *S. matensis*, *S. albus*, *S.azureus*, *S. caesius*, and *S. violaceoruber* (Anderson and Wellington 2001). Reclassification of the genus *Streptoverticillium* within *Streptomyces* was done by the data of Korn-Wendisch and Schneider (1992); hence, phage typing can be used for classification and identification at the genus and species level (Wellington and Williams 1981).

Serology

Anderson and Wellington (2001) reported that serological tests involved genus-specific and species specific; this was based on results obtained after the application of antisera raised against mycelia of streptomycetes. The antisera was raised against the mycelia from *Streptomycetes*, *streptoverticillia* and *Nocardiopsis* sp. by Ridell et al. (1986). The study confirmed the close relationship between streptomycetes from cluster 61 (*Streptomyces lavendulae*) and the *streptoverticillia*, and cross-reactivity occurred by one of *Nocardiopsis dassonvillei* strains against *Streptoverticillium avopersicum* and *Streptomyces griseous* by raising antibodies. Kirby and Rybicki (1986) raised antisera against *Streptomyces griseus* (*Streptomyces anulatus*, cluster 1B of Williams et al. 1983a) and *Streptomyces cattleya* (cluster 47). The antisera developed could be used for streptomycete taxonomy which was genus-specific, and it also showed some degree of species specificity too. This showed the benefit of antisera, but it failed in reproducibility and specificity of monoclonal antibodies. Monoclonal antibodies raised against *Streptomyces lividans*' 1326 spores and antibodies from hybridoma cell lines were screened against *Streptomyces lividans*' 1326 (Wipat et al. 1994). It was detected that the antibodies were specific for the cluster group containing *Streptomyces lividans* (cluster 21, Williams et al. 1983a), thus supporting the grouping put forward by Williams et al. (1983a). Drawback of using monoclonal antibodies for classification of streptomycetes from soil is that the expression of surface antigens might change due to the low nutrient environment affecting the antibody binding. Similar pattern was established for Gram-negative microorganisms and poorly evidenced in some Gram-positive microorganisms (Nelson et al. 1991; Smith et al. 1991).

6.2.2.4 Genotypic Approach

The genotypic approach involves those methods where there is the involvement of RNA or other DNA molecules. Analysis of bacterial genomes with the use of various techniques of molecular biology has contributed significantly to the field of taxonomy involving bacteria. The establishment of technology such as polymerase chain reaction (PCR) in 1983 by Mullis and restriction enzymes has played a significant part in the field of taxonomy, which are based on the genotypic approach. Techniques involving gene-specific sequencing have also helped in the understanding of the study of evolutionary relationships among prokaryotes at various taxonomic ranks.

Random Amplified Polymorphic DNA Fingerprinting (RAPD)

Random amplified polymorphic DNA (RAPDs) are fragments of DNA which are amplified through polymerase chain reaction (PCR) with the help of 10 bp of length with arbitrary sequence of small synthetic oligonucleotides (primers). The key

benefit of RAPDs key is that they are simple and fast to examine. Since there is the involvement of PCR, only small amounts of the DNA template are needed. RAPDs are arbitrarily scattered all over the genome and contain very high genomic profusion. The primary intension in using RAPDs is that they can be used in studies where individual species are involved and studies where closely associated species are involved (Hadrys et al. 1992).

RAPD-PCR is the most convenient method in order to quickly screen various strains of *Streptomyces* on the basis of similarity. But this method requires tough regularization of the response boundaries. Boundaries such as composition of buffer, sequence of the primers, DNA template quality and concentration, and annealing temperature are included. Some of the *Streptomyces* species including *S. ambofaciens*, *S. coelicolor*, *S. lividans*, *S. glaucescens*, *S. rimosus*, and *S. pristinaespiraliss* have been classified with the help of his technique (Martin et al. 2000).

Ribotyping

Identification and categorization of bacteria can be done by a molecular technique called ribotyping. Ribotyping makes use of phylogenetic studies where there is the involvement of rRNA (Madigan et al. 2014). Ever since the establishment of gene restriction patterns of rRNA as a tool for taxonomical studies in 1986, the technique ribotyping has become an eminent method to use in various studies such as to study diversity of genome of microorganisms and to study taxonomy and epidemiology and studies involving ecological population (Pal 2014). The technique ribotyping comprises of four phases: first phase is where the bacterial chromosome is digested with an endonuclease, for example, EcoRI, SalI, or ClaI with the help of restriction digestion method; the second phase is where the resultant fragments or the digests are separated by gel electrophoresis method; the third phase is where the restriction digests are transferred on nylon membrane by Southern blotting technique; and the fourth phase is where the gel is hybridized with a marked probe which is complementary to the 23S and 16S rDNA (Grimont and Grimont 1986). Doering-Saad et al. (1992) were the first one to apply ribotyping technique who observed 40 isolates of *Streptomyces* which are not pathogenic and also which are causing potato scab. The resultant patterns indicated greater variety with slight relationship with consequent restriction fragment length polymorphism (RFLP) and data from taximetrics studies. Most of the types of stains which differed in the sequences of 16S rDNA sequence were outlined by distinct ribogroups (Lanoot 2004).

DNA-DNA Hybridization

The first molecular method which was used often to assess the scale of similarity of the genomes was DNA-DNA hybridization method. It was also the first method to be largely accepted for enhancing the classification of bacteria. The DNA-DNA

hybridization method for total chromosomal DNA has been used to ascertain the identical species within the genus *Streptomyces*. It is mentioned in publications as the "gold standard" for the description of species (Stackbrandt and Goebel 1994; Wayne et al. 1987). The DNA-DNA hybridization method is executed by examining the reorganization of single strands of DNA from various organisms. In the process of DNA-DNA hybridization, ssDNA from two different strains of bacteria are used, and under distinct conditions of temperature and ionic concentrations, the two single strands of DNA hybridize together to form dsDNA. To calculate the ideal temperature for the hybridization, a formula given by Gillis et al. (1970) is used, which is $T_{OR} = 0.51 \times (G + C) + 47\ ^{\circ}C$. The reproducibility of the outcomes can be impacted by both the parameters: ideal temperature and ionic concentration which in turn ascertain the severity of the reaction. The values of % homology between the heterologous and homologous hybrids can be used to determine the degree of affinity between the two hybrids. The DNA-DNA homology values with $\geq$70% imply a strong relationship between the two strains. The DNA-DNA homology values between 30% and 70% imply a definite relationship that may be coming from the clusters of a gene that encodes related pathways. When the values of DNA-DNA homology are below 30%, it implies no relationship between the two strains (Lanoot 2004).

After 1960, several methods have been established for DNA-DNA hybridization based on whether the DNA is immobilized or in free solution (Stackbrandt and Goebel 1994). These methods include membrane filter method (Gillespie and Spiegelman 1965), the agar gel method (McCarthy and Bolton 1963), and the initial renaturation method (De Ley et al. 1970). In 1989, as the technology became more advanced, another technique was developed by Ezaki et al. (1989) called the fluorescent microplate method. This method was very easy to apply and less time-consuming, and it required very fewer amounts of DNA which is pure when compared to the DNA-DNA hybridization method. The reproducibility levels acquired from the initial renaturation method by Goris et al. (1998) were similar to the results acquired from the fluorescent microplate method, which was based on the reactions where binding was non-covalent. The genus *Streptoverticillium* was merged with the genus *Streptomyces* using the fluorescent microplate method by Witt and Stackebrandt (1990). The values of DNA-DNA hybridization varied from 15% for *Streptomyces coelicolor* and *Streptomyces rimosus* to 97% for *Streptomyces lavendulae* and *Streptomyces colombiensis*. The cross-hybridization values were between 17 and 25% between representatives of the genus *Streptoverticillium* and *streptomycetes*. When determining the correlation between the individual species and groups of species, where there is significant genetic variability in some regions of the chromosome (Redenbach et al. 1993), then the DNA-DNA hybridization technique is not appropriate to use as a taxonomical method (Anderson and Wellington 2001).

6.2.2.5 Phylogeny Approach

rRNA Sequence Analysis

In the technique of 16S rRNA gene sequence analysis, the differences in the sequence (polymorphisms) present in the HVR (hypervariable region) of 16S rRNAgene are recognized. It is a conventional technique which is used for the identification and taxonomy of bacteria. Comparing the sequences of rRNA is principally an effective means in the taxonomy of the genus *Streptomyces*. Comparing the sequences of rRNA sequence have also helped in answering some of the questions which are concerned with the lateral gene transfer (Huddleston et al. 1997) between genera.

In the taxonomy of the genus *Streptomyces*, the investigation of the sequences of the genes of rRNA was done at various taxonomic ranks such as the species, genus, and the strain. Stackebrandt et al. (1992) assessed the purpose of the 16S rRNA sequence comparison by underlining the importance of selected region. The information regarding phylogeny at the low level of hierarchy (between species and intra genus) can easily be generated since there is superior rate of evolution present in the variable regions of the gene 16S rRNA. The use of these variable regions in resolving the relationships of both the intraspecies and interspecies within the genus *Streptomyces* was confirmed by study conducted by Kataoka et al. (1997). By examining a total of 82 strains, it was found that 42 of them had sequences which were unique, and 57 of them were substitutes. From a total of 485 strains of *Streptomyces*, sequencing of gamma regions was done by Kataoka et al. (1997) and was later consigned to the GenBank which became the biggest library where data of *Streptomyces* 16S rDNA was stored (Anderson and Wellington 2001).

Whole-Genome Sequence Analysis

To determine the full sequence of the genome of an organism, whole-genome sequencing (WGS) is used. It is a cheap and rapid method which not only has significantly improved the knowledge in the field of biotechnology but also played its part in making clinical advancements. To inspect organisms which have newly diverged, a modern tool, bioinformatics, is used. This tool can be used to determine the sequences of the entire genome and later to compare the results with other genomes. Whole-genome sequence analysis reflects better subsequent evolutionary changes concerning some of the factors, including content of gene, organization of the genome, and the average identity. The following information can be obtained after the whole-genome sequence analysis: information on regulation of pathways, metabolism, pathogenicity, and information on the structure. This information is useful in the development of drugs for new diseases which are infectious (Lanoot 2004; Procópio et al. 2012). Examples of some of the strains which are sequenced before are listed in Table 6.2.

Table 6.2 *Streptomyces* members with their available genome sequences

Organism	Size (in Mb)	% GC	Protein	GenBank	References
S. griseus	8.54	72.20	7.138	AP009493.1	Ohnishi et al. (2008)
S. scabiei	10		8.746	FN554889.1	Bignell et al. (2010)
S. coelicolor	9.05	72.00	7.825	AL645882.2	Bentley et al. (2002)
S. cattleya	8.1			NC 016111	Barbe et al. (2011)
S. bingchenggensis	11.93	70.80	10.023	CP002047	Wang et al. (2009)
Streptomyces atratus ZH16	*9.641*	*69.5*		CP027306	Li et al. (2018)
Streptomyces sp. F1	*8.142*	*72.65*	*7.262*	FKJI03000000	Melo et al. (2017)
S. rochei 7434AN4	*8.36*	*71.7*	*7.568*	AP018517	Nindita et al. (2019)
Streptomyces sp. GS93–23	*8.24*	*72*	*7.188*	NZ_CP019457	Heinsch et al. (2019)
S. albus CAS922	8.06	72.59	6.776	CP048875	Tippelt et al. (2020)

6.3 Diversity

The genus *Streptomyces* is highly diversified taxa within the bacteria domain which are scattered around substantial terrestrial sections and contains around 591 species (Euzeby 2012). *Streptomyces* are obtained in all kinds of habitats, but it is mostly obtained in soil which represents 1–20% of the overall viable count (Kumar and Jadeja 2016). Spores produced from the *Streptomyces* remain unaffected by UV light, starvation, and dehydration, which clearly indicates the distribution of *Streptomyces* in all kinds of habitats. A large-scale assessment of the genus *Streptomyces* showed variation in the phenotypes of *Streptomyces* at different habitats, which may have been aroused due to the interaction of various species (Schlatter and Kinkel 2014). This assessment suggested proper inspection of collected strains of *Streptomyces* is required in order to search for other phenotypes.

Some of the strains of *Streptomyces* were also obtained from manures and some foods, especially obtained in legumes after spontaneous combustion. Some of the examples are *S. albus* and *S. griseus* which are thermotolerant (Goodfellow and Simpson 1987). *Streptomyces* have also been obtained from aquatic environments and in freshwater which may be present naturally or may have aroused from neighboring soils (Goodfellow and Simpson 1987). *Streptomyces* represented 2–5% of the population of microbes in the sediments of seaside swamp which occurred naturally. Bentley and Meganathan (1981) observed that *Streptomyces* can also be found in reservoirs of drinking water that affects the quality of water by causing foul odor. This is because of the production of secondary products: geosmin and methylisoborneol.

Katsifas et al. (1999) studied the relationship among the number of species by identifying different phenotypes of *Streptomyces* which were obtained from various Greek terrestrial environments. Numerous strains of *Streptomyces* were obtained from various sites in the Greek territory. These sites included spoilt agronomic regions and regions of conserved forests (Derry et al. 1999). To study the diversity of *Streptomyces*, selection of field is a very important aspect. Once an appropriate field is selected, a suitable field stations based on the soil conditions and agricultural practices are selected for the collection of samples. After the sample is collected, proper processing of samples is crucial. Processing is done by removing the stones and debris and air dried for 3–4 days at room temperature. The air-dried samples are refilled in the respective polythene bags and stored at 4 °C for further studies. Once the sample processing is completed, analysis of soil is done to analyze organic matter, pH, moisture content, N, P, K, Zn, Fe, Cu, lime, and SiO_2. In order to get better isolation of the *Streptomyces*, enrichment of samples is done by three different methods (Agate and Bhat 1963; Pridham et al. 1957), viz., calcium carbonate treatment, phenol treatment, and heat treatment. After the enrichment of the samples, *Streptomyces* are isolated. Once the *Streptomyces* are isolated and enumerated, its morphological characterization, biochemical characters, and physiological properties are determined (Fig. 6.4). Organic matter, moisture, pH, and other mineral contents of the soils are thought to regulate the diversity of actinomycetes in the soils. It is not common to detect highly varied physicochemical and nutritious characteristics of soils in diverse geographical regions widespread across the world. Naturally the diversity of actinomycetes in different soils is varied worldwide. Choice of natural materials, such as soils, may be based on the assumption that samples from broadly different locations are most expected to yield novel isolates and, therefore, novel bioactive molecules. Actinomycete populations from acidic soils were found to be different than those from natural soils (Williams et al. 1971).

6.4 Biotechnological Applications

The greatest role of *Streptomyces* is its capacity to generate biologically active specialized products, for instance, antivirals, antihypertensives, anticancer, fungicides, immunosuppressors, and most importantly antibiotics. Because of this property, *Streptomyces* find its use for various biotechnological applications. Generally, the products or metabolites produced can be segregated into four groups: first group consists of factors that regulate growth, agents that causes a cell to change its shape (morphogenetic agents), and molecules that are iron-chelating (siderophore). The second group consists of agents that inhibit physiological actions (antagonist) and agents that restrict the growth of protozoan (antiprotozoal agent), bacteria (antibacterial), fungi (antimycotic), and also virus (antiviral). The third group consists of agents that kill insects (insecticide), agents that are used to control pests (pesticide), and agents that restrict the growth of unwanted plants (herbicide). The last group consists of agents that help in controlling neurological disorders, agents

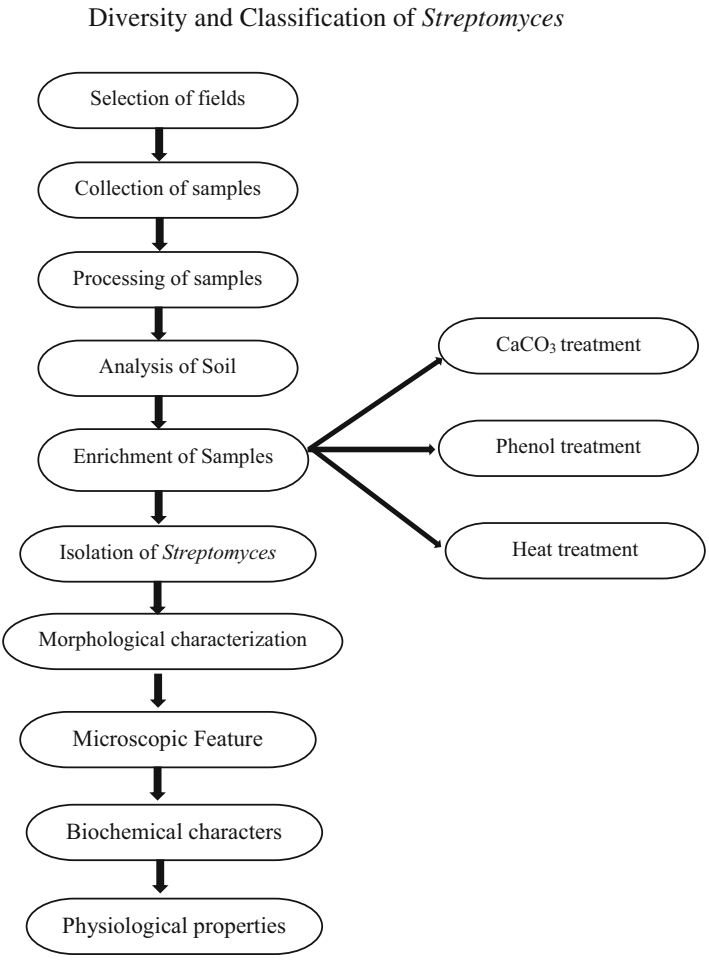

Fig. 6.4 Flow chart showing general steps involved for studying the diversity of *Streptomyces*

that help regulate immune system (immunomodulator), agents that restrict the growth of tumor (anticancer), and agents that decrease enzyme activity (enzyme inhibitors) (Harir et al. 2018).

Seventy to eighty percent of the naturally available biologically active products which have uses in pharmaceutical industries are produced by *Streptomyces* (Bérdy 2005; Manteca et al. 2008). Antibiotics were the first and most vital product of *Streptomyces* (Watve et al. 2001). Since 1955, *Streptomyces* has been a critical source for the production of novel antibiotics (Hwang et al. 2001). Many agents such as anticancer, antibacterial, antiviral, antifungal, agrobiologicals, and pharmacological agents have all been obtained from *Streptomyces*. Enzymes are the second most vital product obtained from *Streptomyces* (Nascimento et al. 2002) such as proteases,

Table 6.3 Various antibiotics and enzymes produced from *Streptomyces* species and their applications

Type of product	Name of compound	*Streptomyces* species	Application	References
Antibiotic	Anthracyclines	*S. galilaeus*	Antitumor	Hori et al. (1977)
Antibiotic	Hygromycin	*S. hygroscopicus*	Antimicrobial; immunosuppressive	Palaniappan et al. (2009)
Antibiotic	Pristinamycine	*S. pristinaespiralis*	Antibacterial	Folcher et al. (2001)
Antibiotic	Streptomycin	*S. griseus*	Antimicrobial	Whiffen (1948)
Antibiotic	Rapamycin	*S. hygroscopicus*	Immunosuppressive; antifungal	Sehgal et al. (1975)
Antibiotic	Streptozotocin	*S. achromogenes*	Diabetogenic	Agarwal (1980)
Antibiotic	Avermectin	*S. avermitilis*	Antiparasitic	Dutton et al. (1991)
Antibiotic	Bleomycin	*S. verticillus*	Anticancer	Kong et al. (2018)
Antibiotic	Actinomycins V, X_2, and D	*S. antibioticus* strain M7	Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE)	Sharma and Manhas (2019)
Enzyme	L-asparaginase	*S. karnatakensis*, *S. halstedii*	The treatment of acute lymphoblastic leukemia	El-Sabbagh (2013)
Enzyme	Pectinase	*S. lydicus*	Clarification, mashing, scouring	Jacob et al. (2008)
Enzyme	*N*-acetylmuramidase	*S. globisporus*	Bacteriostatic enzymes	Lichenstein et al. (1990)
Enzyme	Alkaline protease	*Streptomyces* sp. Al-Dhabi-82	Industrial application	Al-Dhabi et al. (2020)
Enzyme	L-glutaminase	*Streptomyces canarius* FR	Antitumour	Reda (2015)
	Keratinase	*Actinomadura keratinilytica* strain Cpt29	Poultry compost	Habbeche et al. (2014)

lipases, cellulases, amylases, pectinases, and xylanases (Table 6.3) (Harir et al. 2018).

Streptomyces are not only useful for the production of antibiotics and enzymes, but they are also useful for production of herbicides and pigments (Table 6.4). They are also useful in controlling diseases in plants and in the production of odor and flavor compounds.

Table 6.4 Examples of bioherbicides and pigments produced by *Streptomyces* species

Type of product	Name of compound	*Streptomyces* species	References
Bioherbicide	Carbocyclic coformycin and hydantocidin	*S. hygroscopicus*	Duke et al. (2002)
Bioherbicide	Bialaphos	*S. viridochromogenes*	Hara et al. (1991)
Herbicide	Methoxyhygromycin	*Streptomyces* sp. 8E-12	Lee et al. (2003)
Bioherbicide	Herbicidines and herbimycins	*S. saganonensis*	Al-Tawaha et al. (2008)
Pigment	Undecylprodigiosin, metacycloprodigiosin	*S. longispororuber* DSM 40599	Stankovic et al. (2014)
Pigment	Granaticin	*S. olivaceus*	Kutzner and Waksman (1959)
Pigment	Undecylprodigiosin	*Streptomyces* sp. strain JAR6	Abraham and Chauhan (2018)

6.5 Conclusion

The taxon *Actinobacteria* is a highly diversified unit of taxonomy within the domain of bacteria. *Actinobacteria* display a massive range in relation to the structural, metabolic, and physiology competencies. The systematics or the taxonomy of the genus *Streptomyces* has a complex history and remains such nowadays. *Streptomyces* has received enormous attention because of its capacity to generate specialized products, including antibiotics, enzymes, etc. Between the 1950s and 1970s, a huge number of novel species (more than 300) had been characterized based on structural and functional attributes. These attributes still govern the present-day methods for taxonomy. Regardless of continuous advancement in the approach for taxonomy, there is still a very high number of species (more than 500), which has become a major hurdle in order to study the exact associations between the species. The genus *Streptomyces* has been evolving continuously throughout the years, and most of the species have evolved to live in mixed communities. By exploration of various habitats such as the soil, compost, freshwater, marine environments, caves, etc., more and more *Streptomyces* species are being discovered, and it has become of utmost importance to understand the association of these species.

References

Abraham J, Chauhan R (2018) Profiling of red pigment produced by *Streptomyces* sp. JAR6 and its bioactivity. 3 Biotech *8*(1):1–9

Agarwal MK (1980) Streptozotocin: mechanisms of action. FEBS Lett 120(1):1–3

Agate AD, Bhat JV (1963) A method for the preferential isolation of actinomycetes from soils. Antonie Van Leeuwenhoek 29(1):297–304

Al-Dhabi NA, Esmail GA, Ghilan AKM, Arasu MV, Duraipandiyan V, Ponmurugan K (2020) Characterization and fermentation optimization of novel thermo stable alkaline protease from

Streptomyces sp. Al-Dhabi-82 from the Saudi Arabian environment for eco-friendly and industrial applications. J King Saud Univ Sci 32(1):1258–1264

Al-Tawaha A, Odat N, Gzawi A, Al-udatt M, Turk M, Ababneh F (2008) Genomic and chemical approaches to weed control in pasture. Am Eurasian J Agric Environ Sci 3(2):187–193

Anderson A, Wellington E (2001) The taxonomy of *Streptomyces* and related genera. Int J Syst Evol Microbiol 51(3):797–814

Barbe V, Bouzon M, Mangenot S, Badet B, Poulain J, Segurens B, Vallenet D, Marlière P, Weissenbach J (2011) Complete genome sequence of Streptomyces cattleya NRRL 8057, a producer of antibiotics and fluorometabolites. J Bacteriol 193(18):5055–5056

Bentley R, Meganathan R (1981) Geosmin and methylisoborneol biosynthesis in *Streptomycetes*. FEBS Lett 125(2):220–222

Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 9 417(6885):141–147

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58(1):1–26

Bignell DR, Seipke RF, Huguet-Tapia JC, Chambers AH, Parry RJ, Loria R (2010) Streptomyces scabies 87-22 contains a coronafacic acid-like biosynthetic cluster that contributes to plant–microbe interactions. Mol Plant-Microbe Interact 23(2):161–175

Bradley SG, Anderson DL, Jones LA (1961) Phylogeny of actinomycetes as revealed by susceptibility to actinophage. Dev Ind Microbiol 2:223–237

Chater K (2016) Recent advances in understanding *Streptomyces*. F1000Research 5:2795

Chater KF, Lomovskaya ND, Voeykova TA, Sladkova IA, Mkrtumian NM, Muravnik GL (1986) *Streptomycesè* C31-like phages: cloning vectors, genome changes and host range. In: Szabo G, Biro S, Goodfellow M (eds) Biological, biochemical and biomedical aspects of actinomycetes. Akademiai Kiado, Budapest, pp 45–54

Chater KF, Biró S, Lee KJ, Palmer T, Schrempf H (2010) The complex extracellular biology of *Streptomyces*. FEMS Microbiol Rev 34(2):171–198

Cox K, Baltz R (1984) Restriction of bacteriophage plaque formation in *Streptomyces* spp. J Bacteriol 159(2):499–504

Derry AM, Staddon WJ, Kevan PG, Trevors JT (1999) Functional diversity and community structure of micro-organisms in three arctic soils as determined by sole-carbon-source-utilization. Biodivers Conserv 8(2):205–221

Dietz A, Mathews J (1971) Classification of *Streptomyces* spore surfaces into five groups. Appl Microbiol 21(3):527–533

Doering-Saad C, Kämpfer P, Manulis S, Kritzman G, Schneider J, Zakrzewska-Czerwinska J, Schrempf H, Barash I (1992) Diversity among Streptomyces strains causing potato scab. Appl Environ Microbiol 58:3932–3940

Duke S, Dayan F, Rimando A, Schrader K, Aliotta G, Oliva A, Romagni J (2002) Chemicals from nature for weed management. Invited Paper. Weed Sci 50(2):138–151

Dutton C, Gibson S, Goudie A, Holdom K, Pacey M, Ruddock J (1991) Novel avermectins produced by mutational biosynthesis. J Antibiot 44(3):357–365

El-Sabbagh S (2013) L-Asparaginase production by *Streptomyces halstedii* isolated from Egyptian Soil. J Faculty Sci Menoufia Univ XXVI:101–130

Euzeby JP (2012) List of prokaryotic names with standing in nomenclature—genus *Streptomyces*. http://www.bacterio.cict.fr/s/*Streptomyces*b.html. Accessed 20 Feb 2012

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39(3):224–229

Folcher M, Gaillard H, Nguyen LT, Nguyen KT, Lacroix P, Bamas-Jacques N, Rinkel M, Thompson CJ (2001) Pleiotropic functions of a *Streptomyces pristinaespiralis* autoregulator receptor in development, antibiotic biosynthesis, and expression of a superoxide dismutase. J Biol Chem 276(47):44297–44306

Foor F, Roberts G, Morin N, Snyder L, Hwang M, Gibbons P, Paradiso M, Stotish R, Ruby C, Wolanski B, Streicher S (1985) Isolation and characterization of the *Streptomyces cattleya* temperate phage TG1. Gene 39(1):11–16

Gillespie D, Spiegelman S (1965) A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J Mol Biol 12(3):829–842

Gillis M, Ley J, Cleene M (1970) The determination of molecular weight of bacterial genome DNA from renaturation rates. Eur J Biochem 12(1):143–153

Goodfellow M (2000) Microbial systematics: background and uses. In: Priest FG, Goodfellow M (eds) Applied microbial systematics. Kluwer Academic, Dordrecht, pp 1–18

Goodfellow M, O'Donnell AG (1993) Roots of bacterial systematic. In: Handbook of new bacterial systematics. Academic Press, London, pp 3–56

Goodfellow M, Simpson KE (1987) Ecology of streptomycetes. Front Appl Microbiol 2:97–125

Goris J, Suzuki K, Vos P, Nakase T, Kersters K (1998) Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. Can J Microbiol 44(12): 1148–1153

Grimont F, Grimont P (1986) Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Annales de l'Institut Pasteur/Microbiologie 137(1):165–175

Habbeche A, Saoudi B, Jaouadi B, Haberra S, Kerouaz B, Boudelaa M, Badis A, Ladjama A (2014) Purification and biochemical characterization of a detergent-stable keratinase from a newly thermophilic actinomycete Actinomadura keratinilytica strain Cpt29 isolated from poultry compost. J Biosci Bioeng 117(4):413–421

Hadrys H, Balick M, Schierwater B (1992) Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol 1(1):55–63

Hara O, Murakami T, Imai S, Anzai H, Itoh R, Kumada Y et al (1991) The bialaphos biosynthetic genes of *Streptomyces viridochromogenes*: cloning, heterospecific expression, and comparison with the genes of *Streptomyces* hygroscopicus. J Gen Microbiol 137(2):351–359

Harir M, Bendif H, Bellahcene M, Fortas, Rebecca Pogni Z (2018) *Streptomyces* secondary metabolites. In: Basic biology and applications of actinobacteria. IntechOpen, London

Heinsch SC, Hsu SY, Otto-Hanson L, Kinkel L, Smanski MJ (2019) Complete genome sequences of Streptomyces spp. isolated from disease-suppressive soils. BMC Genomics 20(1):1–13

Hori S, Shirai M, Hirano S, Oki T, Inui T, Tsukagoshi S, Ishizuka M, Takeuchi T, Umezawa H (1977) Antitumor activity of new anthracycline antibiotics, aclacinomycin-A and its analogs, and their toxicity. Gann 68(5):685–690

Hori H, Higo K, Osawa S (1987) The rates of evolution in some ribosomal components. J Mol Evol 9(3):191–201

Huddleston A, Cresswell N, Neves M, Beringer J, Baumberg S, Thomas D, Wellington E (1997) Molecular detection of streptomycin-producing Streptomycetes in Brazilian soils. Appl Environ Microbiol 63(4):1288–1297

Hwang B, Lim S, Kim B, Lee J, Moon S (2001) Isolation and in vivo and in vitro antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. Appl Environ Microbiol 67(8):3739–3745

Jacob N, Poorna CA, Prema P (2008) Purification and partial characterization of polygalacturonase from *Streptomyces lydicus*. Bioresour Technol 99(14):6697–6701

Kampfer P, Glaeser SP, Parkes L, van Keulen G, Dyson P (2014) The Family Streptomycetaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30138-4_184

Kämpfer P, Kroppenstedt R, Dott W (1991) A numerical classification of the genera *Streptomyces* and Streptoverticillium using miniaturized physiological tests. J Gen Microbiol 137(8): 1831–1891

Kataoka M, Ueda K, Kudo T, Seki T, Yoshida T (1997) Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus *Streptomyces*. FEMS Microbiol Lett 151(2):249–255

Katsifas EA, Giannoutsou EP, Karagouni AD (1999) Diversity of streptomycetes among specific Greek terrestrial ecosystems. Lett Appl Microbiol 29(1):48–51

Kirby R, Rybicki E (1986) Enzyme-linked immunosorbent assay (ELISA) as a means of taxonomic analysis of *Streptomyces* and related organisms. Microbiology 132(7):1891–1894

Klaus S, Krugel H, Suss F, Neigenfind M, Zimmermann I, Taubeneck U (1981) Properties of the temperate actinophage SH10. Microbiology 123(2):269–279

Kong J, Yi L, Xiong Y, Huang Y, Yang D, Yan X, Shen B, Duan Y, Zhu X (2018) The discovery and development of microbial bleomycin analogues. Appl Microbiol Biotechnol 102(16): 6791–6798

Korn-Wendisch F, Schneider J (1992) Phage typing – a useful tool in actinomycete systematics. Gene 115:243–247

Kumar RR, Jadeja VJ (2016) Isolation of actinomycetes: a complete approach. Int J Curr Microbiol Appl Sci 5(5):606–618

Kutzner HJ, Waksman SA (1959) Streptomyces coelicolor Mueller and Streptomyces violaceoruber Waksman and Curtis, two distinctly different organisms. J Bacteriol 78(4):528–538

Langham C, Williams S, Sneath P, Mortimer A (1989) New probability matrices for identification of *Streptomyces*. Microbiology 135(1):121–133

Lanoot B (2004) Improved taxonomy of the genus *Streptomyces*. PhD thesis. University Gent, pp 1–321

Lanoot B, Vancanneyt M, Dawyndt P, Cnockaert M, Zhang J, Huang Y, Liu Z, Swings J (2004) BOX-PCR fingerprinting as a powerful tool to reveal synonymous names in the genus Streptomyces. Emended descriptions are proposed for the species Streptomyces cinereorectus, S. fradiae, S. tricolor, S. colombiensis, S. filamentosus, S. vinaceus and S. phaeopurpureus. Syst Appl Microbiol 27(1):84–92

Lechevalier M, Lechevalier H (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20(4):435–443

Lee HB, Kim CJ, Kim JS, Hong KS, Cho KY (2003) A bleaching herbicidal activity of methoxyhygromycin (MHM) produced by an actinomycete strain Streptomyces sp. 8E-12. Lett Appl Microbiol 36(6):387–391

Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12(1):133–142

Li Y, Zhang C, Liu C, Ju J, Ma J (2018) Genome sequencing of Streptomyces atratus SCSIOZH16 and activation production of nocardamine via metabolic engineering. Front Microbiol 9:1269

Lichenstein HS, Hastings AE, Langley KE, Mendiaz EA, Rohde MF, Elmore R, Zukowski MM (1990) Cloning and nucleotide sequence of the N-acetylmuramidase M1-encoding gene from *Streptomyces globisporus*. Gene 88(1):81–86

Ludwig W, Euzéby J, Schumann P, Busse HJ, Trujillo ME, Kämpfer P, Whitman WB (2012) Road map of the phylum actinobacteria. In: Bergey's manual® of systematic bacteriology. Springer, New York, pp 1–28

Madigan MT, Martinko JM, Bender KS, Buckley DH, Stahl DA, Brock T (2014) Brock biology of microorganisms. Pearson, London

Manchester L, Pot B, Kersters K, Goodfellow M (1990) Classification of *Streptomyces* and Streptoverticillium species by numerical analysis of electrophoretic protein patterns. Syst Appl Microbiol 13(4):333–337

Manteca A, Alvarez R, Salazar N, Yagüe P, Sanchez J (2008) Mycelium differentiation and antibiotic production in submerged cultures of *Streptomyces coelicolor*. Appl Environ Microbiol 74(12):3877–3886

Marchant R, Banat I (2012) Microbial biosurfactants: challenges and opportunities for future exploitation. Trends Biotechnol 30(11):558–565

Marinelli F (2009) Antibiotics and *Streptomyces*: the future and antibiotic discovery. Microbiol Today 2:20–23

Martin P, Dary A, André A, Decaris B (2000) Identification and typing of *Streptomyces* strains: evaluation of interspecific, intraspecific and intraclonal differences by RAPD fingerprinting. Res Microbiol 151(10):853–864

McCarthy B, Bolton E (1963) An approach to the measurement of genetic relatedness among organisms. Proc Natl Acad Sci 50(1):156–164

Melo RRD, Persinoti GF, Paixão DAA, Squina FM, Ruller R, Sato HH (2017) Draft genome sequence of Streptomyces sp. strain F1, a potential source for glycoside hydrolases isolated from Brazilian soil. Braz J Microbiol 48(4):612–614

Mikulík K, Janda I, Weiser J, Jiráňová A (1982) Ribosomal proteins of *Streptomyces* aureofaciens producing tetracycline. Biochim Biophys Acta 699(3):203–210

Nascimento RP, Coelho RRR, Marques S, Alves L, Gırio FM, Bon EPS, Amaral-Collaço MT (2002) Production and partial characterisation of xylanase from Streptomyces sp. strain AMT-3 isolated from Brazilian cerrado soil. Enzym Microb Technol 31(4):549–555

Nelson D, Bathgate A, Poxton I (1991) Monoclonal antibodies as probes for detecting lipopolysaccharide expression on Escherichia coli from different growth conditions. J Gen Microbiol 137(12):2741–2751

Nguyen TM, Kim J (2015) Streptomyces gilvifuscus sp. nov., an actinomycete that produces antibacterial compounds isolated from soil. Int J Syst Evol Microbiol 65(10):3493–3500

Nindita Y, Cao Z, Fauzi AA, Teshima A, Misaki Y, Muslimin R, Yang Y, Shiwa Y, Yoshikawa H, Tagami M, Lezhava A (2019) The genome sequence of Streptomyces rochei 7434AN4, which carries a linear chromosome and three characteristic linear plasmids. Sci Rep 9(1):1–11

O'Farrell PH (1975) High-resolution two-dimensional electrophoresis of proteins. J Biol Chem 250(10):4007–4021

Ochi K (1989) Heterogeneity of ribosomal proteins among *Streptomyces* species and its application to identification. Microbiology 135(10):2635–2642

Ochi K (1992) Polyacrylamide gel electrophoresis analysis of ribosomal protein: a new approach for actinomycete taxonomy. Gene 115(1–2):261–265

Ochi K, Hiranuma H (1994) A taxonomic review of the genera Kitasatosporia and Streptoverticilliumby analysis of ribosomal protein AT-L30. Int J Syst Bacteriol 44(2):285–292

Ochi K (1995) A taxonomic study of the genus Streptomyces by analysis of ribosomal protein AT-L30 Int. J Syst Bacteriol 45:507–514

Ohnishi, Y., Ishikawa, J., Hara, H., Suzuki, H., Ikenoya, M. And Ikeda, H. 2008. Genome sequence of the streptomycin-producing microorganism *Streptomyces* griseus IFO 13350. J Bacteriol, 190(11), pp. 4050–4060.

Pal P (2014) Ribotyping as a molecular biological technique for studying diversity in *Shigella* isolates—a review. Int Lett Nat Sci 14(2014):84–92

Palaniappan N, Dhote V, Ayers S, Starosta A, Wilson D, Reynolds K (2009) Biosynthesis of the aminocyclitol subunit of hygromycin A in *Streptomyces hygroscopicus* NRRL 2388. Chem Biol 16(11):1180–1189

Paradis E, Goyer C, Hodge N, Hogue R, Stall R, Beaulieu C (1994) Fatty acid and protein profiles of *Streptomyces* scabies strains isolated in eastern Canada. Int J Syst Bacteriol 44(3):561–564

Pot B, Vandamme P, Kersters K (1994) Analysis of electrophoretic whole-organism protein fingerprints. In: Goodfellow M, O'Donnell AG (eds) Modern microbial methods. Chemical methods in prokaryotic systematics. Wiley, Chichester, pp 493–521

Pridham TG, Anderson P, Foley C, Lindenfelser LA, Hesseltine CW, Benedict RG (1957) A selection of media for maintenance and taxonomic study of streptomycetes. A selection of media for maintenance and taxonomic study of streptomycetes. Antibiot, Ann

Pridham T, Hesseltine C, Benedict R (1958) A guide for the classification of *Streptomycetes* according to selected groups. Placement of strains in morphological sections. Appl Microbiol 6(1):52–79

Procópio REDL, Silva IRD, Martins MK, Azevedo JLD, Araújo JMD (2012) Antibiotics produced by *Streptomyces*. Braz J Infect Dis 16(5):466–471

Reda FM (2015) Kinetic properties of Streptomyces canarius L-glutaminase and its anticancer efficiency. Braz J Microbiol 46(4):957–968

Redenbach M, Flett F, Piendl W, Glocker I, Rauland U, Wafzig O, Kliem R, Leblond P, Cullum J (1993) The *Streptomyces lividans* 66 chromosome contains a 1 MB deletogenic region flanked by two amplifiable regions. Mol Gen Genet 241–241(3–4):255–262

Ridell M, Wallerström G, Williams S (1986) Immunodiffusion analyses of phenetically defined strains of *Streptomyces*, Streptoverticillium and Nocardiopsis. Syst Appl Microbiol 8(1–2): 24–27

Saddler G, Goodfellow M, Minnikin D, O'Donnell A (1986) Influence of the growth cycle on the fatty acid and menaquinone composition of *Streptomyces cyaneus* NCIB 9616. J Appl Bacteriol 60(1):51–56

Saddler G, O'Donnell A, Goodfellow M, Minnikin D (1987) SIMCA pattern recognition in the analysis of streptomycete fatty acids. Microbiology 133(5):1137–1147

Sanglier J, Whitehead D, Saddler G, Ferguson E, Goodfellow M (1992) Pyrolysis mass spectrometry as a method for the classification, identification and selection of actinomycetes. Gene 115(1–2):235–242

Schlatter D, Kinkel L (2014) Global biogeography of *Streptomyces* antibiotic inhibition, resistance, and resource use. FEMS Microbiol Ecol 88(2):386–397

Sehgal S, Baker H, Vezina C (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. J Antibiot 28(10):727–732

Sharma M, Manhas RK (2019) Purification and characterization of actinomycins from Streptomyces strain M7 active against methicillin resistant Staphylococcus aureus and vancomycin resistant Enterococcus. BMC Microbiol 19(1):1–14

Smith D, Wilcox M, Williams P, Finch R, Denyer S (1991) Characterization of cell envelope proteins of *Staphylococcus* epidermidis cultured in human peritoneal dialysate. Infect Immun 59(2):617–624

Stackbrandt E, Goebel B (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44(4):846–849

Stackebrandt E, Liesack W, Witt D (1992) Ribosomal RNA and rDNA sequence analyses. Gene 115(1–2):255–260

Stackebrandt E, Rainey F, Ward-Rainey N (1997) Proposal for a new hierarchic classification system, actinobacteria classis nov. Int J Syst Bacteriol 47(2):479–491

Stankovic N, Senerovic L, Ilic-Tomic T, Vasiljevic B, Nikodinovic-Runic J (2014) Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Appl Microbiol Biotechnol 98(9):3841–3858

Taguchi S, Kojima S, Miura K, Momose H (1996) Taxonomic characterization of closely related *Streptomyces* spp. based on the amino acid sequence analysis of protease inhibitor proteins. FEMS Microbiol Lett 135(2–3):169–173

Tippelt A, Nett M, Gurovic MSV (2020) Complete genome sequence of the lignocellulose-degrading actinomycete Streptomyces albus CAS922. Microbiol Resour Announ *9*(21): e00227–e00220

Truper HG, Schleifer KH (1991) Principles of characterization and identification of prokaryotes. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer, New York, pp 126–149

van der Aart L, Nouinoui I, Kloosterman A, Ingual J, Willemse J, Goodfellow M, van Wezel G (2018) Classification of the gifted natural product producer *Streptomyces* sp. nov. by polyphasic taxonomy. *BioRxiv*, p 310888.

Vandamme P, Pot B, Gillis M, De Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438

Wang XJ, Wang XC, Xiang WS (2009) Improvement of milbemycin-producing Streptomyces bingchenggensis by rational screening of ultraviolet-and chemically induced mutants. World J Microbiol Biotechnol 25(6):1051–1056

Wang Y, Jiang Y (2016) Chemotaxonomy of actinobacteria. In: Dhanasekaran D (ed) Actinobacteria—basics and biotechnological applications. IntechOpen, London, p 61482

Watve M, Tickoo R, Jog M, Bhole B (2001) How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 176(5):386–390

Wayne L, Moore W, Stackebrandt E, Kandler O, Colwell R, Krichevsky M, Truper H, Murray R, Grimont P, Brenner D, Starr M, Moore L (1987) Report of the Ad Hoc Committee on reconciliation of approaches to bacterial systematics. Int J Syst Evol Microbiol 37(4):463–464

Wellington EMH, Williams ST (1981) Host ranges of phages isolated to *Streptomyces* and other genera. ZentralblattfürBakteriologie, Mikrobiologie und Hygiene Abteilung Supplement 11:93–98

Whiffen A (1948) The production, assay, and antibiotic activity of actidione, an antiobiotic from *Streptomyces griseus*. J Bacteriol 56(3):283–291

Williams S, Davies F, Mayfield C, Khan M (1971) Studies on the ecology of actinomycetes in soil II. The pH requirements of streptomycetes from two acid soils. Soil Biol Biochem 3(3):187–195

Williams S, Goodfellow M, Alderson G, Wellington E, Sneath P, Sackin M (1983a) Numerical classification of *Streptomyces* and related genera. Microbiology 129(6):1743–1813

Williams S, Goodfellow M, Wellington E, Vickers J, Alderson G, Sneath P, Sackin M, Mortimer A (1983b) A probability matrix for identification of some *Streptomycetes*. Microbiology 129(6): 1815–1830

Williams ST, Goodfellow M, Alderson G (1989) *Streptomyces* Waksman and Henrici 1943, 339AL. In: Williams ST, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, pp 2452–2492

Wipat A, Wellington EM, Saunders VA (1994) Monoclonal antibodies for Streptomyces lividans and their use for immunomagnetic capture of spores from soil. Microbiology 140(8):2067–2076

Witt D, Stackebrandt E (1990) Unification of the genera Streptoverticillum and *Streptomyces*, and amendation of Streptomyces Waksman and Henrici 1943, 339AL. Syst Appl Microbiol 13(4): 361–371

Chapter 7
Diversity and Classification of Rare Actinomycetes

Anil Kumar S Katti, Shilpa AK, and Sulochana B Mudgulkar

Abstract Rare groups of actinobacterial species are widely distributed in soil and water habitats. Even though soil consists of enormous actinobacteria, they can also be isolated from water, plants, sediments, limestone quarry, and animals. In any environment, various factors like physicochemical and biochemical reactions define the diversity and distribution. Environmental parameters such as soil type, soil conductivity, humus content, and characteristics of the humic acid content also affect the soil microbial community. One of the significant ways to explore rare actinomycetes lies in sampling the underexplored or unexplored environments, and these habitats provide unparalleled chemical diversity and potential novel communities. Several environments are yet to be explored to determine the productive types of rare actinobacteria. Recognition of unusual environments is crucial in isolating different groups of rare actinobacteria, and understanding the complex ecological interactions among these microbes is to be defined. There has been a significant advancement in isolation, identification, and characterization of the bioactive producing rare *Actinomycetes* gaining more importance.

Keywords Rare actinobacteria · Diversity · Distribution · Classification · Ecological study · Identification

7.1 Diversity of Rare *Actinomycetes*

Rare actinobacteria are generally classified as strains other than *Streptomyces* (Berdy 2005). The frequency of isolation of actinobacterial strains under normal parameters is significantly less (Baltz 2006). Compared to *Streptomyces,* the growth of

A. K. S. Katti (✉)
Department of Biotechnology, Walchand College of Arts and Science, Solapur, Maharashtra, India

S. AK · S. B. Mudgulkar
Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India

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non-*Streptomyces is* usually slow and requires very complicated procedures for isolation, cultivation, and preservation in some genera (Lazzarini et al. 2000). Terrestrial and aquatic ecosystems harbor a wide variety of rare *Actinomycetes,* but the primary habitat of rare *Actinomycetes is* soil. These organisms were also isolated from various niches such as sediments, stones, water, plants, and animals (Groth et al. 1999). The rare *Actinomycetes* diversity and distribution in individual habitat are affected by several physicochemical parameters that are soil type pH, humus type, and humus content (Tiwari and Gupta 2013). The rare *Actinobacteria* genera isolated by the research team from Egypt include *Micromonospora*, *Actinoplanes*, and *Actinomadura* from Egypt soil samples (Abd-allah et al. 2012). Another report confirmed the rare *Actinobacteria* isolated from Trondheim Fjord [Norway] of shallow-water sediments including *Actinocorallia, Actinomadura, Micromonospora*, *Glycomyces*, *Nocardia*, *Nocardiopsis, Pseudonocardia*, *Streptosporangium, Nonomuraea,* and genera of *Rhodococcus* (Bredholdt et al. 2007). Rare *Actinobacteria* biodiversity belongs to the genera *Micromonospora* reported in Lake Baikal's water (Terkina et al. 2002). Rare *Actinobacteria* can sustain their lives in extreme ecological habitats, such as caves with high relative humidity, low amounts of organic nutrients, high mineral concentrations, and low temperatures. The genera of *Nocardia* and *Micromonospora* were isolated from El Gola cave, Sinai, Egypt (Mansour 2003). Besides, the Altamira Cave, Cantabria, Spain, was the source of *Nocardia altamirensis* (Jurado et al. 2008). The extreme drought condition of hyper-arid deserts is often associated with lower water activity, excessive radiation, and high-temperature conditions (Horikoshi et al. 2011). The isolation of *Micromonospora and Kribbella* genera from the Sinai Desert, Egypt, was reported by Amin et al. (Tolba et al. 2013). The rare *Actinomycetes* physiology and genetics were poorly understood, while the discovery of these microorganisms may lead to the isolation of novel chemicals (Tiwari and Gupta 2012b). According to the previous reports, the actinobacteria were also isolated from very soil layers, but it decreases gradually with an increase of depth (Takahashi and Omura 2003).

7.2 Rare *Actinomycetes* from the Soil

Actinomycetes can be seen abundantly in all soil types around the globe, such as desert alkaline soil, salt pans, and snowcaps (Agarwal and Mathur 2016).

The rhizosphere soil samples from Madhya Pradesh, India, can be considered important sources for the bioactive pigment-producing *Actinomycetes* (Parmar and Singh 2018). An excellent producer of extracellular xylanases by a moderately thermotolerant *Streptomyces atrovirens* subspecies [strain WJ-2] was isolated from Jeju Island, Korea soil sample (Kim et al. 2016).

The total number of *Actinomycetes* of 1191 was isolated selectively from 10 different soil samples obtained from five regions of Egyptian Governorates, including Qalubiya, Giza, Alexandria, Asuit, and Sinai. The types of soil samples collected from various places in Egypt were sandy, clay, cultivated, and uncultivated soils.

The soil samples numbered 2, 3, 6, 8, 9, and 10 were clay cultivated soil, while those numbered 4, 5, and 7 were sandy soil. The collected soil samples represented that the diversity of rare *Actinomycetes* genera was distributed throughout the study area. The site number 1 recorded highest value of genera diversity, which was followed by sites numbered 2, 3, 5, and 9. The less diversity in genera has occurred on site 7. To enhance the non-*streptomycete Actinomycetes* isolation from the soil sample, increase their relative number on the agar plates, and inhibit the fungal and bacterial competition, different types of selective pretreatments and antibiotics are used which act as a selective condition (Hayakawa 2008). The clay uncultivated soil [170,000 CFU/g soil] found in site number 1 showed highest count of rare *Actinomycetes* followed by sites numbered 9, 8, 3, 6, and 2, while the lowest population of 35,000 CFU/g soil was recorded in sites 10 and 5. The soil sample obtained from site 7 [Barmine cave, sandy soil] showed no rare *Actinomycetes*. The high concentration of salts and ions may be the reason for this (Abd-allah et al. 2012). Hozzein et al. (2008) studies found that with the increase in the concentration of salts and ions in the soil, the rare *Actinomycetes* colony count decreases. The sandy soil has a lower population of rare *Actinomycetes* than clay soil. This can be due to environmental factors such as dryness, higher temperature, root exudates, physical parameters of soil particles, and the absence or presence of root exudates in the rhizosphere (Xu et al. 1996). The high diversity of rare *Actinomycetes* was found in uncultivated soil than cultivated soil and hugely affected by soil properties. According to Tolba et al. (2002), in uncultivated soil the diversity increases more than in the current orchards and apple soil. The most diverse group of microorganisms are found in equilibrated, stabilized biotopes as stated by Burges and Raw (Burges and Raw 1967). This view showed that the organisms of the rhizosphere should be less differentiated than that of root-free soil because the rhizosphere is subjected to root secretions effect and the antagonistic type interactions among microorganisms which lead to dominancy of the selected group of microorganisms. From the sandy, cultivated, and uncultivated clay soil, the members of the genus *Micromonospora* and *Actinomadura* were isolated. In contrast, from cultivated and wild clay soil, organisms of genus *Actinoplanes* were isolated. As the desert temperature reaches 70 °C during the day, *Actinoplanes* sporangia cannot show resistance to desiccation, and so only genera *Nocardioides* of high temperature were isolated from sandy soil. From uncultivated clay soil, the genus *Saccharomonospora* was finally isolated.

7.3 Rare *Actinomycetes* from Aquatic Environments

As physicochemical parameters such as pH, temperature, salinity, and nutrient loads vary in aquatic environments, the distribution of inhabiting microbial communities also vary (El-Gayar et al. 2017). Actinomycetes are predominant in habitats like lakes, rivers, and marine (Subramani and Aalbersberg 2013).

7.3.1 Freshwater Environments

The water and mud from freshwater lakes are the natural sources for a large number of indigenous *Micromonospora*; 10–50% can be isolated from lake sediments of the total population of microbial inhabitants in lake water. About 15% of *Micromonospora* out of 3300 bacteria per mL were from Nebish Lake, and 3600 bacteria per mL with 16% *Micromonospora* from Crystal Lake were reported. The aquatic environments are the indigenous inhabitant of the representatives belonging to *Thermoactinomyces*, *Streptomyces,* and *Rhodococcus* (Cross 1981). Xu and Jiang contemplated populations of *Actinomycete* from 12 lakes. They found that the *Micromonospora* was the dominant genus at the central plateau of Yunnan, China, that revealed 39–89% of *Actinomycetes* in the sediments of the above lakes (Xu and Jiang 1996). Moreover, the second most abundantly found genus in the sediments of the lake was *Streptomyces*. Lake sediments likewise have been accounted for members of rare genera such as *Actinomadura, Micropolyspora, Actinoplanes, Microbispora, Nocardia, Microtetraspora, Rhodococcus, Saccharomonospora, Mycobacterium, Nocardiopsis, Promicromonospora, Streptosporangium, Thermoactinomyces, Thermomonospora, Saccharopolyspora,* and *Thermopolyspora* (Xu and Jiang 1996). Many researchers declared the occurrence of *Micromonospora* in rivers and lake sediments. *Micromonospora* plays a vital role in the turnover of lignin, cellulose, and chitin (Chavan et al. 2013). Ten *Actinomycetes were* isolated from an estuary in India; out of which five were chosen for secondary metabolite screening and reported important antibacterial activity against *Proteus mirabilis* and *Enterobacter aerogenes*. The selected *Streptomyces* sp. ES2 demonstrated potent activity against elected microbes (Al-Ansaria et al. 2019). The rare aquatic *Actinomycetes* were good candidates for exploring new bioactive molecules isolated from Fetzara Lake (Benhadj et al. 2018). The sediments from shrimp ponds are an excellent resource for the isolation of promising *Actinomycetes* (Aly et al. 2019).

7.3.2 Marine Environments

The marine environments have several distinct habitats, including seagrass beds, numerous fish species, mangroves, salt pans, coral reefs, salt marshes, and various communities of microbes (Abdelfattah et al. 2016). Many natural habitats that are underexplored can be considered as an important source for the isolation of rare *Actinomycetes* (Tiwari and Gupta 2012a). Recently unexplored marine environments have currently become a prevalent research area due to the presence of enormous resources. The latest report (Stach and Bull 2005) of the deep-sea sediments microbial diversity has shown that they might possess greater than 1300 diverse actinobacterial taxonomic units and are expected to represent a high percentage of novel genera and species. As compared to terrestrial soils, sea sediments

consist of a lesser amount of easily available organic material, with more chitin and cellulose as carbon sources occurring in intricate form. On the other hand, the culture-independent studies revealed that the sea sediment ecosystem contains *Actinomycetes* of broad diversity and various distinctive taxa, which highly differ from their terrestrial counterparts (Stach et al. 2003). Besides, according to culture-dependent studies, marine *Actinomycetes* are found to be ubiquitous in marine sediments (Jensen et al. 2005). In 2005, seawater-obligate marine *Actinomycete* species was isolated, which belongs to genera *Salinispora* (Maldonado et al. 2005); and which was further led by the finding of following genera such as *Solwaraspora, Demequina, Marinispora, Marinactinospora, Lamerjespora, Aeromicrobium, Salinibacterium, Serinicoccus, Sciscionella,* and *Williamsia*. In marine habitats, rare *Actinomycetes* are extensively present (Subramani and Aalbersberg 2012). In addition, until now, very few marine obligate species were isolated (Goodfellow 2010). The habitats such as seawater, marine sediments, symbiotic and mangrooves deep sea sediments (Emery 1969) covered 63.5% of the earth's surface and denotes under explored marine habitat (Butman and Calton 1995). The very first obligatory marine *Actinomycetes* belonged to the novel genus *Salinispora* (Maldonado et al. 2005) was described and then documented due to its strict prerequisite of seawater for growth and development. Another marine actinobacterial genus *Sciscionella* that can withstand growth in up to 13% of high salt concentrations was described by Tian et al. (2009). To date, marine milieu has been used for the identification of more than 14 new actinobacterial genera (Goodfellow and Fiedler 2010; Kurahashi et al. 2010; Chang et al. 2011). Marine ecosystems have become an obvious essential indigenous microflora for *Actinomycetes*.

From 2007 to 2013, from sea sediments, overall 38 new rare *Actinomycete* taxa were identified, belonging to 15 varied actinomycete families. Of these, nine unique genera, such as *Sciscionella, Actinotalea, Marisediminicola, Spinactinospora, Miniimonas,* and *Demequina,* persisted and were reported. In marine sediments, the reported families in that period were *Nocardioidaceae* [4 novel species], *Propionibacteriaceae* [3 novel species], *Streptosporangiaceae* [1 novel species], *Pseudonocardiaceae* [5 novel species], *Nocardiopsaceae* [2 novel species], *Promicromonosporaceae* [2 novel species], *Intrasporangiaceae* [2 novel species], *Micrococcineae* [suborder] [5 novel species], *Nocardiaceae* [2 novel species], *Cellulomonadaceae* [1 novel species], *Beutenbergiaceae* [1 novel species], *Micrococcaceae* [2 novel species], *Micromonosporaceae* [5 novel species], *Microbacteriaceae* [2 novel species], and *Geodermatophilaceae* [1 novel species]. The cultivable types of microbes from marine sediments [0.25%] are substantially greater than seawater [0.001–0.10%] (Amann et al. 1995). From 2007 to 2013, a total of 11 novel, uncommon *Actinomycete* spp. belonging to 6 varied *Actinomycete* families were described from marine water. Among them *Ornithinibacter, Marihabitans,* and *Oceanitalea* were the three new genera described in seawater. The families reported between 2007 and 2013 in seawater were *Nocardioidaceae* [4 novel species], *Intrasporangiaceae* [2 novel species], *Micrococcaceae* [2 novel species], *Propionibacteriaceae* [1 novel species], *Bogoriellaceae* [1 novel species],

and *Micrococcineae* [suborder] [1 novel species]. More than two-thirds of the Earth's surface is covered with marine ecosystems. Therefore, the marine habitats are inexhaustible store for the under-used, uncommon, unique *Actinomycetes isolation.*

7.3.3 Symbionts as the Source of Rare Actinomycetes

Symbiotic microorganisms, especially *Actinomycetes* (Schneemann et al. 2010*;* Izumi et al. 2010) from aquatic invertebrates, animals, and plants, are progressively rising for application in the process of drug development (Ganachari et al. 2018; Piel 2009). The symbiotic microbial population is vastly diverse and novel which shows the sequential geographic variation in species composition (Webster and Hill 2001). As a result, very less information is available about the taxonomic relationship of marine symbiotic microorganisms (Friedrich et al. 1999). The widely occurred symbionts are still unculturable, even with significant advancements in cultivation-independent techniques used for studying these bacteria. These methods will have a huge impact on the upcoming chemical analysis of symbionts because many symbionts are still unidentified (Piel 2009). Interestingly, from the sea cucumber, *Holothuria edulis,* two novel families such as *Euzebyaceae* (Kurahashi et al. 2010) and *Iamiaceae* (Kurahashi et al. 2009) in actinobacteria were reported. Between 2007 and 2013, in plants and animals, 17 novel and rarely occurring *Actinomycete* species associated with 11 different families of *Actinomycete* have been reported, respectively. Of these, five new genera belonging to *Phycicola, Labedella, Iamia, Koreibacter,* and *Euzebya* have been reported in marine animals and alga. From 2007 to mid-2013, the families described in marine animals and plants are *Nocardioidaceae* [2 novel species], *Pseudonocardiaceae* [1 novel species], *Microbacteriaceae* [3 novel species], *Tsukamurellaceae* [1 novel species], *Euzebyaceae* [1 novel species], *Micrococcineae* [suborder] [3 novel species], *Micrococcaceae* [1 novel species], *Nocardiopsaceae* [2 novel species], *Alteromonadaceae* [1 novel species], *Micromonosporaceae* [1 novel species], and *Iamiaceae* [1 novel species].

Mangroves are woody plants that are a unique community in subtropical and tropical zones, situated between the transition of the sea and land region (Holguin et al. 2001; Kathiresan and Bingham 2001). The mangroves play a very vital role for many organisms in providing shelter, nourishment, breeding areas, and support a large food web, this is mainly based on the organic matter produced by the decomposition of organisms. The ecosystem of mangrove varies from others because of seasonal flooding and changes in environmental factors such as salinity and nutrient availability that result in metabolic pathway adjustment that could produce very uncommon biomolecules. This idea resulted in the increased exploitation of the resources from microorganisms thriving in the mangrove ecosystem (Long et al. 2005). Fourteen new rare Actinomycete species belonging to seven diverse families are reported in mangrove sediments during 2007 to mid-2013. From those families,

Ilumatobacter and *Lysinimicrobium,* two novel genera, were reported from mangrove sediments. The reported seven families are Micrococcineae [suborder] [1 novel species], *Micromonosporaceae* [7 novel species], *Promicromonosporaceae* [1 novel species], *Streptosporangiaceae* [2 novel species], *Acidimicrobiaceae* [1 novel species], *Demequinaceae* [1 novel species], and *Thermomonosporaceae* [1 novel species]. A new family of *Actinomycetes* was reported from sediments of mangroves by Hamada et al. (2012). Therefore, sediments of mangrove are very rich resource for the *Actinomycetes* to produce various antimicrobial molecules and enzymes (Subramani and Narayanasamy 2009).

7.4 Rare Actinomycetes from Plants

Several rare *Actinomycetes* were isolated from various parts of the plant (Matsumoto et al. 1998; Shellikeri et al. 2018; Janso and Carter 2010), for the purpose of finding novel microbial resources for regular screening of novel bioactive molecules (Inahashi et al. 2011). For example, spoxazomicin, a new antitrypanosomal compound, was found in the culture broth of a novel endophytic actinomycete *Streptosporangium oxazolinicum* sp. nov. strain K07-0460T (Inahashi et al. 2011). This strain is phylogenetically related to the genus *Streptosporangium* which was isolated from the variety of orchid roots. *Actinophytocola oryzae* GMKU 367T and *Phytohabitans suffuscus* K07-0523T, two novel genera, were also discovered (Inahashi et al. 2010; Indananda et al. 2010). Therefore, plant roots are confirmed to be a potential source for the discovery of new *Actinomycetes*.

The inner tissues of higher plants are relatively an overlooked niche. Previous studies have shown that some actinobacteria form a close association with plants and inhabit their internal tissues. *Streptomyces scabies* and *Frankia* species can penetrate their hosts and establish either endophytic or pathogenic associations (Benson and Silvester 1993; Doumbou et al. 1998). The *Actinomycetes* that occur in the plant tissues and do not damage the plants are called as endophytic actinobacteria (Hallmann et al. 1997). These actinobacteria are comparatively least studied and are likely sources of new natural products for utilization in industry, agriculture, and medicine (Strobel et al. 2004). In recent years, endophytic actinobacteria have gained attention, with increasing reports of isolates from a variety of plant types, including crop plants (rice and wheat, as well as citrus, carrots, potatoes, and tomatoes) (Araujo et al. 2002; Coombs and Franco 2003; Sessitsch et al. 2004; Surette et al. 2003; Tian et al. 2007) and medicinal plants (Taechowisan et al. 2003; Zin et al. 2007). The endophytic culturable actinobacteria from these plant types fell within a narrow species distribution in that *Streptomyces* spp. were the major species, and common genera were *Micromonospora*, *Microbispora*, *Streptosporangium, Nocardioides,* and *Nocardia*.

Relatively, endophytic *Actinomycetes* are a new source for novel species and new bioactive molecules. By using special selective media and techniques, endophytic *Actinomycetes* were isolated and their diversity from medicinal plants in

Xishuangbanna, China, of the tropical rain forests studied (Qin et al. 2009). Thirty-two different genera have shown an unexpected level of diversity. It was the first report of *Saccharopolyspora, Dietzia, Blastococcus, Actinocorallia, Promicromonospora, Oerskovia, Jiangella,* and *Dactylosporangium* species isolation from endophytes (Tiwari and Gupta 2012a).

7.5 Extreme Environments

Actinomycetes, like other microorganisms, adapt and grow in different ecological niches such as deep sea, low temperatures in glaciers, alkaline pH, acidic in the industrial and mine wastewater effluents, extreme desiccation in deserts, high levels of radiation, the high salt concentration in lakes, thermal vents, and high temperatures in hot springs (Mahajan and Balachandran 2017). The microorganisms present in extreme environments have received tremendous interest because of their unique adaptation mechanisms to their harsh environments and also due to the production of unusual compounds (Meklat et al. 2011). Irrespective of the appeal, however, there has been little research carried out on *Actinomycetes* present in extreme habitats: An accidentally discovered pioneer was *Actinopolyspora halophila* (Gochnauer et al. 1975). In recent years, several new *Actinomycetes* were discovered from basic soils and salt in Qinghai and Xinjiang, the People's Republic of China, by research scholars from the Yunnan Institute of Microbiology at Yunnan University (Jiang and Xu 1996; Jiang et al. 2006). They reported a novel family *Yaniaceae*, many new genera of *Streptomonospora, Naxibacter, Jiangella, Myceligenerans,* and a vast number of novel species of the genera *Halomonas, Amycolatopsis, Isoptericola, Citricoccus, Massilia, Nocardia, Microbacterium, Prauserella, Jonesia, Kribbella, Nocardiopsis, Kocuria, Rhodococcus, Marinococcus, Saccharopolyspora, Virgibacillus, Liuella, Saccharomonospora, Nesterenkonia, Sphingomonas,* and *Thermobifida.* Recently, by use of a polyphasic approach, a wide range of halophilic *Actinomycetes* were evaluated and reported by Meklat et al., which revealed the occurrence of a new genus and many new species of the *Nocardiopsis, Actinopolyspora, Streptomonospora, Saccharopolyspora,* and *Saccharomonospora* genera. In addition, their discovery of *Nocardiopsis* strains which had a high number of NRPS genes could be an indicator of great potential *Actinomycetes* of halophilic nature for the production of enormous active biological molecules (Meklat et al. 2011). One new family, eight new genera, and more than 30 new species of alkalophilic and halophilic actinomycetes from alkaline and saline habitats, respectively, were isolated by Kavita Tiwari and Rajinder Gupta (2012a). *Actinomadura, Nocardiopsis,* and *Micromonospora* were isolated from soda salty soils of transient saline lakes in Buryatiya (Lubsanova et al. 2014).

Bacterial populations inhabiting Roopkund Glacier, Himalayan Mountain, were studied, and actinobacteria are the primary class, followed by β-proteobacteria (Rafiq et al. 2017). As these habitats being the rich diversity of culturable actinomycetes, the recent study revealed that the occurrence of novel *Streptomyces* spp.

from the Antarctic regions (Sivalingam et al. 2019). Two novel selected strains ZLN712T and ZLN81T belonging to actinomycetes were isolated from a frozen soil sample collected from the Arctic region (Kamjam et al. 2019). Some actinomycetes were isolated from rhizosphere soil from Lachung, Himalaya region, and exhibit antimicrobial activity (Singh et al. 2019). Bacterial diversity was explored and screened for several hydrolytic enzymes from soil samples of Dras, India, the coldest place after Siberia. Phylogenetic analysis showed that 40 different bacteria were grouped into three major phylum, *Firmicutes, Actinobacteria,* and *Proteobacteria,* differentiated into 17 diverse genera (Rafiq et al. 2017).

Some microbiologically specialized and diverse habitats for the isolation of thermophilic actinomycetes are hot springs, desert soil, thermal industrial wastes, and volcanic eruptions (Agarwal and Mathur 2016). In recent years, due to the economic potential of thermophilic actinomycetes, researchers have shown great interest in them, either in useful biological processes such as biodegradation or in the production of antibiotics and enzymes. Thermoactinomyces belong to the genus *Microbispora, Saccharopolyspora, Thermoactinomyces*, *Streptomyces,* and *Thermomonospora*. Among these, thermophilic actinomycetes of the genus *Thermoactinomyces* have clinical and industrial value. Few *Thermoactinomyces* strains are recognized as effective protease producers (Agarwal and Mathur 2016). Thermotolerant actinobacteria produce various enzymes of hydrolytic action like amylase, cellulase, and xylanase, which show their activity at elevated temperatures of 50–65 °C (Mohammadipanah and Wink 2016).

For the discovery of new actinomycetes and the bioactive compounds, the hot spring sediments are an excellent source (Thawai 2012). The strain YIM 78087T was isolated from a sediment sample collected from Hehua hot spring in Yunnan province, southwest China, during a study on thermophilic actinobacterial resources from hot springs. The isolate YIM 78087T represents a novel species of the genus *Streptomyces* named *Streptomyces calidiresistens* sp. nov. as indicated from the experimental data obtained (Duan et al. 2014). Actinomycetes were collected from the sediments of a hot spring pond located in Krabi and Trang province, Thailand. By studying the morphological properties and 16S rRNA gene sequence analysis, these actinomycetes strains were identified and classified. They belong to the member of genera *Planosporangium, Streptomyces, Micromonospora,* and *Microbispora* (Aly et al. 2019). Overall, 20 samples of hot spring sediment and soil samples from West Anatolia in Turkey were examined for the existence of thermophilic actinomycetes. Strains were grown at a temperature of 55 °C. Sixty-seven thermophilic actinomycete isolates are classified under *Thermoactinomyces sacchari* and *T. thalpophilus* species. The maximum isolates are found to be extracellular protease producers, among them (Agarwal and Mathur 2016). From hot water springs, actinomyces species that produce a remarkable amount of thermostable amylase and cellulose are active at acidic and alkaline pH (Chaudhary and Prabhu 2016).

In two actinomycetes strains, LC2T and LC11T, isolated from a filtration substrate made from Japanese volcanic soil, their taxonomic position was determined using a polyphasic approach (Agarwal and Mathur 2016). From a mud volcano in

India, two thermophilic *Streptosporangium* and *Rhodococcus* were isolated (Mohammadipanah and Wink 2016). It is apparent that volcanic spring is one of the extreme habitats on earth and harbors novel microbes as a source of potential drug leads. Although the knowledge of the *Streptomyces* population in volcanic habitat is sparse, there have been few noteworthy studies on the isolation of natural drugs from volcanic *Streptomyces* (Sivalingam et al. 2019).

7.5.1 Caves

Recently, numerous novel *Actinomycetes* species are isolated from caves, including those inhabited by bats in Spain, Reed Flute Cave in China, the Grotta Dei Cervi Cave in Italy, and a gold mine in Korea (Subramani and Aalbersberg 2013). From cave and cave-related habitats, 47 species in 30 genera of actinobacteria were reported (Rangseekaew and Athom-Aree 2019). From a soil sample collected from a karst cave in China, a novel actinobacterium was isolated. It was a novel species of the genus *Nocardioides* identified based on phenotypic, genotypic, and phylogenetic data (Zhang et al. 2018). From small stones collected from caves and agricultural fields, the novel rare actinomycete genera *Beutenbergia* and *Terrabacter*, respectively, have been reported (Subramani and Aalbersberg 2013). The rock walls of caves are often colonized by Actinobacteria. In a study on the biogeochemical role of actinobacteria, actinobacteria-coated spots on the cave walls in Altamira Cave [Spain] were found to uptake carbon dioxide gas, which exists in abundance in the cave. To dissolve rock and subsequently generate crystals of calcium carbonate, this gas is used by the bacteria (Fang et al. 2017).

In general, caves have high humidity, but they are short of nutrients, luminous intensity, and temperature (Schabereiter-Gurtner et al. 2002). The aforementioned factors may promote antagonism, which augments hydrolytic enzymes and antibiotics production, leading to growth inhibition of other microorganisms (Nakaew et al. 2009). Recently, numerous *Actinomycetes* species have been isolated from the caves including the Grotta Dei Cervi Cave in Italy (Jurado et al. 2005a), a gold mine in Korea (Lee et al. 2000; Lee 2006a, b), the Reed Flute Cave in China (Groth et al. 1999), and a bats-occupied cave in Spain (Jurado et al. 2005b). For the foremost time, the *Spirillospora* and *Nonomuraea* isolation from the soil of a cave was reported by Nakaew et al. (2009) and very rare genera such as *Nonomuraea, Catellatospora, Spirillospora,* and *Micromonospora*. From the caves were isolated members of genera *Actinomadura* and *Saccharopolyspora,* and other rare genera *Actinoplanes, Micromonospora, Microbispora, Nocardia, Gordonia, Nonomuraea,* along with principal genus *Streptomyces* by Niyomvong et al. (2012). Above studies validate that the caves may act as a wide source of novel *Actinomycetes* yielding new compounds.

7.5.2 *Actinomycetes from Insects*

For discovering novel and new microorganisms, the insect world is another important unexplored environment such as termites, ants, gall midges, and beetles (Kaltenpoth 2009) for practicing fungi culture. Ant workers also protect their fungal gardens through a combination of grooming and weeding (Little et al. 2006), producing their antimicrobials through metapleural gland secretions (Bot et al. 2002), and the use of weed killers. These weed killers produced by symbiotic Actinomycete bacteria (Haeder et al. 2009) are a natural producer of antimicrobials. However, latest evidence suggests that bacteria from the Actinomycete genera are also associated with attine ants; those genera are *Amycolatopsis* and *Streptomyces* (Mueller et al. 2008). Whether the attine ant associated with *Actinomycetes* produces antifungal compounds mainly remains unknown. Therefore, the world of insects is rapidly flourishing as the source for discovering unusual and novel biologically active molecules from *Actinomycetes*.

7.5.3 *Other Habitats*

From desert soil (Takahashi et al. 1996), *Actinomycetes* of rare genera such as *Nocardia, Saccharothrix, Microbispora, Microtetraspora, Amycolatopsis,* and *Actinomadura* have been isolated successfully. The novel rare *Actinomycetes* genera *Beutenbergia* (Groth et al. 1999) and Terrabacter (Lee et al. 2008) have been reported from small stones collected from caves and agriculture fields respectively. Recently, soils from the nests of solitary wasps and swallow birds (Kumar et al. 2012) and the rare *Actinomycetes* genera such as *Actinomadura, Nocardia, Saccharopolyspora, Thermoactinomyces,* and *Streptosporangium* were isolated.

7.6 Classification of Rare *Actinomycetes*

Among the 18 significant lineages presently documented in the domain *Bacteria, Actinobacteria* is one of the largest units of taxonomy, including five subclasses, six orders, and 14 suborders (Ludwig et al. 2012). The genera of this phylum show a wide diversity in their morphology, physiology, and metabolic capabilities. With the accumulation of knowledge over time, the taxonomy of *Actinobacteria* has evolved significantly. Buchanan (1917) established the order *Actinomycetales,* which belongs to this prokaryotic organisms group.

Based on its position of branching in gene trees of 16S rRNA, the phylum *Actinobacteria* was delineated. However, ambiguity occurs because sequences of rRNA cannot be well differentiated between closely related genera or species. For example, within the family *Streptomycetaceae* the status of taxonomy of

Kitasatospora genus (Omura et al. 1982) has been disputed for many years (Ludwig et al. 2012; Wellington et al. 1992; Zhang et al. 1997), while current details of genetic analysis provided strong confirmation that it should be considered as a separate genus (Girard et al. 2014). A similar type of close relationship does exist between *Salinispora, Micromonospora,* and *Verrucosispora*. For discrimination of closely related genera, rpoB and in recent times ssgB have been used as additional genetic markers (Girard et al. 2013).

In addition, detailed insights into genome evolution and identification of genes specific to organisms at the family and genera level have been provided by the recent massive increase in the availability of information of genome sequence (Kirby 2011). Based on 16S rRNA trees, for the phylum Actinobacteria, an updated status of taxonomy was recently reported (Ludwig et al. 2012). The ranks of the taxonomy of suborders and subclasses were eliminated, and former suborders and subclasses were elevated to levels of orders and classes, respectively, by that update (Gao and Gupta 2012). Actinomycetes are Gram-positive bacteria and have filamentous growth like fungi. They are aerobic and ubiquitous. The DNA of Actinomycetes is rich in G + C content with GC% of 57–75% (Lo et al. 2002). These Gram-positive bacteria have been placed within the phylum Actinobacteria, Class Actinobacteria, subclass Actinobacteridae, and order Actinomycetales, which at present consist of 10 suborders, more than 30 families, and over 160 genera (Chavan et al. 2013). They resemble morphologically with fungi and physiologically with bacteria (Sultan et al. 2002).

According to Bergey's Manual of Systematic Bacteriology, first edition, *Actinobacteria* belonged to the order *Actinomycetales* and was divided into four families *Actinoplanaceae, Mycobacteriacea, Streptomycetaceae,* and *Actinomycetaceae*. With the buildup of information over time, the taxonomy of *Actinobacteria* has considerably evolved. *Actinobacteria* were included separately in the fifth volume in the second edition of Bergey's Manual. The phylum *Actinobacteria* separated into six classes: *Rubrobacteria*, *Actinobacteria, Thermoleophilia, Nitriliruptoria, Acidimicrobiia,* and *Coriobacteriia*. The class *Actinobacteria* subdivided into 16 orders: *Frankiales*, *Actinopolysporales*, *Glycomycetales, Micromonosporales*, *Catenulisporales*, *Actinomycetales, Kineosporiales*, *Jiangellales*, *Bifidobacteriales, Streptosporangiales, Pseudonocardiales*, *Micrococcales*, *Corynebacteriales, Streptomycetales*, *Propionibacteriales*, and *Incertaesedis* (Zhi et al. 2009). The Actinomycetales order is currently limited to the family members of *Actinomycetaceae* (Gao and Gupta 2012).

According to Bergey's Manual, *Archaea and Bacteria,* the phylum *Actinobacteria* includes five classes, 19 orders, 50 families, and 221 genera. However, as many novel taxa are continuously discovered, this listing is certainly unfinished. Based on the 16S rRNA gene, sequence-based groups, and taxon-specific 16S rRNA gene sequences, the class *Actinobacteria* and fundamental taxonomic ranks above the genus level were proposed. This classification showed an apparent change in the classification of *Actinobacteria* above the genus level as it represented that former classifications based on the form and function did not reflect

natural relationships. The rank of a phylum has been assigned to *Actinobacteria* because the phylogenetic depth signified by the lineage resembles that of existing species based on its branching position in 16S rRNA gene trees (Barka et al. 2016). Among the 30 significant species currently recognized within the domain Bacteria, the phylum Actinobacteria represents one of the largest phyla. Until October 2016, 6 classes, 18 orders, 14 suborders, 63 families, and 374 genera have been recorded in this phylum.

7.7 Morphological Classification

The morphology and chemotaxonomy are the two main characteristic features considered to define the *Actinobacteria* taxonomy at the species and genus levels. The latter of the above characteristic features principally relates to whole-cell sugar distribution and composition of the cell wall. However, the composition of phospholipid and type of menaquinone might also be considered for enhancement purpose (Labeda 1987). In a special vegetative form of reproduction, mycelial fragmentation can be considered. However, reproduction by forming asexual spores is primarily the lifestyles of mycelial *Actinobacteria. Actinobacteria* show a broad diversity of morphology, differing mainly concerning the structure and appearance of their spores, the absence or presence of a substrate or aerial mycelium, the mycelium's color, and the ability to produce a diffusible form of pigments of melanoid.

7.7.1 Mycelial Morphology

Actinobacteria from a substrate mycelium in both solid-grown and submerged cultures, except for *Sporichthya* sp., produce an aerial form of hyphae that are uprightly initiated on the medium's surface by using holdfasts. However, many differences form aerial hyphae on solid surfaces, primarily for reproductive spores production (Flardh and Buttner 2009; van Dissel et al. 2014). From a germinating spores outgrowth, the substrate mycelium develops that usually is monopodial, which in few exceptional cases of *Actinobacteria* like *Thermoactinomyce* show branching of dichotomous nature (Kalakoutskii and Agre 1976). Alternatively, a large substrate mycelium with rudimentary or no aerial type mycelium is produced by members of the *Micromonosporaceae* family. *Actinobacteria* display various morphologies, including coccus [*Micrococcus*], coccobacillus [*Arthrobacter*], fragmenting hyphae [*Nocardia* spp.], and the ones with highly differentiated and permanent branching mycelia [e.g., *Streptomyces* spp., *Frankia*] (Atlas 1997). On the substrate, Corynebacteria do not produce mycelia at all, while Rhodococci produces filaments of elongated form but not a true mycelium (Locci and Schaal 1980). However, filaments develop at the apex rather than through the extension of

the lateral wall in the case of other *Actinobacteria* (Flardh 2003; Letek et al. 2008). The development of branched hyphae on substrate breaks to form motile elements with flagella, which is the characteristic of *Actinobacteria* belonging to the genus *Oerskovia* (Prauser et al. 1970). *Rhodococcus* and *Mycobacteria* do not frequently form the hyphae of aerial type (Ochi 1995).

7.7.2 Spore Chain Morphology

In the taxonomy of Actinobacteria, spores are extremely important (Locci and Sharples 1984). The preliminary sporulation steps in many oligosporic *Actinobacteria* could be considered as a process of budding because they show property that satisfies the definition of budding in the other bacteria. The substrate and aerial mycelium form spores of single cells or chains of diverse lengths. Spores may occur in special flagellated vesicles [sporangia], in other cases. Therefore, the formation of spores occurs directly on substrate mycelium in genera *Micropolyspora*, *Micromonospora*, and *Thermoactinomycetes* (Cross and Goodfellow 1973), whereas spores develop out of the aerial mycelium in *Streptomyces.* Motile spores are the characteristic feature of *Actinoplanes* and *Actinosynnema* groups, while unique heat-resistant endospores occur in *Thermoactinomyces* (Cross and Goodfellow 1973). Some other genera of *Actinobacteria* have sclerotia [*Chainia*], synnemas [*Actinosynnem*a], vesicles that contain spores [*Frankia*], or vesicles that are devoid of spores [*Intrasporangium*]. Based on their sporangial morphology, other genera are classified as *Actinoplanes*, *Ampulariella*, *Planomonospora*, *Planobispora*, *Dactylosporangium*, and *Streptosporangium.* The spores of diverse types are found in the Actinomycetes genera. Thus, to characterize the species, the morphology of spores can also be used: they might have spiny, smooth, hairy, rugose, or warty surfaces (Dietz and Mathews 1971).

7.7.3 Spore Chain Length

There exists wide variation from genus to genus in the spores number of every spore chain. The isolated spores are produced by genera *Salinispora*, *Micromonospora*, *Saccharomonospora*, *Thermomonospora*, and *Promicromonospora,* while spores of longitudinal pairs occur in *Microbispora.* Organisms of genera *Sporicthya*, *Saccharopolyspora*, *Actinomadura,* and some *Nocardia* spp. possess short length chain of spores, while the genera *Streptoverticillium, Nocardioides*, *Kitasatospora*, *Nocardia* spp., and *Streptomyces* produce a long length of chains up to 100 spores. Conversely, sporangia are spore-containing bags produced by *Frankia* species. The spore chains of *Streptomycetes* are classified as straight to flexuous [Rectus-

Flexibilis], open loops [Retinaculum-Apertum], open or closed spirals [spira], or verticillate (Pridham et al. 1958).

7.7.4 Based on Melanoid Pigment

Melanins are polymeric with varied molecular structures that are brown or black. They are formed by the oxidative polymerization reaction of phenolic and indolic compounds. Melanins are synthesized by a range of organisms, from humans to bacteria. For a long time, *Actinobacteria* are known for pigments production, depending on the strain, used medium, and culture age, which may be yellow, red-orange, brownish, pink, greenish-brown, distinct brown, black or blue (Lechevalier and Lechevalier 1965). These metabolic polymers are useful in taxonomic studies and are similar to humic substances in soil (Dastager et al. 2006; Manivasagan et al. 2013). In spite of melanins having a role in improving the survival and competitiveness of *Actinobacteria,* they are not indispensable for the growth and development of an organism.

7.8 Chemotaxonomic Classification

Chemotaxonomy is the grouping of organisms according to the similarity in their cellular chemistry based on the distribution of chemical components (Goodfellow and Minnikin 1985; O'Donnell 1988). In this chemotaxonomy, the constituents of cell wall lipids, amino acids, vitamin K2, muramate types, carbohydrates, proteins, and DNAs base composition are considered for grouping the organisms (Goodfellow and O'Donnell 1989; Williams et al. 1989) for gouping the organisms. Chemotaxonomic identification and classification are performed based on information resulting from techniques of chemical fingerprinting of whole organism. The valuable markers of chemotaxonomy that have been reported for the purpose of identification and classification of the *Actinomycetes* are discussed further (Ludwig et al. 2012). As the composition of cell walls differs between the suborders, this characteristic is valuable taxonomically for *Actinobacteria* analysis (Berd 1973).

Particularly, the information about the chemical structure of cell walls peptidoglycan is useful to classify actinomycetes because it promotes discrimination between *Actinobacteria* groups above the genus level. Several differentiating characteristics in relation to their composition and structure of peptidoglycans are identified (Willey et al. 2010). Non-proteinogenic amino acid 2,6-diaminopimelic acid [DAP] present in the cell wall of bacteria of Gram-positive nature is an important chemotaxonomical characteristic. Depending on the genus, the peptidoglycan may be DL or LL [*meso*]-DAP in *Actinobacteria.* By considering DAP isomerism, Lechevalier and Lechevalier had identified nine different chemotypes of the cell wall in *Actinobacteria* (Lechevalier and Lechevalier 1980). On the other

hand, diverse *Actinobacteria* groups share the same profile of DAP. For instance, in spite of differences in families and morphologies of *Streptomyces*, *Arachnia*, *Streptoverticillium*, and *Nocardioides* genera, they all share identical chemotype, i.e., chemotype I. Therefore, for assessing diversity in the phenotype of *Actinobacteria,* profiling of DAP and other genotypic or phenotypic criteria should be used (Bouizgarne and Ait Ben Aoumar 2014). Thus, a system was proposed to classify *Actinobacteria* on the basis of both their chemical and morphological characteristics (Lechevalier and Lechevalier 1965).

For the identification of specific *Actinobacteria* genera, patterns of fatty acid in cell are useful indicators of chemotaxonomy (Kroppenstedt 1985). Generally, fatty acids in bacteria have carbon chain length of C2 to over C90, but only C10 to C24 have taxonomic value (Suzuki et al. 1993). In *Actinobacteria,* majorly 3 types of fatty acid profiles have been reported (Kroppenstedt 1985). In bacteria, various isoprenoid quinone types are characterized (Collins et al. 1985), of which menaquinones are found in cell envelopes of actinomycetes (Kroppenstedt 1985; Suzuki et al. 1993; Collins et al. 1985; Collins 1994). Menaquinone analysis has provided valuable information for taxonomical classification of *Streptomyces, Actinomadura* and *Microtetraspora* strains (Kroppenstedt 1985; Collins et al. 1988; Kroppenstedt et al. 1990; Yamada et al. 1982). Additionally, menaquinones of cyclic form occur in *Nocardia* genus members (Goodfellow 1992; Tindall et al. 2006), while cyclic menaquinones with full saturation occur in *Pyrobaculum organotrophum* (Tindall et al. 2006). In the *Actinomycetes'* cytoplasmic membranes, different types of phospholipids are unevenly distributed, which provide information for the identification and classification of genera of Actinomycete (Williams et al. 1989; Goodfellow 1989). On the basis of semi-quantitative analysis of important phospholipid markers present in extracts of whole organism, *Actinobacteria* are classified into five phospholipid groups (Lechevalier 1977; Lechevalier et al. 1977, 1981).

In the identification of *Aeromicrobium* (Yokota and Tamura 1994) and *Dietzia* (Rainey et al. 1995), this classification system was used. It has been reported that the same type of phospholipid occurs in a population of the same genus of *Actinobacteria.* For chemotaxonomy, analysis of the composition of sugar is vital. One of the major constituents of cell envelope of actinomycete is neutral sugars, which is a useful marker of taxonomy at the suprageneric level. *Actinomycetes* can be divided into five groups based on the discontinuous distribution of major diagnostic sugars. The group A species contain galactose and arabinose in the cell wall; group B cell wall has madurose [3-*O*-methyl-D-galactose]; species with no diagnostic sugars are clustered in group C; cell wall of group D species contains xylose and arabinose; rhamnose and galactose are present in the cell wall of group E species (Labeda 1987; Lechevalier and Lechevalier 1970). Additionally, for the classification of some actinomycete taxa, the occurrence of 3-*O*-methyl-rhamnose in *Catellatospora* (Asano et al. 1989) and tyvelose in *Agromyces* (Maltsev et al. 1992) has been reported.

7.9 Molecular Classification

More recently, by the rapid advancement of genome sequencing, the classification of actinomycetes becomes easy by molecular taxonomic data. Notably, based on molecular analysis, recently, some organisms have been reclassified as they were previously placed in inappropriate taxonomic groups (Zhi et al. 2009). Recently, genome sequencing gave the final classification of *Kitasatospora* as a distinct genus within *Streptomycetaceae* (Girard et al. 2013) which provided a solution to a debate of a long time about the relationship of this group with genus *Streptomyces* (Zhang et al. 1997; Girard et al. 2014; Ichikawa et al. 2010; Kim et al. 2004). At present, without genetic analysis based on sequencing the 16S rRNA gene and DNA-DNA hybridization, even genome sequencing a new species cannot be claimed. The criteria of chemical and molecular composition have been used to group the order *Actinomycetales* into 14 suborders: *Actinomycineae*, *Pseudonocardineae*, *Corynebacterineae*, *Propionibacterineae,* *Jiangellineae*, *Actinopolysporineae*, *Kineosporineae*, *Streptomycineae*, *Micromonosporineae*, *Frankineae*, *Glycomycineae*, *Catenulisporineae,* *Micrococcineae*, and *Streptosporangineae* (Euzeby 1997). Moreover, 16S rRNA gene sequencing led to the identification of 130 genera and 39 families. Based on these molecular and chemical criteria, all the groups that were previously assigned to the taxonomic rank of "order" have been recovered as strictly being monophyletic. Still, some paraphyletic groups are found within the rank "suborder."

Berdy (2005) reported that rare actinomycetes produce highly unique, diverse, and rarely complicated compounds with tremendous antibacterial activity and low toxicity. Currently, more than 50 rare actinomycete taxa are reported to produce 2500 bioactive compounds (Fig. 7.1). These bioactive compounds can be used for pharmaceutical and biotechnological applications (Kurtboke 2010). The investigation of secondary metabolites from rare actinomycetes has been less frequent than

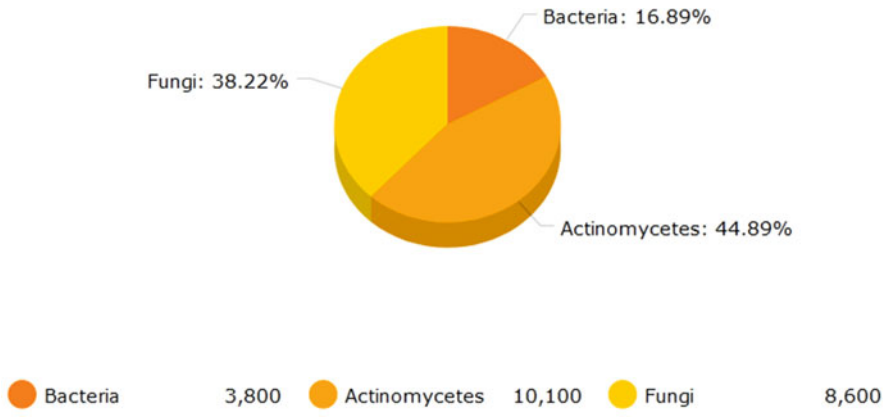

Fig. 7.1 Bioactive compounds of microbial origin

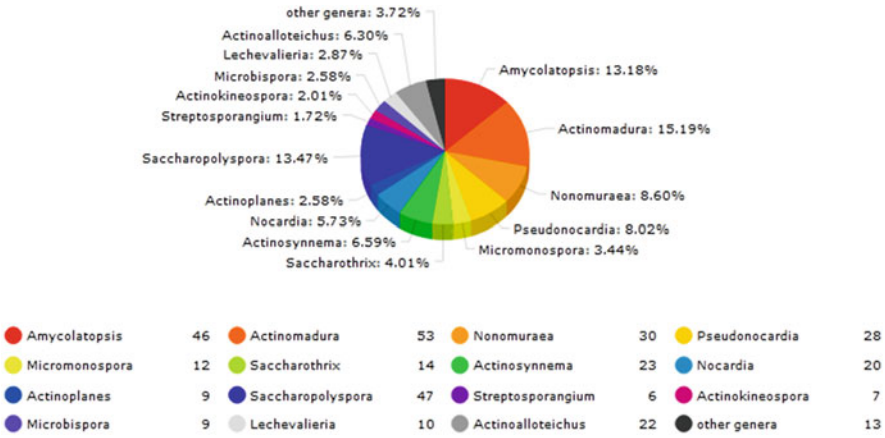

Fig. 7.2 The secondary metabolites of rare actinomycetes

Streptomyces. This has made rare actinomycetes a significant resource for finding new secondary metabolites with biological activity. A number of secondary metabolites discovered from 2008 to 2018 in 21 genera of rare actinomycetes isolated mainly from soil and insects were shown in Fig. 7.2 (Ding et al. 2019).

References

Abd-allah N, Tolba S, Hatem D (2012) Selective isolation of rare actinomycetes from different types of Egyptian soil. Egypt J Exp Biol 8(2):175–182

Abdelfattah MS, Elmallah MIY, Hawas UW, Abou El-Kassema LT, Eid MAG (2016) Isolation and characterization of marine-derived actinomycetes with cytotoxic activity from the Red Sea coast. Asian Pac J Trop Biomed 6(8):651–657

Agarwal A, Mathur N (2016) Thermophilic actinomycetes are potential source of novel bioactive compounds: a review. EJPMR 3(2):130–138

Al-Ansaria M, Alkubaisi N, Vijayaragavan P, Murugan K (2019) Antimicrobial potential of *Streptomyces* sp. to the gram positive and gram negative pathogens. J Infect Public Health 12(6):861–866

Aly MM, Bahamdain LA, Aba SA (2019) Unexplored extreme habitats as sources of novel and rare actinomycetes with enzyme and antimicrobial activities. J Pharm Biol Sci 14(6):45–54

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Araujo WL, Marcon J, Maccheroni W Jr, Van Elsas JD, Van Vuurde JW, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914

Asano K, Masunaga I, Kawamoto I (1989) *Catellatospora matsumotoense* sp. nov. and *C. tsunoense* sp. nov., actinomycetes found in woodland soils. Int J Syst Bacteriol 39:309–313

Atlas R (1997) Principles of microbiology. WCB McGraw-Hill, New York

Baltz RH (2006) Marcel Faber roundtable: is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? J Ind Microbiol Biotechnol 33:507–513

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clement C, Ouhdouch Y, van Wezeld GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80(1):1–43

Benhadj M, Gacemi-Kirane D, Menasria T, Guebla K, Ahmane Z (2018) Screening of rare actinomycetes isolated from natural wetland ecosystem (Fetzara Lake, northeastern Algeria) for hydrolytic enzymes and antimicrobial activities. J King Saud Univ Sci 31(4):706–712

Benson DR, Silvester WB (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev 57:293–319

Berd D (1973) Laboratory identification of clinically important aerobic actinomycetes. J Appl Microbiol 25:665–681

Berdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ (2002) Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insect Soc 49:363–370

Bouizgarne B, Ait Ben Aoumar A (2014) Diversity of plant associated *Actinobacteria*. In: Maheshwari DK (ed) Bacterial diversity in sustainable agriculture. Springer International, Heidelberg, pp 41–100

Bredholdt H, Galatenko OA, Engelhardt K, Faervik E, Terekhova LP, Zotchev SB (2007) Rare actinomycete bacteria from the shallow water sediments of the Trondheim Fjord, Norway: isolation, diversity and biological activity. Environ Microbiol 9(11):2756–2764

Buchanan RE (1917) Studies in the nomenclature and classification of the bacteria. II The primary subdivisions of the Schizomycetes. J Bacteriol 2:155–164

Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO (eds) (2011) Extremophiles handbook

Burges A, Raw F (1967) Soil biology. Academic Press, New York, p 532

Butman CA, Carlton JT (1995) Marine biological diversity—some important issues, opportunities and critical research needs. Rev Geophys 33:1201–1209

Chang XB, Liu WZ, Zhang XH (2011) *Spinactinospora alkalitolerans* gen. Nov. sp. nov., an actinomycete isolated from marine sediment. Int J Syst Evol Microbiol 61:2805–2810

Chaudhary N, Prabhu S (2016) Thermophilic actinomycetes from hot water spring capable of producing enzymes of industrial importance. Int J Res Stud Biosci (IJRSB) 4(6):29–35

Chavan DV, Mulaje SS, Mohalkar RY (2013) A review on actinomycetes and their biotechnological applications. Int J Pharm Sci Res 4(5):1730–1742

Collins MD (1994) Isoprenoid quinones. In: Goodfellow M, O'Donnell AG (eds) Chemical methods in prokaryotic systematics. Wiley, Chichester, pp 265–309

Collins MD, Goodfellow M, Minnikin DE, Alderson G (1985) Menaquinone composition of mycolic acid-containing actinomycetes and some sporoactinomycetes. J Appl Bacteriol 58: 77–86

Collins MD, Smida J, Dorsch M, Stackebrandt E (1988) *Tsukamurella* gen. Nov., harboring *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. Int J Syst Bacteriol 38: 385–391

Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608

Cross T (1981) Aquatic Actinomycetes: a critical survey of the occurrence, growth and role of Actinomycetes in aquatic habitats. J Appl Bacteriol 50:379–423

Cross T, Goodfellow M (1973) Taxonomy and classification of the actinomycetes. Soc Appl Bacteriol Symp Ser 2:11–112

Dastager S, Dayanand LWJA, Tang SK, Tian XP, Zhi XY, Xu LH, Jiang C (2006) Separation, identification and analysis of pigment (melanin) production in *Streptomyces*. Afr J Biotechnol 5: 1131–1134

Dietz A, Mathews J (1971) Classification of *Streptomyces* spore surfaces into five groups. Appl Microbiol 21:527–533

Ding T, Yang L-J, Zhang W-D, Shen Y-H (2019) The secondary metabolites of rare actinomycetes: chemistry and bioactivity. RSC Adv 9:21964–21988

Doumbou CL, Akimov V, Beaulieu C (1998) Selection and characterization of microorganisms utilizing thaxtomin a, a phytotoxin produced by *Streptomyces scabies*. Appl Environ Microbiol 64:4313–4316

Duan YY, Ming H, Dong L, Yin YR, Zhang Y, Zhou EM, Liu L, Nie GX, Li WJ (2014) *Streptomyces calidiresistens* sp. nov., isolated from a hot spring sediment. Antonie Van Leeuwenhoek 106(2):189–196

El-Gayar KE, Al-Abboud MA, Essa AMM (2017) Characterization of thermophilic bacteria isolated from two hot springs in Jazan, Saudi Arabia. J Pure Appl Microbiol 11:743–752

Emery KO (1969) The continental shelves. Sci Am 221:106–122

Euzeby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 47:590–592

Fang BZ, Salam N, Han MX, Jiao JY, Cheng J, Wei DQ, Xiao M, Li WJ (2017) Insights on the effects of heat pretreatment, pH, and calcium salts on isolation of rare Actinobacteria from karstic caves. Front Microbiol 8:1535

Flardh K (2003) Growth polarity and cell division in *Streptomyces*. Curr Opin Microbiol 6:564–571

Flardh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol 7:36–49

Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence in situ hybridization (FISH). Mar Biol 134:461–470

Ganachari S, Yaradoddi J, Somappa S, Mogre P, Tapaskar R, Salimath B, Venkataraman A, Viswanath V (2018) Green nanotechnology for biomedical, food and agricultural applications. In: Martínez LMT, et al (ed) Handbook of ecomaterials. Springer. https://doi.org/10.1007/978-3-319-68255-6_184

Gao B, Gupta RS (2012) Phylogenetic framework and molecular signatures for the main clades of the phylum *Actinobacteria*. Microbiol Mol Biol Rev 76:66–112

Girard G, Traag BA, Sangal V, Mascini N, Hoskisson PA, Goodfellow M, van Wezel GP (2013) A novel taxonomic marker that discriminates between morphologically complex actinomycetes. Open Biol 3:130073

Girard G, Willemse J, Zhu H, Claessen D, Bukarasam K, Goodfellow M, van Wezel GP (2014) Analysis of novel kitasatosporae reveals significant evolutionary changes in conserved developmental genes between *Kitasatospora* and *Streptomyces*. Antonie Van Leeuwenhoek 106: 365–380

Gochnauer MB, Leppard GG, Komaratat P, Kates M, Novitsky T, Kushner DJ (1975) Isolation and characterization of *Actinopolyspora halophila*, gen. Et sp. nov., an extremely halophilic Actinomycete. Can J Microbiol 21:1500–1511

Goodfellow M (1989) The genus *Rhodococcus* Zopf 1891. In: Williams ST, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 4, 1st edn. Williams & Wilkins, Baltimore, MD, pp 2362–2371

Goodfellow M (1992) The family *Nocardiaceae*. In: Balows A, Truper HG, Dworkin M, Harder W, Scheifer K-H (eds) The prokaryotes. Springer, New York, pp 1188–1212

Goodfellow M (2010) Selective isolation of actinobacteria. In: Baltz RH, Davies J, Demain AL (eds) Manual of industrial microbiology and biotechnology. Section 1: isolation and screening of secondary metabolites and enzymes. Bull AT, Davies JE (section eds), vol 3. ASM, Washington, DC, pp 13–27

Goodfellow M, Fiedler HP (2010) A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek 98:119–142

Goodfellow M, Minnikin DE (1985) Chemical methods in bacterial systematics. Academic Press, London

Goodfellow M, O'Donnell AG (1989) Search and discovery of industrially-significant actinomycetes. In: Baumberg S, Hunter IS, Rhodes PM (eds) Microbial products: new approach. Cambridge University Press, Cambridge, pp 343–383

Groth I, Schumann P, Schuetze B, Augsten K, Kramer I, Stackebrandt E (1999) *Beutenbergia cavernae* gen. Nov., sp. nov., an L-lysine-containing *actinomycete* isolated from a cave. Int J Syst Bacteriol 49(Pt 4):1733–1740

Haeder S, Wirth R, Herz H, Spiteller D (2009) Candicidin-producing Streptomyces support leaf-cutting ants to protect their fungus garden against the pathogenic fungus Escovopsis. Proc Natl Acad Sci U S A 106:4742–4746

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hamada M, Tamura T, Yamamura H, Suzuki K, Hayakawa M (2012) *Lysinimicrobium mangrovi* gen. Nov., sp. nov., an Actinobacterium isolated from the rhizosphere of a mangrove. Int J Syst Evol Microbiol 62:1731–1735

Hayakawa M (2008) Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica 22:12–19

Holguin G, Vazquez P, Bashan Y (2001) The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biol Fertil Soils 33:265–278

Hozzein WN, Ali MIA, Rabie W (2008) A new preferential medium for enumeration and isolation of desert actinomycetes. World J Microbiol Biotechnol 24:1547–1552

Ichikawa N, Oguchi A, Ikeda H, Ishikawa J, Kitani S, Watanabe Y, Nakamura S, Katano Y, Kishi E, Sasagawa M, Ankai A, Fukui S, Hashimoto Y, Kamata S, Otoguro M, Tanikawa S, Nihira T, Horinouchi S, Ohnishi Y, Hayakawa M, Kuzuyama T, Arisawa A, Nomoto F, Miura H, Takahashi Y, Fujita N (2010) Genome sequence of *Kitasatospora setae* NBRC 14216T: an evolutionary snapshot of the family *Streptomycetaceae*. DNA Res 17:393–406

Inahashi Y, Matsumoto A, Danbara H, Omura S, Takahashi Y (2010) *Phytohabitans suffuscus* gen. Nov., sp. nov., an actinomycete of the family *Micromonosporaceae* isolated from plant roots. Int J Syst Evol Microbiol 60:2652–2658

Inahashi Y, Matsumoto A, Omura S, Takahashi Y (2011) *Streptosporangium oxazolinicum* sp. nov., a novel endophytic *Actinomycete* producing new antitrypanosomal antibiotics, spoxazomicins. J Antibiot 64:297–302

Indananda C, Matsumoto A, Inahashi Y, Takahashi Y, Duangmal K, Tamchaipenet A (2010) *Actinophytocola oryzae* gen. Nov., sp. nov., isolated from the roots of tai glutinous rice plants, a new member of the family *Pseudonocardiaceae*. Int J Syst Evol Microbiol 60:1141–1146

Izumi H, Gauthier MEA, Degnan BM, Ng YK, Hewavitharana AK, Shaw PN, Fuerst JA (2010) Diversity of Mycobacterium species from marine sponges and their sensitivity to antagonism by sponge derived rifamycin synthesizing actinobacterium in the genus *Salinispora*. FEMS Microbiol Lett 313:33–40

Janso JE, Carter GT (2010) Biosynthetic potential of phylogenetically unique endophytic *Actinomycetes* from tropical plants. Appl Environ Microbiol 76:4377–4386

Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W (2005) Culturable marine actinomycetes diversity from tropical Pacific Ocean sediments. Environ Microbiol 7:1039–1048

Jiang CL, Xu LH (1996) Diversity of soil Actinomycetes in Yunnan, China. Appl Environ Microbiol 62:244–248

Jiang Y, Li WJ, Xu P, Tang SK, Xu LH (2006) Study on Actinomycete diversity under salt and alkaline environments. Wei Sheng Wu Xue Bao 46:191–195

Jurado V, Groth I, Gonzalez JM, Laiz L, Saiz-Jimenez C (2005a) *Agromyces salentinus* sp. nov. and *Agromyces neolithicus* sp. nov. Int J Syst Evol Microbiol 55:153–157

Jurado V, Groth I, Gonzalez JM, Laiz L, Saiz-Jimenez C (2005b) *Agromyces subbeticus* sp. nov., isolated from a cave in southern Spain. Int J Syst Evol Microbiol 55:1897–1901

Jurado V, Boiron P, Kroppenstedt RM, Laurent F, Couble A, Laiz L et al (2008) *Nocardia altamirensis* sp. nov., isolated from Altamira cave, Cantabria, Spain. Int J Syst Evol Microbiol 58(9):2210–2214

Kalakoutskii LV, Agre NS (1976) Comparative aspects of development and differentiation in actinomycetes. Bacteriol Rev 40:469–525

Kaltenpoth M (2009) Actinobacteria as mutualists: general healthcare for insects? Trends Microbiol 17:529–535

Kamjam M, Nopnakorn P, Zhang L, Peng F, Deng Z, Hong K (2019) *Streptomyces polaris* sp. nov. and *Streptomyces septentrionalis* sp. nov., isolated from frozen soil. Antonie Van Leeuwenhoek 112:375

Kathiresan K, Bingham BL (2001) Biology of mangroves and mangrove ecosystems. Adv Mar Biol 40:81–251

Kim BJ, Kim CJ, Chun J, Koh YH, Lee SH, Hyun JW, Cha CY, Kook YH (2004) Phylogenetic analysis of the genera *Streptomyces* and *Kitasatospora* based on partial RNA polymerase beta-subunit gene (*rpoB*) sequences. Int J Syst Evol Microbiol 54:593–598

Kim DS, Bae CH, Yeo JH, Chi WJ (2016) Identification and biochemical characterization of a new Xylan-degrading *Streptomyces atrovirens* subspecies WJ-2 isolated from soil of Jeju Island in Korea. Microbiol Biotechnol Lett 44(4):512–521

Kirby R (2011) Chromosome diversity and similarity within the *Actinomycetales*. FEMS Microbiol Lett 319:1–10

Kroppenstedt R (1985) Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Chemical methods in bacterial systematics. SAB technical series, vol 20. Academic Press, London, pp 173–199

Kroppenstedt RM, Stackebrandt E, Goodfellow M (1990) Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. Syst Appl Microbiol 13:148–160

Kumar V, Bharti A, Gupta VK, Gusain O, Bisht GS (2012) Actinomycetes from solitary wasp mud nest and swallow bird mud nest: isolation and screening for their antibacterial activity. World J Microbiol Biotechnol 28:871–880

Kurahashi M, Fukunaga Y, Sakiyama Y, Harayama S, Yokota A (2009) *Iamia majanohamensis* gen. Nov., sp. nov., an actinobacterium isolated from sea cucumber *Holothuria edulis*, and proposal of Iamiaceae fam. Nov. Int J Syst Evol Microbiol 59:869–873

Kurahashi M, Fukunaga Y, Sakiyama Y, Harayama S, Yokota A (2010) Euzebya tangerina gen. Nov., sp. nov., a deeply branching marine actinobacterium isolated from the sea cucumber Holothuria edulis, and proposal of Euzebyaceae fam. Nov., Euzebyales Ord. Nov. and Nitriliruptoridae subclass nov. Int J Syst Evol Microbiol 60:2314–2319

Kurtboke DI (2010) Biodiscovery from microbial resources: Actinomycetes leading the way. Microbiol Aust 31:53–57

Labeda D (1987) Actinomycete taxonomy: generic characterization. Dev Ind Microbiol 28:115–121

Lazzarini A, Cavaletti L, Toppo G, Marinelli F (2000) Rare genera of actinomycetes as potential sources of new antibiotics. Antonie Van Leeuwenhoek 78(399):405

Lechevalier MP (1977) Lipids in bacterial taxonomy: a taxonomist's view. Crit Rev Microbiol 5: 109–210

Lechevalier HA, Lechevalier MP (1965) Classification des *actinomycetesaerobiesbasee* sur leurmorphologie et leur composition chimique. Ann Inst Pasteur 108:662–673. (In French)

Lechevalier MP, Lechevalier H (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443

Lechevalier MP, Lechevalier HA (1980) The chemotaxonomy of actinomycetes, p 225 292. In: Dietz A, Thayer DW (eds) Actinomycetes taxonomy, vol A6. Virginia Society of Industrial Microbiology, Arlington, VA

Lechevalier MP, De Bievre C, Lechevalier HA (1977) Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem Syst Ecol 5:249–260

Lechevalier MP, Stern AE, Lechevalier HA (1981) Phospholipids in the taxonomy of actinomycetes. In: Schaal KP, Pulverer G (eds) Actinomycetes. Gustav Fischer Verlag, Stuttgart, pp 111–116

Lee SD (2006a) *Amycolatopsis jejuensis* sp. nov. and *Amycolatopsis halotolerans* sp. nov., novel Actinomycetes isolated from a natural cave. Int J Syst Evol Microbiol 56:549–553

Lee SD (2006b) *Nocardia jejuensis* sp. nov., a novel Actinomycetes isolated from a natural cave on Jeju Island, Republic of Korea. Int J Syst Evol Microbiol 56:559–562
Lee SD, Kang SO, Hah YC (2000) Catellatospora koreensis sp. nov., a novel Actinomycete isolated from goldmine cave. Int J Syst Evol Microbiol 50:1103–1111
Lee SD, Lee DW, Kim JS (2008) *Nocardioides hwasunensis* sp. nov. Int J Syst Evol Microbiol 58: 278–281
Letek M, Ordonez E, Vaquera J, Margolin W, Flardh K, Mateos LM, Gil JA (2008) Diva is required for polar growth in the MreB-lacking rod-shaped actinomycete *Corynebacterium glutamicum*. J Bacteriol 190:3283–3292
Little AEF, Murakami T, Mueller UG, Currie CR (2006) Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. Biol Lett 2:12–16
Lo CW, Lai NS, Cheah HY, Wong NKI, Ho CC (2002) Actinomycetes isolated from soil samples from the Crocker range Sabah. In: ASEAN review of biodiversity and environmental conservation, pp 1–7
Locci R, Schaal KP (1980) Apical growth in facultative anaerobic actinomycetes as determined by immunofluorescent labeling. Zentralbl Bakteriol A 246:112–111
Locci R, Sharples G (1984) Morphology. In: Goodfellow M, Mordarski M, Williams ST (eds) The biology of *Actinomycetes*. Academic Press, London, pp 165–199
Long H, Xiang W, Zhuang T, Lin P (2005) Microorganism resource of mangrove ecosystems. Chin J Ecol 24:696–702
Lubsanova DA, Zenova GM, Kozhevin PA, Manucharova NA, Shvarov AP (2014) Filamentous Actinobacteria of the saline soils of arid territories. Moscow Univ Soil Sci Bull 69:88–92
Ludwig W, Euzeby J, Schumann P, Buss HJ, Trujillo ME, Kampfer P, Whiteman WB (2012) Road map of the phylum Actinobacteria. In: Goodfellow M, Kampfer P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 5. Springer-Verlag, New York, pp 1–28
Mahajan GB, Balachandran L (2017) Sources of antibiotics: hot springs. Biochem Pharmacol 134: 35–41
Maldonado LA, Fenical W, Jensen PR, Kauffman CA, Mincer TJ, Ward AC, Bull AT, Goodfellow M (2005) *Salinispora arenicola* gen. Nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. Int J Syst Evol Microbiol 55:1759–1766
Maltsev II, Kalinovskii AI, Zgurskaya HI, Evtushenko LI (1992) Tyvelose in *Agromyces* cell walls. Syst Appl Microbiol 15:187–189
Manivasagan P, Venkatesan J, Sivakumar K, Kim SK (2013) Marine actinobacterial metabolites: current status and future perspectives. Microbiol Res 168:311–332
Mansour SR (2003) The occurrence and distribution of soil actinomycetes in Saint Catherine area, South Sinai, Egypt. Pak Biol Sci 6(7):721–728
Matsumoto A, Takahashi Y, Mochizuki M, Seino A, Iwai Y, Omura S (1998) Characterization of *Actinomycetes* isolated from fallen leaves. Actinomycetologica 12:46–48
Meklat A, Sabaou N, Zitouni A, Mathieu F, Lebrihi A (2011) Isolation, taxonomy, and antagonistic properties of halophilic Actinomycetes in Saharan soils of Algeria. Appl Environ Microbiol 77: 6710–6714
Mohammadipanah F, Wink J (2016) Actinobacteria from arid and desert habitats: diversity and biological activity. Front Microbiol 6:Article 1541
Mueller UG, Dash D, Rabeling C, Rodrigues A (2008) Coevolution between attine ants and Actinomycete bacteria: a reevaluation. Evolution 62:2894–2912
Nakaew N, Pathom-aree W, Lumyong S (2009) Generic diversity of rare Actinomycetes from Thai cave soils and their possible use as new bioactive compounds. Actinomycetologica 23:21–26
Niyomvong N, Pathom-aree W, Thamchaipenet A, Duangmal K (2012) Actinomycetes from tropical limestone caves. Chiang Mai J Sci 39:373–388

O'Donnell AG (1988) Recognition of novel actinomycetes. In: Goodfellow MM, Williams ST, Mordarski M (eds) Actinomycetes in biotechnology. Academic Press, London, pp 69–88
Ochi K (1995) Phylogenetic analysis of mycolic acid-containing wall chemotype IV actinomycetes and allied taxa by partial sequencing of ribosomal protein AT-L30. Int J Syst Bacteriol 45:653–660
Omura S, Takahashi Y, Iwai Y, Tanaka H (1982) *Kitasatosporia*, a new genus of the order *Actinomycetales*. J Antibiot 35:1013–1019
Parmar RS, Singh CA (2018) Comprehensive study of eco-friendly natural pigment and its applications. Biochem Biophys Rep 13:22–26
Piel J (2009) Metabolites from symbiotic bacteria. Nat Prod Rep 26:338–362
Prauser H, Lechevalier MP, Lechevalier H (1970) Description of *Oerskovia* gen. n. To harbor Orskov's motile *Nocardia*. Appl Microbiol 19:534
Pridham TG, Hesseltine CW, Benedict RG (1958) A guide for the classification of streptomycetes according to selected groups; placement of strains in morphological sections. Appl Microbiol 6: 52–79
Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, Xu LH, Li WJ (2009) Isolation, diversity, and antimicrobial activity of rare Actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176–6186
Rafiq M, Hayat M, Anesio AM, Umair S, Jamil U, Hassan N, Shah AA, Hasan F (2017) Recovery of metallotolerant and antibiotic resistant psychrophilic bacteria from Siachen glacier, Pakistan. PLoS One 12(7):e0178180
Rainey FA, Klatte S, Kroppenstedt RM, Stackebrandt E (1995) *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. Int J Syst Bacteriol 45:32–36
Rangseekaew P, Athom-Aree W (2019) Cave Actinobacteria as producers of bioactive metabolites. Front Microbiol 10:387
Schabereiter-Gurtner C, Saiz-Jimenez C, Pinar G, Lubitz W, Rolleke S (2002) Altamira cave Paleolithic paintings harbour partly unknown bacterial communities. FEMS Microbiol Lett 211:7–11
Schneemann I, Nagel K, Kajahn I, Labes A, Wiese J, Imhoff JF (2010) Comprehensive investigation of marine Actinobacteria associated with the sponge *Halichondria panicea*. Appl Environ Microbiol 76:3702–3714
Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field-grown potato plants and their plant-growth promoting and antagonistic abilities. Can J Microbiol 50:239–249
Shellikeri A, Kaulgud V, Yaradoddi J, Ganachari S, Banapurmath N, Shettar A (2018) Development of neem-based bioplastic for food packaging application. IOP Conf Ser Mater Sci Eng 376:012052. https://doi.org/10.1088/1757899X/376/1/012052
Singh L, Sharma H, Sahoo D (2019) Actinomycetes from soil of Lachung, a pristine high altitude region of Sikkim Himalaya, their antimicrobial potentiality and production of industrially important enzymes. Adv Microbiol 9:750–773
Sivalingam P, Hong K, Pote J, Prabakar K (2019) Extreme environment Streptomyces: potential sources for new antibacterial and anticancer drug leads? Int J Microbiol 2019:5283948, 20 pages
Stach JE, Bull AT (2005) Estimating and comparing the diversity of marine actinobacteria. Antonie Van Leeuwenhoek 87:3–9
Stach JEM, Maldonado LA, Ward AC, Goodfellow M, Bull AT (2003) New primers for the class actinobacteria: application to marine and terrestrial environments. Environ Microbiol 5:828–841
Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268
Subramani R, Aalbersberg W (2012) Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res 167:571–580
Subramani R, Aalbersberg W (2013) Culturable rare Actinomycetes: diversity, isolation and marine natural product discovery. Appl Microbiol Biotechnol 97:9291–9321

Subramani R, Narayanasamy M (2009) Screening of marine Actinomycetes isolated from the bay of Bengal, India for antimicrobial activity and industrial enzymes. World J Microbiol Biotechnol 25:2103–2111

Sultan MZ, Khatune NA, Sathi ZS, Bhuiyan SAMD, Sadik GM, Choudury MA, Gafur MA, Rahman AAMD (2002) *In vitro* antibacterial activity of an active metabolite isolated from *Streptomyces* species. Biotechnology 1:100–106

Surette MA, Sturz AV, Lada RR, Nowak J (2003) Bacterial endophytes in processing carrots (*Daucus carota* L. var. *sativus*): their localization, population density, biodiversity and their effects on plant growth. Plant Soil 253:381–390

Suzuki K, Goodfellow M, O'Donnell AG (1993) Cell envelopes and classification. In: Goodfellow M, O'Donnell AG (eds) Handbook of new bacterial systematics. Academic Press, London, pp 195–250

Taechowisan T, Peberdy JF, Lumyong S (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World J Microbiol Biotechnol 19:381–385

Takahashi Y, Omura S (2003) Isolation of new actinomycete strains for the screening of new bioactive compounds. J Gen Appl Microbiol 49(3):141–154

Takahashi Y, Matsumoto A, Seino A, Iwai Y, Omura S (1996) Rare Actinomycetes isolated from desert soils. Actinomycetologica 10:91–97

Terkina I, Drukker V, Parfenova V, Kostornova TY (2002) The biodiversity of actinomycetes in Lake Baikal. Microbiology 71(3):346–349

Thawai C (2012) Isolation and characterization of antibiotic-producing Actinomycetes from hot spring sediment of Thailand. In: International conference on BioScience: biotechnology and biodiversity—step in the future. The fourth joint UNS-PSU conference, Novi Sad, Serbia, 18–20 June 2012. Conference proceedings, pp 215–219

Tian XL, Cao LX, Tan HM, Han WQ, Chen M, Liu YH, Zhou SN (2007) Diversity of cultivated and uncultivated actinobacterial endophytes in the stems and roots of rice. Microb Ecol 53:700–707

Tian XP, Zhi XY, Qiu YQ, Zhang YQ, Tang SK, Xu LH, Zhang S, Li WJ (2009) *Sciscionella marina* gen. Nov., sp. nov., a marine *Actinomycete* isolated from a sediment in the northern South China Sea. Int J Syst Evol Microbiol 59:222–228

Tindall BJ, Kampfer P, Euzeby JP, Oren A (2006) Valid publication of names of prokaryotes according to the rules of nomenclature: past history and current practice. Int J Syst Evol Microbiol 56:2715–2720

Tiwari K, Gupta RK (2012a) Diversity and isolation of rare actinomycetes: an overview. Crit Rev Microbiol 39:256–294

Tiwari K, Gupta RK (2012b) Rare actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol 32:108–132

Tiwari K, Gupta RK (2013) Diversity and isolation of rare actinomycetes: an overview. Crit Rev Microbiol 39(3):256–294

Tolba S, Egan S, Kallifidas D, Wellington EM (2002) Distribution of streptomycin resistance and biosynthesis genes in *Streptomycetes* recovered from different soil sites. FEMS Microbiol Ecol 42(2):269–276

Tolba ST, Nagwa AAA, Hatem D (2013) Molecular characterization of rare actinomycetes using 16S rRNA-RFLP. Afr J Biol Sci 9:185–197

van Dissel D, Claessen D, van Wezel GP (2014) Morphogenesis of *Streptomyces* in submerged cultures. Adv Appl Microbiol 89:1–45

Webster NS, Hill RT (2001) The culturable microbial community of the great barrier reef sponge *Rhopaloeidesodorabile* is dominated by an α-Proteobacterium. Mar Biol 138:843–851

Wellington EM, Stackebrandt E, Sanders D, Wolstrup J, Jorgensen NO (1992) Taxonomic status of *Kitasatosporia*, and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and Henrici 1943, 339AL. Int J Syst Bacteriol 42:156–160

Willey JM, Sherwood LM, Woolverton CJ (2010) Prescott's microbiology, 7th edn. McGraw-Hill, New York

Williams ST, Goodfellow M, Alderson G (1989) Genus *Streptomyces* Waksman and Henrici 1943, 339AL. In: Williams ST, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 4, 1st edn. Williams & Wilkins, Baltimore, MD, pp 2452–2492

Xu LH, Jiang CL (1996) A study on diversity of aquatic Actinomycetes in lakes of the middle plateau, Yunnan, China. Appl Environ Microbiol 62:249–253

Xu L, Li Q, Jiang C (1996) Diversity of soil actinomycetes in Yunnan, China. Appl Environ Microbiol 62(1):244–248

Yamada Y, Aoki K, Tahara Y (1982) The structure of hexa-hydrogenated isoprenoid sidechain menaquinone with nine isoprene units isolated from *Actinomadura madurae*. J Gen Appl Microbiol 28:321–329

Yokota A, Tamura T (1994) Transfer of *Nocardioides fastidiosa* Collins and Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. Int J Syst Bacteriol 44: 608–611

Zhang Z, Wang Y, Ruan J (1997) A proposal to revive the genus *Kitasatospora* (Omura, Takahashi, Iwai, and Tanaka 1982). Int J Syst Bacteriol 47:1048–1054

Zhang LY, Ming H, Zhao ZL, Ji WL, Salam N, Jiao JY, Fang BZ, Li WJ, Nie GX (2018) *Nocardioides allogilvus* sp. nov. a novel actinobacterium isolated from a karst cave. Int J Syst Evol Microbiol 68:2485–2490

Zhi XY, Li WJ, Stackebrandt E (2009) An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int J Syst Evol Microbiol 59:589–608

Zin NM, Sarmin NIM, Ghadin N, Basri DF, Sidik NM, Hess WM, Strobel GA (2007) Bioactive endophytic streptomycetes from the Malay peninsula. FEMS Microbiol Lett 274:83–88

Chapter 8
Identification of Novel Actinomycetes

Jayachandra S. Yaradoddi, Merja H. Kontro, Nagaraj R. Banapurmath, Sharanabasava V. Ganachari, and M. K. Umesh

Abstract Recent technological developments have accelerated the vast collection of genome sequence data**.** There are nearly 8000 bacterial genomes available in various databases. Among them, actinobacteria genomes contributed significantly. The present methods in the classification of prokaryotes have relied on the integrated approaches of phenotypic, biochemical, and genotypic information, popularly known as polyphasic taxonomy. The advancement of molecular biology techniques using 16S rRNA, cellular G + C content analysis, phospholipid, and DNA-DNA hybridization analysis has gained much attention in the description of novel taxa and defined even at the species level. Particularly, the development in polyphasic taxonomy has played a remarkable role in classifying and describing most of the actinobacteria. Despite these advancements, there are several issues related to the species belonging to the genus *Streptomyces that* remains the same. However, the determination through 16S rRNA and phenotypic analysis is not enough to describe the novel taxon. A recent report has evidenced that there is scope in the classification of morphologically closely related actinomycetes within the families Micromonosporaceae and Streptomycetaceae.

J. S. Yaradoddi (✉)
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

M. H. Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

N. R. Banapurmath
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

M. K. Umesh
Department of Microbiology, Gulbarga University, Kalaburagi, Karnataka, India

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8.1 Introduction

Research work on actinobacteria is at its nascent stage, and original technological advancements will add to accumulate the genome sequence data of the microbial genomes (Shendure and Lieberman 2012). According to a latest report, more than 8000 bacterial genomes are available in public nucleotide databases; among them, many bacterial species confirm within the phylum actinobacteria (Goodfellow 2012). *Streptomycetes* contribute around 70% of recognized antibiotics in connection with this genus, so that these organisms can be used efficiently towards novel emerging antibiotic-resistant microorganisms (Berdy 2012; Hopwood 2007). Subsequently the announcement of genome sequences of the typical actinobacteria *Streptomyces coelicolor* A3(2) a decade ago (Bentley et al. 2002). However, many gene sequences of *Streptomyces* and other actinobacterial genomes are publicly accessible (http://www.genomesonline.org). These advancements feature the necessity for the fast and parallelly precise classification of environmentally and commercially remarkable organisms. Current approaches or methods in identification of such type of bacteria are mainly based on the multi-step process by a combination of phenotypic, biochemical, genomic, and phylogenetic information, known as polyphasic taxonomy (Schleifer 2009; Tindall et al. 2010; Yaradoddi et al. 2020; Vandamme et al. 1996; Yaradoddi and Sulochana 2020). This particular approach is being determined by gradual advancements in molecular biology, as perceived through the influence of 16S rRNA gene sequence analysis and DNA-DNA hybridization and its relative values are significant methods in the description of taxa, especially at the species level (Rossello-Mora and Amann 2001; Sutcliffe et al. 2012). The extensive utilization of the polyphasic taxonomy approach has led to enormous improvements regarding the classification of various taxonomic groups belonging to the phylum Actinomycetes. Despite the present advancements, few important issues remain unsolved. Through several related species under genus especially *Streptomyces*, the results offered by 16S rRNA and other related phenotypic markers are not constantly adequate to identify the novel taxon.

There is a specific method that needs to be established in the classification of the closely related genera in phenotypically complex actinomycetes, especially those classified under the families *Streptomycetaceae* and *Micromonosporaceae* (Ludwig et al. 2012a; Kämpfer 2012). For example, there is a persistent prerequisite in the determination of whether the genera *Streptomyces* is paraphyletic or the inclusion of species *Streptacidiphilus* and *Kitasatospora* within the evolutionary relatedness of this taxon simply reflects deficient variation in the entire 16S rRNA gene sequences (Labeda et al. 2012).

Certainly, the circumscription of genera, as divergent to species, is presently very particular within entire prokaryotes (Stackebrandt 2006).

8.2 Strains and Medium

Few important physicochemical parameters are considered vital in recovering actinomycetes from being obtained (Table 8.1). Girard and coworkers in 2013 (Girard et al. 2013) isolated *Streptomyces* strains such as Che1, Gre19, Gre54, and Che26 from French forest soil and maintained in Loire department and L13 strain from the soil in Canary Island Lanzarote. During the recovery of actinobacteria, serial dilution was employed for the soil samples, and diluted samples were spread onto plates containing humic acid contained agar (Hayakawa and Nonomura 1987), and the medium was added with antifungal antibiotic nystatin (50 mg mL) and antibacterial agent of nalidixic acid (10 mg mL) of these microorganisms sporulated profusely on regularly used medium MM (minimal medium), R2YE, or SFM contained agar plates added with 1% glycerol as the solitary carbon source (Kieser et al. 2000). *Streptomycetes* were cultured using the normal medium conditions as defined by Kiejser et al. (Keijser et al. 2000). To determine the potential *Streptomycetes* forms sporulation under submerged culture, they are usually allowed to grow in tryptic soy broth with sucrose (TSBS) or modified MM which was supplemented with mannitol (Girard et al. 2013); the TSBS grown bacterial cultures were exposed to nutritional shift down, which stimulate the submerged sporulation process (Colson et al. 2008). Afterwards, cultures were centrifuged, washed in MM, and transferred to MM containing mannitol or glycerol (1% w/v) as the single carbon source. The submerged spores were then collected, and the mycelial biomass was removed, tested for their ability of germination, and plated succeeding to the native strains to ensure its identity. Microscopy was employed as per a previous report (Kendrick and Ensign 1983). Meanwhile, the cultures were checked at regular periods of time using the phase-contrast microscope; conversely, the colony morphology was

Table 8.1 Physiological and biochemical requirements in cultivation of actinobacteria

Parameters	Characteristic features of actinobacteria
Temperature	Can be classified as slight, mesophilic, extremophilic
pH	Occurs as alkalophilic, acidophilic, and neutrophilic
Osmotic pressure	Few actinobacterial species are extremely halophilic in nature
Sugar fermentation and acid production	They are capable of utilizing organic acids, monosaccharides, disaccharides, polysaccharides, alcohol, etc.
Utilization of nitrogen source	They can use peptone, protein, nitrogen, amino acids, other inorganic matter, etc.
Antimicrobial activity	They are potential producers of the antibacterial, antifungal, anti-yeast activities, etc.
Growth factors	Mainly required vitamins, amino acids, hemin and nicotinamide adenine dinucleotide (NAD), etc.
Susceptibility	Sensitive to dyes, antibiotics, potassium cyanide, antimicrobial agents, etc.
Enzymes	Urease, oxidase, catalase, etc.

determined using a Zeiss Lumar V-12 stereo microscope, the above method can be followed in recovery of potential *Streptomyces* strains.

8.3 Taxonomic Description of Genera *Salinispora, Verrucosispora,* and *Micromonospora*

The SsgB (Sporulation specific cell division) proteins of various genera includes *Micromonospora*, *Actinoplanes*, *Verrucosispora,* and *Salinispora* were almost similar, as the outcome of close association detected between these members of the *Micromonosporaceae* family (Ahmed et al. 2013; Genilloud 2012a). The very next nearest relative can be *Stackebrandtia nassauensis* DSM 44728^T, employing around 65% amino acid (aa) identity to that of SsgB from *Micromonosporaceae*. The researchers could not witness the significant changes in the evolutionary relatedness of the *Micromonosporaceae* by means or without the sequences derived from NCBI accession numbers. *Stack. Nassauensis*, with the slight homology of SsgB, is interesting; this is due to genera *Stackebrandtia* designated to the order of *Glycomycetales*, whereas it is roughly related with the order *Micromonosporales* constructed on 16S rRNA gene sequence information (Ludwig et al. 2012b). Such highly diverse SsgB orthologues among the closely associated genus permit quick discrepancy between the phenotypically close actinobacteria. This reliable result concluded by analyzing the concatenated gene sequences of about 35 widely dispersed proteins as the type strain of *Stack. Nassauensis* that form a clade with *Micromonospora aurantiaca* ATCC 27029^T and a typical genera *Salinispora*; this species was supported by a 100% bootstrap value similarity (Gao and Gupta 2012).

SsgB proteins of *Micromonospor*a strain L5 and *M. aurantiaca* ATCC 27029^T are similar; nevertheless, two aa variations were observed among *Micromonospora* strain ATCC 39149. Interestingly, *Verrucosispora* and *Micromonospora* species have similar SsgB proteins; while 25 polymorphic nucleotides occur among genes, this is comparable to changes instituted among SsgB orthologues via numerous *Streptomyces* sp. Astoundingly *Verrucosispora* and *Micromonospora* have similar SsgB proteins, while 25 polymorphic nucleotides occur among the genes, the comparative difference between SsgB orthologues, since various *Streptomyces* species (maximum single amino acid change, and 25–30 polymorphic nucleotides). The SsgB gene sequences propose that *Verrucosispora* and *Micromonospora* strains may belong to the same genera. *Salinispora tropica* SsgB has a solitary variation related to the SsgB from *Micromonospora, Salinispora arenicola*, and *Verrucosispora* profiles (Wang et al. 2013; Genilloud 2012b). Lastly, members of the genera *Polymorphospora* mainly correlated to *Micromonospora* (Tamura et al. 2006), which could be exciting to understand how meticulously associated with the other *Micromonosporaceae*, and in specific to the genera *Micromonospora*, in terms of the SsgB gene sequence homologies and their significances for phylogeny. The significant difference between *S. tropica* and *S. arenicola* is very less than that

between *S. tropica* and the other genera, demonstrating that *Salinispora* species deviated from *Verrucosispora* and *Micromonospora* may arranged into a distinct clade. The three genera can also be differentiated from one another based on comparisons of menaquinone, fatty acid, and sugar profiles. The difficulties involved in classifying closely related actinomycetes at the genetic level are demonstrated via the genus *Kitasatospora*, which was initially proposed by Omura et al. (1982). Later, they changed the genus as *Streptomyces* (Wellington et al. 1992) and, at that point, re-established it as a distinct genus (Zhang et al. 1997).

The phylogenetic position of the genera *Kitasatospora* has yet needs to be determined (Kämpfer 2012), as demonstrated by the fact that even though associated two genera from corresponding clades after using conserved rpoB gene sequence data, *Kitasatospora* species relating to a huge statistically unconfirmed clade among *Streptomyces* sp.16S rRNA gene tree (Labeda et al. 2012). Labeda and colleagues recognized that *Kitasatospora* can only be seen as taxonomically authenticated of the genera *Streptomyces* genera and should be verified using polyphasic taxonomy. To resolve this interesting taxonomic dilemma, Ichikawa et al., in 2010, compared SsgB orthologue (KSE_14600) and described the genome of *Kitasatospora setae* KM-6054^T (Ichikawa et al. 2010) related to streptomycetes. There are three to four amino acid variations identified within the *Streptomyces* SsgB orthologues and several 50 nT variations were associated with the ssgB DNA consensus sequence. The observed divergence is significant, provided that only a sole amino acid replacement was observed in all SsgB orthologues of *streptomycetes*; nevertheless, it does not offer definite substantiation where *Kitasatospora* must maintain its position with distinct genera.

Primarily, the taxonomic classification of actinomycetes is mainly based on morphological features such as cell morphology, nutritional requirements, and pathogenicity (Lehmann and Neumann 1896). Furthermore, the biochemical and physiological properties of bacteria were also crucial in identification purposes (Sulochana et al. 2014a, b; Anil Kumar et al. 2010); methods such as DNA-DNA hybridization (Minnikin et al. 1975; Ezaki et al. 1989) and chemotaxonomy (Christensen et al. 2000) were also extensively used. The advantages of DNA amplification and 16S rRNA gene sequence analysis enabled the vital criteria for the determination of the taxonomic position of prokaryotes (Gándara et al. 2001; Coenye and Vandamme 2004; Konstantinidis and Tiedje 2007); nowadays, there has been tremendous improvement in discovering novel taxon (Chun and Rainey 2014). 16S rRNA gene analysis is an excellent method in defining the phylogenetic relationship among different species among the same genera or from organisms of other genera; 16S rRNA gene contains functionally persistent and composed of variable and highly conserved regions. Several additional molecular tools have been used in the identification of prokaryotic organisms, majorly MLST (multilocus sequencing typing) (Maiden et al. 1998; Sullivan et al. 2005), SDS-PAGE analysis of whole-cell proteins (Tan et al. 1997), secondary structure, and conserved nucleotides analysis of different regions among the 16S rRNA gene (Bouthinon and Soldano 1999; Akutsu 2000). Though the genomic age of organisms representing some genomic features also has good potential in determining the taxonomic

position of archaea and bacteria that is through molecular percentage content of G + C (Guanosine and cytosine) and also using DNA-DNA hybridization methods (Chun and Rainey 2014; Ramasamy et al. 2014; Amaral et al. 2014; Meier-Kolthoff et al. 2014).

8.4 Extraction and Purification of Actinobacterial Genomic DNA

It is a well-known fact that DNA is the main transporter of genetic information and which is also the foundation for the gene expression system. Molecular phylogenetic analysis also plays a key role in the identification of actinobacteria. Proceeding with the genetic-based techniques, the primary step involved in the extraction and purification of DNA, the DNA's purity defines the success or failure of the method followed.

8.4.1 Principles Involved

The purity of the DNA in the preparation of the DNA sample is very significant. If not, it is not easy to obtain the proper outcome. For confirming the purity of the DNA, subsequent conditions must be maintained; they are as follows: evading exposure towards higher temperature; balanced pH (pH between 5.0 and 9.0); maintenance of the ionic strength of the buffer; critical to keep the space configuration of DNA; and reduction of disruption of DNA concerning physical factors involved in extraction processes, such as mixing, freezing, thawing, and high-speed oscillation. In the natural environment, there are many DNA and RNA enzymes that are meant for the digestion of the biomolecules. Therefore the constituents used in the extraction process need to be sterilized and require a quantity of the enzyme inhibitors, that can be supplemented to the extraction buffer. Apart from this, circumventing contamination caused by exogenous DNA is also a critical factor.

8.4.2 Key Steps Involved

8.4.2.1 Cell Disruption

To extract the intracellular DNA, the first step is to disrupt the cell. To disrupt the microbial biomass, a list of the following methods is generally used: grinding with liquid nitrogen, ultrasonic vibrations, freezing, thawing, alkali treatment, surfactant treatment, microwave preparation.

8.4.2.2 Elimination of Nucleoprotein from the Extracted Genomic DNA

The main forces involved in the binding of protein and nucleic acid are hydrogen bond, Van der Waals interactions, and electrostatic forces. The vital process involved in extracting genomic DNA is separation of directly associated protein by the genomic DNA to avoid the denaturation process. At present numerous generally adapted techniques are available, for example, the addition of a concentrated solution of NaCl, lead to the depolymerization of nucleoprotein. SDS addition forms the protein-free genomic DNA in the phenol/chloroform extraction process. Nevertheless, numerous kits are available in elimination of nucleoprotein within the sample to ensure the quality of DNA.

8.4.2.3 Precipitation

The process of precipitation is recognized to be the best method used to concentrate DNA; moreover, it is widely accepted method. A major benefit of using the precipitation process is to eliminate various salts from the solution. Apart from the above, precipitation plays a key role in the purification of DNA. Isopropanol, ethyl alcohol, and polyethylene glycol are generally used for purification. Ethyl alcohol is the most promising precipitant. Ethanol or ethyl alcohol, usually two times volume, is appropriate for the precipitation of DNA and 2.5 times for RNA if in suitable salt concentration. The benefit of using isopropanol is its requirement; a small volume is enough for a large number of DNA samples. However, it has a disadvantage of salt coprecipitation along with DNA and is tough to volatilize. Consequently, washing with 70% ethanol multiple times for the removal of isopropanol and salt is a must. PEG is used to select the DNA fragments of diverse lengths.

8.4.2.4 Standard Time and Temperature for DNA Precipitation

The most common step is that DNA precipitation can be conducted at a lower temperature, likely −20 °C or −70 °C, kept for few hours or even overnight. Again as long as the type of action is easy to cause salt coprecipitation along with DNA. Thus, incubation at about 0 °C or 4 °C for 30–60 min can be preferred.

8.5 Few Meticulous Processes Employed for the Extraction and Purification of Genomic DNA

8.5.1 Purification of Genomic DNA

Always there is a need for ultrapure DNA in the sequencing process, cellular GC content, and DNA-DNA hybridization. The procedure can be followed is: to 100 mg

of cell mass extracted DNA, add 480μL 1× TE buffer, then to the mixture add 15μL RNase A (400μg/mL) and 5μL of proteinase K (20 mg/mL) and further incubate at 37 °C for about 1 h; 550μL mixture of phenol:chloroform:isoamyl alcohol (with a ratio of 25:24:1) should be mixed to vortex for 2 min, spin for 10 min about 12,000 rpm, and the phase contained DNA is then transferred to another sterile Eppendorf tube subsequent to centrifugation (avoid sucking waste in between); afterwards add 550μL chloroform again, vortex it for 2 min, spin the tube for 10 min (12,000 rpm), and the aqueous phase containing DNA is then transferred to a fresh tube. To this, add 50μL of 3 mol/L sodium acetate (pH 4.8–6.2), vortex it gently and immediately, add 800μL absolute alcohol, vortex it again for about 10 min, and keep it at 4 °C for 30 min for about 2 h. Spin at 12,000 rpm for 10 min, and add 200μL ethanol, slightly vortex, spin for about 5 min at 12,000 rpm, discard the ethanol 2–3 times, the same step has to be repeated, and the sample will be dried at room temperature. Later, add 50μL (quantity depends on the availability of DNA) of deionized water to sample to dilute the DNA.

8.5.2 Amplification of 16S rRNA Gene Sequence

Polymerase chain reaction (PCR) is a useful method employed in the rapid amplification of the target DNA or gene sequence. This technique was invented by Kary Mullis in the year 1983 and for his remarkable work he has won the Nobel Prize in chemistry in 1993. PCR has been explained in several ways since beginning, and today it is generally used for many applications that include cloning, genotyping, mutation, forensics, sequencing, microarray, detection, and patterning testing.

Typically, PCR relies on a three-step process. A sample comprising a diluted concentration of template DNA is combined with a thermostable DNA polymerase, dNTPs (deoxynucleoside triphosphates), primers, and buffer (containing magnesium). During the initial phase of PCR, the sample is heated at a temperature of 94–98 °C for about 3–8 min, which pre-denatures the double-helical structure of DNA, divided into two separate single strands. In the next step, the sample is heated to 94–98 °C temperature for 30–60 s to generate continuous denaturation of double-stranded DNA. In a later stage, the temperature should be decreased to 52–65 °C to permit the primers to anneal in a specific site in the single strand, which is also called a template. At last, the temperature is generally increased to 72 °C providing the DNA polymerase enzyme to react by adding dNTPs that generate a fresh strand of DNA. During the process, the extension is diverse based on the extent of the sample sequence and the type of polymerase used. Normally, the extension speed of the Taq-DNA polymerase is 1 kb/min. The final step involves the final extension process, preceded with repair leads to seal some gaps created during step two, and the rate of reaction reaches a peak during this step.

8.5.3 Amplification of 16S rRNA

It is reported that there are universal primers available for the amplification of 16S rRNA of the actinobacteria; they are as follows:

27F: AGAGTTTGATCCTGGCTCAG
1492R: GGTTACCTTGTTACGACTT

The 16S rRNA gene sequence can be the finest molecules to study the evolutionary relationships among prokaryotic organisms. Due to its existance in almost all bacterial species, they consists of highly conserved regions and they are variable among different species but functionally they are constant. As designated above, 16S rRNA gene sequence analysis is normally carried out as a foremost step in identifying new species. The basic constituents for amplification of 16S rDNA are listed in Tables 8.2 and 8.3.

8.5.4 Issues During the Amplification of 16S rRNA Gene Sequence

1. Both negative and positive controls can be used each time; if the negative controls turn to positive, then the amplification process needs to be repeated.
2. Higher temperatures kept for a long reaction duration to obtain higher G + C content are optional, since the increased dosage of polymerase and template DNA against the number of cycles. Through annealing process temperature can be reduced, and it is beneficial. But, examine system update is essential.

Table 8.2 Constituents in amplification of 16S rDNA gene sequence

Components	Quantity (μL)
10× PCR buffer	5.0
dNTPs	4.0
27F (25 pmol/μL)	1.0
1492R (25 pmol/μL)	1.0
Template DNA	1.0
Taq DNA polymerase	0.3
Dd H_2O	37.7
Total volume	50.0

Table 8.3 Normal condition for 16S rRNA amplification

Number of steps	Temperature and incubation time
1	94 °C 4 min
2	94 °C 45 s
3	55 °C 45 s
4	72 °C 90 s
5	Repeat steps 2–4 for 35 times
6	72 °C 10 min
7	4 °C hold (optional)

3. Unclear products exists instead of no products; lower dose of polymerase and template DNA leads to annealing temperature.
4. Impurities like phenol or increased salt may affect the outcome if no PCR product or nonspecific products.
5. Contaminated template DNA could lead to sequencing failure, or there is a appearance of double peak interfere with pure strain identification.
6. If PCR product is wrongly labeled, there is a possibility of causing template mutations, incorrect primer, contagion, and inaccurate annealing temperature.
7. Using many primers may lead to the annealing themselves.

8.5.5 Recognition of Polymerase Amplification Products

The discovery of primogenital electrophoresis trial was done for closely 200 years ago, the technology of electrophoresis has been constantly progressed and improvised. In recent times electrophoresis is one of the most largely used techniques for the detection of macromolecules of biological origin and which is really exciting. Electrophoresis is a method that is involved in the purification of various biological macromolecules such as nucleic acids and proteins, which generally have differences among their charge, size, and conformation. Once the charged molecules are under an electric field, these molecules travel towards the negative or positive pole based on their charge. Nucleic acids such as DNA and RNA are electrophoresed using a gel or matrix. Generally, the matrix is cast according to the shape of thin slabs through wells for loading the sample. The gel is dissolved in an electrophoresis buffer (TBE or TAE) that gives rise to ions to carry a current and maintain the pH to a fairly constant value. The gel is usually made up of either polyacrylamide or agarose, every one of which has credits appropriate to specific errands: agarose is a polysaccharide obtained from the seaweeds. It is ideally utilized in a small quantity between 0.5–2%. Agarose gels are incredibly simple to prepare: just pour agarose powder in a buffer, dissolve it by warming, and pour the gel. Additionally it is non-harmful. Agarose gels have a huge scope of partition, however, with moderately low resolving power. By fluctuating the convergence of agarose, parts of DNA from around 100 bp to 50,000 bp can be isolated utilizing standard electrophoretic procedures. Polyacrylamide is a cross-connected polymer of acrylamide. The length of the polymer chains is directed by the grouping of acrylamide utilized, which is normally somewhere in the range of 3.5% and 20%. Polyacrylamide gels are fundamentally more complex to prepare than agarose gels and have a somewhat little scope of division, yet with high settling power. Since oxygen restrains the polymerization cycle, they should be poured between glass plates (or chambers). Acrylamide is an intense neurotoxin and has a small range of separation. Wear expendable gloves while handling the acrylamide and a cover when weighing out powder. On account of DNA, polyacrylamide is utilized for isolating fragments of under 500 bp. In any case, under appropriate conditions, fragments of DNA complementary long single straded base pair are effectively settled. Rather than agarose, polyacrylamide gels are utilized broadly for isolating and describing mixtures of proteins.

8.6 Conclusion

Along with the morphological and biochemical characterization, 16S rRNA gene sequence analysis, chemo biosystematics, and DNA-DNA hybridization studies help to identify bacteria or actinobacteria at the species level (Anil Kumar et al. 2010). It is a well-known fact that actinobacteria inherently have the potential to produce bioactive compounds (Fig. 8.1) and other unique metabolites, which have industrial applications. Most advanced methods like DNA-DNA hybridization can be employed in the identification of potential strains at the species level (Fig. 8.2). However, extensive studies are much essential in the exploration of these bacteria to

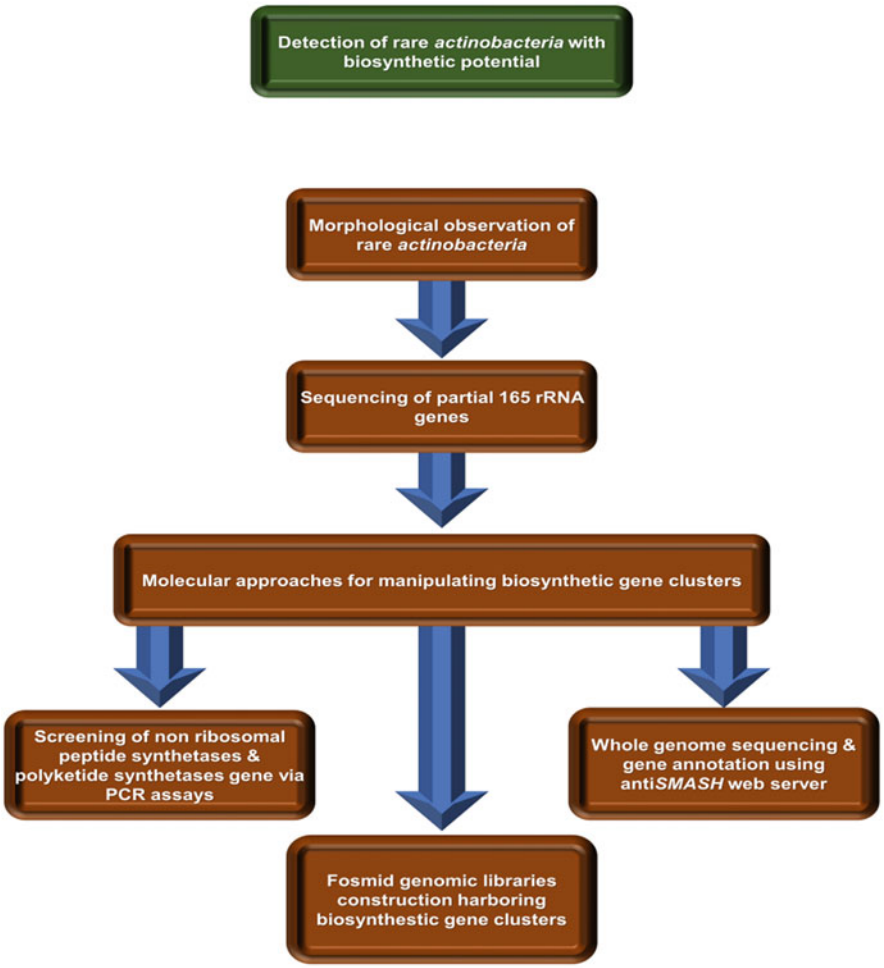

Fig. 8.1 Identification potential biosynthetic gene clusters from the Actinobacteria

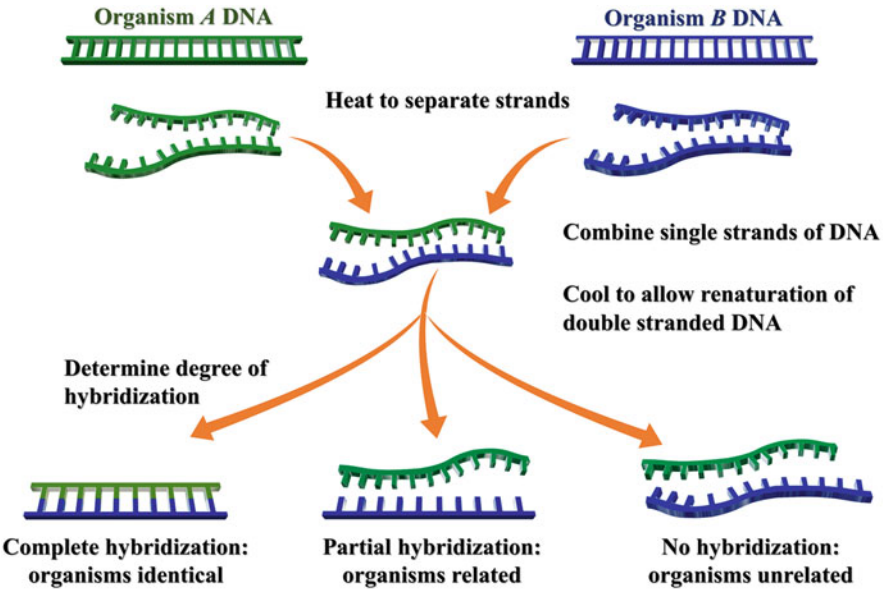

Fig. 8.2 Schematic representation of DNA-DNA hybridization of Actinomycetes

attain new heights in the biotechnological sector. In addition, in silico studies against the target identification for binding sites are under progress (Mohan et al. 2015a, b; Prabhu et al. 2015; Khieu et al. 2015; Yaradoddi et al. 2019; Manikprabhu et al. 2016; Wang et al. 2017).

References

Ahmed L, Jensen PR, Freel KC, Brown R, Jones AL, Kim BY, Goodfellow M (2013) *Salinispora pacifica* sp. nov., an actinomycete from marine sediments. Antonie Van Leeuwenhoek 103: 1069–1078. https://doi.org/10.1007/s10482-013-9886-4

Akutsu T (2000) Dynamic programming algorithms for RNA secondary structure prediction with pseudoknots. Discret Appl Math 104:45–62. https://doi.org/10.1016/S0166-218X(00)00186-4

Amaral GR, Dias GM, Wellington-Oguri M, Chimetto L, Campeão ME, Thompson FL, Thompson CC (2014) Genotype to phenotype: identification of diagnostic vibrio phenotypes using whole genome sequences. Int J Syst Evol Microbiol 64:357–365. https://doi.org/10.1099/ijs.0.057927-0

Anil Kumar S, Arunashri R, Jayachandra SY, Sulochana MB (2010) Screening of extracellular hydrolytic enzymes from *Marinobacter hydrocarbonoclasticus* strain AK5. Int J Bioscan 5(1): 97–99

Bentley SD et al (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141–147. https://doi.org/10.1038/417141a

Berdy J (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot (Tokyo) 65:385–395. https://doi.org/10.1038/ja.2012.27

Bouthinon D, Soldano H (1999) A new method to predict the consensus secondary structure of a set of unaligned RNA sequences. Bioinformatics 15:785–798. https://doi.org/10.1093/bioinformatics/16.10.785

Christensen H, Angen Ø, Mutters R, Olsen JE, Bisgaard M (2000) DNA-DNA hybridization determined in micro-wells using covalent attachment of DNA. Int J Syst Evol Microbiol 50: 1095–1102. https://doi.org/10.1099/00207713-50-3-1095

Chun J, Rainey FA (2014) Integrating genomics into the taxonomy and systematics of the bacteria and archaea. Int J Syst Evol Microbiol 64:316–324. https://doi.org/10.1099/ijs.0.054171-0

Coenye T, Vandamme P (2004) Use of the genomic signature in bacterial classification and identification. Syst Appl Microbiol 27:175–185. https://doi.org/10.1078/072320204322881790

Colson S, van Wezel GP, Craig M, Noens EE, Nothaft H, Mommaas AM, Titgemeyer F, Joris B, Rigali S (2008) The chitobiose-binding protein, DasA, acts as a link between chitin utilization and morphogenesis in Streptomyces coelicolor. Microbiology 154:373–382. https://doi.org/10.1099/mic.0.2007/011940-0

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyri-bonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229. https://doi.org/10.1099/00207713-39-3-224

Gándara B, Merino AL, Rogel MA, Martínez-Romero E (2001) Limited genetic diversity of Brucella spp. J Clin Microbiol 39:235–240. https://doi.org/10.1128/JCM.39.1.235-240.2001

Gao B, Gupta RS (2012) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76:66–112. https://doi.org/10.1128/MMBR.05011-11

Genilloud O (2012a) Current challenges in the discovery of novel antibacterials from microbial natural products. Recent Pat Antiinfect Drug Discov 7:189–204. https://doi.org/10.2174/157489112803521968

Genilloud O (2012b) Genus *Micromonospora*. In: Busse H-J, Goodfellow M, Kämpfer P, Ludwig W, Suzuki K, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 1035–1038

Girard G, Traag BA, Sangal V, Mascini N, Hoskisson PA, Goodfellow M, van Wezel GP (2013) A novel taxonomic marker that discriminates between morphologically complex actinomycetes. Open Biol 3:130073. https://doi.org/10.1098/rsob.130073

Goodfellow M (2012) Phylum XXVI. Actinobacteria phyl. Nov. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 1–2083

Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferm Technol 65:501–509. https://doi.org/10.1016/0385-6380(87)90108-7

Hopwood DA (2007) Streptomyces in nature and medicine: the antibiotic makers. Oxford University Press, New York

Ichikawa N et al (2010) Genome sequence of Kitasatospora setae NBRC 14216T: an evolutionary snapshot of the family *Streptomycetaceae*. DNA Res 17:393–406. https://doi.org/10.1093/dnares/dsq026

Kämpfer P (2012) Family 1. Streptomycetaceae Waksman and Henrici 1943, 339AL emend. Rainey, Ward-Rainey and Stackebrandt, 1997, 486 emend. Kim, Lonsdale, Seong and Goodfellow 2003b, 113 emend. Zhi, Li and Stackebrandt 2009, 600. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 1446–1454

Keijser BJ, van Wezel GP, Canters GW, Kieser T, Vijgenboom E (2000) The ram-dependence of *Streptomyces lividans* differentiation is bypassed by copper. J Mol Microbiol Biotechnol 2: 565–574

Kendrick KE, Ensign JC (1983) Sporulation of Streptomyces griseus in submerged culture. J Bacteriol 155:357–366

Khieu T-N, Liu M-J, Nimaichand S, Quach N-T, Chu-Ky S, Phi Q-T, Thu-Trang V, Nguyen T-D, Xiong Z, Manikprabhu D, Jun-Li W (2015) Characterization and evaluation of antimicrobial

and cytotoxic effects of *Streptomyces* sp. HUST012 isolated from medicinal plant *Dracaena cochinchinensis* lour. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00574

Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. The John Innes Foundation, Norwich

Konstantinidis KT, Tiedje JM (2007) Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead. Curr Opin Microbiol 10:504–509. https://doi.org/10.1016/j.mib.2007.08.006

Labeda DP et al (2012) Phylogenetic study of the species within the family *Streptomycetaceae*. Antonie Van Leeuwenhoek 101:73–104. https://doi.org/10.1007/s10482-011-9656-0

Lehmann KB, Neumann RO (1896) Atlas und Grundriss Der Bakteriologie und Lehr-buch Der SpeziellenBakteriologischenDiagnostik, 1st edn. Lehmann JF, Munchen

Ludwig W, Euzeby J, Schumann P, Busse H-J, Trujillo ME, Kämpfer P, Whitman WB (2012a) Roadmap of the phylum Actinobacteria. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 1–28

Ludwig W, Euzeby J, Schumann P, Busse H-J, Trujillo ME, Kämpfer P, Whitman WB (2012b) Road map of the phylum Actinobacteria. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 1–28

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95:3140–3145

Manikprabhu D, Cheng J, Chen W, Sunkara AK, Mane SB, Kumar R, Hozzein WN, Duan Y-Q, Li W-J (2016) Sunlight mediated synthesis of silver nanoparticles by a novel actinobacterium (*Sinomonas mesophila* MPKL 26) and its antimicrobial activity against multi drug resistant *Staphylococcus aureus*. J Photochem Photobiol B Biol 158:202–205. https://doi.org/10.1016/j.jphotobiol.2016.01.018

Meier-Kolthoff JP, Klenk HP, Göker M (2014) Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64:352–356. https://doi.org/10.1099/ijs.0.056994-0

Minnikin DE, Alshamaony L, Goodfellow M (1975) Differentiation of mycobacterium, Nocardia, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. J Gen Microbiol 88:200–204. https://doi.org/10.1099/00221287-88-1-200

Mohan RK, Siva DS, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant Exo-Inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan RK, Siva DS, Jayachandra SY, Parameshwar AB, Agsar D, Bikshapathi E, Sulochana MB (2015b) In *Silico* structural analysis for exo-Inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol App Sci 4(11):858–867

Omura S, Takahashi Y, Iwai Y, Tanaka H (1982) Kitasatosporia, a new genus of the order Actinomycetales. J Antibiot (Tokyo) 35:1013–1019. https://doi.org/10.7164/antibiotics.35.1013

Prabhu DM, Quadri SR, Cheng J, Liu L, Chen W, Yang Y, Hozzein WN, Lingappa K, Li W-J (2015) *Sinomonas mesophila* sp. nov., isolated from ancient fort soil. J Antibiot 68:318–321

Ramasamy D, Mishra AK, Lagier JC, Padhmanabhan R, Rossi M, Sentausa E, Raoult D, Fournier PE (2014) A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 64:384–391. https://doi.org/10.1099/ijs.0.057091-0

Rossello-Mora R, Amann R (2001) The species concept for prokaryotes. FEMS Microbiol Rev 25:39–67. https://doi.org/10.1016/S0168-6445(00)00040-1

Schleifer KH (2009) Classification of bacteria and archaea: past, present and future. Syst Appl Microbiol 32:533–542. https://doi.org/10.1016/j.syapm.2009.09.002

Shendure J, Lieberman AE (2012) The expanding scope of DNA sequencing. Nat Biotechnol 30: 1084–1094. https://doi.org/10.1038/nbt.2421

Stackebrandt E (2006) Defining taxonomic ranks. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, 3rd edn. Springer, Berlin, pp 29–57

Sullivan CB, Diggle MA, Clarke SC (2005) Multilocus sequence typing: data analysis in clinical microbiology and public health. Mol Biotechnol 29:245–254. https://doi.org/10.1385/MB:29:3:245

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Sutcliffe IC, Trujillo ME, Goodfellow M (2012) A call to arms for systematists: revitalizing the purpose and practises underpinning the description of novel microbial taxa. Antonie Van Leeuwenhoek 101:13–20. https://doi.org/10.1007/s10482-011-9664-0

Tamura T, Hatano K, Suzuki K (2006) A new genus of the family Micromonosporaceae, Polymorphospora gen. Nov., with description of Polymorphospora rubra sp. nov. Int J Syst Evol Microbiol 56:1959–1964. https://doi.org/10.1099/ijs.0.64046-0

Tan ZY, Xu XD, Wang ET, Gao JL, Martinez-Romero E, Chen WX (1997) Phylogenetic and genetic relationships of Mesorhizobium tianshanense and related rhizobia. Int J Syst Bacteriol 47:874–879. https://doi.org/10.1099/00207713-47-3-874

Tindall BJ, Rossello-Mora R, Busse HJ, Ludwig W, Kampfer P (2010) Notes on the characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Microbiol 60:249–266. https://doi.org/10.1099/ijs.0.016949-0

Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438

Wang X et al (2013) *Xiangella phaseoli* gen. Nov., sp. nov., a member of the family *Micromonosporaceae*. Int J Syst Evol Microbiol 63:2138–2145. https://doi.org/10.1099/ijs.0.045732-0

Wang H-F, Li Q-L, Xiao M, Zhang Y-G, Zhou X-K, Rao MPN, Duan Y-Q, Li W-J (2017) *Streptomyces capparidis* sp. nov., a novel endophytic actinobacterium isolated from fruits of *Capparis spinosa* L. Int J Syst Evol Microbiol 67:133–137. https://doi.org/10.1099/ijsem.0.001586

Wellington EM, Stackebrandt E, Sanders D, Wolstrup J, Jorgensen NO (1992) Taxonomic status of *Kitasatosporia,* and proposed unification with Streptomyces on the basis of phenotypic and 16S rRNA analysis and emendation of Streptomyces Waksman and Henrici 1943, 339AL. Int J Syst Bacteriol 42:156–160. https://doi.org/10.1099/00207713-42-1-156

Yaradoddi J, Kontro M, Ganachari S, Sulochana M, Agsar D, Tapaskar R, Shettar A (2019) Protein nanotechnology (Dr. Kharisov BI (Editor-in-Chief) Handbook of ecomaterials). Springer. https://doi.org/10.1007/978-3-319-68255-6_192

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12)

Yaradoddi JS, Sulochana MB, Kontro Merja H, Parameshwar AB, Dayanand A (2020) The Occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Zhang Z, Wang Y, Ruan J (1997) A proposal to revive the genus Kitasatospora (Omura, Takahashi, Iwai, and Tanaka 1982). Int J Syst Bacteriol 47:1048–1054

Chapter 9
Screening of Novel Metabolites from Actinobacteria

Prabhurajeshwar, H. M. Navya, Jayshree Uppin, Seema J. Patel, and Chandrakanth Kelmani

Abstract Exploration of untapped environments in discovering novel bioactive metabolites is one of the priority areas of research. Different environments and ecosystems like alkaline, mangrove ecosystems, marine sponges, hypersaline lakes, alkaline soils, medicinal plants, and insect intestine have explored for the recovery of many bioactive metabolites. Many culture-based studies have revealed that actinobacteria recovered from the hot spring environments comprise substantial synthesis potentials for various biological active compounds. The potential actinobacterial variety and distribution endemic to the hot spring environments can be explored in recovering novel metabolites and compounds. In the present scenario, the discovery of novel antagonist drugs in controlling the multidrug-resistant bacteria and biofilm forming bacteria is in high demand. However, due to the use of similar and repeated screening methods, results in previously reported compounds and are the major limitations to endure. Nowadays, integrated molecular networking approaches such as mass spectrometry profiles using the unknown complex samples have gained considerable interest in discovering novel metabolites. Despite the use of these advanced techniques, researchers should also look to explore untapped ecosystems for the discovery of potential therapeutic applications.

Keywords Actinobacteria · Secondary metabolites · Bioactive compounds · Ecosystems · Screening of novel secondary metabolites

Prabhurajeshwar · H. M. Navya · S. J. Patel
Department of Studies in Biotechnology, Davangere University, Davangere, Karnataka, India

J. Uppin · C. Kelmani (✉)
Department of Biotechnology, Gulbarga University, Gulbarga, Karnataka, India

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9.1 Introduction

Microorganisms have always been interesting organisms for human endeavor and their applications. Microbes are involved in vast variety of reactions and adjust to environments, allowing them to be transplanted from nature to the laboratory flask, where they can be grown on limited carbon and nitrogen sources to produce effective biological compounds (Demain and Adrio 2008). However, discovering bioactive compounds from biological origin against multidrug-resistant organisms is an age-old practice. Almost all living beings can produce antimicrobial agents and secondary metabolites. The secondary metabolite production ability is quite different among the different species involved. In the prokaryotic and plant kingdom, various organisms including unicellular bacteria, eukaryotic fungi, and filamentous actinobacteria are the most frequent and most widely used producers. The filamentous Actinomycetales species has over 10,000 biologically active compounds, 7600 are derived from the genus *Streptomyces,* and 2500 are called rare Actinomycetes species, representing the largest group (45%) of bioactive microbial metabolites. Still majority of Actinobacteria are not cultivated and could be an important source of bioactive metabolites. Moreover, the increased emergence of antimicrobial resistance among pathogenic microorganisms has led to the discovery of a new antibiotic from actinobacteria.

Actinobacteria are predominant and among the mixed group of bacteria in the environment. Actinobacteria are known to produce secondary metabolites. In 1940 Selman Waksman discovered the soil bacteria *actinomycin*; he received a Nobel Prize for this discovery. Since then, hundreds of naturally occurring antibiotics have been discovered in these terrestrial microorganisms, especially from the genus *Streptomyces*. The single genera of *Streptomyces* reports for almost 5% of the ~16,000 indicated bacterial species (Parte 2018). Actinobacteria have a good impact on human health and serve as a source for many new metabolites as essential drugs, including most antibiotics (Hopwood 2007) and their biological activity like antibacterial, antifungal antiviral, antiparasitic, herbicides, pesticides, antioxidant, and antitumor activity.

9.2 Introduction to Actinobacteria

The phylum Actinobacteria is a pivotal taxonomical classification among the significant heredities directly perceived inside the microbial domain (Ludwig et al. 2012). The genetic material of actinobacteria sequenced till today is important to human and veterinary medicines, biotechnology, and biology (Ventura et al. 2007). The prevalence of actinobacteria are free-living that is widely spread in both earthbound and sea-going environments. Some Actinobacteria form branching filaments, which fairly resemble the mycelia of the unrelated fungi, among which they were originally classified under the old name *Actinomycetes*. Most members are

aerobic, but a few, such as *Actinomyces israelii*, can grow under anaerobic conditions. It encompasses the Gram-positive type of bacterium with more G + C content in their genetic material, i.e., DNA, which ranges from 51.00% in some Corynebacterium to 70.00% or above in Streptomyces and Frankia.

Actinobacteria are Gram-positive microorganisms with a high G-C content in their genetic material. Actinomycetes were estimated between structures among growths and microorganisms. Positively, as filamentous parasites, numerous actinobacteria produce mycelium, and a significant number of these mycelial actinomycetes reproduce by sporulation. Nonetheless, the relationship to parasites is shallow; like all microorganisms, actinomycetes cells are slim like a chromosome dispersed in a nucleoid of prokaryotic and a peptidoglycan layer; besides, these cells are helpless antibacterial activities (Ludwig et al. 2012).

9.2.1 Ecology of Actinobacteria

Most of the actinobacteria, particularly Streptomyces, are saprophytic, soil-dwelling organisms that use most of their life cycles as semi-dormant spores and are predominant under inadequate nutritional conditions (Mayfield et al. 1972). Despite the fact that the genera have adjusted to a broad scope of natural climate, actinomycetes are additionally present in muds, alkaline water, and in the atmosphere. They are more copious in muds than other media, predominantly in antacid muds and muds advanced with a genuine issue, where they make a significant part out of the microbial populace.

Actinobacteria are located on the surface of the mud, and at more than 2 m deep inside the ground. The population of actinobacteria is based on their niche and inhabiting environmental circumstances. These organisms usually occur in a density of 10^6 to 10^9 cells per gram of soil sample (Good Fellow and Williams 1983); the soil population is governed by the *Streptomyces*, which signifies over 95% of the actinobacterial strains isolated from soil (Williams 1989).

Different variables, for example, temperature, pH, and soil dampness, affect the development of actinobacteria. Likewise, other soil actinobacteria are mostly mesophilic, with good reproduction at 25–30 °C; thermophilic actinobacteria grow at 50–60 °C (Edwards 1993). Nonsexual multiplication of actinobacteria in the dirt is favored by low stickiness, and significantly spores are lowered in the water. In dry soils where the dampness strain is more noteworthy, development is controlled and might be stopped. Most actinobacteria develop in soils with a nonpartisan pH. They grow best at a pH somewhere between 6 and 9, with the most extreme development around nonpartisan conditions (Fig. 9.1).

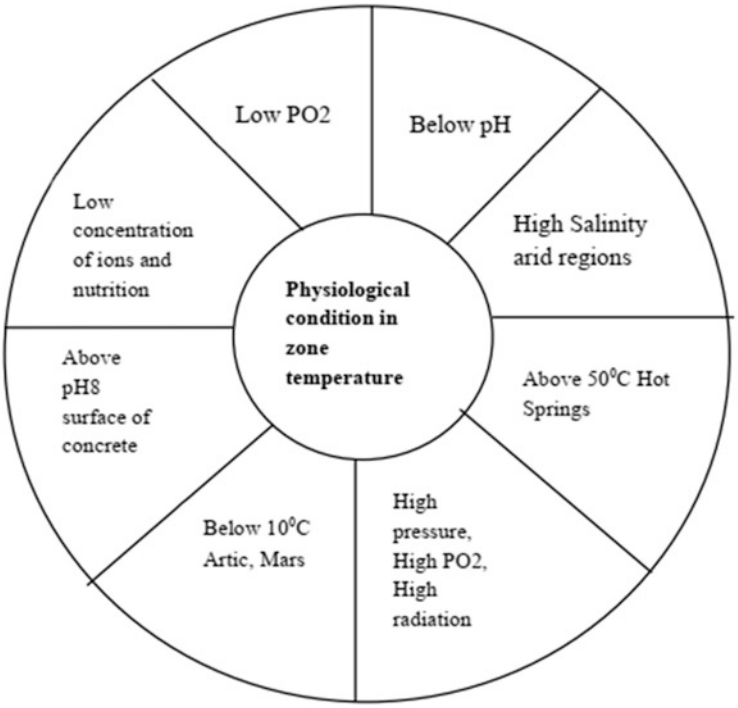

Fig. 9.1 Ecology and habitat distribution of actinobacteria

9.2.2 Taxonomy of Actinobacteria

Actinobacteria involve one of the significant taxonomic units among 18 major lineages presently documented within the bacterial domain, together with five subclasses, six orders, and 14 suborders (Ludwig et al. 2012). The genera of this phylum demonstrate major diversity in terms of their morphology, physiology, and metabolic activities. The taxonomy of actinobacteria has developed remarkably over time with the count of knowledge (Buchanan 1917). The phylum Actinobacteria is defined based on its branching position in 16S rRNA gene sequencing (Omura et al. 1982; Ludwig et al. 2012; Zhang et al. 1997; Girard et al. 2014). A comparable relationship remains alive between *Micromonospora*, *Verrucosispora*, and *Salinispora*. Therefore, supplemental genetic markers have been utilized to recognize firmly related genera, including rpoB, and the most recent one, ssgB, which is nearly useful for observing between firmly related genera (Girard et al. 2013). Moreover, the tremendous ongoing increment in the accessibility of genome succession data has been given recorded into genome development and made it conceivable to recognize qualities explicit to life forms at the degree of genera and family (Kirby 2011; Ludwig et al. 2012; Gao and Gupta 2012). The phylum is accordingly sorted into six classes: Actinobacteria, Acidimicrobiia, Coriobacteriia, Nitriliruptoria, Rubrobacteria, and Thermoleophilia. The class Actinobacteria

contain 16 orders including Actinomycetales and Bifidobacteriales (Gao and Gupta 2012; Goodfellow et al. 2012).

9.2.3 Secondary Metabolites (SM)

Microbial secondary metabolites are mixes created for the most part by actinobacteria and organisms, generally late in their growth cycle (idiophase). Even though antimicrobials are the most popular auxiliary metabolites, there are others with a gigantic scope of other organic exercises principally in fields like pharmaceuticals, beautifiers, and horticulture. These incorporate mixes with antifungal, calming, hypotensive, antitumor, and bug sprays alongside plant development controllers (Fig. 9.2) (Abdelmohsen et al 2014).

9.2.4 Mechanism of Elicitation for Secondary Metabolism in Actinobacteria

Actinomycetes (Phylum: Actinobacteria) are important, which have an effective capacity to deliver an abundance of normal items with basic multifaceted nature

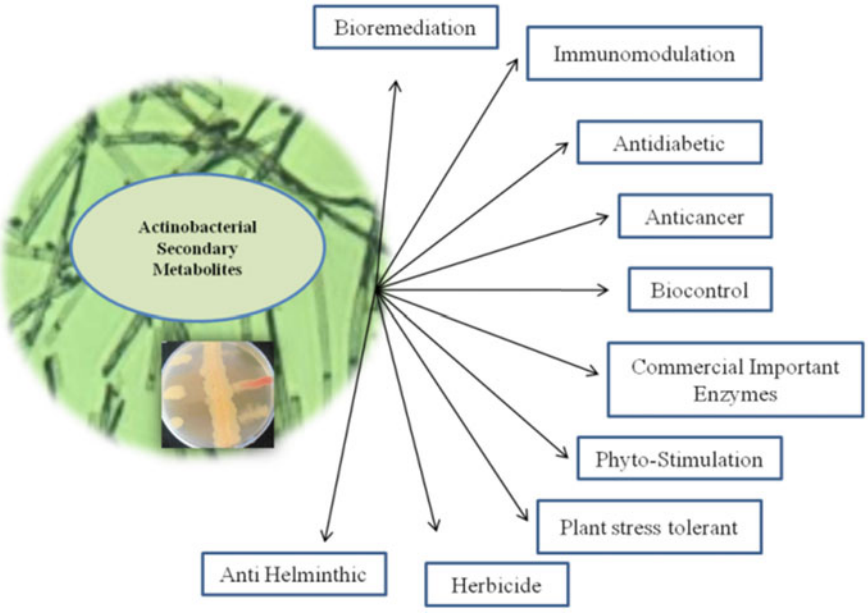

Fig. 9.2 Applications of secondary metabolites production by actinobacteria

and with assorted organic exercises (Abdelmohsen et al 2014; Yaradoddi et al. 2021; Shellikeri et al. 2018; Hopwood 2007; Nett et al. 2009). Notwithstanding, an excess of secondary metabolites encoded in the actinomycete genomes stays unfamiliar apparently because these qualities are not translated under traditional research facility environments (Bentley et al. 2002; Hertweck 2009; Pettit 2011; Seyedsayamdost 2014). A great deal of the numerical models has anticipated a large number of unmapped antimicrobials from actinomycete genomes (Caboche 2014; Cimermancic et al. 2014; Watve et al. 2001). Critical examination exertion are needed to resilience the hidden quality groups with the plan to recognize new common items and atomic frameworks.

Since the revelation of the β-lactam antimicrobial penicillin by Alexander Fleming in 1928, co-development or blended maturation has been perceived as an amazingly effective methodology for disclosing organically dynamic regular items. A few late examinations have uncovered methodologies to initiate secretive qualities, which incorporate "compound, organic, and atomic elicitation" (Dashti et al. 2014; Nett et al. 2009; Hertweck 2009; Luo et al. 2013; Zhu et al. 2014). Conventional methodologies, for example, co-cultivation and changes in aging conditions (media organization, pH, and temperature), are for some time known to prompt noteworthy changes in the microbial metabolome (Marmann et al. 2014). Control of the maturation conditions is known as the OSMAC approach and speaks to a viable open door for initiating quiet or ineffectively communicated metabolic pathways (Paranagama et al. 2007). Co-cultivation has been utilized in the creation of nourishments: food additives, catalysts, mass and fine synthetic concoctions, bioremediation, and debasement of lignocelluloses (Bader et al. 2010).

Testing bacterial cells with outside signs, alleged elicitors (Table 9.1), is a perceived methodology for creating novel, naturally dynamic metabolic compounds (Chiang et al. 2011; Müller and Wink 2014). Detecting the information by the maker strain brings about an intuitive reaction that may prompt a modified metabolite profile in the microbial genome. The test with little particle synthetic elicitors can empower the distinguishing proof of various reactions in microorganisms, for example, the expanded form of pressure (Romero et al. 2007), biofilm development acceptance, and balance of harmfulness articulation (Bajaj et al. 2014; Nouaille et al. 2009). In certain uncommon cases, elicitation may bring about unaltered metabolite profiles or even suppress the looked-for after metabolites (Marmann et al. 2014). There are several reports on ratifying quiet quality bunches in progressions (Brakhage 2013; Chiang et al. 2009), just as in *myxobacteria* (Krug et al. 2008; Wenzel and Müller 2009); but re-markably little is known about the activation of gene clusters in actino-mycetes by elicitation approaches. Given that the large number of available actinobacterial genome sequences have shown the hiddennatural product biosynthetic capacity of the phylum actinobacteria (Bentley et al. 2002; Doroghazi et al. 2014; Jensen et al. 2014; Zazopoulos et al. 2003), an endeavor has been made in this writing audit which centers around elicitation of optional digestion in actinomycetes. The concoction and molecular intricacy of the elicitation cycle and its system have been ineffectively perceived. To date, the instrument of the activity of "elicitors" is most prevalent in plants and contagious

Table 9.1 List of secondary metabolites elicitation with cell derivative constituents

Elicitor	Strain	Secondary metabolites induced	Mechanism of elicitation
N-acetylglucosamine (under poor nutrient conditions)	*Streptomyces coelicolor*	Actinorhodin, Undecylprodigiosin	Confining of glucosamine-6-phosphate to fructose 6-phosphate repression in antibiotic synthesis
AS 3.5163	*S. natalensis*, HW-2	Pimaricin	Low atomic weight particle from stock of *P. chrysogenum* AS 3.5163 showed an elicitation impact
N-butyryl-DL-homoserine lactone (HSL)	*Aspergillus fumigatus* MBC-F1–10 (P)	Emestrin A Emestrin B	–
PI factor	*S. natalensis*	Pimaricin	Hydrophilic autoinducer atom-directed antimicrobial creation
Glycerol-1,2–Propanediol 1,3 Propanediol ethylene glycol	*S. natalensis*-npi287	Pimaricin	Glycerol-adjusted film porous in nature and led
Oligosaccharides and polysaccharides	*S. rimosus, Penicillium chrysogenum*	Unknown auxiliary metabolites	Inhibition of ROS leads to overproduction of auxiliary metabolites

cells (Nutzmann et al. 2011; Radman et al. 2003, 2006; Murphy et al. 2011). The elicitor can either legitimately impact the record of the optional metabolite quality group or instigate a transcriptional activator of the objective quality bunch (Rigali et al. 2008; Nair et al. 2009; Tanaka et al. 2010).

9.3 Biological Elicitation

9.3.1 Elicitation by Microbial Co-Cultivation

Actinomycetes live as gatherings alongside different organisms in different environments. The experience of microorganisms in their condition may turn on baffling quality groups, accordingly setting off the creation of new metabolites in the local microscopic organisms. This communication could initiate quiet pathways that may bring about the combination of novel auxiliary metabolites and eventually can prompt substance guard of the creating microorganisms. The last wonder occurs on transformative time scales, and any progressions are forever fixed on the beneficiary genome. Aside from these components, the inducer strain could likewise initiate epigenetic adjustments in the maker strain (Marmann et al. 2014).

Notwithstanding, in the above conditions, the association of inducer strain with the strain eventually impacts auxiliary metabolite in the strain. Co-cultivation studies additionally dependent on the arrangements of strains being co-refined as (1) co-cultivation of actinomycetes with different actinomycetes; (2) co-cultivation of actinomycetes with other microbes, and (3) co-cultivation of actinomycetes with organisms.

9.3.2 Co-Cultivation of Actinobacteria with Actinobacteria

In one of the examinations, co-cultivation of two sponge derived actinomycetes, in particular *Actinokineospora* sp. EG49 and *Nocardiopsis* sp. RV163, brought about the creation of three metabolic compounds not delivered; the strains were grown freely. Among three mixes, one of the compounds demonstrated apparent organic movement beside *Bacillus* sp. p25 and *Trypanosoma brucei* just as against *Actinokineospora* sp. EG49 itself. These discoveries recommend that the recently integrated atoms are delivered by the strains in blended culture (Dashti et al. 2014).

Another investigation uncovers the adjustment in the common item biosynthesis that happened in various soil *Streptomyces* strains upon co-cultivation with the mycolic corrosive. The creation of red colors comparing to polypeptide actinorhodin and tripyrrole undecylprodigiosin by *Streptomyces lividans* TK-23 was utilized as a marker to focus on the inducer strain. A dialysis test affirmed that this impact was a result of physical cell-to-cell communications. Thus, it was derived that co-development with mycolic corrosive-containing microorganisms is one exquisite approach to initiate quiet quality groups (Onaka et al. 2011). Kurosawa and his colleagues have considered the co-culture of multi-antimicrobial safe freak strain of *Rhodococcus fascians* with *Streptomyces padanus,* which prompted the rise of another *Rhodococcus strain* that was found to incorporate a huge section of genomic DNA from *S. padanus* (Kurosawa et al. 2008).

9.3.3 Co-Cultivation of Actinomycetes with Bacteria

Perez et al. (2005) contemplated that those predator microorganisms that slaughter and feed on other live bacterial cells by retaining their supplements can likewise actuate the statement of anti-infection agents in maker life forms. *Myxococcus xanthus*, a predator of *S. coelicolor*, appeared to incite the blue polyketide anti-toxin actinorhodin in the maker living being, just as triggering ethereal mycelium creation on strong media (Perez et al. 2005). Moreover, actinorhodin delivering cells were not encircled by *M. xanthus* showing that *S. coelicolor* could utilize actinorhodin as an anti-agent signal against *M. xanthus*.

Consequently, it was found from this examination that the utilization of predator strains is likewise an approach to unwind the statement of metabolites that stay quiet

or ineffectively communicated in axenic societies. In continuation with the elicitation component, co-refined in one of the producer strains *Streptomyces griseorubiginosus* and *Pseudomonas maltophilia* brought about a 60-fold increment of the cyclic peptide anti-infection biphenomycin A when contrasted with the unadulterated solitary culture, which delivered low degrees of anti-toxin. Treatment of *S. griseorubiginosus* with the cell-free concentrate of *P. maltophilia* additionally prompted the creation of the above cyclic peptide anti-infectives. These chemicals changed over the antecedent of biphenomycin A to its dynamic structure in the co-culture, and these proteins were believed to be missing in the maker *Streptomyces* strain inferable from the creation of low degrees of the compound. Later on, the peptide antecedent of biphenomycin A was disengaged as biphenomycin C and fundamentally recognized from the blended culture (Perez et al. 2005).

Further, the impact of the rivalry between 53 marine microorganisms with the istamycin delivering marine bacterium *Streptomyces tejamariensis* was studied to examine the job of the anti-toxin istamycin in serious cooperations between organisms under normal conditions. Subsequently, 12 of the 53 strains initiated the creation of istamycin in co-culture with *S. tejamariensis*. The co-culture multiplied the creation of istamycin when contrasted with the axenic monoculture of *S. tejamariensis* (Slattery et al. 2001).

9.3.4 Co-Cultivation of Actinobacteria with Fungi

In one of the co-cultivation studies revealed by Isaka et al. of actinomycetes with organisms, blended aging of *Streptomyces peucetius* and *Aspergillus fumigatus* yielded two new metabolic compounds-specific xanthocillin like fumiformamide and diformamide alongside two recently known N-formyl subordinates and another particle (Isaka et al. 2007). The compound diformamide showed harmful impacts on an expansive range of cell lines, and one of the subordinates showed moderate cytotoxicity. In contrast, cytotoxic effects for fumiformamide and other new compounds were absent (Zuck et al. 2011).

In relationship with our investigation, one of the reports portrayed anti-multi-drug resistant *S. aureus* (MRSA) cyclic depsipeptides, emericellamides A and B, which were acquired from the marine-inferred growth *Emericella* sp. strain in co-culture with the marine actinomycete *Salinispora arenicola* strain.

9.3.5 Elicitation with Microbial Lysates

If there should arise an occurrence of elicitation with microbial lysates study, one of the examinations gave the yield of undecylprodigiosin, which is an antimicrobial with immune suppressive properties and had apoptotic impacts against bosom malignancy cells, which expanded upon treatment with live and heat executed

cells of *B. subtilis* by *S. coelicolor* A3. At the same time, unadulterated societies of *S. coelicolor* A3 created just very low convergences of antimicrobials (Williamson et al. 2006). We should go with heat executed *S. aureus* over *B. subtilis* may identify with the way that the human skin-related *S. aureus* is more unfamiliar to *S. coelicolor* than *B. subtilis*, the last of, which are regular soil inhabitants. Subsequently, re-enacting the nearness of a contending domain with abiotic elicitor is an advanced approach. It can be utilized as the main impetus for mysterious bioactive metabolite quality articulation in actinomycetes.

9.3.6 Elicitation with Microbial Cell Components

Cell wall constituents, microbial hormones or signaling particles, carbohydrates, or biopolymers from cells could be utilized as elicitors in inciting auxiliary digestion. Zhu et al. (2014), have reported in their investigation that the healthful status of the microbial condition may likewise impact the creation of antimicrobials. The cell wall segment and the chitin monomer N-acetyl glucosamine (GlcNAc) inspired actinorhodin and undecylprodigiosin by *S. coelicolor* under helpless supplement conditions, though GlcNAc ruined anti-infection creation under rich supplement conditions. Strikingly, this supplement-based elicitation of GlcNAc was discovered to be related with the DNA-restricting protein DasR. DasR-responsive components (dre) (the DNA restricting locales of DasR) were discovered upstream to the activators of these antimicrobials. Likewise, obstruction with DasR action could incite anti-toxin creation. GlcNAc incited anti-infection creation in *Streptomyces clavuligerus*, *Streptomyces collinus*, *S. griseus*, *S. hygroscopicus,* and *Streptomyces venezuelae*, when developed on helpless supplement medium, demonstrating their boundless anti-toxin elicitation impact inside the Streptomyces class (Rigali et al. 2008; Zhu et al. 2014). Along these lines, it was surmised that treatment of actinomycetes with GlcNAc under explicit supplement conditions is one novel approach to stir obscure optional digestion gene operon.

Wang and his team have investigated the biosynthesis of the antifungal polyethylene antibiotic natamycin (or pimaricin) by *Streptomyces natalensis* HW-2, which was improved upon elicitation with a basically unidentified contagious elicitor from *Penicillium chrysogenum* AS strain. Both the warmth executed biomass and the aging stock of *P. chrysogenum* improved the creation of the above antibiotic. This parasitic elicitor from *P. chrysogenum* upgraded the yield of natamycin as much as 200% when contrasted with the untreated control. The essential quantitative examination uncovered that the elicitor from the aging stock of the above parasitic strain was discovered to be a low subatomic weight particle with extremity practically identical to that of butanol.

9.3.7 Chemical Elicitation

Chemical elicitation measure includes non-organic source for elicitation which brings about changes in the metabolomics profile of life forms. Substance elicitors incorporate inorganic mixes, uncommon earth components, weighty metal particles, and so forth.

Craney et al. reported that the screening of *Streptomycetes* with a Canadian compound assortment incorporating 30,569 little particles prompted the noticeable proof of 19 compounds that changed the auxiliary digestion pathway in actinomycetes (Craney et al. 2012). It repressed unsaturated fat biosynthesis through the hindrance of transporter protein reductase FabI, a compound that catalyzes the rate restricting and the last advance in unsaturated fat biosynthesis. Unsaturated fat combination (essential digestion) and polyketide union (optional digestion) shares certain forerunners (like malonyl-CoA and acetyl-CoA). Accordingly, it is proposed that ARC2 may act through the fractional restraint of this transporter protein reductase FabI prompting an end in the essential digestion and occupying simultaneously the antecedent stream toward anti-infection creation. ARC2 treatment additionally instigated the declaration of some obscure metabolites in *S. peucetius*, in this manner clarifying the job of the elicitor in the disclosure of new metabolites (Craney et al. 2012; Zhu et al. 2014). Subsequently, it was closed that reconfiguring the digestion by little particles like ARC2 and triclosan which hinder unsaturated fat biosynthesis or essential digestion is a compelling methodology in improving polyketide amalgamation and initiating quiet polyketide quality groups in actinomycetes (Table 9.2).

9.3.8 Molecular Elicitation

Out of the developmet, elicitation approaches referenced over, the strategies for recognizing the secondary metabolic items from the silent gene bunches of microorganisms distinguished through genomic mining has previously surveyed (Bentley et al. 2002). Digging microbial genomes for distinguishing silent gene bunches and the ensuing portrayal of the concealed items through quality take-out examinations combined with expository methods like HPLC-MS and MALDI-TOF imaging offers another stage to uncover the shrouded capability of actinomycetes.

Newfound and recently announced natural items encoded by these silent gene bunches could be uncovered by the constitutive quality articulation of putative controllers present in a similar genetic group. For example, Laureti et al. have contemplated the constitutive over articulation of a pathway explicit controller protein (LuxR) family that had a place with the obscure kind I polyketide synthase (PKS) quality bunch in *Streptomyces ambofaciens* strain which coordinated the statement of four 51 membered glycosylated macrolides anti-infection agents, Stambomycins A, D. In this investigation, Stambomycins A, D indicated moderate

Table 9.2 Secondary metabolites of chemical elicitation

Elicitor	Strain	Secondary metabolites induced	Mechanism of elicitation
ARC2	*S. coelicolor* *S. peucetius*	Germicidins A–C Actinorhodin	Hindrance of unsaturated fat biosynthesis by restraint of enoyl-acyl transporter protein reductase FabI
Scandium (Sc3+)	*S. coelicolor, S. antibioticus, and* *S. parvulus, S. griseus* *Bacillus subtilis* 168	Actinorhodin Actinomycin D Streptomycin Bacilysin	Upregulation of antimicrobial activator records and reduction in the bacterial alarmones ppGpp levels by accurate of scandium (Sc3+) to ribosome changes anti-toxin creation
Scandium, Lanthanum	*S. coelicolor*–A3	Actinorhodin	Nine genes upregulated
DMSO, Ethanol, Dimethyl sulfone	*S. venezuelae* ATCC-10712*S. glaucescensS. aureus* ATCC14921*S. lividansS. glaucescensS. venezuelae* ATCC10712	ChloramphenicolTetracenomycin CThiostreptonProdigiosinTetracenomycin CChloramphenicol	Effect at the translational level
Ethanol	*S. venezuelae* ISP5230	Jadomycin-B	Induced either a heat shock response, or served as a metabolite precursor, or altered membrane permeability in *S. venezuelae*-ISP5230
Sodium butyrate	*S. coelicolor*	Actinorhodin	HDAC inhibitor

antibacterial action against Gram-positive microscopic organisms and powerful antiproliferative exercises against human cell lines (Laureti et al. 2011; Zhu et al. 2014).

9.4 Bioactivities of Secondary Metabolites of Actinobacteria

The Actinobacteria are rich in diverse metabolic compounds significant for humans in terms of antibiotics, antifungal, anticancer agents, and other secondary metabolic compounds that can be used in medicine or to induce plant growth and resistance pattern to diseases (Table 9.3); they are also capable of biocontrol of pests and act as plant growth promoters.

9.4.1 Antibacterial Activity

About 80% of the world's antibiotics are of microbial origin, and among those genera, *Streptomyces* and *Micromonospora* of actinomycetes contributed extensively. Antibiotics, viz. Alnumycin, munumbicins A to D, and corona mucins, have been reported from species of endophytic *Streptomyces* (Bieber et al. 1998; Castillo et al. 2002). Generally, Actinobacteria are known to be great producers of antibiotics. Besides, rare actinobacteria such as *Sinomonas, Pseudarthrobacter, Lefsonia* and *Gordonia* are pivotal cradles in the drug-discovery field (Tiwari and Gupta 2012). Omura and group reported a new group of secondary metabolites called mangromicins from *Lechevalieria aerocolonigenes* K10–0216 during the last decade (Nakashima et al. 2014a, b, 2015).

Siddharth and Rai (2019) conducted research work on the isolation of "4-Bromophenol" and "Bis-(2-ethylhexyl)-phthalate" from the genus *Nocardiopsis* and the auxiliary metabolites produced by *Nocardiopsis* sp. SCA21 reported alpha-glucosidase and alpha-amylase inhibition activities concurrently reported antioxidant and antibacterial properties. However, a moderate and mild growth inhibition of *M. luteus* and *B. subtilis* was observed with some strains of *Nocardiopsis* as observed by Zhang et al. (2016).

Significant antibacterial activity was exhibited against Gram-positive and Gram-negative bacteria by cultivable Streptomyces species isolated from deposits of a moonmilk speleothems of limestone cave in Belgium (Maciejewska et al. 2016).

A new, promising natural product called anthracimycin with broad antibacterial nature was found effective in inhibiting the growth of bacterial isolates including *Bacillus anthracis*, *E. faecalis, S. pneumoniae, H. influenzae, Moraxella catarrhalis,* and *vancomycin-resistant S. aureus* and others. A differential antibacterial activity was displayed by Quadoctomycin, isolated from *Streptomyces* sp. MM168-141F8, where it showed potent antibacterial activity toward Gram-positive bacteria S. aureus. However, it does not exhibit any antimicrobial activity with Gram-negative bacteria (Sawa et al. 2018). *Streptomyces cheonanensis* VUK-A isolated

Table 9.3 List of actinobacterial species with their bioactive metabolites

Compound/bioactive metabolite	Source	Activity	Reference
Aureoverticillactam	*Streptomyces aureoverticellatus*	Antitumor	Mitchell et al. (2004)
Pyrroles	*Cephalosporium acremonium*	Antibacterial	Manimegalai et al. (2013)
Peptide-cyclo	*Streptomyces* sp. *YIM64018*	Antibacterial	Yang et al. (2015)
Peptide-cyclo	*Streptomyces* sp. *YIM64018*	Antibacterial	Yang et al. (2015)
δ-Lactone	*Streptomyces hygroscopicus TP-A0451*	Antitumor	Igarashi et al. (2006)
Arylcoumarins	*Streptomyces aureofaciens*	Antifungal, antitumor	Taechowisan et al. (2005) and Taechowisan et al. (2007)
Spirotetronate—polyketide	*Micromonospora* sp. *GMKU326*	Antibacterial	Igarashi et al. (2011)
Hydro-xypyrone—polyketide	*Streptomyces sundarbansensis WR1L1S8*	Antibacterial activity	Djinni et al. (2014)
6-Alkylsalicylic acids	*Streptomyces laceyi*	Antitumor	Kim et al. (2006)
Macrolides	*Streptomyces* sp. *CS*	Antimicrobial	Lu and Shen (2007)
Pyranonaphthoquinone	*Streptomyces thermoviolaceus NT1*	Antibacterial (anti-MRSA)	Roy and Banerjee (2015)
Anthraquinones	*Micromonospora lupini*	Antitumor	Igarashi et al. (2007)
di-O-prenylated flavone	*Streptomyces* sp. MA-12	Antifungal activity	Ding et al. (2013)
β-Carboline	*Microbispora* sp. *LGMB259*	Antimicrobial, anticancer	Savi et al. (2015)
Arenamides	*Salinispora arenicola* CNT-088	Human colon carcinoma	Asolkar et al. (2009)
Chalcomycin A	*Streptomyces* sp. *strain M491; Streptomycetes isolate B7064*	Human cervix carcinoma	Wu et al. (2007)
Manumycin, Chinikomycin A, B	*Streptomyces* sp. isolate M045	Renal cancer, mammary cancer	Li et al. (2005)

from mangrove sediment produced diethyl phthalate (DEP), which exhibited antibacterial activity against *Streptomyces epidermidis* (Mangamuri et al. 2016). Asenjonamide C, a derivative of the β-diketone family of polyketides, showed antibacterial activity against *E. coli*, *E. faecium,* and methicillin-sensitive *S. aureus* (MSSA) (Abdelkader et al. 2018).

9.4.2 Antifungal Activity

A variety of bioactive compounds have been isolated from numerous actinobacteria. However, studies are still ongoing in the identification of novel bioactive compounds against pathogenic fungal species. Diethyl phthalate (DEP) was found effective in suppressing *Streptomyces albidoflavus* (Roy et al. 2006) and *Streptomyces melanosporpfaciens* (Ahsan et al. 2017) growth. The same compound from *Streptomyces cheonanensis* VUK-A possesses antimicrobial activity against *S. epidermidis*. Furthermore, innovative phthalate referend "2-methyl butyl propyl phthalate" isolated from *S. epidermidis* unveiled very strong antifungal and cytotoxic activities (Mangamuri et al. 2016). Furthermore, literature reports evidenced phthalate esters "Bis-(2-ethylhexyl) phthalate" and "Bis-(5-ethylheptyl) phthalate" from *Nocardia levis*-MK-VL_113 possess potential antimicrobial properties (Kavitha et al. 2009). Antibacterial and antifungal activity against *Curvularia oryzae, F. oxysporum, Helminthosporium oryzae, Pyricularia oryzae, R. oryzae-sativae,* and *R. solani* infecting rice crop was evidenced with members of genera *Streptomyces* and *Janibacter*. Non-polyenic compound from *Streptomyces* spp. has recorded antifungal activity against *C. albicans* (Belyagoubi et al. 2018). Similarly, significant growth inhibition activity of *C. glabrata* ATCC 90030 was observed with endopeptides A, B, and C from *Streptomyces* sp. (Chen et al. 2017). *Spongia officinalis,* a marine bacteria, produces pyrrolo[1–a]pyrazine-1, 4-dione, hexahydro-3-(2-methyl propyl), and potent antibacterial and antifungal activity.

9.4.3 Anticancer Activities

Many researchers have reported the anticancer properties of bioactive compounds obtained from actinomycetes for treating cancer and other infectious diseases. Mangamuri et al. (2016) observed, in addition to intense antifungal activity, a new compound reported as phthalate analog "2-methyl butyl propyl phthalate" from *Streptomyces cheonanensis* VUK-A, which displayed cytotoxic potentials against cancer cells, viz."MDA– MB – 231," "Hela," and "OAW 42." Another phthalate compound called "Dioctyl phthalate" (DIOP) from *S. parvus* is also found to possess potential in inhibiting the cancer cell lines (Abd-Elnaby et al. 2006; Zhang et al. 2010).

Anticancer and antioxidant metabolites pyrrolo[1–a]pyrazine-1, 4-dione, and hexahydro-3-(2-methylpropyl) are identified in *Streptomyces* sp. MUM 256 and *Streptomyces malaysiense* sp. MUSC 136 (Tan et al. 2015). This compound exhibited potent antioxidant activity and high cytotoxic activity against HCT-116 cells (Ser et al. 2016) and potentially active against lung (A549) and cervical (HeLa) cancer cells in a dose-dependent manner (Lalitha et al. 2016).

Maklamicin obtained from endophytic *Micromonospora* sp. GMKU326 showed moderate cancer cell cytotoxicity. It also displayed potent antimicrobial activity against Gram-positive bacteria (Igarashi et al. 2011; Ser et al. 2016).

9.4.4 Antioxidant Effect

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against various infections and degenerative diseases (Sharma and Gupta 2008). Modern research is now directed toward natural antioxidants from plants and microorganisms, which serve as safe therapeutics (Suriyavathana and Nandhini 2010). Recent studies are focusing on the response of the antioxidant system of bacteria, which is important in terms of biotechnology, such as *Streptomyces* growth in various oxidative stress conditions (Schweder et al. 2005).

Cytotoxicity and antioxidant activity of a novel compound 5-(2, 4-dimethyl benzyl) pyrrolidin-2-one extracted from *Streptomyces* VITSVK5 spp. was reported by Saurav and Kannabiran (2012). Daryamides, antifungal polyketides isolated from the culture broth of a Streptomyces strain, CNQ-085, have been shown to exhibit moderate cytotoxicity against the human colon carcinoma cell line HCT-116 and moderate antifungal activities against *Candida albicans* (Asolkar et al. 2009).

9.5 Conclusion and Future Approach

Actinobacteria are rich sources of natural products with discrete pharmaceutical and biotechnological applications. The rapidly growing number of Actinobacteria genome sequences reveals their potential for the biosynthesis of secondary metabolites that are much higher than the actual number of compounds produced during classical fermentation.

In modernized literature survey to date, most of the elicitation and mixed fermentation studies are carried out on actinobacteria from terrestrial soils. Thus, marine environment, in particular, offers a novel and chemically rich actinomycete species; it is still less explored in terms of elicitation and co-cultivation. Furthermore, challenging metabolically active microorganisms with various elicitors might be a successful approach to identify the chemical novelty. Therefore, the identification of mysterious gene clusters by correlating genome mining and gene expression analyses with elicitation approaches will provide a new avenue to the fortune of natural products from actinomycetes. On the other hand, actinobacteria have numerous clear potential benefits for humans as sources of novel antibiotics, antifungals, anticancer agents, and other secondary metabolites that might be used in medicine or to improve plant growth and resistance to diseases. Actinobacteria are capable of biocontrol of pests and act as plant growth promoters. With the rapid developments

in the fields of genomics, synthetic biology, and ecology and the strong requirement for new antimicrobial compounds to combat antimicrobial resistance, the biology of the actinobacteria is a highly dynamic research field, and we expect to see many new advances in this field in the years to come.

References

Abdelkader MSA, Philippon T, Asenjo JA et al (2018) Asenjonamides A–C, antibacterial metabolites isolated from *Streptomyces asenjonii* strain KNN 42.f from an extreme-hyper arid Atacama Desert soil. J Antibiot (Tokyo) 71:425–431

Abdelmohsen UR, Bayer K, Hentschel U (2014) Diversity, abundance and natural products of marine sponge-associated actinomycetes. Nat Prod Rep 31(3):381–399

Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS et al (2014) Elicitation of secondary metabolism in actinomycetes. Biotechnol Adv 33:798811

Abd-Elnaby H, Abo-Elala G, Abdel-Raouf U, Abd-Elwahab A, Hamed M (2006) Antibacterial and anticancer activity of marine *Streptomyces parvus*: optimization and application. Biotechnol Eq 30:180–191

Ahsan T, Chen JG, Zhao XX, Irfan M, Wu YH (2017) Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Expr 7(1):54

Asolkar RN, Freel KC, Jensen PR, Fenical W, Kondratyuk TP, Park E-J (2009) Arenamides A-C, cytotoxic NF-κB inhibitors from the marine Actinomycete Salinispora arenicola. J Nat Prod 72(3):396–402

Bader J, Mast-Gerlach E, Popovic MK, Bajpai R, Stahl U (2010) Relevance of microbial co-culture fermentations in biotechnology. J Appl Microbiol 109:371–387

Bajaj I, Veiga T, van Dissel D et al (2014) Functional characterization of a Penicillium chrysogenum mutanase gene induced upon cocultivation with Bacillus subtilis. BMC Microbiol 14:114130

Belyagoubi L, Belyagoubi-Benhammou N, Jurada V, Dupont J, Lacost S, Djebbah F et al (2018) Antimicrobial activities of culturable microorganisms (actinomycetes and fungi) isolated from Chaabe cave, Algeria. Int J Speleol 47:189–199

Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL et al (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3 (2). Nature 417:141–147

Bieber B, Nuske J, Ritzau M, Grafe U (1998) Alnumycin, a new naphthoquinone antibiotic, produced by an endophytic *Streptomyces* sp. J Antibiot 51:381–382

Brakhage AA (2013) Regulation of fungal secondary metabolism. Nat Rev Microbiol 11:21–32

Buchanan RE (1917) Studies in the nomenclature and classification of the bacteria. II The primary subdivisions of the Schizomycetes. J Bacteriol 2:155–164

Caboche S (2014) Biosynthesis: bioinformatics bolster a renaissance. Nat Chem Biol 10:798–800

Castillo UFG, Strobel A, Ford EJ, Hess WM, Porter H et al (2002) Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. Microbiology 148:2675–2685

Chen Y, Liu R-H, Li T-X, Huang S-S, Kong L-Y, Yang M-H (2017) Enduspeptides A-F, six new cyclic depsipeptides from a coal mine derived *Streptomyces* sp. Tetrahedron 73:527–531

Chiang YM, Lee KH, Sanchez JF, Keller NP, Wang CC (2009) Unlocking fungal cryptic natural products. Nat Prod Commun 4:1505–1510

Chiang YM, Chang SL, Oakley BR, Wang CC (2011) Recent advances in awakening silent biosynthetic gene clusters and linking orphan clusters to natural products in microorganisms. Curr Opin Chem Biol 15:137–143

Cimermancic P, Medema MH, Claesen J, Kurita K et al (2014) Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 158:412–421
Craney A, Ozimok C, Pimentel-Elardo SM, Capretta A, Nodwell JR (2012) Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. Chem Biol 19(8):10201027
Dashti Y, Grkovic T, Abdelmohsen UR et al (2014) Production of induced secondary metabolites by a co-culture of sponge associated actinomycetes, Actinokineospora sp. EG49 and Nocardiopsis sp. RV163. Mar Drugs 12:3046–3059
Demain AL, Adrio JL (2008) Contributions of microorganisms to industrial biology. Mol Biotechnol 38(1):41–55
Ding WJ, Zhang SQ, Wang JH, Lin YX, Liang QX et al (2013) A new diO-prenylated flavone from an actinomycete Streptomyces sp. MA-12. J Asian Nat Prod Res 15(2):209–214
Djinni I, Defant A, Kecha M, Mancini I (2014) Metabolite profile of marine-derived endophytic Streptomyces sundarbansensis WR1L1S8 by liquid chromatography mass spectrometry and evaluation of culture conditions on antibacterial activity and mycelial growth. J Appl Microbiol 116(1):39–50
Doroghazi JR, Albright JC, Goering AW et al (2014) A roadmap for natural product discovery based on largescale genomics and metabolomics. Nat Chem Biol 10:963–968
Edwards C (1993) Isolation properties and potential applications of thermophillic actinomycetes. Appl Biochem Biotechnol 42:161–179
Gao B, Gupta RS (2012) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76:66–112
Girard G, Traag BA, Sangal V, Mascini N et al (2013) A novel taxonomic marker that discriminates between morphologically complex actinomycetes. Open Biol 3:130073
Girard G, Willemse J, Zhu H, Claessen D et al (2014) Analysis of novel kitasatosporae reveals significant evolutionary changes in conserved developmental genes between Kitasatospora and Streptomyces. Antonie Van Leeuwenhoek 106:365–380
Good Fellow M, Williams ST (1983) Ecology of actinomycetes. Annu Rev Microbiol 37:189–216
Goodfellow M, Kampfer P, Busse HJ et al (2012) Bergey's manual of systematic bacteriology. The Actinobacteria, part A and B, vol 5. Springer, New York
Hertweck C (2009) Hidden biosynthetic treasures brought to light. Nat Chem Biol 5:450–452
Hopwood DA (2007) Nature and medicine: the antibiotic makers. Oxford University Press, New York
Igarashi Y, Miura SS, Fujita T, Furumai T (2006) Pterocidin, a cytotoxic compound from the endophytic Streptomyces hygroscopicus. J Antibiot 59(3):193–195
Igarashi Y, Trujillo ME, Martínez-Molina E, Yanase S, Miyanaga S et al (2007) Antitumor anthraquinones from an endophytic actinomycete Micromonospora lupine sp. nov. Bioorg Med Chem Lett 17(13):3702–3705
Igarashi Y, Ogura H, Furihata K, Oku N, Indananda C et al (2011) Maklamicin, an antibacterial polyketide from an endophytic Micromonospora sp. J Nat Prod 74(4):670–674
Isaka M, Berkaew P, Intereya K, Komwijit S, Sathitkunanon T, Sathitkunanon T (2007) Tetrahedron 63:6855–6860. https://doi.org/10.1016/j.tet.2007.04.062
Jensen PR, Chavarria KL, Fenical W, Moore BS, Ziemert N (2014) Challenges and triumphs to genomics based natural product discovery. J Ind Microbiol Biotechnol 41:203–209
Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y (2009) Production of bioactive metabolites by *Nocardia levis* MK-VL_113. Lett Appl Microbiol 49:484–490
Kim N, Shin JC, Kim W, Hwang BY, Kim BS et al (2006) Cytotoxic 6 alkylsalicylic acids from the endophytic Streptomyces laceyi. J Antibiot 59(12):797–800
Kirby R (2011) Chromosome diversity and similarity within the Actinomycetales. FEMS Microbiol Lett 319:1–10
Krug D, Zurek G, Revermann O, Vos M, Velicer GJ, Müller R (2008) Discovering the hidden secondary metabolome of Myxococcus xanthus: a study of intraspecific diversity. Appl Environ Microbiol 74:3058–3068

Kurosawa K, Ghiviriga I, Sambandan TG, Lessard PA et al (2008) Rhodostreptomycins, antibiotics biosynthesized following horizontal gene transfer from *Streptomyces padanus* to *Rhodococcus fascians*. J Am Chem Soc 130(4):1126–1127

Lalitha P, Veena V, Vidhyapriya P, Lakshmi P, Krishna R, Sakthivel N (2016) Anticancer potential of pyrrole (1, 2, a) pyrazine 1, 4, dione, hexahydro 3-(2-methyl propyl) (PPDHMP) extracted from a new marine bacterium, Staphylococcus sp. strain MB30. Apoptosis 21:566–577

Laureti L, Song L, Huang S, Corre C, Leblond P et al (2011) Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in Streptomyces ambofaciens. Proc Natl Acad Sci 108(15):62586263

Li Y, Ling H, Li W, Tan H (2005) Improvement of nikkomycin production by enhanced copy of sanU and sanV in Streptomyces ansochromogenes and characterization of a novel glutamate mutase encoded by sanU and sanV. Metab Eng 7:165–173

Lu C, Shen Y (2007) A novel ansamycin, naphthomycin K from Streptomyces sp. J Antibiot 60(10):649–653

Ludwig W, Euzeby J, Schumann P et al (2012) Road map of the phylum Actinobacteria. In: Bergey's manual® of systematic bacteriology. Springer, New York, pp 1–28

Luo Y, Huang H, Liang J, Wang M, Lu L, Shao Z et al (2013) Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster. Nat Commun 4:28–94

Maciejewska M, Adam D, Martinet L, Naome A, Calusiriska M, Delfosse P et al (2016) A phenotypic and genotypic analysis of the antimicrobial potential of cultivable Streptomyces isolated from cave moonmilk deposits. Front Microbiol 7:14–55

Mangamuri U, Muvva V, Poda S, Naragani K, Munaganti RK, Chitturi B, Yenamandra V (2016) Bioactive metabolites produced by Streptomyces cheonanensis VUKA from Coringa mangrove sediments: isolation, structure elucidation and bioactivity. 3 Biotech 6:63–68

Manimegalai K, Asha Devi NK, Padmavathy S (2013) Marine fungi as a source of secondary metabolites of antibiotics. Int J Biotechnol Bioeng Res 4(3):275–282

Marmann A, Aly AH, Lin W, Wang B, Proksch P (2014) Co-cultivation—a powerful emerging tool for enhancing the chemical diversity of microorganisms. Mar Drugs 12:1043–1065

Mayfield CI, Williams ST, Ruddick SM et al (1972) Studies on the ecology of actinomycetes in soil. IV. Observations on the form and growth of streptomycetes in soil. Soil Biol Biochem 4:79–91

Mitchell SS, Nicholson B, Teisan S, Lam KS, Barbara C (2004) Aureoverticillactam, a novel 22-atom macrocyclic lactam from the marine actinomycete Streptomyces aureoverticillatus. J Nat Prod 67(8):1400–1402

Müller R, Wink J (2014) Future potential for anti-infectives from bacteria — how to exploit biodiversity and genomic potential. Int J Med Microbiol 304:3–13

Murphy TM, Nilsson AY, Roy I, Harrop A, Dixon K et al (2011) Enhanced intracellular Ca2+ concentrations in *Escherichia coli* and *Bacillus subtilis* after addition of oligosaccharide elicitors. Biotechnol Lett 33:985–991

Nair R, Roy I, Bucke C, Keshavarz T (2009) Quantitative PCR study on the mode of action of oligosaccharide elicitors on penicillin G production by Penicillium chrysogenum. J Appl Microbiol 107:1131–1139

Nakashima T, Kamiya Y, Iwatsuki M, Takahashi Y, Omura S (2014a) Mangromicins, six new antioxidative agents isolated from a culture broth of the actinomycete, *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 67(7):533–539

Nakashima T, Iwatsuki M, Ochiai J et al (2014b) Mangromicins a and B: structure and antitrypanosomal activity of two new cyclopentadecane compounds from *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 67(3):253–260

Nakashima T, Kamiya Y, Iwatsuki M et al (2015) Mangromicin C, a new analog of mangromicin. J Antibiot 68(3):220–222

Nett M, Ikeda H, Moore BS (2009) Genomic basis for natural product biosynthetic diversity in the actinomycetes. Nat Prod Rep 26:1362–1384

Nouaille S, Even S, Charlier C, Le Loir Y et al (2009) Transcriptomics response of Lactococcus lactis in mixed culture with Staphylococcus aureus. Appl Environ Microbiol 75:4473–4482

Nutzmann HW, Reyes Dominguez Y, Scherlach K et al (2011) Bacteria induced natural product formation in the fungus Aspergillus nidulans requires Saga/Ada mediated histone acetylation. Proc Natl Acad Sci U S A 108:14282–14287

Omura S, Takahashi Y, Iwai Y, Tanaka H (1982) Kitasatosporia, a new genus of the order Actinomycetales. J Antibiot 35:1013–1019

Onaka H, Mori Y, Igarashi Y, Furumai T (2011) Mycolic acid containing bacteria induce natural product biosynthesis in *Streptomyces* species. Appl Environ Microbiol 77:400–406

Paranagama PA, Wijeratne EMK, Gunatilaka AAL (2007) Uncovering biosynthetic potential of plant associated fungi: effect of culture conditions on metabolite production by Paraphaeosphaeria quadriseptata and Chaetomium chiversii. J Nat Prod 70:1939–1945

Parte AC (2018) LPSN-list of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. Int J Syst Evol Microbiol 68:1825–1829

Perez J, Bax L, Escolano C (2005) Roadmap report on nanoparticles. Willems and Van Den Wildenberg, Barcelona

Pettit RK (2011) Small molecule elicitation of microbial secondary metabolites. J Microbial Biotechnol 4:471–478

Radman R, Saez T, Bucke C, Keshavarz T (2003) Elicitation of plants and microbial cell systems. Biotechnol Appl Biochem 37(1):91–102

Radman R, Bland EJ, Sangworachat N et al (2006) Effects of oligosaccharides and polysaccharides on the generation of reactive oxygen species in different biological systems. Biotechnol Appl Biochem 44:129–133

Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA et al (2008) Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces. EMBO Rep 9:670–675

Romero D, de Vicente A, Rakotoaly RH et al (2007) The iturin and fengycin families of lip peptides are key factors in antagonism of Bacillus subtilis toward Podosphaera fusca. Mol Plant Microbe Interact 20:430–440

Roy S, Banerjee D (2015) Broad spectrum antibacterial activity of granaticinic acid, isolated from Streptomyces thermoviolaceus NT1; an endophyte in Catharanthus roseus (L.) G. Don. J Appl Pharm Sci 5(7):6–11

Roy RN, Laskar S, Sen SK (2006) Dibutyl phthalate, the bioactive compound produced by 724 *Streptomyces albidoflavus* 321.2. Microbiol Res 161:121–126

Saurav K, Kannabiran K (2012) Cytotoxicity and antioxidant activity of 5-(2,4-dimethylbenzyl) pyrrolidin-2-one extracted from marine *Streptomyces VITSVK5* spp. Saudi J Biol Sci 19:81–86

Savi DC, Haminiuk CWI, Sora GTS, Adamoski DM, Kenski J et al (2015) Antitumor, antioxidant and antibacterial activities of secondary metabolites extracted by endophytic actinomycetes isolated from Vochysia divergens. Int J Pharm 5(1):347–356

Sawa R, Kubota Y, Umekita M, Hatano M, Hayashi C et al (2018) Quadoctomycin, a 48-membered macrolide antibiotic from *Streptomyces* sp. MM168-141F8. J Antibiot (Tokyo) 71:91–96

Schweder T, Lindequist U, Lalk M (2005) Screening for new metabolites from marine microorganisms. Adv Biochem Eng Biotechnol 96:1–48

Ser HL, Palanisamy UD, Yin WF, Chan KG, Goh BH, Lee LH (2016) *Streptomyces malaysiense* sp. nov.: A novel Malaysian mangrove soil actinobacterium with Antioxidative activity and cytotoxic potential against human cancer cell lines. Sci Rep 6:24247

Seyedsayamdost MR (2014) High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. Proc Natl Acad Sci 111(20):72667271

Sharma SK, Gupta VK (2008) *In vitro* antioxidant studies of Ficus racemosa Linn root. Pharmacogn Mag 13:70–74

Shellikeri A, Kaulgud V, Yaradoddi J, Ganachari S, Banapurmath N, Shettar A (2018) Development of neem-based bioplastic for food packaging application. IOP Conf Ser: Mater Sci Eng 376:012052. https://doi.org/10.1088/1757899X/376/1/012052

Siddharth S, Rai RV (2019) Isolation and characterization of bioactive compounds with antibacterial, antioxidant and enzyme inhibitory activities from marine-derived rare Actinobacteria, *Nocardiopsis* sp. SCA21 microbial pathogenesis. Microb Pathog 137:103775

Slattery M, Rajbhandari I, Wesson K (2001) Competition mediated antibiotic induction in the marine bacterium *Streptomyces tenjimariensis*. Microb Ecol 41(2):9096

Suriyavathana M, Nandhini K (2010) *In vitro* antioxidant profile of liv-PRO-08 oral ayurvedic formulation. J Pharm Res 3:873–876

Taechowisan T, Lu C, Shen Y, Lumyong S (2005) 4-arylcoumarins from endophytic Streptomyces aureofaciens CMUAc130 and their antifungal activity. Ann Microbiol 55:63–66

Taechowisan T, Lu C, Shen Y, Lumyong S (2007) Antitumor activity of 4-arylcoumarins from endophytic Streptomyces aureofaciens CMUAc130. J Cancer Res Ther 3:86–91

Tan LTH, Ser HL, Yin WF, Chan KG, Lee LH, Goh BH (2015) Investigation of antioxidative and anticancer potentials of *Streptomyces* sp. MUM256 isolated from Malaysia mangrove soil. Front Microbiol 6:13–16

Tanaka Y, Hosaka T, Ochi K (2010) Rare earth elements activate the secondary metabolite biosynthetic gene clusters in Streptomyces coelicolor A3 (2). J Antibiot 63:477–481

Tiwari K, Gupta RK (2012) Rare actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol 32(2):108–132

Ventura M, Canchaya C, Fitzgerald GF et al (2007) Genomics as a means to understand bacterial phylogeny and ecological adaptation: the case of bifidobacteria. Antonie Van Leeuwenhoek 91(4):351–372

Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus Streptomyces? Arch Microbiol 176(5):386390

Wenzel SC, Müller R (2009) Myxobacteria–'microbial factories' for the production of bioactive secondary metabolites. Mol BioSyst 5:567–574

Williamson NR, Fineran PC, Leeper FJ, Salmond GP (2006) The biosynthesis and regulation of bacterial prodiginines. Nat Rev Microbiol 4(12):887899

Wu SJ, Fotso S, Li F, Qin S, Laatsch H (2007) Amorphane sesquiterpenes from a marine Streptomyces sp. J Nat Prod 70(2):304–306

Yang X, Peng T, Yang Y, Li W, Xiong J et al (2015) Antimicrobial and antioxidant activities of a new benzamide from endophytic Streptomyces sp. YIM 67086. Nat Prod Res 29(4):331–335

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2021) Bio-based material from fruit waste of orange peel for industrial applications. J Mater Res Technol. https://doi.org/10.1016/j.jmrt.2021.09.016

Zazopoulos E, Huang K, Staffa A, Liu W et al (2003) A genomics guided approach for discovering and expressing cryptic metabolic pathways. Nat Biotechnol 21:187–190

Zhang Z, Wang Y, Ruan J (1997) A proposal to revive the genus Kitasatospora (Omura, Takahashi, Iwai, and Tanaka 1982). Int J Syst Bacteriol 47:1048–1054

Zhang H, Sun GS, Li X, Pan HL, Zhang YS (2010) A new Geldanamycin analogue from Streptomyces hygroscopicus. Molecules 15:1161–1167

Zhang H, Saurav K, Yu Z, Mandi A, Kurtán T, Li J et al (2016) α–Pyrones with diverse hydroxy substitutions from three marine-derived Nocardiopsis strains. J Nat Prod 7:1610–1618

Zhu H, Sandiford SK, van Wezel GP (2014) Triggers and cues that activate antibiotic production by actinomycetes. J Ind Microbiol Biotechnol 41:371–386

Zuck KM, Shipley S, Newman DJ (2011) Induced production of N-formyl alkaloids from *Aspergillus fumigatus* by co-culture with *Streptomyces peucetius*. J Nat Prod 74(7):1653–1657

Chapter 10
Scope of *Actinobacteria* in Bioengineering

Jayachandra S. Yaradoddi, Merja H. Kontro, Sharanabasava V. Ganachari, Nagaraj R. Banapurmath, Manzoore Elahi M. Soudagar, and Mahesh Divatar

Abstract In the present situation, it is interesting to find out novel bioactive metabolites with desired characteristic features of a novel drug. However, similar compounds are frequently rediscovered due to repeated samples from known environments and the use of conventional screening methods. Certainly, revitalization in exploring different groups of bacteria is very much essential in functional genomics and annotations yielding potential bioactive molecules with novel structures. The major challenge behind this particular genome-driven approach lies in metabolic engineering of the stress to utter the biosynthetic pathways that aims to synthesize the desired molecules in higher quantity. Surprisingly, genetic engineering or manipulation of actinobacteria is not easy when compared with other model organisms such as *E. coli* and *Saccharomyces cerevisiae*. It is because they unlike genomic content with higher GC content in their genomes. One of the previous

J. S. Yaradoddi (✉)
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

M. H. Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

N. R. Banapurmath
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

M. E. M. Soudagar
Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur, Malaysia

M. Divatar
Viral Research and Diagnostic Laboratory, Gulbarga Institute of Medical Sciences, Gulbarga, Karnataka, India

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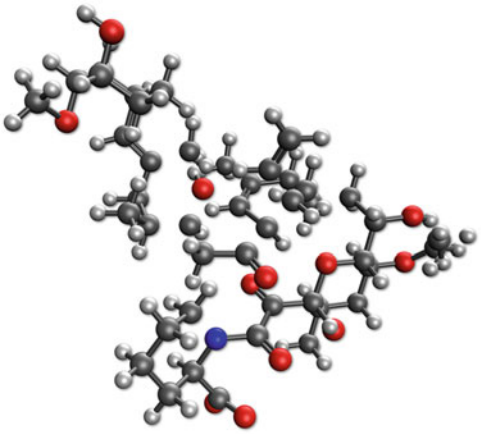
reports suggests that the standard method for replacing actinomycete gene utilizing RCA facilitated twofold crossover actions with non-replicative or temperature-sensitive plasmids. However, the efficiency of these methods has significantly enhanced by the templates through most similar regions, like the "ReDirect method" or using meganuclease I-SceI to introduce DNA DSBs a positive selection marker.

Keywords *Actinobacteria* · Genome · Bioengineering · Genetic manipulation · Secondary metabolites · Plasmids

10.1 Introduction

From the past few years, there were intensive research conducted toward the discovery and delivery of the most prominence natural products, and these products were later employed like scaffolds toward obtaining a huge amount of antibiotics which are presently under the clinical trials (Berdy 2012; Landwehr et al. 2016). In spite of the previous success, the current proliferation of multidrug resistance toward numerous dreadful pathogens (examples: *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* sp., and *Acinetobacter* sp.) and the scarcity of therapeutic agents' advancements to address the emergent infections in the hospitals were very hard to treat and turned out into main health concerns, and that needs a novel and sustainable approach to satisfy the need for novel antibiotics (Fischbach and Walsh 2009). The main challenge for the development of new drugs in the field of medicine occurred due to even massive group of companies leaving the burden of the high cost of research and development, and also a very poor encouragement led to the formation of the broken economic model that remained as an academic group, and now a smaller group or bio-tech startup companies can be the key players in original discovery field. Research groups are always challenged in the identification of the novel class of antibiotics that they have the ability to expand it individually equal to the market (Editorial 2018). Global initiatives were established during past several years which support research and development of new antibiotics and rejuvenate the preclinical and clinical trials. However, fundamental research and previous findings endeavor yet continue indigent addressed by these programs, and the prevailing investigation gaps are not strengthening the formation of a sustainable previous discovery channel for the betterment in the recovery of novel compounds using advanced techniques that can be seen from the past few decades (Simpkin et al. 2017). These inadequate numbers of clinical advancement programs are directly discrete to the dynamic and productive action that has been established in the area of secondary microbial metabolites during the few years (Brown and Wright 2016). Research on natural products has changed in the direction of signifying one of the vital sources for discovering potential novel drugs; this is when compared to the inefficiency of synthetic compounds. Relatively deprived reliability of artificial libraries that are employed in selecting the molecules as of target-built in laboratory, or to synthesize

rational antibiotics (drug) designs created on ligand binding lacking of appropriate physiochemical properties against piercing bacterial cell membranes was widely described in previous reports (Lewis 2017; Sulochana et al. 2014a; b; Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020; Yaradoddi et al. 2013).



Tacrolimus

Regardless of the discovery issues for previously known metabolites, they can be characterized as part of the main load for sustained asset related to conventional novel product discovery paths reported in the past. Advancements in DNA sequencing, whole-genome sequencing, and bioinformatics have provided a collective wealth of information that helps in understanding the biosynthetic mechanism pathways of natural products; the wide untapped potential biosynthetic types of microbes, especially actinobacteria, are also revealed (Baltz 2017). These likely filamentous organisms are indicated and bear formerly unpredicted diverse biosynthetic gene pools; initially, there is a new opportunity in the identification of novel classes of metabolites or compounds that are expecting to be discovered (Rutledge and Challis 2015). Innovative progression in culturing methods using new microbial habitats can provide ample scope in determining actinobacterial diversity and acquiring the broader space of secondary metabolites. The metabolomics, comparative proteomics and genomics, and progression in growth of a novel analysis tools are creating a wealth of combined information, which is really encouraging for the advent of new plans that are targeted for obtaining a restored understanding of the physiology and the production of natural products by the actinomycetes (Mohan Reddy et al. 2015a; b). Novel strategies were developed to mine genomes and in the prediction of structures of compounds; however, to activate silent clusters and empower the pathway engineering and to dictate metagenomic gene groups in heterologous hosts provided that the required tools to lead the foundation for a novel antibiotic discovery paradigm. Most of the latest reviews reveal each and individual facets of this progressing area and its impact on the recognition and classification of novel antibiotics (Genilloud 2017).

10.2 Exploration of Biodiversity of a Cultured Community of the Actinomycetes

The sustained research interest in the exploration of the different niches to untap the new sources of the microbial populations brings up wide potential investigations upon different range of sources that cover between particular terrestrial environmental conditions and exceptional microbial communities especially under marine ecosystems and plant-host association.

The dispersal of some important microbial species offers biogeographic patterns; they can be typically identified by microenvironmental surroundings. The freshest reports were focused on the exploration of yet inadequately studied niches to rediscover novel chemical versatility. Investigation of extreme environmental situation, mainly arid and desert ecosystems, such as the desert of Atacama usually comprising higher levels of UV radiations and salinity, has allowed isolating the novel taxon among the actinomycetes that are well adjusted to such a harsh condition (Bull et al. 2016; Mohammadipanah and Wink 2016). The cave habitats provide another untapped ecosystem that has often been exploited in search of new potential microbes, and most of them have emphasized the isolation of novel taxon belonging to actinomycetes that produce secondary metabolites (Ghosh et al. 2017). The actinomycete biodiversity in calcite moonmilk deposits is being considered latest example displaying the capacity to synthesize novel products with antimicrobial activities (Adam et al. 2018) (Table 10.1). The use of diffusion chamber techniques in growing uncultured soil bacterial communities has allowed isolating novel antibiotics, mostly lassomycin (Gavrish et al. 2014). The marine environments have been naturally an additional source of marine actinobacteria (Fenical and Jensen 2006; Bull and Stach 2007). The wide variety of marine habitats, extending from shallow waters, mangroves, associated invertebrates, and deep-sea sediments, has continuously gaining the attention of microbiologists (Anil et al. 2010; Jayachandra et al. 2013). Their isolation procedures have confirmed exhaustive research in discovering novel cultures producing novel metabolites or novel analogs by their potential bioactivity (Fenical and Jensen 2006; Bull and Stach 2007; Choudhary et al. 2017; Dhakal et al. 2017; Xu et al. 2018; Jose and Jha 2017). In spite of this inexhaustible deliberation on marine-oriented strains and novel antibiotic producers, these ecosystems continued to be poorly studied with respect to microbial diversity and also in terms of functional diversity. Marine sediments in recent times have shown that the presence of actinobacteria population is limited in this niche compared to other groups of bacteria. In addition, these discoveries have clearly indicated that synthesis of most of bioactive molecules has a profound influence on the existence of microbial population composition (Sarmiento-Vizcaíno et al. 2017). No doubt, there are parallel advancements during the metagenomic library estimation of microbial abundance have permitted researchers to understand molecular dynamics within microorganism of interest among the communities were rigorously reviewed.

Table 10.1 List of antimicrobial compounds produced by actinomycetes

S. no.	Bioactive compounds	Microbial source	Activity
1	Rhamnose	*Saccharopolyspora spinosa*	Essential component of insect control agent
2	Rapamycin	*Streptomyces hygroscopicus*	Antifungal
3	Oxytetracycline	*Streptomyces rimosus*	Antibacterial
4	Asukamycin	*Streptomyces nodosus* subsp. *asukaensis*	Antibacterial
5	Fosfomysin	*Streptomyces fradiae*	Antibacterial
6	Rimocidin	*Streptomyces diastaticus* var. 108	Antifungal
7	Chloramphenicol	*Streptomyces venezuelae*	Antibacterial
8	Amythiamicins	*Amycolatopsis* sp.	Antibacterial
9	Rifamycin	*Amycolatopsis mediterranei* U-32	Antibacterial
10	Erythromycin	*Saccharopolyspora erythraea*	Antibacterial
11	Nanchangmycin	*S. nanchangensis*	Insecticidal
12	Nikkomycins	*S. ansochromogenus*	Antifungal
13	Tubelactomicin A	*Nocardia* sp.	Antibacterial
14	Lomofungin	*Streptomyces lomodensis*	Antifungal, antibacterial
15	Axenomycins	*Streptomyces lisandri* nov. sp.	Antifungal
16	Streptomycin	*Streptomyces griseus*	Antibacterial
17	MM461156	*Actinomadura pelletieri*	Antiviral, antibacterial
18	Musacin C	*Streptomyces griseovirdis*	Antiviral
19	Fattiviracin A1	*Streptomyces microÀDYXV*	Antiviral
20	FK 506	*Streptomyces tsukubaensis*	Antiviral
21	Apramycin	*Streptomyces tenebrabrius* UD2	Antibacterial
22	Leptomycin	*Streptomyces lividans*	Antifungal
23	Gentamicin	*Micromonospora purpurea* var. *violaceae*	Antibacterial
24	Dunaimycins	*Streptomyces diastatochromogenes*	Antimicrobial
25	Tetracycline	*Streptomyces aureofaciens*	Antibacterial

Above techniques and advancements are uncovering new opportunities in the determination of their potential roles of the microbes in antibacterial compounds on especially microbiome composition. These detailed investigations can be useful in the recovery and selection of most potential strains from these microbial populations and also screening for the production, purification, and applications of new bioactive compounds.

10.3 Genomics-Driven Discovery

Parallelly, logarithmic growth in the amount of partial or whole-genome sequencing programs against the actinobacterial species existing in the public database ensures their wide biosynthetic multiplicity among the different lineages but then again allowed exhaustive bioengineering techniques that can be utilized to untap the novel natural product scaffolds. Determining novel biosynthetic pathways concerning to exciting novel compounds has stimulated the process of discovery for novel BGCs (biosynthetic gene clusters) upon current genomes that is to be considered as much-improvised bioinformatics tool in gene cluster recognition and gene annotation, such as AntiSMASH (Patin et al. 2017; Medema and Fischbach 2015; Weber and Kim 2016). Additionally, increased figures of most of the whole genomes have allowed to study extensively on genomic analyses of potential candidature among bacterial communities and the recognition of the genomic composition and the phylogenetic history of various taxa (Blin et al. 2017; Sánchez-Hidalgo et al. 2018; Adamek et al. 2018). These relative studies are disclosing the existence of a core genome within several members of actinobacteria along with diversity of BGCs among the various lineages. Above reports have provided the source for understanding mechanism of functional evolution of typical taxa as indicated for *Streptomyces* (Zhou et al. 2012; Kim et al. 2015). One more important characteristic feature of the increase in a number of BGC sequence information on antibiotic discovery is an opportunity in developing precisely targeted genome engineering searchers in the genome libraries that depends on particular genomic signatures concerned with biosynthesis of beneficial scaffolds and functionalization which may be driving force in discovering novel metabolites and chemical compounds (Ju et al. 2015; Tang et al. 2015). The combination of genomics, metabolomics, transcriptomics, and proteomics has provided whole information for assessment of functional position of actinobacterial species. This information is really interesting and influences the progression of novel methods majorly engineering and expression of these biosynthetic pathways and the recognition of novel bioactive compounds from cultured actinobacteria not only from unexplored environments but also using huge microorganism assemblages which are yet unexploited treasurers of biosynthetic multiplicity.

Some of the main challenges that persisted during this situation are effective cloning and expression of biosynthetic cluster formerly silent and partially expressed within host subsequently through reengineering by the replacements among regulatory molecules and additional discovery of synthesized compounds (Myronovskyia and Luzhetskyy 2016; Weber et al. 2015). So far, numerous various in vitro DNA assemblages or direct detain techniques were described to clone BGCs in heterologous hosts (Bonet et al. 2014; Yang et al. 2015; Ren et al. 2017; Li et al. 2017; Tong et al. 2018). Most of the BGCs may not be detected by the rule-based bioinformatics tool in the absence of the signature genes; however, the main advantages of the prediction tool based on the occurrence of Pfam domains seen in the BGCs have a better potential in identifying added clusters (Weber and Kim 2016). In a novel

genomic bacterial artificial chromosome (BAC) constructed from large 100-kb fragments of *Streptomyces* sp., the genomic DNA can be utilized in carrying out high-throughput functional screening methods in identification of BGCs through heterologous expression systems (Xu et al. 2016).

10.4 Description of the Production Pathways

Gene activation for BGCs among identified potential bacterial cultures through direct gene engineering will be a substituted method, where bacterial strains should be open up for the genetic engineering process. The latest effective example has been described with the advent of advanced genetic engineering techniques in the modification of metabolic pathways, manipulating metabolic variability through blockage of irrelevant metabolic pathways, transcriptional repressors inactivation, and overexpression of activator genes or also the multiplication of BGC copies within the unique potential strains to increase their concentration or titers (Tong et al. 2018; Hwang et al. 2014; Horbal et al. 2018; Goh et al. 2002). More recently, the progression in optimal ribosomal binding sequences along with strong terminator beneficial to control metabolic activities of actinomycetes has unveiled the new avenues in the modulation of gene expression and to denote most gifted method in metabolic manipulation or metabolic engineering (Horbal et al. 2018).

Regardless of this accomplishment, a huge group of natural type and important industrial strains is yet intractable besides genetic manipulation and as long as BGCs may not straight away activate using the genetic tools. Culture-dependent methods rely on malnutrition situations that have been one of the most techniques adopted to define the media constituents required for the synthesizing novel metabolites. One of the fundamental processes involves tiny molecules as an elicitor through disturbing biological system, and their signaling pathways have been preexisted for some decades, which has been broadly utilized for activation of weekly expressed pathways by a wide variety of tiny molecules that include the subinhibitory quantities of antibiotics (Goh et al. 2002; Rosen and Seyedsayamdost 2017). Very first large-scale elicitor screening process was assessed for more than 30,000 varieties of compounds by *S. coelicolor*, and it enables to identify a small number of compounds which are capable to enhance the production capacities concerned to secondary metabolites by numerous times (Subramani and Aalbersberg 2012). Several instances of hormesis (which means dose response) have been revealed along with subinhibitory concentrations of antibiotics and extra familiar bioactive compounds, which have triggered a response that is concerned to the major activators of secondary metabolism, in production of new compounds and inducing cryptic gene clusters (Larose et al. 2010). The activation consequence ineffectively foreseen from the antibiotic means of action and the absence of universal effectors to activate all silent BGCs has extra stage intricacy in the identifications of novel effector substances (Rosen and

Seyedsayamdost 2017). The similar effect is followed by cocultivation, which is a technique that has been utilized widely with several diverse types of culturing methods and strain consortium. This method takes advent of the consequence that even little amount of signaling molecules using selected strains may have an additional strain. Major problem in large-scale approach as overall method is concerned to the unlikely assumption for combination results for effective response, which usually does not apply for more than 20% of the case studies (Okada and Seyedsayamdost 2017). Mycolic acids involved in the physical interaction leads to stimulation of few silent pathways. Novel antibacterial agents, for example, arcyriafavin E, alchivemycin A and B, or ciromicins, were well defined subsequently; mycolic acid has proven role in physical interlinkage and the stimulation of silent metabolic pathways. Novel antibacterial compounds, mainly arcyriaflavin E or ciromicins and alchivemycin A and B, were designated through cocultivation of *Rhodococci* along with the genera *Tsukamurella*, *Streptomyces*, and *Nocardiopsis* (Onaka 2017; Derewacz et al. 2015). Additionally, the inductance usually does not necessitate the cell-cell interaction which is only facilitated by diffusing a tiny effector molecule, for instance, the synthesis of the natural compound keyicin through cocultivating the producers such as *Micromonospora* strain along with the *Rhodococcus* (Zhang et al. 2014). Greatest part of the research carried out on discrepancy metabolomic analysis in reply to cultivatable conditions indicating the chemical ability of actinobacteria is so far for its complete characterization (Xu et al. 2014). Since the advancement's direction, the latest LC-MS analysis and NMR analysis techniques and modified metabolomics analysis have been used for the detection of production of potential new bioactive compounds in a multifaceted mixture and mapping the exact response toward the external stimuli. Relative metabolomics can be proficiently utilized in identification of the enhanced expression of bioactive compounds from *S. Coelicolor* ambiguous genes resulted from the exposure toward complex agitation and in identification of subdivision of primary and secondary metabolites which give response to a likewise huge diversity of inducements (Goodwin et al. 2015). The main challenge among these techniques is concerning the identification of unknown secondary metabolites inside the complicated metabolomic profile. Novel dereplication and identification and classification methods are uninterruptedly being progressed, which are grounded on the resemblance of MS/MS forms in natural product databases and NMR-based metabolomics (Wang et al. 2016; Pérez-Victoria et al. 2016; Wu et al. 2017). The proteo mining is other technique used to help the identification process. The analysis connects naturally occurring compounds to biosynthetic enzymes as it is corresponding to the protein expression pattern of the enzymes in metabolome concerning potential strains based on the statistical evaluation of isolates which were cultivated under varied circumstances (Gubbens et al. 2014; Chao Du and van Wezel 2018).

10.5 Exploration and Regulation of Primary and Secondary Metabolisms

Production of bioactive molecules using actinobacteria is critically controlled and tends toward bringing specific response against external stimulation from the atmosphere. This regulation activity directed through the various channels—in particular the regulator fragments involve two-component systems, extra-cytoplasmic pathway specific regulator or sigma factors, for example, the utmost described LmbU family (Hou et al. 2018). Which stimulates expression of BGCs genes continuously channelize transcriptional response through master regulators employed in international metabolic regulator networks and they are not permanently path specific (Hoskisson and Fernández-Martínez 2018). Knowing the right mixtures of regulatory essentials and transcriptional factors that control a BGC has been projected as the "cracking the code" tactic and can be followed in identification of the key regulatory signals which generates the signals that are required to establish the cultural conditions to stimulate a specific BGC (Rigali et al. 2018). There is an important program developed in identification of signaling pathways which is known as regulon predictor PRE-Detector, which is presumed to be part of the in silico searches of regulatory elements; it will reveal the transcription factors involved in primary metabolism and plays key role in controlling pathway-specific regulators (Hiard et al. 2007).

In order to reach the imperative pharmaceutical requirements of potential drug candidates, advanced methods are very much needed in the improvisation of the efficacy of novel natural product research (Baltz 2008). Typically, identical types of secondary metabolites were synthesized through gene clusters that comprise homologous genes (Liu et al. 2003). The occurrence of marker genes, likely non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes, and manipulation of genes concerning to these enzymes can be designated for the production of secondary metabolites. Thus, PCR-based marker gene is considered to be an effective route in discovery of most potential natural products from the natural environment (Hornung et al. 2007). The process of halogenation induced by halogenases is a vital engineering phase for bioactivities of most of the natural products (Saadoun and Gharaibeh 2003; Yaradoddi et al. 2020b). Halogenated secondary compounds extend from simple halogenated terpenes, indoles, and phenols to intricate bioactivities and structures (Smith et al. 2013). Among them, several compounds are used for decades for pharmaceutical purposes. For instance, the much familiar antibiotic chloramphenicol (Podzelinska et al. 2010), the antitumor agent rebeccamycin, and antifungal agent pyrrolnitrin (Linares et al. 2006) were widely used in clinics. Thus, investigation on halo metabolites could be an imperative and the most significant method to discover novel drugs. Furthermore, promising biotechnological advantages, antibiotics especially halo metabolites, remain to be biological weapons against the pathogens from the native ecosystems through regulation of homeostasis of the microbial populations (Wagner et al. 2009).

Halogenases recovered from the deep sea and polar regions were minutely examined than from terrestrial and marine environments. The PCR screening methods of FDHs have been directed toward microbial consortia discovery associated with marine sponge (Bayer et al. 2013) and also from actinobacteria from different terrestrial and other habitats (Gao and Huang 2009). $FADH_2$-based halogenases have been investigated in depth from soil and marine actinobacteria, such as *Streptomyces* (Jia et al. 2006) and *Amycolatopsis*, in concerning to associated gene clusters. However, FDHs from the polar environments are limitedly studied. The Arctic regions are most fascinating, comprising peculiar environmental conditions, and premeditatedly important.

The Arctic regions were explored for the discovery of commercially viable drug or medically important compounds for biotechnological applications (Zerbe et al. 2002). However, several reports have been revolving around halogenases. Recent report (Svenson 2012) realized the halogen genes originating from the Arctic actinobacteria deprived of gene sequencing and cloning. Further, they screened FDHs from actinobacteria which were derived from marine sediments of the Arctic region, and sequencing is carried out for the first time. The new FDH genes were later cloned to acquire complete coding sequences for the study. Apart from these, the whole-genome sequencing was carried out to find out a cryptic gene cluster forecast to synthesize an unidentified halo metabolite. The investigation also shows that the halogenase genes are truly advantageous in bioprospecting among the Arctic actinobacteria. Interestingly the great genetic ability of halo metabolite synthesis is mostly anticipated from the Arctic actinomycetes.

10.6 Bioprospective of Halogenase from the *Actinobacteria*

PCR-gated studies are one of the most effective for bioprospecting of halogenase embedded actinobacteria. In one of the studies, it was observed that out of 60 strains, 9 were found positive for the halogenase gene. Among these, most of the isolated strains belonged to *Streptomyces*. However, few exceptional microbes belonged to *Pseudonocardia*, *Brevibacterium*, and *Nocardiopsis*. Reasonable amount of halogenase was detected in actinobacteria from Arctic region, in comparison with actinobacteria originating from other geographical locations (Yuan et al. 2014). It was mentioned in the report that these actinobacterial strains grow well at 15 °C in ISPII medium containing yeast extract (0.4%), malt extract (1.0%), and glucose (0.4%) at optimum temperature about 20–25 °C. There were no such significant phylogenetic variation and geographical distribution found. And the nine halogenase comprising strains were belonged to two genera: *Nocardiopsis* (two isolates) and *Streptomyces* (seven isolates). This report is observed as same with the previous studies, indeed the main genera consisting of halogenase producer and also producers of halo metabolites (Xiang et al. 2010). *Nocardiopsis* were rarely found along

with halogenase producers. Certainly, zero halometabolites were isolated from *Nocardiopsis* as per the report is concern.

10.6.1 Analysis Associated with the Complete Halogenase Genes

Meanwhile the partial gene sequences of halogenase indicated significant novelty and diversity; the type of microbes was selected to clone complete coding gene sequences. Nearly four full-length sequences of halogenase gene were cloned depending on genome ambiguity of partial gene sequence from the *Streptomyces* strains 604F and 551F and for *Nocardiopsis* genera, strains 597F and 531F. Together, *Streptomyces* sp. 551F and *Nocardiopsis* strains were recovered from deep-sea sediments in the high Arctic, though it was reportedly recovered from narrower depths. Halogenases were described and normally can be seen in gene groups for biosynthesis for concerned metabolites. Therefore, an evolutionary analysis using phylogenetic analysis was carried out for the four full halogenases and the formerly recognized halogenases from known biosynthetic gene clusters. The full halogenase gene sequence (hal551) of strain *Streptomyces* sp. 551F was alike to Trp-halogenase intricate in a biosynthesis of familiar halometabolites. Accordingly, hal551 gene sequence contains nearly 1605 base pairs that encode 534 amino acids, which has shared almost 85% of amino acid identity by numerous presumed Trp-halogenases from genome sequencing of *Streptomyces* and 71% amino acid similarity with the Thal of *Streptomyces albogriseolus* [GenBank: ABK79936] (Xu et al. 2014). Characteristically the Thal was a 6-Trp-halogenase which is regioselectively cleaved by bromination or chlorination at the sixth position of tryptophan in order to synthesize thienodolin, a growth stimulating factor in plants. The evolutionary tree indicated that Hal551 was phylogenetically linked to RebH (67% amino acid identity) since *Lechevalieria aerocolonigenes* ATCC 39243 [GenBank: CAC93722] (Seibold et al. 2006).

The Trp-halogenase chooses in bringing up the catalyzing activity of halogenation of seventh position of tryptophan during the synthesis of rebeccamycin. Furthermore, for Hal551 clustered by additional known Trp-halogenases, it regioselectivity acts upon the five, six, and seven position of tryptophan. Thus, Hal551 is forecasted to be regioselective catalyzer of tryptophan that produces ultimate halometabolites. It is also indicated that gene depending on bioprospecting approach has allowed fastidious discovery of microorganisms with respect to halometabolite production. Added to this, that can also enhance the robustness of halometabolite discovery of silent or cryptic gene clusters. Besides, it is also recommended that Arctic marine actinobacteria are unique targets for the discovery of novel halogenases and halo metabolites.

10.7 Functional Genomics

The functional genomics of *Streptomyces* sp. MP131-18 of certain proteins with the bactNOG subset by the eggnog v4 databases (using protein BLAST with an expectation value cutoff of 0.001) (Powell et al. 2014) gives rise to the identification of likely orthologous gene by several biological functions designated for 4533 (64.3%) among 7054 proteins. Among the residue, 986 CDSs (14%) have zero hits on bactNOGs, and 1535 proteins (21.8%) required hits on proteins deprived of functional category (*function unknown*). Among the proteins through functional obligations, about 2018 (28.6%) are occupied in metabolism, as well as comprising 170 (2.4%) contributing in metabolism.

10.7.1 Evaluation of Bioactive Compound Gene Clusters

There are certain tools involved in analysis purposes, such as antiSMASH3.0 analysis for the *Streptomyces* sp. MP131-18, and the genome that exposed about 36 gene clusters projected to be secondary metabolism of the isolates dwell 8.4% of the chromosome. Still, the reevaluation of antiSMASH3.0 consequences exposed that cluster 1 is utmost may be encoding two different pathways for biosynthesis of polyketide and terpenes. Inside the genome of *Streptomyces* sp. MP131-18, six gene clusters by type I PKS (polyketide synthase) genes were forecasted, greatest part of them coding for biomodular PKS. The gene cluster 35 is perhaps concerning to the synthesis of compound that contains an α-pyridine ring, depending on higher identity to PKS and the post-PKS enzymes from the piericidin A1 biosynthesis gene cluster (Liu et al. 2012). Another type I PKS gene cluster has no similarity in sequences of bacterial genomes which are accessible under public databases, and their products can be somewhat foreseen from the genome. The gene cluster 1b is coding for type II PKS utmost producing molecules with angucycline group. The occurrence of glycosyltransferase encoded with genes in the 1b groups acclaims the glycosylation of the synthesized polyketide. Gene cluster 7 corresponds to the type III PKS that looks like α-pyrone sort of PKS from plants and fungi (Lin et al. 2013). An indistinguishable kind of IIIPKS additionally saw in the hybrid quality gatherings 3, both with monomodular type I PKS encoding quality. Cluster 19 extended to uncover the biosynthetic pathways for a polyunsaturated aryl polyene-like compound. Three non-ribosomal peptide synthase (NRPS) quality assemblies were seen among the genome of *Streptomyces* sp. MP13118 comparing to cluster 2 indistinguishable from the coelibactin quality group.

Cluster 2 is like the coelibactin quality group from *S. coelicolor* (Bentley et al. 2002). The structure of this compound isn't known, yet it is thought to go about as zincophore. Four quality groups—13, 14, 30, and 34—join type I PKS and NRPS-encoding qualities. Groups 13, 14, and 30 incorporate qualities coding for discrete

adenylation area proteins that most presumably are providing the amino corrosive starter units for the PKS segments. Five important gene clusters (1a, 8, 16, 18, 29) inside the genome of *Streptomyces* sp. MP131-18 are dedicated to terpene biosynthesis, including cluster 18 for the sesquiterpene geosmin. Cluster 23 is coding for a sort I PKS and a terpene synthase/cyclase. It likewise contains a glycosyltransferase proposing that the putative hybrid item may be glycosylated. An additional enormous secondary metabolism gene sequence is coding for ribosomal orchestrated, posttranslationally altered peptides (RiPPs) (Ortega and van der Donk 2016). Clusters 6, 21, 25, and 27 are coding for lantipeptide-type complexes. Cluster 6, alongside the lanM homolog, likewise harbors a quality for thiazole ring arrangement. Cluster 20 is similar to formerly identified actinomycetes gene cluster for lasso peptide biosynthesis (Elsayed et al. 2015).

10.8 Conclusion

Nowadays, it has created a high demand for the potential bioactive compounds producing prokaryotes due to the disastrous effects of Covid-19, which is famously known as coronavirus. These actinobacteria are the most versatile groups of organisms; they can make plenty of secondary metabolites that do have potential immunosuppressive, immunostimulatory, and therapeutic enzymes (Table 10.2) and antiviral activities. Much research work needs to be carried out to explore the unique communities of actinomycetes.

Table 10.2 Potential immunosuppressive, immunostimulatory, and therapeutic enzymes produced by actinobacteria

Immunosuppressive agent producers	Bioactive agents/drugs
Streptomyces filipinensis	Hygromycin
Nocardia brasiliensis	Brasilicardin
Streptomyces filipinensis	Pentalenolactone
Immunostimulatory agent producers	
Streptomyces olivoreticuli	Bestatin
Nocardia rubra	Rubratin
Kitasatospora kifunense	FR-900494
Therapeutic enzymes	
S. olivochromogenes	L-Glutaminase
S. albidoflavus	L-Asparaginase

References

Adam D, Maciejewska M, Naômé A, Martinet L, Coppieters W, Karim L, Baurain D, Rigali S (2018) Isolation, characterization, and antibacterial activity of hard-to-culture actinobacteria from cave moonmilk deposits. Antibiotics 7:28–48

Adamek M, Alanjary M, Sales-Ortells H, Goodfellow M, Bull AT, Winkler A, Wibberg D, Kalinowski J, Ziemert N (2018) Comparative genomics reveals phylogenetic distribution patterns of secondary metabolites in *Amycolatopsis* species. BMC Genomics 19:426

Anil Kumar S, Arunashri R, Jayachandra SY, Sulochana MB (2010) Screening of extracellular hydrolytic enzymes from *Marinobacter hydrocarbonoclasticas* strain AK5. Int J Bioscan 5(1): 97–99

Baltz RH (2008) Renaissance in antibacterial discovery from actinomycetes. Curr Opin Pharmacol 8:557–563

Baltz RH (2017) Gifted microbes for genome mining and natural product discovery. J Ind Microbiol Biotechnol 44:573–588

Bayer K, Scheuermayer M, Fieseler L, Hentschel U (2013) Genomic mining for novel FADH2-dependent halogenases in marine sponge-associated microbial consortia. Mar Biotechnol 15: 63–72

Bentley SD et al (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141–147

Berdy JJ (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot 65:385–395

Blin K, Medema MH, Kottmann R, Lee SY, Weber T (2017) The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters. Nucleic Acids Res 45:D555–D559

Bonet B, Teufel R, Cruüsemann M, Ziemert N, Moore BS (2014) Direct capture and heterologous expression of *Salinispora* natural product genes for the biosynthesis of enterocin. J Nat Prod 78: 539

Brown ED, Wright GD (2016) Antibacterial drug discovery in the resistance era. Nature 529:336–343

Bull AT, Stach JEM (2007) Marine actinobacteria: new opportunities for natural product search and discovery. Trends Microbiol 15:491–499

Bull AT, Asenjo JA, Goodfellow M, Gomez-Silva B (2016) The Atacama desert: technical resources and the growing importance of novel microbial diversity. Annu Rev Microbiol 70: 215–234

Chao Du C, van Wezel GP (2018) Mining for microbial gems: integrating proteomics in the postgenomic natural product discovery pipeline. Proteomics 18(18):e1700332

Choudhary A, Naughton LM, Montánchez I, Dobson ADW, Rai DK (2017) Current status and future prospects of marine natural products (MNPs) as antimicrobials. Mar Drugs 15:272

Derewacz DK, Covington BC, McLean JA, Bachmann BO (2015) Mapping microbial response metabolomes for induced natural product discovery. ACS Chem Biol 10:1998–2006

Dhakal D, Pokhrel AR, Shrestha B, Sohng JK (2017) Marine rare actinobacteria: isolation, characterization, and strategies for harnessing bioactive compounds. Front Microbiol 8:1106

Editorial (2018) Wanted: a reward for antibiotic development. Nat Biotechnol 36:555

Elsayed SS et al (2015) Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyperarid Atacama desert. J Org Chem 80:10252–10260

Fenical W, Jensen PR (2006) Developing a new resource for drug discovery: marine actinomycete bacteria. Nat Chem Biol 2:666–673

Fischbach MA, Walsh CT (2009) Antibiotics for emerging pathogens. Science *325*:1089–1093

Gao P, Huang Y (2009) Detection, distribution, and organohalogen compound discovery implications of the reduced flavin adenine dinucleotide dependent halogenase gene in major filamentous actinomycete taxonomic groups. Appl Environ Microbiol 75:4813–4820

Gavrish E, Sit CS, Cao S, Kandror O, Spoering A, Peoples A, Ling LA, Hughes D, Bissell A, Torrey H et al (2014) Lassomycin, a ribosomally synthesized cyclic peptide, kills mycobacterium tuberculosis by targeting the ATP-dependent protease ClpC1P1P2. Chem Biol 21:509–518

Genilloud O (2017) Actinomycetes: still a source of novel antibiotics. Nat Prod Rep 34:1203–1232

Ghosh S, Kuisiene N, Cheeptham N (2017) The cave microbiome as a source for drug discovery: reality or pipe dream? Biochem Pharmacol 134:18–34

Goh EB, Yim G, Tsui W, McClure J, Surette MG, Davies J (2002) Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. Proc Natl Acad Sci USA 99:17025–17030

Goodwin CR, Covington BC, Derewacz DK, McNees CR, Wikswo JP, McLean JA, Bachmann BO (2015) Structuring microbial metabolic responses to multiplexed stimuli via self-organizing metabolomics maps. Chem Biol 22:661–670

Gubbens J, Zhu H, Girard G, Song L, Florea BI, Aston P, Ichinose K, Filippov DV, Choi YH, Overkleeft HS et al (2014) Natural product proteomining, a quantitative proteomics platform, allows rapid discovery of biosynthetic gene clusters for different classes of natural products. Chem Biol 21:707

Hiard S, Marée R, Colson S, Hoskisson PA, Titgemeyer F, van Wezel GPB, Wehenkel JL, Rigali S (2007) PREDetector: a new tool to identify regulatory elements in bacterial genomes. Biochem Biophys Res Commun 357:861–864

Horbal L, Siegl T, Luzhetskyy A (2018) A set of synthetic versatile genetic control elements for the efficient expression of genes in actinobacteria. Sci Rep 8:491

Hornung A, Bertazzo M, Dziarnowski A, Schneider K, Welzel K, Wohlert S-E, Holzenkämpfer M, Nicholson GJ, Bechthold A, Süssmuth RD, Vente A, Pelzer S (2007) A genomic screening approach to the structure-guided identification of drug candidates from natural sources. ChemBioChem 8:757–766

Hoskisson PA, Fernández-Martínez LT (2018) Regulation of specialised metabolites in actinobacteria–expanding the paradigms. Environ Microbiol Rep 10:231–238

Hou B, Lin Y, Wu H, Guo M, Petkovic H, Tao L, Zhu X, Ye J, Zhang H (2018) The novel transcriptional regulator LmbU promotes lincomycin biosynthesis through regulating expression of its target genes in *Streptomyces lincolnensis*. J Bacteriol 200:e00447–e00417

Hwang K-S, Kim HU, Charusanti P, Palsson BO, Lee SY (2014) Systems biology and biotechnology of *Streptomyces* species for the production of secondary metabolites. Biotechnol Adv 32: 255–268

Jayachandra SY, Mohanreddy K, Paramesh B, Shouche YS, Sulochana MB (2013) Screening of halophilic bacteria extracellular enzymes production from west coast of Karnataka, India. Int J Univ Pharm Biosci 3(1):31–45

Jia X-Y, Tian Z-H, Shao L, Qu X-D, Zhao Q-F, Tang J, Tang G-L, Liu W (2006) Genetic characterization of the chlorothricin gene clusteras a model for spirotetronate antibiotic biosynthesis. Chem Biol 13:575–585

Jose PA, Jha B (2017) Intertidal marine sediment harbours actinobacteria with promising bioactive and biosynthetic potential. Sci Rep 7:10041

Ju K-S, Gao J, Doroghazi JR, Wang K-KA, Thibodeaux CJ, Lia S, Metzger E, Fudala J, Su J, Zhang JK et al (2015) Discovery of phosphonic acid natural products by mining the genomes of 10,000 actinomycetes. Proc Natl Acad Sci U S A 112:12175–12180

Kim J-N, Kim Y, Jeong Y, Roe J-H, Kim B-G, Cho B-K (2015) Comparative genomics reveals the core and accessory genomes of *Streptomyces* species. J Microbiol Biotechnol 25:1599–1605

Landwehr W, Wolf C, Wink J (2016) Actinobacteria and myxobacteria—two of the most important bacterial resources for novel antibiotics. Curr Top Microbiol Immunol 398:273–302

Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, Vogel TM (2010) Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. Extremophiles 14:205–212

Lewis K (2017) New approaches to antimicrobial discovery. Biochem Pharmacol 134:87–98

Li L, Zhenga G, Chena J, Gec M, Jianga W, Lu Y (2017) New strategies and approaches for engineering biosynthetic gene clusters of microbial natural products. Metab Eng 40:80–92
Lin ZJ et al (2013) A bacterial source for mollusk pyrone polyketides. Chem Biol 20:73–81
Linares JF, Gustafsson I, Baquero F, Martinez JL (2006) Antibiotics as inter microbial signaling agents instead of weapons. Proc Natl Acad Sci U S A 103:19484–19489
Liu W, Ahlert J, Gao Q, Wendt-Pienkowski E, Shen B, Thorson JS (2003) Rapid PCR amplification of minimal enediyne polyketide synthase cassettes leads to predictive familial classification model. Proc Natl Acad Sci U S A 100:11959–11963
Liu Q et al (2012) Elucidation of piericidin A1 biosynthetic locus revealed a thioesterase-dependent mechanism of alpha-pyridonering formation. Chem Biol 19:243–253
Medema MH, Fischbach MA (2015) Computational approaches to natural product discovery. Nat Chem Biol 11:639–648
Mohammadipanah F, Wink J (2016) Actinobacteria from arid and desert habitats: diversity and biological activity. Front Microbiol 6:1541
Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363
Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867
Myronovskyia M, Luzhetskyy A (2016) Native and engineered promoters in natural product discovery. Nat Prod Rep 33:1006–1020
Okada BK, Seyedsayamdost MR (2017) Antibiotic dialogues: induction of silent biosynthetic gene clusters by exogenous small molecules. FEMS Microbiol Rev 41:19–33
Onaka H (2017) Novel antibiotic screening methods to awaken silent or cryptic secondary metabolic pathways in actinomycetes. J Antibiot 70:865–870
Ortega MA, van der Donk WA (2016) New insights into the biosynthetic logic of ribosomally synthesized and post-translationally modified peptide natural products. Cell Chem Biol 23:31–44
Patin NV, Schorn M, Aguinaldo K, Lincecum T, Moore BS, Jensen PR (2017) Effects of actinomycete secondary metabolites on sediment microbial communities. Appl Environ Microbiol 83:e02676-16
Pérez-Victoria I, Martín J, Reyes F (2016) Combined LC/UV/MS and NMR strategies for the dereplication of marine natural products. Planta Med 82:857–871
Podzelinska K, Latimer R, Bhattacharya A, Vining LC, Zechel DL, Jia Z (2010) Chloramphenicol biosynthesis: the structure of CmlS, flavin-dependent halogenase showing a covalent flavin-aspartate bond. J Mol Biol 397:316–331
Powell S et al (2014) EggNOGv4.0: nested orthology inference across 3686 organisms. Nucleic Acids Res 42:231–239
Ren H, Wang B, Zhao H (2017) Breaking the silence: new strategies for discovering novel natural products. Curr Opin Biotechnol 48:21–27
Rigali S, Anderssen S, Naômé A, van Wezel GP (2018) Cracking the regulatory code of biosynthetic gene clusters as a strategy for natural product discovery. Biochem Pharmacol 153:24–34
Rosen PC, Seyedsayamdost MR (2017) Though much is taken, much abides: finding new antibiotics using old ones. Biochemistry 56:4925–4926
Rutledge PJ, Challis GL (2015) Discovery of microbial natural products by activation of silent biosynthetic gene clusters. Nat Rev Microbiol 13:509–523
Saadoun I, Gharaibeh R (2003) The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J Arid Environ 53:365–371
Sánchez-Hidalgo M, González I, Díaz-Muñoz C, Martínez G, Genilloud O (2018) Comparative genomics and biosynthetic potential analysis of two lichen-isolated *Amycolatopsis* strains. Front Microbiol 9:369

Sarmiento-Vizcaíno A, González V, Braña AF, Palacios JJ, Otero L, Fernández J, Molina A, Kulik A, Vázquez F, Acuña JL et al (2017) Pharmacological potential of phylogenetically diverse actinobacteria isolated from deep-sea coral ecosystems of the submarine Avilés canyon in the Cantabrian sea. Microb Ecol 73:338–352

Seibold C, Schnerr H, Rumpf J, Kunzendorf A, Hatscher C, Wage T, Ernyei AJ, Dong C, Naismith JH, van Pée K-H (2006) Aflavin-dependent tryptophan 6-halogenase and its use in modification of pyrrol nitrin biosynthesis. Biocatal Biotransform 24:401–408

Simpkin VL, Renwick MJ, Kelly R, Mossialos E (2017) Incentivising innovation in antibiotic drug discovery and development: progress, challenges and next steps. J Antibiot 70:1087–1096

Smith DRM, Grüschow S, RJM G (2013) Scope and potential of halogenases in biosynthetic applications. Curr Opin Chem Biol 17:276–283

Subramani R, Aalbersberg W (2012) Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res 167:571–580

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Svenson J (2012) MabCent: arctic marine bioprospecting in Norway. Phytochem Rev 12:567–578

Tang X, Li J, Millán-Aguiñaga N, Zhang JJ, O'Neill EC, Ugalde JA, Jensen PR, Mantovani SM, Moore BS (2015) Identification of thiotetronic acid antibiotic biosynthetic pathways by target-directed genome mining. ACS Chem Biol 10:2841–2849

Tong Y, Robertsen HL, Blin K, Weber T, Lee SY (2018) CRISPR-Cas9 toolkit for actinomycete genome editing. In: Synthetic metabolic pathways. Humana Press, New York, pp 163–184

Wagner C, ElOmari M, König GM (2009) Biohalogenation: nature's way to synthesize halogenated metabolites. J Nat Prod 72:540–553

Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapono CA, Luzzatto-Knaan T et al (2016) Sharing and community curation of mass spectrometry data with GNPS. Nat Biotechnol *34*:828–837

Weber T, Kim HU (2016) The secondary metabolite bioinformatics portal: computational tools to facilitate synthetic biology of secondary metabolite production. Synth Syst Biotechnol 1:69–79

Weber T, Charusanti P, Musiol-Kroll EM, Jiang X, Tong Y, Hyun Uk Kim HU, Lee SY (2015) Metabolic engineering of antibiotic factories: new tools for antibiotic production in actinomycetes. Trends Biotechnol 33:15–26

Wu C, Du C, Ichinose K, Choi YH, van Wezel GP (2017) Discovery of C-glycosyl pyranonaphthoquinones in *Streptomyces* sp. MBT76 by a combined NMR-based metabolomics and bioinformatics workflow. J Nat Prod 80:269

Xiang W-S, Zhang J, Wang J-D, Jiang L, Jiang B, Xiang Z-D, Wang X-J (2010) Isolation and identification of chlorinated genistein from *Actinoplanes* sp. HBDN08 with antioxidant and antitumor activities. J Agric Food Chem 58:1933–1938

Xu S, Yan L, Zhang X, Wang C, Feng G, Li J (2014) Nocardiopsis fildesensis sp. nov., an actinomycete isolated from soil. Int J Syst Evol Microbiol 64:174–179

Xu M, Wang Y, Zhao Z, Gao G, Huang S-X, Kang Q, He X, Lin S, Pang X, Deng Z et al (2016) Functional genome mining for metabolites encoded by large gene clusters through heterologous expression of a whole-genome bacterial artificial chromosome library in *Streptomyces* spp. Appl Environ Microbiol 82:5795–5805

Xu D, Han L, Li C, Cao Q, Zhu D, Barrett NH, Harmody D, Chen J, Zhu H, McCarthy PJ et al (2018) Bioprospecting deep-sea actinobacteria for novel anti-infective natural products. Front Microbiol 9:787

Yang C, Huang C, Zhang W, Zhu Y, Zhang C (2015) Heterologous expression of fluostatin gene cluster leads to a bioactive heterodimer. Org Lett 17:5324–5327

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi JS, Anil Kumar S, Shouche YS, Sulochana MB (2013) Culturable diversity of extremely halotolerant bacteria from Arabian Sea Karnataka, India. Int J Biol Pharm Allied Sci 2(2): 391–405

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Yuan M, Yu Y, Li H-R, Dong N, Zhang X-H (2014) Phylogenetic diversity and biological activity of actinobacteria isolated from the Chukchi Shelf marine sediments in the Arctic Ocean. Mar Drugs 12:1281–1297

Zerbe K, Pylypenko O, Vitali F, Zhang W, Rouset S, Heck M, Vrijbloed JW, Bischoff D, Bister B, Sussmuth RD, Pelzer S, Wohlleben W, Robinson JA, Schlichting I (2002) Crystal structure of OxyB, acytochrome P450 implicatedinan oxidative phenol coupling reaction during vancomycin biosynthesis. J Biol Chem 277:47476–47485

Zhang G, Cao T, Ying J, Yang Y, Ma L (2014) Diversity and novelty of actinobacteria in Arctic marine sediments. Antonie Van Leeuwenhoek 105:743–754

Zhou Z, Gu J, Li Y-Q, Wang Y (2012) Genome plasticity and systems evolution in *Streptomyces*. BMC Bioinform 13:S8

Chapter 11
Recent Trends of Actinomycetes in Nanotechnology

Jayachandra S. Yaradoddi, Nagaraj R. Banapurmath, Merja H. Kontro, Sharanabasava V. Ganachari, Shankar Hallad, Manzoore Elahi M. Soudagar, and Venkatesh Ramaswamy

Abstract Nanotechnology is one of the advanced areas of research and found applications in science and technology. Nanoparticles possess interesting properties, mainly physiochemical, magnetic, and optoelectronic, making them the best choice among the other materials. Nanoparticles are very small but have a relatively large surface area to volume ratio. They likely to have considerably good biological, catalytic, mechanical, thermal, melting point, optical absorption, and electrical conductivity properties; generally, they do not occur in other materials in bulk form. In the initial stage, there was considerable interest in physical and chemical methods in the preparation of various materials—size, shape, and structure. However, the materials developed using the physical and chemical methods are not good to use for clinical applications due to the release of toxic by-products, chemicals to the environment, and harsh reaction conditions. Consequently, there is a requirement for a clean, nontoxic, biocompatible, and environmentally friendly green technologies. They can be synthesized with good precision concerning their approach in synthesizing nanomaterials.

J. S. Yaradoddi (✉)
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

N. R. Banapurmath · S. Hallad · V. Ramaswamy
Biomaterials Laboratory, Centre for Material Science, KLE Technological University (Formerly Known as B.V. Bhoomaraddi College of Engineering and Technology), Hubballi, Karnataka, India

M. H. Kontro
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland

S. V. Ganachari (✉)
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

M. E. M. Soudagar
Faculty of Engineering, University of Malaya, Kuala Lumpur, Malaysia

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Simultaneously, the nanoparticle synthesis using the biological route contributes the same. In recent times actinomycetes recovered from different habitats have been identified as potential synthesizers of metal nanoparticles, and biosynthesis of nanoparticles has been reported in *Streptomyces hygroscopicus*, *Streptomyces naganishii*, *Rhodococcus* sp., *Thermomonospora* sp., *Streptomyces viridogens*, *Nocardia farcinica*, *Nocardiopsis* sp., *Streptomyces* sp., and *Streptomyces avidinii*.

Keywords Gold nanoparticles · Palladium nanoparticles · Platinum nanoparticles · Biomedical applications · Pharmaceuticals

11.1 Introduction

The nanoparticle has versatile properties and beneficial applications in many areas such as nutrition, energy, and medicine (Chandran et al. 2006). The biological synthesis of monodispersed nanoparticles with specific shapes and sizes has been a substantial opportunity in biomaterial science. In addition, this has produced significant advantages in pharmaceutical industries in order cure various viral, fungal, and bacterial diseases (Song and Kim 2009). Biological synthesis methods have more useful than the conventional synthesis process because of their different entity and eco-friendly procedures. Extensive biodiversity and sufficient availability of plant, bacterial, and fungal resources (Fig. 11.1) have been hugely explored for nanomaterials synthesis (Monda et al. 2011). In recent times the biosynthesis of nanoparticles, flowers, wires, and tubes was revealed. These biosynthesized nanomaterials have received broad applications in various fields such as diagnostics, treatment, and commercial manufacturing processes in developing surgical nanodevices, etc. (Bar et al. 2009). The main area of nanomedicine has produced an enormous impact in the healthcare industry in treating unusual chronic diseases. Therefore, the eco-friendly synthesis of nanoparticles is measured as an ingredient in impending generations to control diverse diseases (Cruz et al. 2010). Nanotechnology has received several applications in various fields (Fig. 11.2).

Crude extracts of plants consist of new secondary metabolites like flavonoids, phenolic acid, alkaloids, and terpenoids. These metabolites are the primary reason for reducing the ionic form into bulk metallic types of nanoparticle formation (Aromal and Philip 2012). Both primary and secondary metabolites are implicated in the redox reaction mechanisms in synthesizing desired sized and eco-friendly nanoparticles. Numerous investigations have demonstrated the biologically synthesized nanoparticles enduring efficient controlled oxidative stress, apoptosis, and genotoxicity-associated changes (Kim et al. 2007). In addition, these nanoparticles have found extensive applications in plant sciences and agriculture industries. For example, the nanoparticle, through bioprocess technology, translates the food and agriculture wastes into energy and other useful by-products. Based on previous studies, the biosynthesized metallic nanoparticles using plant derivatives and their use in medical and commercial sectors have application in fields such as cosmetics, food industries, and wastewater treatment. The constant and overuse of chemical pesticides and fertilizers in agriculture practices

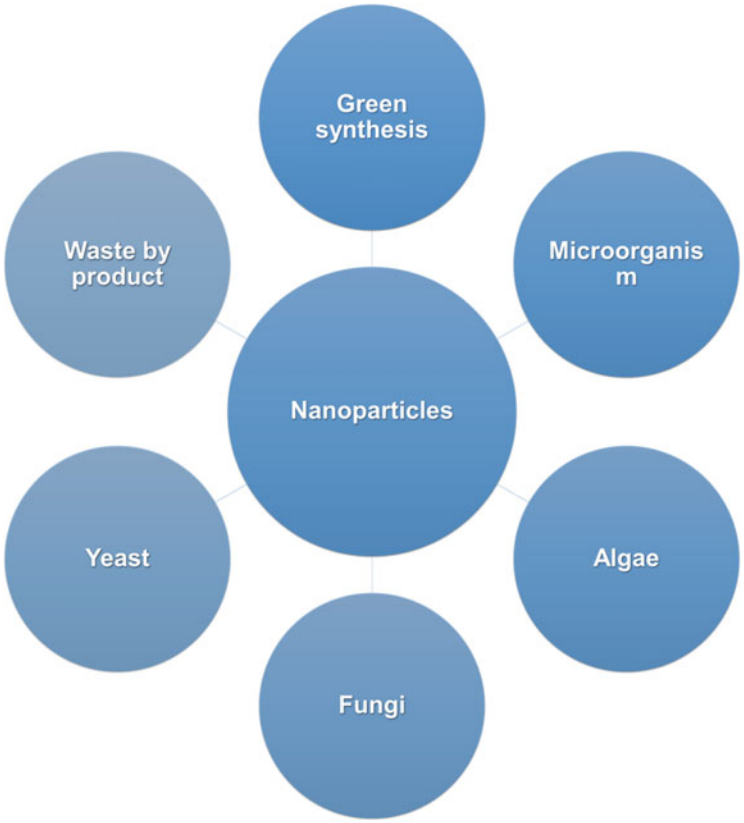

Fig. 11.1 Potentials green synthesis of nanoparticles using microbes

raises a relentless concern in health and productivity among soil, plants, human beings, and on the atmosphere from few decades.

The main issues concerning the use of microbes emerge as a suitable alternative to chemical pesticides due to their inexpensive availability, environmental friendly nature, and flexible continual nutrients available for plants and soils (Jeffries et al. 2003). The soil contains beneficial microbial consortia such as fungi, bacteria, actinomycetes, etc. The actinobacteria are generally characterized as Gram-positive, aerobic bacteria that bear thread-like structures under soil (Elliott and Lynch 1995). However, the actinobacteria possess both fungi and bacteria's characteristic features, yet it is considered true bacteria by their visible similarity to fungi (Bhatti et al. 2017). Actinobacteria are ubiquitous (Srinivasan et al. 1991); however, they are a large group of microbes under dry and alkaline soil conditions. These actinobacteria can serve as exceptional microbes, they play a significant role within the soil nutrient cycle, in the synthesis of a wide variety of bioactive molecules like amino acids, antimicrobial compounds, vitamins, enzymes, and other industrially important compounds (Boer et al. 2005; Sulochana et al. 2014a, b; Yaradoddi et al. 2020b; Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020).

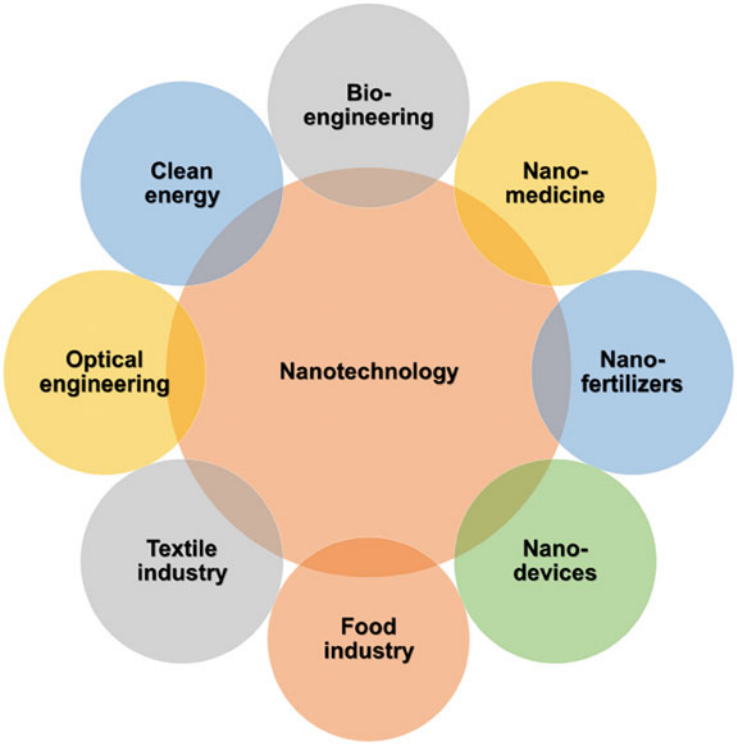

Fig. 11.2 Indicated applications of nanotechnology in various fields

11.2 Importance of Actinobacteria in Nanotechnology

In general, the name nanotechnology corresponds to the synthesis and utilization of materials within the nanometer range. Because they occur as small in size, the character and properties of the materials are under nano-range (10^{-9}), and they are pretty alike compared to other constituents. Rapid advancements in nanotechnology in various disciplines of science are due to the promotion of electronics, energy production, drug discovery, and gene transfer technology (Rao and Cheetham 2001; Yaradoddi et al. 2019a, b, c). A huge improvement among the established techniques together with the advent of new technology therefore, a necessary factor in the production of nanomaterials for vital applications in food, human health, agriculture, and cosmetics. Various biological, physical, and chemical methods are utilized in the design and synthesis of nanomaterials (Iravani et al. 2014). The majority of physiochemical methods were found to be lavish and hazardous. Besides the beneficial effects within biological processes in synthesizing nanomaterials (bionanotechnology) are quite inexpensive, non-hazardous, and eco-friendly. Consequently, they have a profound application in exploring various

biological agents that include bacteria, fungi, algae, actinobacteria, and plants in fabrications of diverse nanomaterials that are gaining prominence among researchers worldwide. Actinobacteria were earlier considered rare fungi; however, later developments in microbiological methods and technology advancements in instrumentation were described in different groups of Actinobacteria.

In spite of their widespread occurrence in certain environmental conditions, these microbes have also been described from harsh environments such as pressure, temperature, organic environment, heavy metals, pH, and salinity (Sastry et al. 2003). The availability of a varied group of actinobacteria under adverse environments is mainly based on the unique physiological and biochemical mechanisms, which help in fundamental biological processes. An explicit life-supporting phenomenon is employed to maintain the structure and function of the cell membrane, enzymes, and intracellular factors. The requirement of metal nanoparticles is steadily raising their prominence in various industries. There are a plenty of studies and reports on the intracellular and extracellular synthesis of nanoparticles of various metals such as iron, copper, gold, zinc, silver, palladium, selenium, cadmium, titanium, and platinum-based biological organization. The hunt for novel biological methods in the synthesis of versatile nanoparticles is yet challenging. Ahmad et al. (2003) initially confirmed the biosynthesis of gold nanoparticles using extremophilic actinobacteria *Thermomonospora* sp. The nanoparticles produced have the a typical size of 8 nanometers and had a good dispersion. The complete extracellular reduction of $AuCl_4$ solution results in the production of nanoparticles attained upon incubation of sample solution for about 5 days. In recent times Nabila and Kannabiran (2018) reported the production of CuO nanoparticles for the first time using actinobacteria; they successfully tested their efficiency against the seven bacterial pathogens which had been previously affecting both fishes and human beings. The average size of the nanoparticles was characterized by TEM analysis, which was 61.7 nm. Thus, fabricated CuO nanoparticles have indicated excellent activity against the tested bacterial pathogens compared with the cell-free extract. The characterization studies on nanoparticles by the dynamic light scattering method have revealed that the zeta potential is equivalent to 31.1 mV. The crystalline forms of nanoparticles were further confirmed through X-Ray diffraction. Among the investigated bacteria, the *Bacillus cereus* has shown significant susceptibility. The implication of actinobacteria (*Streptomyces griseoruber*)-based extracellular synthesis of selenium nanoparticle was described by Ranjitha and Ravishankar (2018). Bioactive molecules in the filtrate of the suspension medium formed by actinomycetes were involved in reducing selenium to produce nanoparticles. These selenium nanoparticles have an absorption maximum at 575 nm by a size range between 100 and 250 nm as per characterization results by UV-Vis spectrophotometry and transmission electron microscopy (TEM). The biological way of fabrication of selenium nanoparticles involves cytotoxic properties against the colon cancer line HT-29. Also, reduced cell viability of cell lines through an increase in the concentration of nanoparticles was recognized and indicates their role in designing chemotherapeutic agents. The biological synthesis of nanoparticles of diverse metals, for example, selenium, silver, gold, iron, zinc, copper, and manganese, can be used as

potential antimicrobial agents against pathogens such as fungi, bacteria, and parasites. Hassan et al. (2018) described the production of copper nanoparticles from endophytic actinobacteria *Streptomyces capillispiralis* Ca-1 obtained from *Convolvulus arvensis,* that changes color of the culture medium in presence of copper was showed the formation of nanoparticles. They were further confirmed by using UV-Vis spectroscopy. Good quality dispersion of the biosynthesized nanoparticles as illuminated by TEM analysis ranging between 36 and 59 nm indicated stability. The occurrence of various functional groups related to actinobacteria induced nanoparticle synthesis, as indicated in FTIR analysis mainly dependable for copper nanoparticle production. Biosynthesized copper nanoparticle has indicated potential applications in controlling human pathogens also in plant disease management. Al-Dhabi et al. (2018) evaluated the antibacterial activity of silver nanoparticle produced by using the extract of *Streptomyces* sp. Al-Dhabi-87 strain. The spherical-sized nanoparticles range between 10 and 17 nm. MIC (minimum inhibitory concentration) of nanoparticles tested against pathogens such as *Klebsiella pneumoniae*, *Escherichia coli,* and *Pseudomonas aeruginosa* showed 78, 152, and 39μg/mL, respectively. Besides this antibacterial activity of nanoparticles, they showed good inhibitory activity even against drug-resistant bacteria including *Staphylococcus aureus* and *Acinetobacter baumannii,* thus opening new avenues towards their important role in medical sciences. Vijayabharathi et al. (2018) reported the extracellular biosynthesis of silver nanoparticles by rhizospheric actinobacteria *Streptomyces griseoplanus* SAI-25 recovered from rice. The extracellularly synthesized nanoparticle displayed absorption maxima ranging from 413 to 417 nm. The systematic study of cell-free extract by using FTIR analysis exposed biologically important functional groups such as alcohol, phenol, and amine, thus catalyzing the reduction and leading to the production of silver nanoparticles. The nanoparticle produced by the actinobacteria has exhibited antifungal action against the phytopathogen mainly *Macrophomina phaseolina* inscribed by applying silver nanoparticles as biopesticides to manage the loss during the crop production. Applications of actinobacteria for both intracellularly and extracellularly synthesized metal nanoparticles appear to be green, ecofriendly, very simple, nontoxic, and cost-effective. Nanoparticles could be good candidates in treating numerous life-threatening diseases because of the altered morphology, larger surface area, higher activity, new composition, and useful properties (Manimaran and Kannabiran 2017).

The intracellular synthesis generally occurs on the surface of mycelia because of the electrostatic binding of Ag^+ ions towards the negatively charged carboxyl groups within the enzyme, which is there on the cell wall of mycelium. The silver ions were further reduced by the activity of enzymes, and that produces silver nuclei. Acquisition of silver nuclei steers the production of nano-sized silver particles (Sunitha et al. 2013). New alkalotolerant actinobacteria, *Rhodococcus* sp., were previously used for the synthesis of gold nanoparticles. As a result of TEM (transmission electron microscope), imaging indicated the existence of nanoparticles on the cell walls surface of the actinobacteria, and the intracellular production of gold nanoparticles was confirmed (Ahmad et al. 2003). The intracellular mode of

synthesis of silver nanoparticles using the *Rhodococcus* NCIM 2891 was described (Otari et al. 2012). In the wet culture of *Streptomyces hygroscopicus* when exposed to $HAuCl_4$ for about 72 h, the original yellow-colored biomass was converted to pink color, thus confirming the extracellular production of gold nanoparticles. The *Streptomyces* sp. HBUM171191 was revealed as potential producers of manganese, zinc, and silver nanoparticles upon wet biomass/culture exposed towards subsequent metal solutions. Variations in the color of biomass from yellow to dark brown-yellow confirm the production of zinc, manganese, and silver nanoparticles (Waghmare et al. 2011). However, the extracellular production of nanoparticles can be ascribed to enzymes that are involved in the nitrogen cycle. This could be responsible for the electron shuttle enzymatic metal reduction method (Waghmare et al. 2014). The α-NADH depends on the nitrate reductase, which plays a crucial role in reducing the silver ions into silver nuclei. Actinobacterial-based extracellular production of nanoparticles was reported from *Streptomyces glaucus* 71MD (Tsibakhashvili et al. 2011). There is also a report that the *Streptomyces* sp. ERI-3 has reduced the colorless silver nitrate solution into reddish-brown color within 12 h (Tsibakhashvili et al. 2011; Zonooz and Salouti 2011), which indeed showed the production of silver nanoparticles.

11.3 Metal and Metal Oxide Nanoparticles from Actinobacteria as Nano-Antibiotics

11.3.1 Antibacterial Properties of the Nanoparticles from Actinobacteria

The silver nanoparticle can be utilized as an antimicrobial agent from the olden days. These silver nanoparticles are recognized for their catalytic properties, good conductivity, and chemical stability. Since the silver in limited concentrations is non-hazardous to human health, they have pretty good properties, and the silver metal is an excellent selection in the synthesis of silver nanoparticles, especially in biomedical sectors. Actinobacteria are well identified for their ability to synthesize novel chemical compounds/secondary metabolites, which could potentially serve as antibiotics. Yet, actinobacterial populations are a major source of antibacterial compounds, and there are copious reports of actinobacteria-based metal and metal oxide nanoparticles production and their antimicrobial properties. Thus, the silver nanoparticles synthesized using actinobacteria have been shown to possess many antibacterial properties. The silver nanoparticle recovered using *Streptomyces* sp. BDUKAS10 indicated 13, 15, and 16 mm zone of inhibition against *Bacillus cereus, Pseudomonas aeruginosa*, and *Staphylococcus aureus,* respectively (Sivalingam et al. 2012). Silver nanoparticles were biosynthesized by *Streptomyces Rochei* between concentrations of 10^{-3} to 100^{-4} $AgNO_3$ to determine the antibacterial properties. The zone of inhibition of 31, 25, 21, 22, and 28 mm against

Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, *Escherichia coli*, and *Pseudomonas aeruginosa* respectively were reported (Selvakumar et al. 2012). Production of silver nanoparticles by *Rhodococcus* sp. and *Streptomyces* sp. their antibacterial activities with a zone of inhibition of about 26 mm against *Pseudomonas aeruginosa*, *Klebsiella Pneumoniae* (21 mm), *E. coli* (19 mm), and *Staphylococcus aureus* (23 mm) were described (Sukanya et al. 2013). Extracellular production of silver nanoparticles from *Streptomyces parvulus* SSNP11 displayed zone of inhibition of 26, 21, 26, and 30 mm against *Klebsiella pneumonia, Pseudomonas putida, Salmonella typhi*, and *Bacillus subtilis*, respectively (Prakasham et al. 2014). The intracellular gold nanoparticle produced from *Streptomyces viridogens* strain HM10 has shown a 16 and 14 mm inhibition zone against *Escherichia coli* and *Staphylococcus aureus* (Balagurunathan et al. 2011).

11.3.2 Antifungal Properties of Nanoparticles from Actinobacteria

The biogenic synthesized silver nanoparticles from the strain *Streptomyces* sp. JAR1 exhibited significant zone of inhibition of 16 mm and 21 mm against the fungal pathogens *Aspergillus terreus* JAS1 and *Fusarium* sp. respectively by using 100µl of nanoparticles (Chauhan et al. 2013). The silver nanoparticles produced using *Streptomyces* sp. VITBT7 have indicated selective inhibition against *Aspergillus niger* (MTCC1344) and *Aspergillus fumigatus* (MTCC3002) with 22 and 20 mm that was superior to the activity of the cell-free supernatant (Subashini and Kannabiran 2013).

Similarly, silver nanoparticles produced using *Streptomyces* sp. strain VITSTK7 have indicated the antifungal index of 62, 67, and 75% against *Aspergillus flavus, Aspergillus niger,* and *Aspergillus fumigates,* respectively (Thenmozhi et al. 2013). The silver nanoparticles produced using *Streptomyces* sp. VITPK1 indicated inherent anticandidal activity against *Candida krusei, Candida tropicalis,* and *Candida albicans* with a zone of inhibition of 16, 18, and 20 mm, respectively (Sanjenbam et al. 2014). Besides this, the gold nanoparticles produced from *Streptomyces* sp. VITDDK3 have displayed potential antifungal activity against *Trichophyton rubrum* and *Microphyton gypseum* of 13 and 10 mm, respectively (Gopal et al. 2013). These metal nanoparticles were also observed to be potentially active against different human pathogenic fungi such as *Saccharomyces cerevisiae, C. tropicalis, C. krusei, Candida albicans,* and also diverse species associated with the genera *Aspergillus* that includes *A. fumigatus, A. terreus, A. flavus, A. niger,* and *A. brasiliensis.* The synthesized nanoparticles have shown potential activity against dermatophytes *T. tonsurans*, *Scedosporium* sp., *Trichophyton rubrum,* and *Ganoderma* sp. (Manivasagan et al. 2013).

11.3.3 Antiparasitic Properties of Nanoparticles from Actinobacteria

The silver nanoparticles produced using actinobacteria (*Streptomyces* sp.) have also been described to have antiparasitic property against *Haemaphysalis bispinosa* and *Rhipicephalus microplus* (Karthik et al. 2014).

11.3.3.1 Synthesis of Biogenic Nanoparticles from Actinobacteria-Derived Compounds

Very limited reports are accessible on the production of biogenic nanoparticles from actinobacteria-derived compounds. A blue pigment, mainly actinorhodin synthesized by *Streptomyces coelicolor*, was separated using biomass through centrifugation and was utilized for the synthesis of silver nanoparticles by the photo-irradiation technique. Thus the produced nanoparticles were tested for antibacterial activity against MRSA (Methicillin-resistant *Staphylococcus aureus*) (Manikprabhu and Lingappa 2013). The silver and gold nanoparticles were synthesized using methyl esters palmitate, myristate, and stearate (thermostable glycolipid) extracted using *Gordonia amicalis* strain HS-11, as cultivated on a medium that consists of *n*-hexadecane as a sole source of carbon. The glycolipid-based synthesis of silver and gold nanoparticles displayed 94.85% and 89.8% hydroxyl radical scavenging action, respectively. The silver and gold mediated using purified glycolipid exhibited 74% and nitric oxide radical scavenging activity of about 67.25%, respectively (Sowani et al. 2016). The amplified effectiveness of nanoparticles produced from actinobacteria-originated compounds is major evidence revealed from the above reports. Each actinobacterial strain had the inherent genetic potential to produce at least 10–20 secondary metabolites (Lam 2006). The metal and metal oxide reduction potential of actinobacteria-derived compounds towards the biological synthesis of nanoparticles and the likely mechanism implicated in the production need to be explored. This kind of nanoparticles would have enhanced efficiency than conventional nanoparticles.

11.4 Challenges for the Use of Metal Oxide and Metal Nanoparticles

Although biologically derived nanoparticles have widespread applications among diverse area, however, they suffer from some limitations that hinder their usage in biomedical and environmental applications. Efficient maintenance is essentially play vital role in preventing the clump formation of nanoparticles because of their higher surface area, and also to safeguard the characteristic features acquired by the produced nanoparticles. The distribution of nanoparticles can be affected through

interaction with proteins by a process known as opsonization, and this will change the properties of the synthesized nanoparticles (Sanvicens and Marco 2008). The mononuclear phagocytic system, also known as RES (reticuloendothelial system), consists of macrophages, and monocytes interact with the nanoparticles. Numerous processes were employed to avoid undesirable connections of nanoparticles in delivery. One among the approach is coating of nanoparticles with polymers. The coating of nanoparticles by the use of PEG (polyethylene glycol) minimizes the undesirable recognition and thus increasing the distribution within the half-life of nanoparticles (Owens III and Peppas 2006). MWCNT (multi-walled carbon nanotubes), when coated with the ammonium/chelator of functional groups, will avoid the uptake through RES.

Meanwhile, water-soluble, thiol stabilizing gold nanoparticles can be produced by using monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol gold nanoparticles were produced and they can be used in various medical applications. The hydrophilic tetraethylene glycol group makes these nanoparticles water-soluble, and also the hydrophobic carbon chain confirms the stability of the nanoparticle (Li et al. 2002). The water-soluble property of nanoparticles enhances their biocompatibility. The hazardous effects of nanoparticles have to be considered during their usage, especially in vivo applications. The non-hazardous or safe effects are concerned with (1) the likely release of (toxic) ions by metallic nanoparticles and (2) the oxidative stress because of the intrinsic features of the nanoparticles (surface charge, size, morphology, and chemical surface composition) (Seabra and Duran 2015). None of the nanoparticles produced by actinobacteria were evaluated widely towards their toxicity. The element-specific toxicity because of the metal oxide or core metal, and in certain cases, the toxicity can be caused by a surface coating, which contributes to the nanotoxicity of the nanoparticles. Other required characteristic features would be their specificity that can be achieved by adding peptides, aptamers, etc., which identify a prostate-specific membrane antigen (Farokhzad et al. 2006). The basic mechanisms of interaction among the nanoparticles and the cells are not accurately understood. This would help researchers produce required metals and metal oxide nanoparticles with better efficacy. The use of magnetic nanoparticles and also quantum dots facilitates real-time imaging and biodistribution of the nanoparticles (Derfus et al. 2007). The major challenges associated with the green synthesis of nanoparticles could be (1) the constraint associated with the large-scale synthesis processes, (2) the reproducibility of the biological-originated method must be improved, (3) the exact mechanism of nanoparticle formation is not precisely elucidated, (4) control on nanoparticles size and distribution needs to be promoted, and (5) the mechanism involved in metal oxide/metal nanoparticles to the targets desired to be investigated. Additionally, the likely hazardous effects on biogenically produced nanoparticles are required to be investigated further. Thus, these are a necessity for further studies on the biological synthesis of metal/ metal oxide nanoparticles by actinobacteria derived secondary metabolites for the stability: shape, specific size, toxicity, and composition to form it as effective bioactive nanoparticles for therapeutic applications.

11.5 Future Prospective

Over the past few decades, there has been increasing attention in actinobacteria-mediated metal nanoparticles synthesis; however, wide studies are yet to be employed in identification and classification of actinobacteria (Mohan et al. 2015a, b; Prabhu et al. 2015; Khieu et al. 2015; Manikprabhu et al. 2016; Wang et al. 2017). Whereas, the biological agents can triggers amplification of nanoparticle production which is generally occurs at slower rates and frequently consuming several hours. For any nanoparticles, surface area, size, and shape are the most vital parameters in analyzing their property. The significance of regulation mechanism have mainly based on various biological processes in controlling the shape and size of the metal nanoparticles is still at nascent stage. The previous investigation have exposed the biological (actinobacteria) mediated synthesis of nanoparticles had flexibility towards the changes related to its structure and functional properties, this is due to reproducibility is the major concern and also future goal. Additionally, researchers need to understand the molecular mechanisms involved in nanoparticle biosynthesis also have to pay attention in reduction of extensive processing time during the intracellular production of nanoparticles, which is also a costly and time-consuming process. The major parameters' impact on the production of nano-sized materials is also very critical; meticulous optimization may be useful for the fabrication of nanoparticles along with desired properties. By removing all associated confines, actinobacteria-mediated nanoparticle synthesis could play an important role in agriculture, food, energy, and environmental applications. Besides, these in silico approaches are essential in developing novel molecules and drug discovery.

References

Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003) Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp. Langmuir 19(8):3550–3553

Al-Dhabi NA, Ghilan AKM, Arasu MV (2018) Characterization of silver nanomaterials derived from marine *Streptomyces* sp. Al-Dhabi-87 and its in vitro application against multi-drug resistant and extended-spectrum beta-lactamase clinical pathogens. Nano 8(5):279

Aromal SA, Philip D (2012) Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size dependent catalytic activity. Spectrochim Acta A 97:1–5

Balagurunathan R, Radhakrishnan M, Rajendran RB, Velmurugan D (2011) Biosynthesis of gold nanoparticles by actinomycetes *Streptomyces viridogens* strain HM10. Indian J Biochem Biophys 48:331–335

Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, PD., Misra, A., 2009.Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. Colloids Surf A Physicochem Eng Asp \,134–139

Bhatti AA, Haq S, Bhat RA (2017) Actinomycetes benefaction role in soil and plant health. Microb Pathog 111:458467

Boer WD, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29(4):795811

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog 22:577–583

Chauhan R, Kumar A, Abraham J (2013) A biological approach to the synthesis of silver nanoparticles with *Streptomyces* sp. JAR1 and its antimicrobial activity. Sci Pharm 81:607–621

Cruz D, Falé PL, Mourato A, Vaz PD, Serralheiro ML, Lino ARL (2010) Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by *Lippia citriodora* (Lemon Verbena). Colloid Surf B 81:67–73

Derfus AM, Chen AA, Min D, Ruoslahti E, Bhatia SN (2007) Targeted quantum dot conjugates for siRNA delivery. Bioconjug Chem 18:1391–1396

Elliott LF, Lynch JM (1995) The international workshop on establishment of microbial inocula in soils: cooperative research project on biological resource management of the Organization for Economic Cooperation and Development (OECD). Am J Altern Agric 10(2):5073

Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci U S A 103:6315–6320

Gopal JV, Thenmozhi M, Kannabiran K, Rajakumar G, Velayutham K, Rahuman AA (2013) Actinobacteriamediatedsynthesisofgoldnanoparticlesusing*Streptomyces* sp. VITDDK3 and its antifungal activity. Mater Lett 93:360–362

Hassan SEL-D, Salem SS, Fouda A, Awad MA, El-Gamal MS, Abdo AM (2018) New approach for antimicrobial activity and bio-control of various pathogens by biosynthesized copper nanoparticles using endophytic actinomycetes. J Radiat Res Appl Sci 11(3):262–270

Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B (2014) Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci 9(6):385

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37(1):116

Karthik L, Kumar G, Kirthi AV, Rahuman AA, Rao KVB (2014) Streptomyces sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application. Bioprocess Biosyst Eng 37(2): 261–267. https://doi.org/10.1007/s00449-013-0994-3

Khieu T-N, Liu M-J, Nimaichand S, Quach N-T, Chu-Ky S, Phi Q-T, Thu-Trang V, Nguyen T-D, Xiong Z, Manikprabhu D, Jun-Li W (2015) Characterization and evaluation of antimicrobial and cytotoxic effects of *Streptomyces* sp. HUST012 isolated from medicinal plant *Dracaena cochinchinensis* Lour. Front Microbiol 6:574. https://doi.org/10.3389/fmicb.2015.00574

Kim JS, Kuk E, Yu KN, Jong-Ho K, Park SJ, Lee HJ, Kim SH (2007) Antimicrobial effects of silver nanoparticles. Nanomedicine 3:95–101

Lam KS (2006) Discovery of novel metabolites from marine actinomycetes. Curr Opin Biotechnol 9:245–251

Li Z, Jin R, Mirkin CA, Letsinger RL (2002) Multiple thiol-anchor capped DNA-gold nanoparticle conjugates. Nucleic Acids Res 30:1558–1562

Manikprabhu D, Lingappa K (2013) Antibacterial activity of silver nanoparticles against methicillin-resistant *Staphylococcus aureus* synthesized using model *Streptomyces* sp. pigment by photo-irradiation method. J Pharm Res 6:255–260

Manikprabhu D, Cheng J, Chen W, Sunkara AK, Mane SB, Kumar R, Hozzein WN, Duan Y-Q, Li W-J (2016) Sunlight mediated synthesis of silver nanoparticles by a novel actinobacterium (*Sinomonas mesophila* MPKL 26) and its antimicrobial activity against multi drug resistant *Staphylococcus aureus*. J Photochem Photobiol B Biol 158:202–205. https://doi.org/10.1016/j.jphotobiol.2016.01.018

Manimaran M, Kannabiran K (2017) Actinomycetes-mediated biogenic synthesis of metal and metal oxide nanoparticles: progress and challenges. Lett Appl Microbiol 64(6):401–408

Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim S (2013) Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel *Nocardiopsis* sp. MBRC-1. Biomed Res Int. https://doi.org/10.1155/2013/287638

Mohan RK, Siva DS, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan RK, Siva DS, Jayachandra SY, Parameshwar AB, Agsar D, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol App Sci 4(11):858–867

Monda S, Roy N, Laskar RA, Sk I, Basu S, Mandal D, Begum NA (2011) Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (*Swietenia mahogani* JACQ.) leaves. Colloid Surf B 82:497–504

Nabila MI, Kannabiran K (2018) Biosynthesis, characterization and antibacterial activity of copper oxide nanoparticles (CuO NPs) from actinomycetes. Biocatal Agric Biotechnol 15:5662

Otari SV, Patil RM, Nadaf NH, Ghosh SJ, Pawar SH (2012) Green biosynthesis of silver nanoparticles from an actinobacteria *Rhodococcus* sp. Mater Lett 72:92–94

Owens DE III, Peppas NA (2006) Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm 307:93–102

Prabhu DM, Quadri SR, Cheng J, Liu L, Chen W, Yang Y, Hozzein WN, Lingappa K, Li W-J (2015) *Sinomonas mesophila* sp. nov., isolated from ancient fort soil. J Antibiot 68:318–321

Prakasham RS, Kumar BS, Kumar YS, Kumar PK (2014) Production and characterization of protein encapsulated silver nanoparticles by marine isolate *Streptomyces parvulus* SSNP11. Indian J Microbiol 54:329–336

Ranjitha VR, Ravishankar VR (2018) Extracellular synthesis of selenium nanoparticles from an actinomycetes *Streptomyces griseoruber* and evaluation of its cytotoxicity on HT-29 cell line. Pharm Nanotechnol 6(1):61–68

Rao CNR, Cheetham AK (2001) Science and technology of nanomaterials: current status and future prospects. J Mater Chem 11(12):28872894

Sanjenbam P, Gopal JV, Kannabiran K (2014) Anticandidal activity of silver nanoparticles synthesized using *Streptomyces* sp. VITPK1. J Med Mycol 24:211–219

Sanvicens N, Marco MP (2008) Multifunctional nanoparticles-properties and prospects for their use in human medicine. Trends Biotechnol 26:425–433

Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85(2):162170

Seabra AB, Duran N (2015) Nanotoxicology of metal oxide nanoparticles. Metals 5:934–975

Selvakumar P, Viveka S, Prakash S, Jasminebeaula S, Uloganathan R (2012) Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived *Streptomyces rochei*. Int J Pharm Biol Sci 3:188–197

Sivalingam P, Antony JJ, Siva D, Achiraman S, Anbarasu K (2012) Mangrove *Streptomyces* sp. BDUKAS10 as nano factory for fabrication of bactericidal silver nanoparticles. Colloids Surf B Biointerfaces 98:12–17

Song JY, Kim BS (2009) Biological synthesis of bimetallic Au/Ag nanoparticles using Persimmon (*Diospyros kaki*) leaf extract. Korean J Chem Eng 25:808–811

Sowani H, Mohite P, Munot H, Shouche Y, Bapat T, Kumar AR, Kulkarni M, Zinjarde S (2016) Green synthesis of gold and silver nanoparticles by an actinomycete *Gordonia amicalis* HS-11: mechanistic aspects and biological application. Process Biochem 51:374–383

Srinivasan MC, Laxman RS, Deshpande MV (1991) Physiology and nutritional aspects of actinomycetes: an overview. World J Microbiol Biotechnol 7(2):171184

Subashini J, Kannabiran K (2013) Antimicrobial activity of *Streptomyces* sp. VITBT7 and its synthesized silver nanoparticles against medically important fungal and bacterial pathogens. Pharm Lett 5:192–200

Sukanya MK, Saju KA, Praseetha PK, Sakthivel G (2013) Therapeutic potential of biologically reduced silver nanoparticles from actinomycete cultures. J Nanosci. https://doi.org/10.1155/2013/940719

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Sunitha A, Isaac RSR, Geo S, Sornalekshmi S, Rose A, Praseetha PK (2013) Evaluation of antimicrobial activity of biosynthesized iron and silver nanoparticles using the fungi *Fusarium oxysporum* and actinomycetes sp. on human pathogens. Nano Biomed Eng 5:39–45

Thenmozhi M, Kannabiran K, Kumar R, Khanna VG (2013) Antifungal activity of *Streptomyces* sp. VITSTK7 and its synthesized Ag2O/Ag nanoparticles against medically important *Aspergillus* pathogens. J Med Mycol 23:97–103

Tsibakhashvili NY, Kirkesali EI, Pataraya DT, Gurielidze MA, Kalabegishvili TL, Gvarjaladze DN, Tsertsvadze GI, Frontasyeva MV, Zinicovscaia II, Wakstein MS, Khakhanov SN, Shvindina NV, Shklover VY (2011) Microbial synthesis of silver nanoparticles by *Streptomyces glaucus* and *Spirulina platensis*. Adv Sci Lett 4:3408–3417

Vijayabharathi R, Sathya A, Gopalakrishnan S (2018) Extracellular biosynthesis of silver nanoparticles using *Streptomyces griseoplanus* SAI-25 and its antifungal activity against *Macrophomina phaseolina*, the charcoal rot pathogen of sorghum. Biocatal Agric Biotechnol 14:166–171

Waghmare SS, Deshmukh AM, Kulkarni W, Oswaldo LA (2011) Biosynthesis and characterization of manganese and zinc nanoparticles. Univ J Environ Res Technol 1:64–69

Waghmare SS, Deshmukh AM, Sadowski Z (2014) Biosynthesis, optimization, purification and characterization of gold nanoparticles. Afr J Microbiol Res 8:138–146

Wang H-F, Li Q-L, Xiao M, Zhang Y-G, Zhou X-K, Rao MPN, Duan Y-Q, Li W-J (2017) *Streptomyces capparidis* sp. nov., a novel endophytic actinobacterium isolated from fruits of *Capparis spinosa* L. Int J Syst Evol Microbiol 67:133–137. https://doi.org/10.1099/ijsem.0.001586

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi J, Kontro M, Ganachari S, Sulochana M, Agsar D, Tapaskar R, Shettar A (2019a) Protein nanotechnology. In: Kharisov BI (Editor-in-Chief) Handbook of eco materials. Springer, New York. https://doi.org/10.1007/978-3-319-682556_192

Yaradoddi J, Kontro M, Ganachari S, Sulochana M, Agsar D (2019b) RNA nanotechnology. In Kharisov BI (Editor-in-Chief) Handbook of ecomaterials. Springer, New York. https://doi.org/10.1007/978-3-319-68255-6_193

Yaradoddi J, Kontro M, Ganachari S, Sulochana M, Agsar D, Tapaskar R, Shettar A (2019c) DNA nanotechnology. In: Kharisov BI (Editor-in-Chief) Handbook of ecomaterials. Springer, New York. https://doi.org/10.1007/978-3-319-682556_191

Yaradoddi JS, Sulochana MB, Kontro Merja H, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from Wst Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Zonooz NF, Salouti M (2011) Extracellular biosynthesis of silver nanoparticles using cell filtrate of *Streptomyces* sp. ERI-3. Sci Iranica 18:1631–1635

Chapter 12
Actinomycetes in Agriculture and Forestry

Merja H. Kontro, Jayachandra S. Yaradoddi, Sharanabasava V. Ganachari, Nagaraj R. Banapurmath, and M. K. Umesh

Abstract Microorganisms are the smallest creatures and are immensely abundant and diverse. These microbes include bacteria, algae, fungi, cyanobacteria, yeast, and actinomycetes, and most of them can decompose organic compounds and convert them into useful nutrients. Further, these nutrients are assimilated by the plants. There are two important groups of microbes found in agricultural soils; they are bacteria and fungi. Among the bacterial communities, actinomycetes grow and utilize the organic residues slowly. These actinomycetes can also be able to degrade the recalcitrant compounds and produce dark black to brown pigments contributing toward the dark color of soil humus. Previous reports have revealed the actinomycete's significant role in phosphate solubilization. Indeed they convert the insoluble hydrogen phosphate to a soluble one. Actinomycetes can be effectively used in the preparation of phosphate fertilizers with reduced cost, and these microbes mobilize the insoluble phosphate fertilizers and increase soil fertility. Actinomycetes are

M. H. Kontro (✉)
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

J. S. Yaradoddi
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

N. R. Banapurmath
Biomaterials Laboratory, Centre for Material Science, Advanced Research in Nanoscience and Nanotechnology, School of Mechanical Engineering, KLE Technological University (Formerly Known as B.V. Bhoomaraddi College of Engineering and Technology), Hubballi, Karnataka, India

M. K. Umesh
Department of Microbiology, Gulbarga University, Kalaburagi, Karnataka, India

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heterotrophic organisms and mainly depend on organic materials for carbon and energy requirements. The symbiotic association of these microbes with the nonleguminous plants can fix soil nitrogen, which is then available to both the host plant and other plants in the surrounding vicinity, and can contribute toward the progression of forestry.

Keywords Actinomycetes · Agriculture · Fertilizer · Phosphate solubilization · Plant nutrients

12.1 Introduction

Actinobacteria have broad and diverse ecological niches in a wide variety of soils, including agricultural and forestry environments. These niches comprise huge global terrestrial ecosystems, as well as their interactions with freshwater, the sea and the atmosphere, eventually covering a large part of the habitats (Singh and Dubey 2018). The terrestrial ecosystem has a huge influence on nearly all types of communities and can be utilized by the world for various purposes, like construction, farming, forestry, mining and recreation (Poff et al. 2011). The peculiar properties of the soil are due to the low mobility of the constituents compared to aquatic habitats (Dotaniya and Meena 2015). The microbial community composition of this habitat is thought to progress as a close connection among each other, and its associated constituents (Zhang et al. 2019; Upchurch et al. 2008).

Microbes are a beneficial source of established and new natural biologically active compounds. Among the producers of commercially viable significant secondary metabolites, soil bacterial species have abundant biosynthetic capacity for such metabolites. Incidentally, a remarkably large portion of species recognized for the production of commercially and biotechnologycally valuable bioactive compounds discovered till today are among the actinobacterial species belonging to the genera *Actinomyces*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Streptomyces*, *Micromonospora*, *Nocardia*, *Actinomadura*, *Streptoverticillium*, and *Arthrobacter* (Bull 2004; Sulochana et al. 2014a; b; Yaradoddi et al. 2020a, b; Yaradoddi and Sulochana 2020). The bioactive molecules synthesized by actinobacteria have a wide variety of biological activities, such as antitumor, herbicidal, antimicrobial, immunosuppressive, antiviral and antioxidant compounds (Van Bergeijk et al. 2020; Sulochana et al. 2014b; Bérdy 2005). Among the bacteria, several agriculturally important microbes are listed in Table 12.1 (Solanki et al. 2008). The actinobacteria have a large biosynthetic ability that is superior to other microbial communities and groups. *Streptomyces* species are responsible for around 7630 bioactive compounds (Bérdy 2005).

Vast numbers of *Streptomyces* spp. were recovered from the soil for some decades around 1950–1980, when the soil was used as a goldfield for antibiotics and associated compounds. Accordingly, the possibilities for recovery of new biologically active compounds producing *Streptomyces* strains from the terrestrial

Table 12.1 Indication for biocontrol activity of actinobacteria

Strains	Potential biocontrol activity
Streptomyces sp.	Controlling rice fungal pathogens
Actinomadura sp., *Micromonospora carbonacea*	Used as a major biocontrol agent against *Phytophthora cinnamomi* in banksia and snapdragon
Streptomyces netropsis	Has potential biocontrol activity against *Verticillium* fungi in cotton
Streptomyces griseus	Used against *Pythium ultimum* causing damping off disease
Micromonospora globusa	Used to kill the *Fusarium udum*
Actinoplanes sp.	Biocontrol activity against *Pythium aphanidermatum*
Actinoplanes utahensis	Potential biocontrol activity against the *Phytophthora* megasperma in soybean
Streptomyces violarus	In controlling damaging effects of *Alternaria alternata* leaf bight disease in groundnut

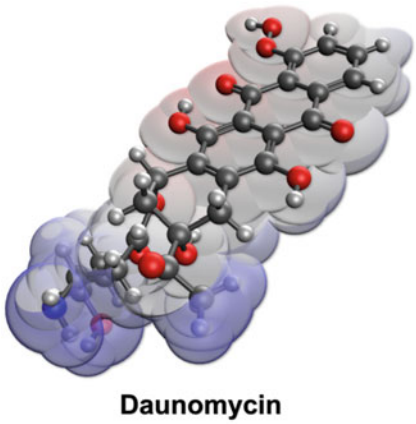

Fig. 12.1 Three-dimensional structure of anticancer agent daunamycin produced by actinobacteria

environment have reduced day by day, the entry and shifting towards the rare actinobacteria has occurred (Watve et al. 2001; Van Bergeijk et al. 2020). The enormous diversity and density of the actinobacterial group, though exhaustively explored, can be the main basis for attracting scientists in the way of discovering new secondary metabolite producers (Jayachandra et al. 2012a, b; Mohan Reddy et al. 2015a, b; Yaradoddi et al. 2018). Many agro-based industries have indicated a clear interest in utilizing actinobacteria as the major source for the PGPR (plant growth promoting rhizobacteria), agro-active compounds, and biocontrol agents (Sulochana et al. 2014a; Běhal 2000; Tanaka and Ōmura 1993) (Table 12.1). They also have the potential of producing anticancer (daunamycin), antibacterial (erythromycin), and antifungal (lomofungin) compounds (Figs. 12.1, 12.2, and 12.3).

The genus *Streptomyces* has been intensely investigated among the reported actinobacteria, and the dominant nature of members of this genus in the control of a wide varieties of fungal as well as bacterial phytopathogens has been most

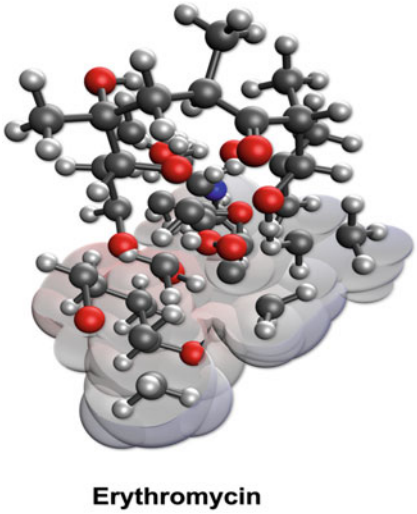

Fig. 12.2 Three-dimensional structure of antibacterial agent erythromycin produced by actinobacteria

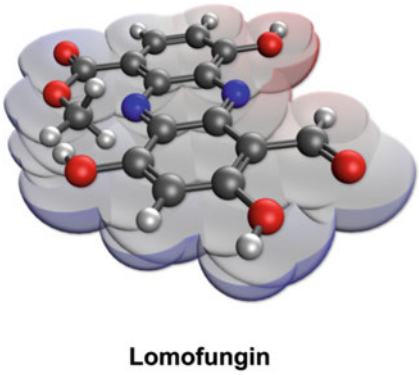

Fig. 12.3 Three-dimensional structure of antifungal agent lomofungin produced by actinobacteria

commonly accepted and endorsed by many researchers. For example, Patil and colleagues (2010) found that the antimicrobials of potential actinobacterial species in the rhizosphere destroyed fungal mycelium without any type of physical interactions with the antagonist, using scanning electron microscopy. The research has shown the contribution of secondary metabolites or bioactive compounds synthesized by antagonistic actinobacteria of the genus *Streptomyces*, and extensive studies may be required for further understanding the observed defense mechanisms. Very few of the taxa of this genus are pathogenic to plants and animals, like *Streptomyces scabies,* which causes potato scab disease in plants that leads to huge commercial losses.

Natural suppressors against *S. scabies* caused potato scab have been observed in long-term potato monoculture in Minnesota soil. The suppressors consisted of antibiotic-producing, non-pathogenic *Streptomyces* spp. (Weller et al. 2002). In addition, oligotrophic conditions with low carbon and nitrogen concentration have

been found to suppress *Streptomyces* spp. causing scab (Sagova-Mareckova et al. 2015). *Streptomyces* spp. have been reported to grow over a wide pH range in media with high concentration of organic compounds, including starch, whereas under nutritionally limited conditions, growth occurred at pH 7 and above. For this reason, oligotrophic conditions in acidic soils may not support the growth of scab-causing *Streptomyces* spp., which would otherwise grow well even at low pH on the surface of starch-rich potatoes (Kontro et al. 2005). Forest soil actinobacteria have been described as acidophilic and neutrophilic, although most commonly actinobacteria have been designated to prefer neutral to alkaline pH. The differences between the representative forest soil isolates that grow well at acidic or neutral pH were not limited to various pH requirements, but also through other physiological properties, and may also be connected to the properties of forest soils (Williams et al. 1971; Khan and Williams 1975).

Actinobacteria are broadly identified for their potentiality of producing extracellular enzymes and antibiotics, which have an interesting role in different sectors of agriculture and forestry. The greatest part of the antibiotics used in farming activities is related to *Streptomyces* spp. Further, Davelos and colleagues (2004) have investigated in prairie soil several groups of *Streptomyces* spp., which can produce multiple types of antibiotic and also have potential resistance capacity to numerous antibiotics, which has made them most competent among all other bacterial groups. The antibiotics synthesized by the genus *Streptomyces* possess the ability to prevent the diverse groups of soilborne microorganisms. Nonetheless, resistance within the *Streptomyces* spp. was more common than antibiotic production, suggesting a greater benefit of resistance. Watve et al. (2001) have also accepted the fact that these particular microorganisms remain a vital source of antibiotics. Their major characteristic features, such as antibiotic synthesis, disease suppressive properties, ability to withstand desiccation and high temperature conditions by their spore-forming abilities, have made them remarkable bioagents.

12.2 Actinobacteria as Bioagents

Actinobacterial species are generally used as bioagents or biocontrolling agents against the broad groups of phytopathogens, such as *Rhizoctonia* (Wu et al. 2019), *Alternaria* (Chattopadhyay and Nandi 1982), *Fusarium* (Abbasi et al. 2019), *Macrophomina* spp. (Hussain et al. 1990), and *Verticillium* (Chen et al. 2021). The members of the genus *Streptomyces* (Table 12.2) have evidenced for their exceptional biocontrol abilities against plant-related diseases, which is confirmed by some good reviews (Newitt et al. 2018; Olanrewaju and Babalola 2019; Law et al. 2017; Ferrer et al. 2018; Bubici 2018; Colombo et al. 2019; Gowdar et al. 2018).

Several researchers have demonstrated the beneficial roles of *Streptomyces* species as biocontrol agents against *Rhizoctonia solani* in concern with their disease suppressive ability. The molecular mechanisms possessed by *Streptomyces* spp.

Table 12.2 Potential herbicidal and antifungal agents produced by the actinobacteria

Herbicide producers	Bioactive agents/drugs
Streptomyces hygroscopicus	Herbimycin
Streptomyces prasinus	Prasinons
Saccharopolyspora sp.	Spinosad
Antifungal agents	
Streptomyces nodosus	Amphotericin B
Streptomyces griseus	Candicidin
Streptomyces sp.	Carboxamycin
Streptomyces galbus	Galbonolides
Streptomyces kasugaensis	Kasugamycin
Streptomyces tendae	Nikkomycin
Streptomyces natalensis	Natamycin
Streptomyces cacaoi	Polyoxin B
Streptomyces lavendulae	Streptothricin
Nocardia transvalensis	Transvalencin

were determined to be the induced systemic resistance by activating the antioxicant enzymes catalase and peroxidase (Abbasi et al. 2019). Elevated expression of lipoxygenase and phenylalanine ammonia lyase in tomato suggested that jasmonic acid and phenyl propanoid signaling pathways could be triggered in plants. *Streptomyces* strains also produced siderophores that can trigger plant defense mechanisms. Increased protease and amylase levels may also be involved in disease suppression (Ebrahimi-Zarandi et al. 2021). Additionally, members of the genus *Streptomyces* have been utilized at a commercial scale in controlling plant diseases, for instance, the *Streptomyces* sp. strain 5406 has been employed in China for more than few decades exclusively for cotton plant protection against the soilborne phytopathogens (Colombo et al. 2019; Valois et al. 1996). Kemira Ltd has described the *Streptomyces griseoviridis* living cell dependent biofungicide to combat the *Alternaria* and *Fusarium* infections (Lahdenperä et al. 1991).

12.3 Basis of *Actinobacteria* for Improved Agriculture

12.3.1 Molecular Mechanisms

As a result of investigations by some classical and modern researchers, it was indicated that several important mechanisms can be seen in plant disease management with an enormous contribution from actinobacterial species. These approaches are precious for the fruitful exploration on a disease management platform, as they uncover the molecular mechanisms involved in the disease suppression potentials of actinobacteria as biological agents. Several molecular mechanisms/associations rely on the antagonism either discretely or in a synergist manner by actinobacteria and the rest of the microbial agents: (a) inhibiting the growth of phytopathogens through

antimicrobial substance production (antibiosis), (b) competing for the iron ion by synthesizing siderophores, (c) competition for characteristic colonization sites and nutritional components supplied through roots and seeds, (d) promotion of plant defense mechanisms, (e) completely inactivating the potential germination factors existing in the root exudates or seed, and (f) deprivation of pathogenicity aspects of the phytopathogen like parasitism. Toxins may be involved in the synthesis of extracellular cell wall digesting enzymes such as chitinase and β-1,3-glucanase, which can lyse the pathogenic cell walls. No single mechanism is essentially synergic and exclusive, as numerous regularly modes of actions can be displayed by an individual actinobacterial strain. Certainly, for a few of the strains, different mechanisms or mixtures of mechanisms can occur during the suppression process connected to the plant disease (Whipps 2001).

12.3.2 PGP Potential

Soil actinobacteria under the rhizospheric regions and endophytes are very well recognized in the synthesis of bioactive compounds that control and minimize external biotic and abiotic adverse effects, among which most of them are agro-important. They promote plant growth either by suppressing pathogen-caused diseases, or by minimizing deleterious effects (Whipps 2001, Singh and Dubey, 2018). Actinobacteria have been reportedly potential contenders for nutrient solubilizing in plant development, such as the insoluble inorganic and organic phosphorus compounds, potassium and zinc. Alternatively, they protect from heavy metal toxicity (Soumare et al. 2020; Sahu et al. 2007; Yadav et al. 2017; Sathya et al. 2017). Members of the genus *Streptomyces* produce siderophores for iron or nutrient acquisition, as well as phytohormones such as IAA (indole acetic acid), which restrict the growth of fungal pathogens such as *Colletotrichum gloeosporioides* (potato dry rot), *Alternaria brassicicola* (rose apple anthracnose), *Penicillium digitatum* (orange green mold), *Sclerotium rolfsii* (damping-off of balsam), and *Fusarium oxysporum* (Chinese cabbage leaf spot), displaying fewer disease symptoms (Khamna et al. 2009). Other inhibitory compounds produced by actinobacteria include ammonia, cyanogens, alcohols, aldehyders, sufides, ketones, cell-wall degrading enzymes, and volatile organic compounds (Sathya et al. 2017; Yadav et al. 2017; Hassani et al. 2018). Several soil actinobacteria related to *Frankia*, *Micromonospora*, *Nocardia,* and *Streptomyces*, etc. have synergetic as well as symbiotic relations with the plant parts, exclusively to the roots. There are several confirmations with respect to actinobacteria associated with nitrogen fixation, like members of the genus *Frankia,* which are in broad-spectrum endophytic actinobacteria, which are synergistically associated within plant roots and help in fixing the atmospheric nitrogen in favor of host plants (Sathya et al. 2017; Benson and Silvester 1993). Sulochana et al. (2014a, b) reported the positive effect of a single actinobacterial strain on plant species by multifunctional features like rock phosphate solubilization, antagonistic properties of fungal phytopathogens,

siderophore production, etc. In one of the studies (Tokala et al. 2002), the process of nitrogen fixation within roots of young pea seedling with symbiotic *Rhizobium* sp. was detected to be enhanced by *Streptomyces lydicus* WYEC108.

12.3.3 Competition for Various Resources

In the soil habitats, competition among the microbial communities for multiple natural resources, like oxygen and nutrients, and the colonization sites occur commonly among different soil-inhabiting organisms. There appears to be a biocontrol process in action when an antagonistic organism straight away competes with pathogens for the resources. The actinobacteria, with on average 10^4–10^6 spores in g of soil, dominate because of their strong physiology, degradation potentials, and biosynthetic and antagonistic capabilities. There are many ways of resource competitions, like root dwelling microbes with major competition for infection sites on the root surfaces, whereas competition for nutrients, specifically for carbon, leads to the inhibition of fungal spore germination process under the soil (Haas and Défago 2005; Sathya et al. 2017; Alabouvette et al. 2006). Likewise, actinobacteria are also involved in the competition for trace elements, such as copper, iron, manganese, zinc etc. along with transformation and accumulation or chelation of heavy metals.

The low molecular weight compounds, such as siderophores synthesized by actinobacteria, have a higher iron affinity than competitively obtained chelated iron ions. Siderophores could function as diffusible fungistatic or bacteriostatic metabolites by creating an iron deficiency. While several researchers have revealed the contribution of siderophores to disease inhibition in certain conditions, it is supposed that siderophore molecules alone are not enough in the suppression of pathogens; if they were, it may be difficult to describe why most of the strains produce siderophores but do not have alone biocontrol abilities. There is a huge necessity in understanding the synergistic mechanisms of these microbes in order to avoid the failing of the practical applications in the field conditions (Khan et al. 2018; Sathya et al. 2017; Alabouvette et al. 2006).

Phosphorus is one of the vital nutrients essential for the plant growth, which contributes the structure of biomass micronutrients, cellular metabolic processes during signal transduction, energy transfer, photosynthesis, respiration chain reactions, and macromolecular biosynthesis (Shenoy and Kalajudi 2005). Unfortunately, the accessibility of phosphorus is limited, and it is regarded as one of the least transportable mineral nutrients of plants in the soil (Takahashi and Anwar 2007). Soils often contain a high reserve of phosphorus, although only about 0.1% of total phosphorus is available for plants in a soluble form (Sharma et al. 2013). Consequently, phosphate fertilizers have been widely used in agricultural fields to maximize the production capacities. The soluble phosphorus in fertilizers can be easily and quickly precipitated into insoluble forms by cations like Fe^{3+}, Co^{2+}, Al^{3+}, or Zn^{2+}; adsorbed to aluminum oxide, calcium carbonate, iron oxide, or aluminum silicate based on the specific properties of the soil; sorbed on the mineral surface; or lost due to erosion and water runoff (Sharma et al. 2013; Del Campillo et al. 1999; Chang and Yang 2009;

Alewell et al. 2020). Therefore, microbial phosphorus solubilization is an important process in soil, and about 20% of actinomycetes, especially those of the genera *Streptomyces* and *Micromonospora*, can release phosphorus in soluble form. The phosphate-solubilizing organisms can convert the insoluble form to a soluble form through chelation, acidification, and exchange reactions (Rodríguez and Fraga 1999). The formulation and use of these microbes in biofertilizers is an attractive option in the future (Sharma et al. 2013; Soumare et al. 2020).

12.3.4 Parasitism and Lytic Enzymes

The parasitism is a relationship in which a parasite or pathogen benefits from a host, such as plant. Actinobacteria can be associated with the physical interaction between a plant pathogen and an antagonist, leading to the death of the pathogen because of the destruction of the cell wall by hydrolytic enzymes synthesized by the antagonist (Adams 1990). The ß-glucans, chitin, as well as glycoproteins are the main structural components in most of the fungi. They can be degraded by extracellular hydrolytic enzymes like cellulase, chitinase, proteases, and glucanases. The enhanced production of extracellular hydrolytic protease by the mutant strain of *Stenotrophomonas maltophilia* W81 resulted in an improved biological control ability of phytopathogenic fungi *Pythium ultimum* (Gow et al. 2017; Dunne et al. 2000). The occurrence of fungi and actinomycetes with high pathogenicity against *Meloidogyne* spp., a plant parasitic nematode that causes tomato root disease, was surveyed. Based on greenhouse biocontrol efficacy tests, seventeen fungal and four actinomycete isolates with high in vitro pathogenicity were able to reduce the root gall index by 13.4 to 58.9% compared to the untreated control (Sun et al. 2006). In a similar way, five *Streptomyces* strains have also been indicated to perform a significant role in mycoparasitism of pathogenic fungi based on their chitobiase and exochitinase producing ability (Gomes et al. 2000). Microbial enzymes have been identified to display hyperparasitic action and attack pathogens under close vicinity by releasing cell wall hydrolytic enzymes (Verma and Suman 2018). Chitinase synthesized by *Streptomyces* sp. strain 385 was majorly involved in the antagonistic mechanism by different modes of action, such as germ tube elongation and inhibition of spore germination, which restrict growth through degradation or lysis of fungal mycelium. Nevertheless, in several actinobacterial strains, chitinolytic activities appear to be less required, as in the case of the genus *Streptomyces*, β-1,3-glucanase is produced rather to lyse the fungal cell wall of *F. oxyxporum* f. sp. *cucumerinum* (Bubici 2018; Kamil et al. 2018; Singh et al. 1999).

12.3.5 Antibiosis

The term antibiosis refers to the inhibition or destruction of a phytopathogen through metabolic derivatives synthesized during the antagonistic activity, including toxic

compounds, volatile compounds, antibiotics, lytic agents, enzymes etc. These are undoubtedly destructive to the growth and development of other microbes at relatively low concentrations (Verma and Suman 2018; Fravel 1988). Among all microbes, actinobacteria are leading by huge contribution to the synthesis of various classes of antibiotics, and their genetic potential is still largely unexplored (Van Bergeijk et al. 2020; Andrade et al. 1994). For example, in the biocontrol activity against the causal banana pathogen *F. oxysporum* f. sp. *cubense, Streptomyces violaceusniger* strain G10 acts as an antagonist with the antibiosis being emphasized (Getha and Vikineswary 2002).

12.3.6 Inactivation of Virulence Factors

One of the biological control phenomena is the inactivation of phytopathogen virulence factors, like detoxification of albicidin toxins synthesized by the strain *Xanthomonas albilineans.* Albicidin causes sugarcane disease, known as leaf scald. The inactivation consists of reversible and irreversible detoxification processes. In the reversible mechanism, the binding protein was produced by *Alcaligenes denitrificans*, which binds in a reversible manner to deactivate the toxin. The irreversible mode of detoxification is facilitated through an esterase enzyme produced by *Pantoea dispersa* (Basnayake and Birch 1995; Zhang and Birch 1997; Saraf et al. 2014). In addition, fusaric acid, a potential toxin synthesized by different *Fusarium* strains, may also be hydrolyzed using enzymes from different microbes, such as *Ralstonia solanacearum* and *Pseudomonas cepacia* (Srinivas et al. 2019; Compant et al. 2005). Several plant growth promoting bacteria (PGPB) can quench the phytopathogen quorum sensing activity through degradative mechanism of autoinducer signals, like n-acylhomoserine lactones, thus hindering the expression of several virulence genes (Newton and Fray, 2004; Uroz et al. 2003). This virulence factor inactivation approach contains the tremendous potential for reducing the disease infectiousness, even after the infection, by remedial action.

12.3.7 Induced Plant Defense Mechanisms

Plants contain a broad range of defensive actions that can be activated in response to parasites and pathogens ranging from smallest viruses to insect herbivores. The effectiveness of such defense actions from the emergence of a parasite, pathogen or some other factor to addressing and combating the challenge is very critical. The plant defense is preceded by an initial infection or treatment process, which outcomes as a resistance or tolerance against the subsequent exposure by the parasite, pathogen or an agent. Most of the plants have natural defense mechanisms against the attachks from pathogens that activate and reduce the severity of the disease. Two main classes of resistance, SAR (systemic acquired resistance) and ISR (induced

systemic resistance) have been elucidated. The presence of stimulation factors that potentiate plants against biotic challenges comprised of a broad range of parasites or pathogens is generally known as SAR (systemic acquired resistance), which can be shown as hardening, leaf necrosis, etc. When rhizobacteria are involved, it is called ISR, which as an inducing plant resistance capacity against a wide variety of the phytopathogens, parasites, and insect pests is normally attained after stimulation through PGPR (plant growth promoting rhizobacteria). Thus, it results in increased resistance because of an inducing agent on infection by a parasite or pathogen; however, these mechanisms can be differentiated depending on the inducing agent, which is rhizobacteria in ISR and another agent in SAR (Vallad and Goodman 2004; Van Loon and Bakker 1998). Identification of the molecules that modulate a cascade of molecular signals that ultimately result in the synthesis of defense compounds by the plant species is utmost important for development of new methods to combat plant diseases without using pesticides (Mhlongo et al. 2018).

12.4 SAR (Systemic Acquired Resistance)

The application of a pathogen or chemical inducer to plants can lead to the promotion of a host defense mechanism called as SAR (systemic acquired resistance). In SAR, resistance is achieved after local, hypersensitive reactions, which accordingly lead to subsequent defense of tissues distant from the first exposure to the same or different pathogen. The defense mechanism generally encompasses a wide range of resistance with long-lasting protection, wherein numerous signal transduction pathways are involved in activation of inducible protection when an attacking pathogen is identified (Klessig et al. 2018; Durrant and Dong 2004). During the activation of the mechanisms, PR (pathogenesis related) proteins accumulate within the infected and uninfected systemic tissues. Some chemicals, comprising of arachidonic acid, INA (2,6-dichloroisonicotinic acid), SA (salicylic acid), and BTH (benzothiadiazole) are known to induce the expression of the similar set of SAR genes as pathogens (Dann et al. 1998; Durrant and Dong 2004; Klessig et al. 2018).

12.5 ISR (Induced Systemic Resistance)

Different rhizobacteria and fungi have been designated to stimulate the ISR in plants and thus provide protection against a wide range of plant pathogenic fungi, bacteria, and viruses as well as herbivore feeding. The plant immune responses associated with the rhizosphere microbes or pathogens involve the recognition of several microbial signature defense molecules by plant extracellular receptors, and the production of signaling and protecting molecules. Some pathogens can down-regulate the immunity-triggered molecules by secreting effector agents, as a result of which the plant begins to produce complementary resistance proteins that

recognize effectors and activate a robust, rapid response called effector-triggered immunity. There are few self-modification processes involved by plant cells, such as thickening of the cell wall, or rapid death of damaged cells has led to necrosis of local tissues as barriers, interrupting the pathogen nutrient absorption and down-regulating the aggressiveness of the pathogen. Then damage-associated molecules are produced, such as cutin monomers, small peptides, and cell wall fragments, which activate the signaling cascades related to the defense. The signaling molecules incude salicylic acid, jasmonic acid, ethylene, phytoalexins, azelaic acid, pipecolic acid, and strigolactones, as well as interactions with other hormones, such as cytokinins, brassinosteroids, auxins, gibberrelins, and abscisic acid, the significance of part of interactions being not well understood. The mechanisms of ISR appear to vary depending on bacterial strains and pathogens, and include communication chemically, between bacteria/fungi, and between bacteria and plant. The ISR is triggered, e.g., by flagella, cell components like lipopolysaccharides, metabolites including siderophores, lipopeptides, volatiles, antibiotics, phenolic compounds, and quorum sensing molecules (Mhlongo et al. 2018; De Vleesschauwer and Höfte 2009; Vallad and Goodman 2004; Ton et al. 2002; Glazebrook et al. 2003). The preliminary signs of PGPB-elicited ISR were detected by Van Peer et al. (1991) on plant carnation (*Dianthus caryophyllus*), in which *Pseudomonas fluorescens* strain WCS417r induced phytoalexin accumulation and reduced sensitivity to wilt disease due to *Fusarium* sp. In addition, Wei et al. (1991) have shown that in *Cucumis sativus* (cucumber) six PGPR strains induced reduced sensitivity toward foliar disease anthracnose caused by *Colletotrichum orbiculare*. In Arabidopsis, the ISR was stimulated in the presence of the non-pathogenic *P. fluorescens* WCS417r, where the inception of quick and strong plant defense was detected upon exposure to the pathogen *Fusarium oxysporum* f. sp. *raphani* or *Pseudomonas syringae* pv. *tomato* without any considerable changes in the pathogenesis-related protein gene expression in distal tissues (Pieterse et al. 1996).

Press et al. (2001) have revealed the importance of iron rhizobacteria facilitated ISR in the cucumber plant. The ISR mediated by *Serratia marcescens* strain 90–166 was dependent on iron concentration in the cucumber pot plantings. The ISR was significantly enhanced when the iron availability of the host plant was reduced by the addition of the iron chelator ethylenediamine-di (o-hydroxyphenylacetic acid) (EDDHA), followed by suppression of fungal disease anthracnose. The ability of the strain to induce resistance was impaired by high iron availability. The approval was obtained through producing a siderophore-negative mutant strain of *S. marcescens* 90–166 that did not show ISR activities, whereas the wild-type strain was able to reduce iron concentration with siderophores, which reduced internal root populations (Press et al. 2001). The combinations of bacterial strains and the host plant are in a key role in the proper expression of ISR, and many reports of PCPR-mediated ISR refer to rhizobacterial strains. Nevertheless, the endophytic bacteria have also been analyzed for ISR activity (Mhlongo et al. 2018; Kilic-Ekici and Yen 2004). Endophytic actinomycetes are important in modulating plant resistance against abiotic and biotic stress under adverse circumtances (Ansari et al. 2020).

Actinobacteria of the genus *Streptomyces* have also been shown to be involved in PCPR-induced type of defense mechanisms. *Streptomyces* GB 4-2 inoculated into roots stimulates the local defense system in Norway spruce roots, and provides the host with better tolerance for the entry of pathogenic organisms through plant parts above the soil surface (Lehr et al. 2007). The role of elicitors in enhanced resistance was confirmed in investigating GB4-2 strain induced resistance on the *Alternaria brassicicola* by stimulating the transcriptional variation of the genes triggering Jasmonic Acid, Salicylic Acid, and ethylene biosynthesis (Schrey and Tarkka 2008).

12.6 Extracellular Enzymatic Screening for Actinobacteria

The media and other appropriate parameters in the screening of actinobacterial species for various extracellular enzyme production have been described (e.g. Raymaekers et al. 2020; Veliz et al. 2017; Hayakawa 2008). Accordingly, the soil is to be pretreated to prevent the growth of other bacteria. Several pre-treatment and following cultivation methods have been developed, including heating, phenol treatment, sodium dodecyl sulfate treatment with yeast extract, calcium carbonate treatment (Hayakawa 2008), specific isolation media for actinomycetes (Suutari et al. 2002), and antibiotic use (Hayakawa and Nonomura 1989). The combinations of these methods have also been commonly used. Isolation of chitin-degrading actinomycetes from tomato rhizosphere soil using the pour plate method on SNA (inorganic salt starch nitrate agar) medium as an example. The soil (10 g) in 100 mL of 0.1% agar water and 20 g of glass beads were first sonicated briefly, and then shaken (250 rpm) for 30 min at 28 °C. After 10-fold serial dilution, 0.2 mL was cultivated on SNA medium amended with cycloheximide and nystatin (50 mg/L), and incubated at 30 °C for 10 days. *Streptomyces* phages could be used to improve the isolation of actinomycetes other than streptomycetes. These isolates were further purified on OMYEA plates (oatmeal agar with 0.1% yeast extract). All these isolates were allowed to grow on petri plates containing CCA medium (colloidal chitin agar) to investigate the chitinolytic enzyme potentials. After 5 days of incubation, the zone of hydrolysis (width of zone) around the inoculation was determined. Three of the 38 strains displayed high chitinase activities and were recognized as related to the genera *Streptomyces* and *Actinoplanes* (Gadelhak et al. 2005). SCNA (starch casein nitrate agar) isolation medium can be used initially to increase the number of chitin- degrading isolates to six out of 13, followed by purification on GGA (glucerol arginine agar) (Das et al. 2014).

The cellulose degrading microbes, including actinobacteria, can utilize naturally available lignocellulosic materials and convert biomass into the absorbable forms of carbon source. The activity of lignocellulose degrading enzymes is a very basic requirement for the successful utilization of the most abundant renewable carbon reservoirs in forestry and agriculture, and in marine algae and plants to replace fossil carbon sources. The field of science has become one of the post prominent area of research for industrial applications, and there is a huge demand for finding effective

cellulose/lignocellulose producers and, on the other hand, degrading enzyme producers (Bettache et al. 2018; Ponnambalam et al. 2011).

To hydrolyse lignocellulose at least xylanases and laccases are required to release hemicellulose and lignin, and to expose cellulose to the hydrolytic enzymes. The β-glucosidases (β-D-glucosideglucohydrolase), endoglucanases (1,4-β-D-glucan-4glucanohydrolase), and cellobiohydrolases (exoglucanases, 1,4-β-D-glucan glucohydrolase) are required for cellulose breakdown. The endoglucanases bring about the random hydrolysis of the β-1,4 bonds within the cellulose molecule and the cellobiohydrolases release a cellobiose unit that is converted to glucose by the action of glucosidases (Bettache et al. 2018; Bhat and Bhat 1997). There is a huge demand in isolating the effective producers of cellulases from natural environments. Cellulose-degrading actinobacteria are typically isolated using CMC (carboxymethyl cellulose) agar medium, on which the 0.1% Congo red zone of clearange assay can be used to visualize the zone of cellulose degradation (Ponnambalam et al. 2011).

12.7 Conclusion

Actinobacteria act as important bioagents in agriculture and forestry. They can promote plant growth potential in the rhizosphere by controlling external biotic and abiotic adverse effects and are associated with induced plant defense mechanisms. In local systemic acquired resistance (SAR), actinobacteria act against a pathogen or chemical inducer, and in induced systemic resistance, they act in the rhizosphere against a variety of phytopathogenic fungi, bacteria, viruses, as well as herbivores. The protective effect of ISR also extends to distant plant structures. Actinobacteria isolated from soil produce a large portion of antibiotic-like compounds that support healthy development in humans and animals. The lytic enzymes of actinobacteria protect plants from pathogens by disrupting cell wall structures and inactivating toxins. Actinobacteria are also involved in protecting plants from heavy metal toxicity, and they regulate the availability of iron, nitrogen, phosphorus, and other nutrients. Actinobacteria are extremely important industrial enzyme producers, e.g., for the controlled hydrolysis of biomass lignocellulose from agriculture to replace fossil carbon sources with the recyclable ones. The progress of efficient molecular tools to elucidate mechanisms underlying actinobacterial activities will allow the development next-generation protective bioagents in agriculture that protect plants by enhancing their natural defence responses, thus reducing the use of anthropogenic pesticides. In all aspects, actinobacteria are an essential, utmost important part of agriculture and forestry.

References

Abbasi S, Safaie N, Sadeghi A, Shamsbakhsh M (2019) Streptomyces strains induce resistance to Fusarium oxysporum f. sp. lycopersici race 3 in tomato through different molecular mechanisms. Front Microbiol:10:1505. https://doi.org/10.3389/fmicb.2019.01505

Adams PB (1990) The potential of mycoparasites for biological control of plant diseases. Annu Rev Phytopathol 28:59–72

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. Eur J Plant Pathol 114:329–341

Alewell C, Ringeval B, Ballabio C, Robinson DA, Ranagos P, Borrelli P (2020) Global phosphorus shortage will be aggravated by soil erosion. Nat Commun 11:4546. https://doi.org/10.1038/s41467-020-18326-7

Andrade OA, Mathre DE, Sands DC (1994) Natural suppression of take-all of wheat in Montana soils. Plant Soil 164:9–18

Ansari WA, Krishna R, Zeyad MT, Singh S, Yadav AK (2020) Endophytic actinomycetes-mediated modulation of defense and systemic resistance confers host plant fitness under biotic stress conditions. In: Singh RP, Manchanda G, Maurya IK, Wei Y (eds) Microbial versatility in varied environments: microbes in sensitive environments. Springer, Singapore

Basnayake WVS, Birch RG (1995) A gene from Alcaligenes denitrificans that confers albicidin resistance by reversible antibiotic binding. Microbiology 141:551–560

Běhal V (2000) Bioactive products from *Streptomyces*. Adv Appl Microbiol 47:113–157

Benson DR, Silvester WB (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev 57:293–319

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Bettache A, Azzouz Z, Boucherba N, Bouiche C, Hamma S, Maibeche R, Gahfif O, Benallaoua S (2018) Lignocellulosic biomass and cellulolytic enzymes of actinobacteria. SAJ Biotechnol 5:203

Bhat MK, Bhat S (1997) Cellulose degrading enzymes and their potential industrial application. Biotechnol Adv 15:583–620

Bubici (2018) Streptomyces spp. as biocontrol agents against Fusarium species. CAB Rev13:050. https://doi.org/10.1079/PAVSNNR201813050

Bull AT (2004) Microbial diversity and bioprospecting. ASM Press, Washington, DC

Chang C-H, Yang S-S (2009) Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Bioresour Technol 100:1648–1658

Chattopadhyay SK, Nandi B (1982) Inhibition of *Helminthosporium oryzae* and *Alternaria solani* by *Streptomyces longisporus* (Krasil'nikov) Waksman. Plant Soil 69:171–175

Chen Q, Bai S, Zhang T, Duan C, Zhao J, Xue Q, Li Y (2021) Effects of seed-coating preparations of living Streptomyces globisporus on plant growth promotion and disease control against Verticillium wilt in cotton. Sustainability 13:6001. https://doi.org/10.3390/su1311600

Colombo EM, Kunova A, Cortesi P, Saracchi M, Pasquali M (2019) Critical assessment of Streptomyces spp. able to control toxigenic Fusaria in cereals: a literature and patent review. Int J Mol Sci 20:6119. https://doi.org/10.3390/ijms20246119

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005

Dann E, Diers B, Byrum J, Hammerschmidt R (1998) Effect of treating soybean with 2,6-dichloroisonicotinic acid and benzothiadiazole (BTH) on seed yields and the level of disease caused by Sclerotinia sclerotiorum in field and greenhouse studies. Eur J Plant Pathol 104:271–278

Das D, Pal P, Chaudhuri S (2014) Interaction of chitin-degrading soil actinomycetes with mycorrhizal colonization of chilli roots. Mycorrhiza News 26:2–4

Davelos AL, Kinkel LL, Samac DA (2004) Spatial variation in frequency and intensity of antibiotic interactions among Streptomycetes from Prairie soil. Appl Environ Microbiol 70:1051–1058

De Vleesschauwer D, Höfte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281. https://doi.org/10.1016/S0065-2296(09)51006-3

Del Campillo MC, Van der Zee SEATM, Torrent J (1999) Modelling long-term phosphorus leaching and changes in phosphorus fertility in excessively fertilized acid sandy soils. Eur J Soil Sci 50:391–399

Dotaniya ML, Meena VD (2015) Rhizosphere effect on nutrient availability in soil and its uptake by plants: a review. Proc Natl Acad Sci India Sect B Biol Sci 85:1–12

Dunne C, Moënne-Loccoz Y, de Bruijn FJ, O'Gara F (2000) Overproduction of an inducible extracellular serine protease improves biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* strain W81. Microbiology 146:2069–2078

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209

Ebrahimi-Zarandi M, Bonjar GHS, Riseh RS, El-Shetehy M, Saadoun I, Barka EA (2021) Exploring twon streptomyces species to control rhizotonia solani in tomato. Agronomy 11:1384. https://doi.org/10.3390/agronomy11071384

Ferrer CM, Olivete JE, Orias SL, Rocas MR, Juan CS, Dungca JZ, Mahboob T, Barusrux S, Nissapatorn V (2018) A review on Streptomyces spp. as platn-growth promoting bacteria (PGPB). Asian J Pharmacognosy 2:32–40

Foster RC (1988) Microenvironments of soil microorganisms. Biol Fertil Soils 6:189203

Fravel DR (1988) Role of antibiosis in the biocontrol of plant diseases. Annu Rev Phytopathol 26: 75–91

Gadelhak GG, El-Tarabily KA, Al-Kaabi FK (2005) Insect control using chitinolytic soil actinomycetes as biocontrol agents. Int J Agric Biol 7:627–633

Getha K, Vikineswary S (2002) Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: indirect evidence for the role of antibiosis in the antagonistic process. J Ind Microbiol Biotechnol 287:303–310

Glazebrook J, Chen W, Estes B, Chang H-S, Nawrath C, Métraux J-P, Zhu T, Katagiri F (2003) Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J 34:217–228

Gomes RC, Semêdo LTAS, Soares RMA, Alviano CS, Linhares LF, Coelho RRR (2000) Chitinolytic activity of actinomycetes from a cerrado soil and their potential in biocontrol. Lett Appl Microbiol 30:146–150

Gow NAR, Latge J-P, Munro CA (2017) The fungal cell wall: structure, biosynthesis, and function. Microbiol Spect 5:FUNK-0035-2016. https://doi.org/10.1128/microbiolspec.FUNK-0035-2016

Gowdar SB, Deepa H, Amaresh YS (2018) A brief review on biocontrol potential and PCPR traits of Streptomyces sp. for the management of plant diseases. J Pharm Phytochem 7:3–7

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

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

Hayakawa M (2008) Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica 22:12–19

Hayakawa M, Nonomura H (1989) A new method for the intensive isolation of actinomycetes from soil. Actinomycetologica 3:95–14

Hussain S, Ghaffar A, Aslam M (1990) Biological controls of *Macrophomina phaseolina* charcoal rot of sunflower and mung bean. J Phytopathol 130:157–160

Jayachandra SY, Parameshwar AB, Mohan Reddy K, Sulochana MB (2012a) Characterization of extracellular hydrolytic enzymes producing extremely halophilic bacterium *Virgibacillus* sp. JS5. World J Sci Technol 2(2):23–26

Jayachandra SY, Anil Kumar S, Merley DP, Sulochana MB (2012b) Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. Recent Res Sci Technol 4(4):46–49

Kamil FH, Saeed EE, El-Tarabily KA, Abu Qamar SF (2018) Biological control of mango dieback disease caused by Lasiodiplodia theobromae using Streptomycete and non-streptomycete actinobacteri in the United Arab Emirates. Front Microbiol 9:829

Khamna S, Yokota A, Lumyong S (2009) Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3 acetic acid and siderophore production. World J Microbiol Biotechnol 25:649–655

Khan MR, Williams ST (1975) Studies on the ecology of actinomycetes in soil – VIII distribution and characteristics of acidophilic actinomycetes. Soil Biol Biochem 7:245–248

Khan A, Singh P, Srivastava A (2018) Synthesis, nature and utility of universal iron chelator – siderophore: a review. Microbiol Res 212–213:103–111. https://doi.org/10.1016/j.micres.2017.10.012

Kilic-Ekici O, Yuen GY (2004) Comparison of strains of *Lysobacter enzymogenes* and PGPR for induction of resistance against *Bipolaris sorokiniana* in tall fescue. Biol Control 30:446–455

Klessig DF, Choi HW, Dempsey DA (2018) Systemic acquired resistance and salicylic acid: past, present and future. Mol Plant-Microbe Interact 31:871–888. https://doi.org/10.1094/MPMI-03-18-0067-CR

Kontro M, Lignell U, Hirvonen M-R, Nevalainen A (2005) pH effects on 10 Streptomyces spp. growth and sporulation dependes on nutrients. Lett Appl Microbiol 41:32–38. https://doi.org/10.1111/j.1472-765X.2005.01727.x

Lahdenperä M-L, Simon E, Uoti J (1991) Mycostop—a novel biofungicide based on *Streptomyces* bacteria. Dev Agric Manag For Ecol 23:258–263

Law JW-F, Ser H-L, Khan TM, Chuah L-H, Pusharajah P, Chan K-G, Goh B-H, Lee L-H (2017) The potential of Streptomyces as biocontrol agents against the rice blast fungus, Magnaporthe oryzae (Pyricularia oryzae). Front Microbiol 8:3. https://doi.org/10.3389/fmicb.2017.00003

Lehr N-A, Schrey SD, Hampp R, Tarkka MT (2007) Root inoculation with a forest soil streptomycete leads to locally and systemically increased resistance against phytopathogens in Norway spruce. New Phytol 177:965–976

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (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

Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867

Newitt JT, Prudence MM, Hutchings MI, Worsley SF (2018) Biocontrol of cereal crop diseases using Streptomycetes. Pathogens 8:78. https://doi.org/10.3390/pathogens8020078

Newton JA, Fray RG (2004) Integration of environmental and host-derived signals with quorum sensing during plant-microbe interactions. Cell Microbiol 6:213–224. https://doi.org/10.1111/j.1462-5822.2004.00362.x

Olanrewaju OS, Babalola OO (2019) Streptomyces: implications and interactions in plant growth promotion. Appl Microbiol Biotechnol 103:1179–1188. https://doi.org/10.1007/s00253-018-09577-y

Patil HJ, Srivastava AK, Kumar S, Chaudhari BL, Arora DK (2010) Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. World J Microbiol Biotechnol 26:2163–2170

Pieterse CMJ, van Wees SC, Hoffland E, van Pelt JA, van Loon LC (1996) Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8:1225–1237

Poff B, Koestner KA, Neary DG, Henderon V (2011) Threats to riparian ecosystems in western North America: an analysis of existing literature. J Am Water Resour Assoc:1–14

Ponnambalam AS, Deepthi RS, Ghosh AR (2011) Qualitative display and measurement of enzyme activity of isolated cellulolytic bacteria. Biotechnol Bioinformatics Bioeng 1(1):33–37

Press CM, Loper JE, Kloepper JW (2001) Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. Phytopathology 91:593–598

Raymaekers K, Ponet L, Holtappels D, Berckmans B, Cammue BPA (2020) Screening for novel biocontrol agents applicable in plant disease management – a review. Biol Control 144:104240. https://doi.org/10.1016/j.biocontrol.2020.104240

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Sagova-Mareckova M, Daniel O, Omelka M, Kristufek V, Divis J, Kopecky J (2015) Determination of factors associated with natural soil suppressivity to paotato common scab. PLoS One 10: e0116291. https://doi.org/10.1371/journal.pone.0116291

Sahu MK, Sivakumar K, Thangaradjou T, Kannan L (2007) Phosphate solubilizing actinomycetes in the estuarine environment: an inventory. J Environ Biol 28:795–798

Saraf M, Pandya U, Thakkar A (2014) Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiol Res 169:18–29. https://doi.org/10.1016/j.micres.2013.08.009

Sathya A, Vijayabharathi R, Gopalakrshnan S (2017) Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech 7:102. https://doi.org/10.1007/s13205-017-0736-3

Schrey SD, Tarkka MT (2008) Friends and foes: streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek 94:11–19

Sharma SB, Sayyed RZ, Trivedi MH, Govi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587

Shenoy VV, Kalagudi GM (2005) Enhancing plant phosphorus use efficiency for sustainable cropping. Biotechnol Adv 23:501–513

Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology 89:92–99

Singh R, Dubey AK (2018) Diversity and application of endophytic actinobacteria of plants in special and other ecological niches. Front Microbiol 9:1767

Solanki R, Khanna M, Lal R (2008) Bioactive compounds from marine actinomycetes. Indian J Microbiol 48:410–431

Soumare A, Boubekri K, Lyamlouli K, Hafidi M, Ouhdouch Y, Kouisni L (2020) From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: status and needs. Font Bioeng Biotechnol 7:425

Srinivas C, Devi DN, Murthy KN, Mohan CD, Lakshmeesha TR, Singh B, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B, Allah EFA, Nayaka SC (2019) Fusarium oxysporum f. sp. lycopersici causal agent of vascular wilt disease of tomato: biology to diversity – a review. Saudi J Biol Sci 26:1315–1324. https://doi.org/10.1016/j.sjbs.2019.06.002

Sulochana MB, Jayachandra SY, Anil Kumar S, Parameshwar AB, Mohan Reddy K, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5): 418–424. https://doi.org/10.1002/jobm.201200770

Sun M-H, Gao L, Shi Y-X, Li B-J, Liu X-Z (2006) Fungi and actinomycetes associated with *Meloidogyne* spp. eggs and females in China and their biocontrol potential. J Invertebr Pathol 93:22–28

Suutari M, Lignell U, Hyvärinen A, Nevalainen A (2002) Media for cultivation of indoor streptomycetes. J Microbiol Methods 51:411–416

Takahashi S, Anwar MR (2007) Wheat grain yield, phosphorus uptake and soil phosphorus fraction after 23 years of annual fertilizer application to an Andosol. Field Crops Res 101:160–171

Tanaka Y, Ōmura S (1993) Agroactive compounds of microbial origin. Annu Rev Microbiol 47: 57–87

Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant–microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68:2161–2171

Ton J, Van Pelt JA, Van Loon LC, Pieterse CMJ (2002) Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. Mol Plant-Microbe Interact 15:27–34

Upchurch R, Chiu C-Y, Everett K, Dyszynski G, Coleman DC, Whitman WB (2008) Differences in the composition and diversity of bacterial communities from agricultural and forest soils. Soil Biol Biochem 40:1294–1305

Uroz S, D'Angelo-Picard C, Carlier A, Elasri M, Sicot C, Petit A, Oger P, Faure D, Dessaux Y (2003) Novel bacteria degrading N-acylhomoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. Microbiology 149:1981–1989

Vallad GE, Goodman RM (2004) Systemic acured resistance and induced systemicresistance in conventional agriculture. Crop Sci 44:1920–1934

Valois D, Fayad K, Barasubiye T, Garon M, Déry C, Brzezinski R, Beaulieu C (1996) Glucanolytic actinomycetes antagonistic to *Phytophthora fragariae* var. *rubi*, the causal agent of raspberry root rot. Appl Environ Microbiol 62:1630–1635

Van Bergeijk EA, Terlouw BR, Medema MH, Van Wezel GP (2020) Ecology and genomics of Actionbacteria: new concepts for natural product discovery. Nat Microbiol Rev 18:546–558

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by pseudomonas sp. strain WCS417r. J Phytopathol 81:728–734

Veliz EA, Martínez-Hidalgo P, Hirsch AM (2017) Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiol 3:689–705. https://doi.org/10.3934/microbiol.2017.3.689

Verma P, Suman A (2018) Wheat microbiomes: ecological significances, molecular diversity and potential bioresources for sustainable agriculture. EC Microbiol 14(9):641–665

Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus Streptomyces? Arch Microbiol 176:386–390

Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508–1512

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Experim Bot 52:487–511

Williams ST, Davies FL, Mayfield CI, han MR (1971) Studies on the eology of actinomycetes in soil II. The pH requirements of streptomycetes from two acid soils. Sil Biol Biochem 3:187–195

Wu Z-M, Yang Y, Li K-T (2019) Antagonistic activity of a novel antifungalmycin N2 from Streptomyces sp. N2 and its biocontrol efficacy against Rhizoctonia solani. FEMS Microbiol Lett 366:fnz018

Yadav AN, Verma P, Singh B, Chauahan VS, Suman A, Saxena AK (2017) Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. Adv Biotech Microbiol 5:555671. https://doi.org/10.19080/AIBM.2017.05.555671

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi JS, Hugar S, Banapurmath NR, Hunashyal AM, Sulochana MB, Shettar AS, Ganachari SV (2018) Alternative and renewable bio-based and biodegradable plastics. In: Martínez LMT,

Kharissova OV, Kharisov BI (eds) Handbook of ecomaterials. Springer, Cham. https://doi.org/10.1007/978-3-319-68255-6_150

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV, Soudagar MEM, Mubarak NM, Hallad S, Hugar S, Fayaz H (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Zhang B, Wu X, Tai X, Sun L, Wu M, Zhang W, Chen X, Zhang G, Chen T, Liu G, Dyson P (2019) Variation in actinobacterial community composition and potential function in different soil ecosystems belonging to the arid Heihe River basin of Northwest China. Front Microbiol 10:2209

Zhang L, Birch RG (1997) The gene for albicidin detoxification from *Pantoea dispersa* encodes an esterase and attenuates pathogenecity of *Xanthomonas albilineans* to sugarcane. Proc Natl Acad Sci U S A 94:9984–9989

Chapter 13
Role of Actinomycetes in Biodegradation of Pesticides

H. Shoba, N. Rajeshwari, H. Yogeeshappa, and Somappa Jaggal

Abstract In agriculture, chemical pesticides are used to control insects, pests, fungi, and plant diseases so as to increase crop yield. Still, the excessive application of these pesticides will affect soil fertility and increase soil toxicity, leading to an imbalance in microbial activity and creating environmental pollution and health hazards. At present suitable alternative methods are much needed in converting the toxic compounds to nontoxic ones. When pesticide accumulates in the soil, it will undergo a wide variety of degradation process through physical, chemical, and biological means by soil microbial action. Especially, degradation by microbial sources serves as an important factor in conversion of toxic pesticides to nontoxic compounds via this biological process called as "Biodegradation." Most pesticides remain on the soil for long duration and are nondegradable using chemicals known as recalcitrants. Several degradation methods like physical, chemical, biological, and enzymatic methods are currently in use. But the most promising, efficient, and cost-effective technology involved in the degradation process is microbial degradation and is thermodynamically more affordable.

H. Shoba (✉)
Agricultural Engineering, College of Horticulture, Munirabad, Karnataka, India

University of Horticulture Sciences, Bagalakot, Karnataka, India

N. Rajeshwari
University of Horticulture Sciences, Bagalakot, Karnataka, India

Spices, Plantation, Medicinal and Aromatic Crops, College of Horticulture, Munirabad, Karnataka, India

H. Yogeeshappa
University of Horticulture Sciences, Bagalakot, Karnataka, India

Soil Science, College of Horticulture, Munirabad, Karnataka, India

S. Jaggal
University of Horticulture Sciences, Bagalakot, Karnataka, India

Department of Crop improvement and Biotechnology, College of Horticulture, Munirabad, Karnataka, India

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Keywords Microorganisms · Biodegradation · Recalcitrant · Health hazards · Pesticides

13.1 Introduction

The world pesticide consumption has reached about two million tones and its utilization outstretch in Europe is 45% followed by USA 24% and 25% among the rest of the world (Uqab et al. 2016). China stands first in the usage of pesticide followed by Korea, Japan, and India. Presently, India stands 12th position in the world production and usage of pesticides, i.e., about 0.5 kg/hectare, in addition to organochlorine pesticides (Bhat and Padmaja 2014; Zhang et al. 2011). In India, Uttar Pradesh stands first in pesticide consumption, followed by Punjab, Haryana, and Maharashtra, and the share of pesticide across agricultural crops accounts 45% for cotton, 25% for rice, 13–24% for vegetables/fruits, 7–8% for plantation crops, 6–7% for cereals/millets/oilseeds, 2–3% for sugarcane, and 1–2% for other crops. Worldwide, air and water are polluted by more than one billion pounds of toxins. Apart from these, roughly 6×10^6 chemical compounds have been produced, and around 1000 new products are synthesized annually whereas commercially, between 60,000 and 95,000 chemicals are used (Shukla et al. 2010).

Agricultural crops are generally harmed by organisms, mainly fungi, bacteria, insects, nematodes, and insects. Hence, in agriculture, pesticides, herbicides, and weedicides are commonly used in controlling their infestation. Among these major pesticides used are fungicides, bactericides, nematicides, and insecticides that kill fungi, bacteria, nematodes, and insects, respectively. These will enhance crop production capacity; however, excess pesticides could limit the export of agricultural commodities.

Finally, the soil became the "sink" for all applied chemical pesticides; this affects the microbial imbalance in the soil. The enormous quantity of soil microbes are vital in scavenging the hazardous substances in different soil forms. Yet, it majorly depends on the chemical properties of the contaminants. To avoid the exposure of humans to pesticides and to maintain soil fertility, some of the key strategies like regulation for pesticide use, safety during the use of pesticides, integrated pest management, and proper application technologies have to be followed. Pesticide manufacturing in the world has to be initiated so that the pesticide should have the ability to destroy target pests and be degradable as early as possible when it reaches the soil. Biodegradation is a natural process (Shellikeri et al. 2018; Yaradoddi et al. 2021; Singh and Walker 2006), where organisms for their own survival they tend to degrade the pesticide and it is the primary strategy but to increase the rate of biodegradation in a short time some modification involves; increase in the microbial process to achieve bioremediation (Chennappa et al. 2019; Megharaj et al. 2011; Paul et al. 2005; Vidali 2001) the knowledge on physiology, biochemistry, and genetics of the desired microbe is required with accurate, limited scope for uncertainty and variability in microbial functioning. To understand the capability of

microbes to degrade a pesticide, gene coding for enzymes has to be identified, and the development of super strain will help bioremediation in a short time with the desired result (Ortiz-Hernandez et al, 2013).

13.1.1 Pest and Classification of Pesticides

According to the WHO, pesticides are chemical compounds mainly used to destroy pests, including rodents, insects, fungal pathogens, pests, and weeds. These pesticides could be a potential threat to the environment as well as for mankind through vector transmitted diseases; for example, mosquitoes, especially in agriculture, can be important vectors in carrying hazardous health pesticides (Yadav and Devi 2017).

With reference to the Insecticides Act 1968 (WHO-World health organization 2004), some substances are under the schedule or similar kind of substance as the central government was discussed with the board through a notification in their followed gazette, included under the schedule from time to time or any research containing any specific single or many of such substances is mainly a pesticide. Pesticides can be divided into various categories as shown in the flowchart (Fig. 13.1).

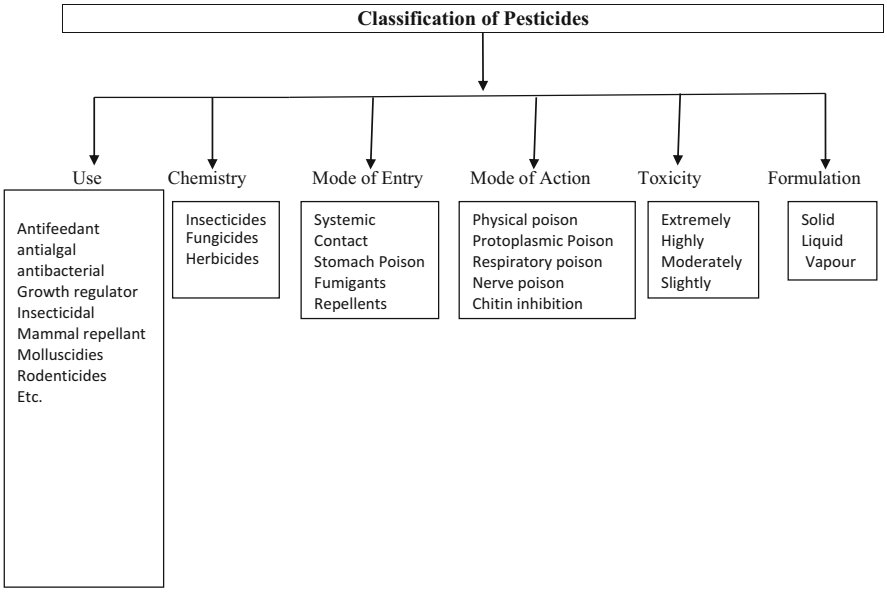

Fig. 13.1 Flowchart for classification of pesticides

13.1.2 Classification of Pesticides Based on Use (Table 13.1)

Table 13.1 The classification of pesticides based on use

SL. No	Type of pesticide	Description	Example
1.	Acaricides	Destroy mites	Carbofuran, methiocarb, abamectin, propoxur, flufenoxuron
2.	Algicides	Potential antagonistic against algae	Isoproturon, simazine, oxyfluorfen, copper sulfate, etc.
3.	Antifeedants	Used to prevent insects from feeding or pests	Fentin, chlordimeform azadirachtin
4.	Avicides	Used to kill birds	Strychnine, fenthion
5.	Bactericides	Potential inhibitors of bacteria growth	Streptomycin, copper hydroxide, tetracycline, etc.
6.	Bird repellants	It is bird repellant chemical	Diazinon and methiocarb are copper oxychloride, thiram, etc.
7.	Chemosterilant	It renders an insect infertile. Hence, it prevents reproduction of insects	Diflubenzuron
8.	Fungicides	Antagonistic effects on fungal pathogens	Metalaxyl-M, metalaxyl, carpropamid carboxin, aureofungin, kasugamycin, streptomycin, etc.
9.	Herbicide softeners	These are chemical softeners that do not prevent herbicides from killing weeds but protect crop from injury by herbicides	Cloquintocet, cyometrinil, benoxacor, cyprosulfamide
11.	Insect growth regulators	This chemical disturbs the growth and development of insects	Diflubenzuron, buprofezin
12.	Insect repellent	It frighten insects from landing on animals or humans	Citronella oil, permethrin
13.	Molluscicides	Chemical that are used to kill snail or slugs	Thiacloprid, metaldehyde, copper sulfate, thiodicarb
14.	Virucide	It will inactivate or kill the viruses	Ribavirin

13.1.3 Classification Based on Chemistry

Pesticides like insecticides, fungicides, herbicides, and rodenticides are further classified based on their chemical composition (Fig. 13.2).

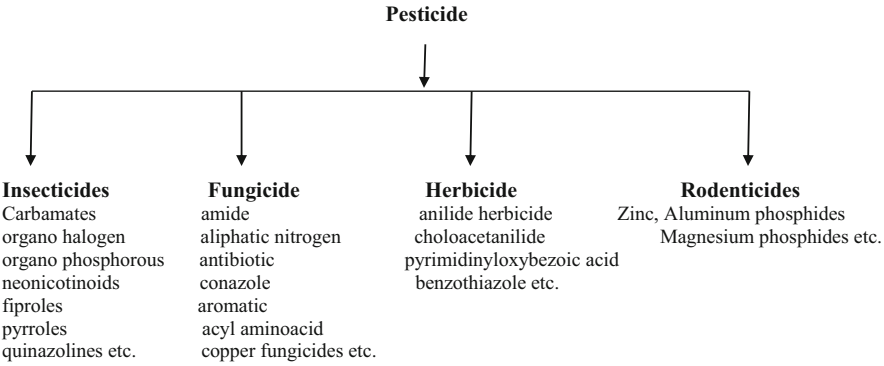

Fig. 13.2 Flowchart for classification of pesticides based on chemistry

13.1.4 Based on Pesticide Formulations

Pesticide formulations are the mixtures of technical-grade pesticides with inert diluents and auxiliary chemicals. These are classified into three main categories: solids, liquids, and gases. Some formulations are available in ready to use form, and others premixing is done before their utility (Damalas 2009; Akashe et al. 2018).

13.2 Effects of Pesticides on Soil and the Environment

Pesticides are extensively used in agriculture to control pests, weeds, and diseases in crops so as to increase crop yield. Still, finally, all applied chemical pesticides ultimately reach to the soil, and it has a direct effect on soil microbiological activities and creates a microbial imbalance (Fig. 13.3). Here are some most important effects which are caused by pesticides:

1. There is a reduction in soil fertility and crop productivity.
2. There is an ecological imbalance in the soil microflora.
3. Continuing the application of a huge amount of pesticides will lead to everlasting changes in soil microflora.
4. The excessive application of soil fumigants like telone, vapam, and ethylene bromide will affect the suppression of nitrifying bacteria like *Nitrosomonas* and *Nitrobacter*.
5. The excessive application of pesticides will inhibit cellulolytic and phosphate solubilizing microorganisms and also N_2 fixing soil microorganisms like *Rhizobium, Azotobacter, and Azospirillum*.
6. There is a nitrogen imbalance in the soil.
7. Occurrence of ammonification interference in soil.

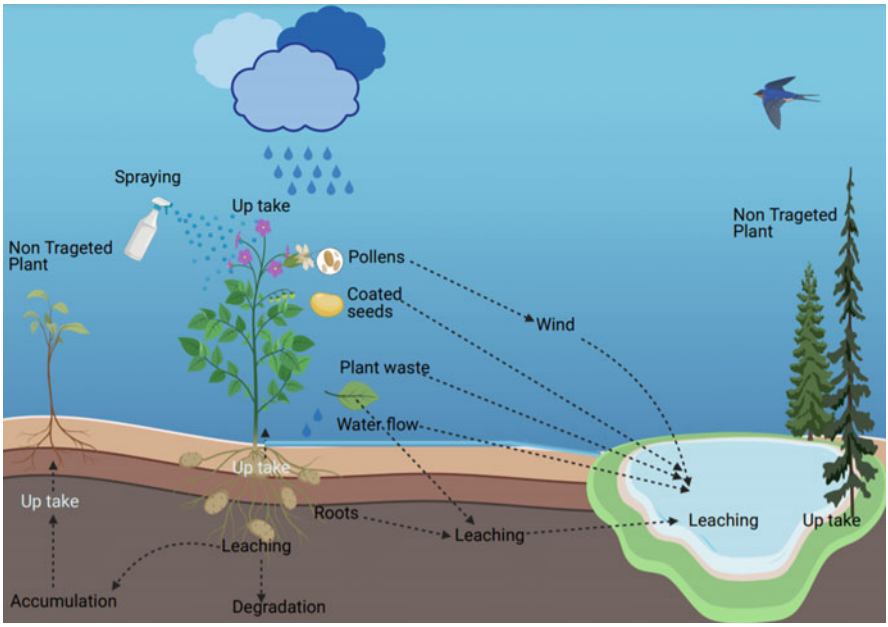

Fig. 13.3 The fate of pesticides in the environment

8. There is an adverse effect on mycorrhizal symbioses in plants and nodulation in legumes, quantitative and qualitative alterations in the rhizosphere microflora.

13.2.1 Effect of Pesticide on Plants

1. There is an increased level of toxicity due to the deposition of chemicals in soil.
2. Plants absorb and deposit the applied pesticides in the edible portions of plant products and root crops. Consumption of these plant products affects human beings as well as livestock perilously.
3. When applied pesticide gets leached by rain or irrigation water, it gets mixed with soil particles which then enters into water stream which leads to soil, water, and air pollutions.

13.2.2 Biodegradation of Pesticides

Pesticides applied to the plants finally reach the soil where they act by several forces. To some extent, physical and chemical forces can also degrade the pesticides, but microorganisms play a major role in converting toxic compound into nontoxic

compounds. This process is known as *Biodegradation.* Not all the applied pesticide chemicals that degraded in the soil but some of the chemicals do not degrade and show complete resistance to biodegradation. These chemicals are called "recalcitrant." Microorganisms can interact, both chemical and physical way, with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders (Briceño et al. 2007).

13.2.2.1 Biodegradation of Pesticides: Classification

- **Detoxification:** It is the conversion of the toxic compound into a nontoxic compound.
- **Degradation:** The degradation process is similar to mineralization. It breaks down or transforms a complex substrate into simpler products which finally leads to mineralization.
  Example: The strain of *Pseudomonas* will degrade the thiram (fungicide) and the degradation products are sulfolipids, dimethlamine, proteins, etc.
- **Conjugation (complex formation or addition reaction):** The addition of amino acid, organic acid, or methyl crown to the substrate organisms will catalyze the reaction and form the additional product, and here organisms make the substrate more complex or combine the pesticide with cell metabolites.
  Example: In the microbial metabolism of sodium dimethyl dithiocarbamate, the organism combines the fungicide with an amino acid molecule normally present in the cell and thereby inactivates the pesticides/chemicals.
- **Activation:** It is the conversion of nontoxic substrate into a toxic molecule; for example, Herbicide, 4-butyric acid (2, 4-D B), and the insecticide Phorate are transformed and activated microbiologically in the soil to give metabolites that are toxic to weeds and insects.
- **Changing the spectrum of toxicity:** Some of the pesticides or fungicides which are in such a way that they control one particular group of pests or organisms but they are metabolized to yield products inhibitory to entirely dissimilar groups of organisms.
  Example: The PCNB fungicide is converted in the soil to chlorinated benzoic acids that kill plants.

13.3 Factors Affecting the Biodegradation

The following important factors are influenced by the soil factors for biodegradation of pesticides/herbicides (Bhattacharya et al. 2006):

- Temperature
- Moisture

- PH
- Organic matter content
- Microbial population and pesticide solubility

Optimum soil moisture, temperature, and organic matter in the soil provide a compatible environment for retention or breakdown of any pesticide added to the soil. Within a period of 3–6 months most of the organic pesticides undergo degradation in tropical conditions, whereas fungi, bacteria, and actinomycetes, their metabolic activities, will play an important role in the degradation of pesticides.

13.3.1 Criteria for Biodegradation

The following aspects are very important criteria for biodegradation of pesticide in soil.

- To degrade contaminants at a faster rate and to bring down the concentration of contaminants, the catabolic activities of organisms are necessary.
- There should be bioavailability for the target contaminant.
- The soil conditions should be favorable in condition for enzymatic activity and plant growth.
- Bioremediation costs should be less than other bioremediation technologies.

13.3.2 Strategies for Bioremediation

The following strategies are required for the successful biodegradation/bioremediation:

- **Passive/Intrinsic Bioremediation:** Here the tile indigenous microorganisms will degrade the contaminant naturally but it is a very slow degradation process.
- **Biostimulation:** To stimulate indigenous microorganisms in soil nitrogen and phosphorus are added.
- **Bioventing:** To stimulate microbial activity as stimulants like oxygen and methane are added by the process/way of Biostimulation.
- **Bioaugmentation:** Here, to facilitate biodegradation in the contaminated site or soil the inoculation or introduction of microorganisms is done.
- **Composting:** Aerobic thermophilic microorganisms can be used to treat the constructed piles of contaminated soils and to improve the microbial activity periodic physical mixing and moistening of piles should be done.
- **Phytoremediation:** It can be achieved indirectly by plants stimulating microorganisms in the rhizosphere or directly by planting plants that hyper-accumulate heavy metals.

- **Bioremediation:** It is the detoxification process by conversion of toxic compounds to nontoxic compounds or detoxification of contaminated chemicals or contaminants in soil or other environment content by using microorganisms.
- **Mineralization:** The group of microorganisms or a group of species complete conversion of an organic contaminant to its inorganic constituent.

13.3.3 Different Techniques of Degradation of Pesticides

The following are some of the most important biodegradation techniques:

- Bacterial degradation
- Fungal degradation
- Enzymatic degradation

1. **Bacterial degradation**: Most of the bacterial species which degrade the pesticides and most of the pesticide undergo partial degradation leading to the formation and accumulation of metabolites.
2. **Fungal degradation**: Fungi degrade pesticides by introducing minor structural changes to the pesticides rendering it nontoxic and are released to soil, where it is susceptible to further biodegradation by bacteria.
3. **Enzymatic degradation:** Enzymes have a great potentiality to effectively transform and detoxify polluting substances because they have been recognized to transform pollutants (Ramakrishnan et al, 2010; Ganachari et al. 2018; Ramakrishnan et al. 2011) at a detectable rate and are potentially suitable to restore polluted environments.

13.3.4 Microbial Degradation Mechanism of Pesticides

Secondary pollution is caused when pesticides in the soil undergo different methods of degradation like physical, chemical, and physico-chemical but recently, the most frequently used method is microbial degradation because most of the pesticides could be utilized by the microbes as a nutrient, and they decompose into some small molecules, such as CO_2 and H_2O. The progress was called enzymatic reaction: here firstly the compound gets into microorganism' body through a certain way, secondly by the action of various enzymes it undergoes a series of physiological and biochemical reactions, and finally pesticides would be completely degraded or broken down into smaller molecular compounds which have no toxicity or less toxicity.

In abnormal environmental conditions, the degrading enzymes were often more resistant than microbial cells that could produce such enzymes, and the degradable efficiency of enzymes was much higher than that of microorganisms, especially for low concentrations of pesticides.

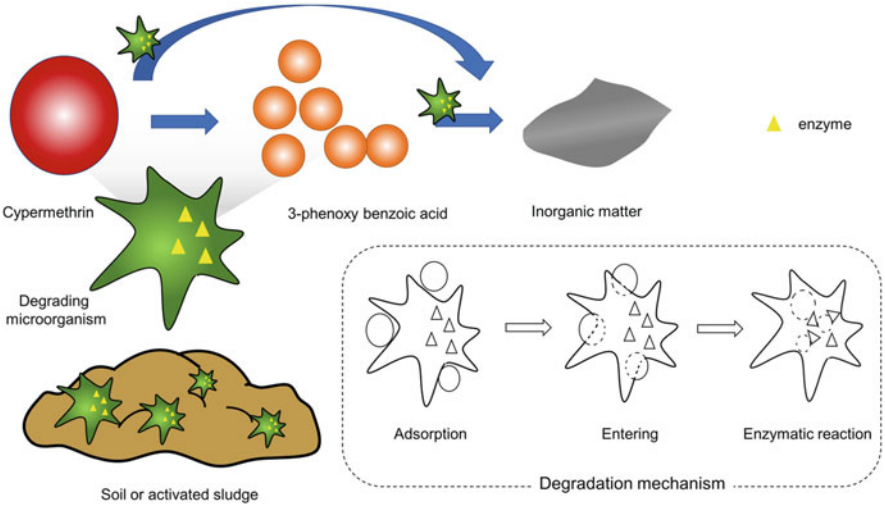

Fig. 13.4 Pesticide degradation mechanism

The mechanism of pesticide microbial degradation was divided into three main parts (Fig. 13.4):

- Firstly, on the surface of the cell membrane, adsorption of the target takes place and was a dynamic equilibrium process that was also critical.
- Secondly, through the surface of the cell membrane target molecules enter into the cell, further the rate of penetration and efficiency is purely based on the molecular structure of the target isomerism.
- Thirdly, the xenobiotic target conducted a rapid enzymatic reaction in the membrane.

13.4 Actinomycetes

Over a 100 years ago, actinomycetes' existence was recognized and these are referred to as filamentous procaryotes because at some stage of their growth, they form hyphae from diverse groups of heterotrophic prokaryotes. In earlier time the prokarotic nature was confined by the determination of their fine structure and chemical composition (Sharma et al. 2014). The application of new taxonomic techniques has led to improvements in the classification and identification of actinomycetes genera and species. Due to their ubiquitous nature, the difference in the structure, production of secondary metabolites and enzymes, and overall importance to man, the actinomycetes are still studied as a group, distinct from other bacteria. Although the actinomycetes have been the subject of extensive literature in recent

years, many aspects of their nature, physiology, genetics, production of secondary metabolites, and especially their role in natural ecosystems are yet to be understood.

13.4.1 Occurrence of Actinomycetes in the Environment

Actinomycetes occur in natural, artificial environments and in all ecological niches such as soil, compost, extreme environments, marshy places, mud marine, and freshwater (Good Fellow and Williams 1983). Some form parasitic or mutualistic associations with plants and animals, but most of them are strictly saprophytes these includes actinomycetes group with different genera are physically and nutritionally distinctly detected in all types of ecosystems. Their ability to degrade almost all natural polymers makes them the ubiquitous group. But only the main limiting factor is their rate of growth is very slow in all types of niches and this can be overcome by producing some secondary metabolites inhibitory to the bacteria and fungi which make them a successful group. The vast majority of cultures of aerobic actinomycetes have originated from soil. Viable counts of over four million g″1 may be obtained from fertile soils (Flaig and Kutzner 1960). Over 20 genera have been isolated from soil with 95% isolates belonging to streptomycetes. The type and population of actinomycetes in soil depend on environmental factors, e.g., numbers of streptomycetes in grasslands were highest in summer (Kuster 1976) and Nocardiae were most numerous in a pasture in winter (Orchard 1981). The distribution and activity of soil actinomycetes mainly depend on pH factor, and most of the actinomycetes isolate pH in the range of 5.0–9.0 and an optimum pH around 7.0. pH behaves as neutrophils whereas in acidic soils pH below 5 neutrophils occurs in less number—however, a species *Streptomyces caeruleus* grows from pH 6.5 to 9.5. Most actinomycetes are mesophilic, and these actinomycetes are active in compost. However, the capacity of exothermic process occur during decomposition often provides ideal conditions for obligate or facultative activities. The presence of thermophilic actinomycetes is also reported, and they grow at temperatures above 40 °C. Some genera (e.g., *Thermoactinomyces, Saccharomonospora*) are strictly thermophilic, while other genera (e.g., *Microbispora, Micropolyspora, Pseudonocardia, Streptomyces, and Thermomonospora*) contain thermophilic species. The actinomycetes from aquatic habitats most frequently present in water are *Actinoplanes, Micromonospora, Nocardia, Rhodococcus, Streptomyces,* and *Thermoactinomyces*. A variety of genera have been isolated from seawater and marine sediments, including *Streptomyces, Micromonospora, Microbispora,* and *Nocardia*.

13.4.2 Importance of Actinomycetes

- Actinomycetes have different morphological, biochemical, physiological, and cultural characters, which are a unique group of organisms in the procaryotes. They are involved in the composting process and they transform many compounds and mediate several biochemical reactions.
- This group is a potential producer of enzyme inhibitors, enzymes, antimicrobial substances, and growth-promoting substances for plants and animals.
- For undergoing degradation of organic matter, inhibition or stimulation of plant growth and other microorganisms, they carry out different activities in soil (Lechevalier 1981).
- In the rhizosphere of diseased plants, the activity against plant pathogen and antagonistic actinomycetes was found (Kundu and Nandi 1985).
- From the free living actinomycetes, plant growth is increased. Vitamin B is produced by actinomycetes isolated from the rhizosphere of pine, since mycorrhizae require these vitamins and it increases the plant growth. Hence, for stimulating plant growth the actinomycetes are contributing indirectly.

13.4.3 Role of Actinomycetes in Degradation of Pesticides

Actinomyces species may form endospores and form fungus-like branched networks of hyphae. Actinomyces species are ubiquitous, occurring in soil and microbiota of animals. They play an important role in soil ecology and have the potential of producing a number of enzymes that intern helps in degradation of organic plant materials, lignin, chitin, and also have a potential role in biotransformation and biodegradation of pesticides (Laura et al. 2011). Members of this group of Gram-positive bacteria have been found to degrade pesticides with widely different chemical structures, including organochlorines, *s*-triazines, triazinones, carbamates, organophosphates, organophosphates, acetanilides, and sulfonylureas. Many Actinomycetes can degrade different pollutants, including several pesticides, and are involved in the transformation of organic and metal substrates and also possess significant bioremediation potential. It can degrade high doses of pesticides, chemical complexes, and heavy metals (Winter et al, 1991).

Actinobacteria could utilize toxic compounds as sole carbon source for their growth and also converts commercially important enzymes, antibiotics, and proteins. The actinobacteria include both mesophilic and thermophilic strains that decompose more complex polymers such as lignocelluloses' plant residue (Goodfellow et al. 1984). Numerous species were used in the conversion of under-utilized agricultural and urban wastes into high-value chemical products. Amylase, cellulase, and ligases activities of actinobacteria do have a high potential for biological conversion of agricultural as well as urban waste, because these are consists of cellulose complexes with various quantities of hemicelluloses and lignin. The metabolic pathways

associated with pesticide degradation by actinobacteria have not been studied extensively; nevertheless, it is a well-known fact that the microorganisms can produce extracellular enzymes that could degrade a wide range of complex organic compounds. A widespread characteristic feature of aerobic Actinobacteria is connected to many types of monooxygenases and dioxygenases. Very limited research work was reported on xenobiotic pesticides mineralized by single isolates; however, often, consortia of bacteria are required for complete degradation. *Actinomyces* species are facultative (except *A. meyeri* and *A. israelii* both obligate anaerobe), and they grow best under anaerobic conditions.

References

Akashe MM, Pawade UV, Nikam AV (2018) Classification of pesticides: a review. Int J Res Ayurveda Pharm 9(4):144

Bhat D, Padmaja P (2014) Assessment of organic pesticides in ground and surface water in Bhopal India. IOSR J Environ Sci Toxicol Food Technol 8:51–52

Bhattacharya J, Islam M, Cheong YW (2006) Microbial growth and action: implications for passive bioremediation of acid mine drainage. J Miner Water Environ 25:233–240

Briceño G, Palma G, Duran N (2007) Influence of organic amendment on the biodegradation and movement of pesticides. Crit Rev Environ Sci Technol 37:233–271. https://doi.org/10.1080/10643380600987406

Chennappa G, Udaykumar N, Vidya M, Nagaraja H, Amaresh YS, Sreenivasa MY (2019) Azotobacter—a natural resource for bioremediation of toxic pesticides in soil ecosystems. Elsevier BV, Amsterdam

Damalas CA (2009) Understanding benefits and risks of pesticide use. Sci Res Essay 4(10): 945–949

Flaig W, Kutzner HJ (1960) Beitrag zur Okologie der Gattung *Streptomyces* Waksman et Henrici. Arch Mikrobiol 35:207–228

Ganachari S, Yaradoddi J, Somappa S, Mogre P, Tapaskar R, Salimath B, Venkataraman A, Viswanath V (2018) Green nanotechnology for biomedical, food and agricultural applications. In: Martínez LMT, et al (ed) Handbook of ecomaterials. Springer. https://doi.org/10.1007/978-3-319-68255-6_184

Good Fellow M, Williams ST (1983) Ecology of actinomycetes. Ann Rev Microbiol 37:189–216

Goodfellow M, Mordarskim M, Williams ST (1984) Introduction to and importance of actinomycetes. In: Goodfellow M, Mordarski M, Williams ST (eds) The biology of the actinomycetes. Academic, London, pp 1–6

Kundu PK, Nandi B (1985) Control of *Rhizoctonia* disease of cauliflower by competitive inhibition of the pathogen using organic amendments in soil. Plant Soil 33:357–362

Kuster E (1976) Ecology and predominance of soil Streptomyces. In: Arai T (ed) Actinomycetales - the boundary microorganisms. Toppan Co., Tokyo, pp 109–121

Laura M, Snchez-Salinas E, Olvera-Velona A, Luis J (2011) Chapter 27 Pesticides in the environment: impacts and its exclude quotes on exclude bibliography on exclude matches off biodegradation as a strategy for residues treatment. IntechOpen

Laura M, Snchez-Salinas E, Gonzlez E, Luisa M (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. https://doi.org/10.5772/56098

Lechevalier MP (1981) Ecological associations involving actinomycetes. Zentralblatt fur Bacteriologie, Mikrobiologie Und Hygiene. I. Abteilung Supplement 11:159–166

Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37:1362–1375

Orchard VA (1981) The ecology of Nocardia and related taxa. Zentralbl Bakteriol Supp 11:167–180

Ortiz-Hernández ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process, chap 10: Biodegradation - life of science, pp 251–287. https://doi.org/10.5772/56098

Paul D, Pandey G, Pandey J, Jain R (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23:135–142

Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R, Sethunathan N (2010) The impacts of environmental pollutants on microalgae and cyanobacteria. Crit Rev Environ Sci Technol 40: 699–821

Ramakrishnan B, Megharaj M, Venkateswarlu K, Sethunathan N, Naidu R (2011) Mixtures of environmental pollutants: effects on microorganisms and their activities in soils. Rev Environ Contam Toxicol 211:63–120

Sharma M, Dangi P, Choudhary M (2014) Actinomycetes: source, identification and their applications. Int J Curr Microbiol Appl Sci 3(2):801–832

Shellikeri A, Kaulgud V, Yaradoddi J, Ganachari S, Banapurmath N, Shettar A (2018). Development of neem based bioplastic for food packaging application. IOP Conf Ser Mater Sci Eng 376:012052. https://doi.org/10.1088/1757899X/376/1/012052

Shukla KP, Singh NK, Sharma S (2010) Bioremediation: developments, current practices and perspectives. Genet Eng Biotechnol J 3:1–20

Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30(3):428–471

Uqab B, Mudasir S, Nazir R (2016) Review on bioremediation of pesticides. J Bioremed Biodegr 7(3):343

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73(7):1163–1172

WHO–World Health Organization (2004) The WHO recommended classification of pesticides by hazard and guidelines to classification: 2009-2010

Winter B, Fiechter A, Zimmermann W (1991) Degradation of organochlorine in spent sulfite bleach plant effluents by actinomycetes. Appl Environ Microbiol 57:2858–2863

Yadav IC, Devi NL (2017) Pesticides classification and its impact on human and environment. In: Environmental science & engineering, vol 6: Toxicology. Studium Press, New Delhi, pp 140–158

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2021) Bio-based material from fruit waste of orange peel for industrial applications. J Mater Res Technol. https://doi.org/10.1016/j.jmrt.2021.09.016

Zhang W, Jiang F, Feng OJ (2011) Global pesticide consumption and pollution: with China as a focus. Proc Int Acad Ecol Environ Sci 1(2):125–144

Chapter 14
Actinomycetes in Environmental Applications

Merja H. Kontro and Jayachandra S. Yaradoddi

Abstract The presence of soil actinomycetes induces plant health by persuading plant growth and development by producing phytohormones. Endophytic actinobacteria such as *Actinoplanes*, *Micromonospora*, and *Nocardiopsis* are the major producers of important phytohormones like indole-3-pyruvic acid (IPYA) and indole-3-acetic acid, whose main function lies in controlling gene regulation and in coordinating cell growth. Describing the microbially produced phytohormone is extremely difficult compared to the phytohormones produced by the host organisms. However, in vitro produced compounds by root colonizing actinobacteria dictate which microorganism is suitable to maintain plant health. Soil actinomycetes, especially *Streptomyces*, usually enhance the nutrient absorption capabilities of plants along with the growth of rhizobia provided with the soil nutrients. Composting with biomass enzymatic hydrolysis may help to alter the physicochemical and biological properties of the soil's nature. This compost can be easily prepared using consortia of microorganisms, and the process involves rapid mixing of different groups of organisms, especially bacteria belonging to actinomycetes. Other unique environments where actinobacteria occur include fungus-farming ants, beewolf wasps offspring protection, and bioweathering of rock minerals. The examples presented illustrate how diverse actinobacteria can occur in different environments. Genetic methods have also revealed that only a small fraction of these actinbacterial functions are known.

Keywords Environmental applications · Composting · Gene regulation · Root colonization · Indole acetic acid

M. H. Kontro (✉)
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

J. S. Yaradoddi
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

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14.1 Introduction (Theory of Composting, Wastewater, and Enzymes)

The aerobic waste treatment called composting supports the organic material modification towards a fertilizer that improves the biological, physical, and chemical properties of the soil. It is the usual process that could be bioaugmented for enhancement using the consortia of microorganisms with biological activity e.g. from old compost (Barthod et al. 2018; Velasco et al. 2004; Sulochana et al. 2014a, b; Yaradoddi et al. 2020a, b; Yaradoddi and Sulochana 2020). This process contains a quick succession of the mixed group of microbial populations. With respect to the dominant processes, the foremost important community of microorganisms majorly belong to bacteria, including actinobacteria, and also fungi (Ryckeboer et al. 2003; Partanen et al. 2010; Yu et al. 2007; Jayachandra et al. 2012a, b; Mohan Reddy et al. 2015a, b; Yaradoddi et al. 2018). The process of composting is generally divided into three or four different phases. In the beginning mesophilic phase, simple organic molecules, such as proteins and carbohydrates, are degraded that results in the decrease in pH due to organic acid production along with the increase of temperature. Thermophilic or thermotolerant organisms are dominated during the middle thermophilic phase through the production of enzymes that are involved in the degradation of complex organic biopolymers, leading to an increase in temperature. The ultimate phase of the composting process could be characterized by analyzing the growth of actinobacteria and also fungal cultures that degrade the majority of composite organic material in higher pH and decreasing temperature conditions. This third stage can also be divided into two different stages, the cooling phase and the maturation phase (Atchley and Clark 1979; Reyes-Torres et al. 2018; Sundberg et al. 2013; Wei et al. 2017).

The raw materials utilized in composting contain lignocellulosic biomass. These lignocellulosic biomasses mainly consist of hemicellulose (15–35%), cellulose (35–50%), and lignin (10–35%) (Karimi 2015). All these three fractions are very closely connected among each other. The composting process proceeds by disintegrating the three constituents with the help of degradative enzymes. A group of enzymes that are essential during the process belong to cellulases, xylanases, and laccases. The hemicellulose constituent holds the cellulose fibers together, whereas the xylanase enzyme acts upon the xylan; and the enzyme laccase eliminates reinforced material through the lignin disintegration process. As soon as hemicellulose and lignin get eliminated, the cellulosic fibers are readily available for the cellulase enzyme and, therefore, degradation of the lignocellulose constituents proceeds throughout the composting. All these enzymes can act in an associated manner as well as in sequence to yield matured compost. The fertilizer formed serves as a dual application, on the one hand, as a product in solid waste management and, on the other hand, as a fertilizer in the modification of soil properties (Ma et al. 2020; Reyes-Torres et al. 2018; Tuomela et al. 2000; Yu et al. 2007).

Composting as the bioremediation method is also the most emerging research field due to the increased amount of hazardous materials disposed to the environment. Because of several serious issues raised by accumulations of waste, released

by industries, a number of nations around the world have adapted limits for the amount of unprocessed fluids being disposed to lakes, rivers, and oceans. Numerous remedial methods have been published, which, however, have been inadequately implemented due to their costs. The biological as well as physiochemical actions were considered to reduce the working capital and increase operational expenses. The biotechnology-based process aimed at reducing the release of toxicants into the environment may be promising in finding inexpensive solutions with respect to thermal issues, increased COD/BOD, or other environmental concerns in the future (Imam et al. 2021; Sayara and Sánchez 2020; Aguelmous et al. 2019; Ventorino et al. 2019).

In addition to municipal wastewater, the major risks exhibited by water bodies are currently formed by the paper and pulp industries. This hazard is directly correlated to the dark-colored effluents (either black or brown), which is the principal outcome of the important stages among the production process, specifically bleaching, pulping, and paper manufacturing. The dark color of fluid waste hinders the light entering into the water system, which lowers the respiration process and the photosynthesis. Discharge from the paper and pulp industries has direct as well as indirect harmful effects on the environment, and they are also threat to human beings. The passage of such polluted water through drinking or cooking may be headed for enhanced chances of getting hazardous diseases. An additional path of imparting major damage to health can be seen by consuming the fishes thriving within such contaminated environments (Kumar et al. 2021; Haq et al. 2020; Viswanath et al. 2014).

Among the enzymes used for the composting process, the laccase enzyme has a huge potential in the bioremediation process; however, it is yet limitedly explored. Laccase is a multicopper enzyme that belongs to a group of blue oxidases. This enzyme can be designated as oxidoreductases as described by Enzyme Commission (EC). The laccase enzyme catalyzes the monoelectronic oxidation of a comprehensive variety of substrates. These substrates consist of polyphenols, *ortho*- and *para*-diphenols, aromatic or aliphatic amines, and aminophenols. The oxidation of the substrates is associated with the four-electron reduction in HO and O. Laccase enzymes are recognized for their capacity to counteract phenolic constituents in lignin, and other common dangerous constituents in effluent PAH (polycyclic aromatic hydrocarbons). Consequently, the laccase enzyme also plays a vital function during the bioremediation process mediated by actinobacteria; bacteria that are generally growing in the form of filaments are filled along with enzymes that are essential for the composting process as well as bioremediation. The filaments produced by the actinobacteria mediate the penetration and also distribution throughout machinery of the organism during their growth, that leads to effective disintegration of these substrate materials. Besides the above, the complex carbohydrates of the compost can stimulate the microbes to discharge extracellular enzymes that degrade the hemicellulose, cellulose, and lignin (Kumar et al. 2021; Haq et al. 2020; Viswanath et al. 2014).

14.2 Actinomycetes and Biomass Hydrolyzing Enzymes

The sediment samples were used in the isolation of actinomycete strains by a very typical method, which is described below. The sampling sites were divided into various categories: mangroves, marine, compost, water spring, lakes sediments, rivers, and soil. The sample collection was carried out by using the sterile spatula, and later these samples were stored in sterilized clean bottles with caps. The sample was treated through an air-drying process for about 48 h, homogenization using pestle and mortar, and it was further sieved. Subsequently, treated samples were properly diluted using saline except for mangrove and marine samples, which were usually diluted using a 1:2 dilution in seawater. Actinobacteria recovering agar at about pH 8 was prepared for isolating the actinobacteria using the sediments. Antibiotics such as streptomycin 100μg/mL and cycloheximide 50μg/mL were added to the media. The inoculated Petri plates were incubated at about 40 °C temperature and further observed for the growth. This incubation time varied between 1 and 3 weeks. The colonies of actinobacteria were recognized based on their phenotypic features using a microscope. The isolation media pH of about 8 and incubation temperature of about 40 °C were maintained in order to obtain a specific class of actinobacterial strains isolated. At this temperature and pH, thermophiles and alkaliphiles normally thrive well. ISP4 medium (International Streptomyces Project medium 4) was utilized for culturing the spore suspension. Spores of about 10^5spores/mL in saline were prepared for inocula (Limaye et al. 2017).

Isolated cultures can be further screened for cellulase, laccase as well as xylanase activities. During the process, cellulases, laccases, and xylanases producing cultures can be identified and classified, and further explored for their potential other hydrolytic activities. The enzyme production medium of pH about 8 at 40 °C can be maintained to confirm that synthesized enzymes exhibited the alkalitolerant and thermotolerant abilities.

Several different plate assay methods are available for measuring xylanase activity, of which the easy-to-use wheat bran agar method is persented here (Limaye et al. 2017; Nagar et al. 2012; Meddeb-Mouelhi et al. 2014). Qualitative plate assay is involved in the analysis of xylanase activity, and the media used for the test should contain wheat bran agar. The wheat bran agar contains (in g/L) wheat bran, 20; beef extract, 3; peptone, 5; and agar, 20, and about 10μL spore suspension (10^5 spores/mL) is added on the media. The medium is incubated for 5 days at 40 °C, and then agar surface is sealed by 1% of Congo red for about 15 min. The overload of Congo red solution is poured off, and the Petri plates are covered by 1 M NaCl for 1 h. The zone of hydrolysis shows the xylanase activity. Further, the microbial strains with the xylanase activity are selected on the basis of the zone produced by them. Typical colonies of xylanase producing *Streptomyces* sp. are shown in Fig. 14.1.

Several different methods are available for screening cellulase-producing microorganisms, one of which is exemplified herein (Kasana et al. 2008; Shanmugapriya et al. 2012; Rasul et al. 2015; Limaye et al. 2017). In analyzing the cellulolytic activity, CMC (carboxy-methyl cellulose) agar medium can be used, which contains (in g/L): CMC, 5; K_2HPO_4, 1; $NaNO_3$, 1; glucose, 1; $MgSO_4$, 0.5; KCl, 1; yeast

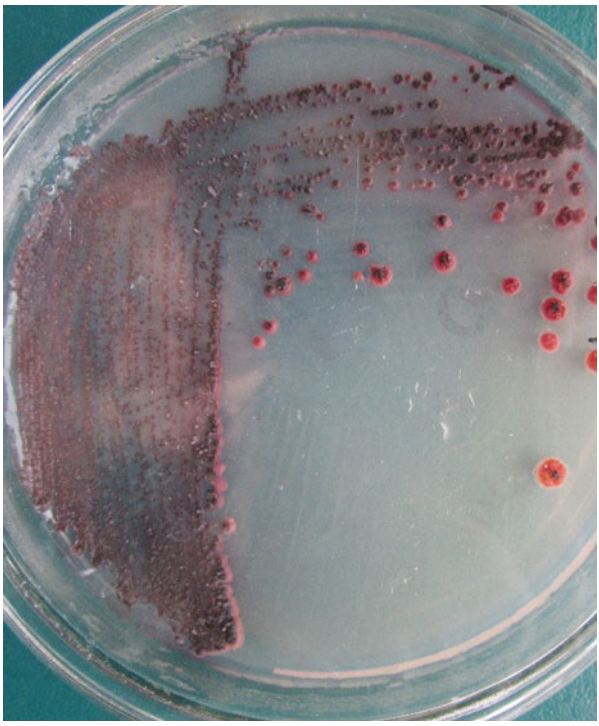

Fig. 14.1 Potential xylanase enzyme-producing *Streptomyces* sp.

extract, 0.5; and agar, 20. To the medium, 10μL (10^5 spores/mL) spore culture was used for inoculation. The inoculated medium was incubated for 5 days at 40 °C temperature; the agar surface was covered by 1% Congo red for about 15 min. The extra solution was removed, and the Petri plates were flooded with 1 M NaCl, kept for 1 h. The hydrolysis zone shows the cellulase enzyme activity. The bacterial cultures with the cellulase activity were selected based on the extent of the zone of clearance.

Various phenolic an non-phenolic compounds have been used to screen laccase enzyme activity, guaiacol being one of the most commonly used substrate in the isolation of actinobacteria, among others (Limaye et al. 2017; Unuofin et al. 2019; Kiiskinen 2004). The qualitative laccase plate assay can be performed using the basal agar medium with the addition of a trace element solution, including 0.01% of guaiacol. The basal agar medium usually consists of (in g/L): $(NH_4)_2SO_4$, 2; KH_2PO_4, 0.5; K_2HPO_4, 1; yeast extract, 1; asparagine, 0.2; maltose, 200 mM; $MgSO_4$, 0.2; and agar, 20. The asparagine amino acid shall be added as a source of nitrogen, and 0.01% guaiacol in ethyl alcohol is added before autoclave, and the sterile trace element containing solution 0.1% v/v can be added to the basal agar after the autoclaving process.

The trace solution composition consists of (in g/L): $ZnSO_4{\cdot}7H_2O$, 0.3; $CuSO_4{\cdot}7H_2O$, 0.025; $MgSO_4{\cdot}7H_2O$, 2.5; $MnCl_2{\cdot}5H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 0.25; $COCl_2{\cdot}6H_2O$, 0.05; and $CaCl_2{\cdot}2H_2O$, 1.5, and this solution should be sterilized

individually through the autoclaving process. Later on, 10μL (10^5 spores/mL) of spore suspension can be added on the media, followed by incubation for about 5 days at 40 °C temperature. The positive strains for laccase enzyme are shown by the appearance of a reddish-brown halo around the colonies in the presence of guaiacol in the medium (Limaye et al. 2017).

14.3 Composting Process for Plant Biomass and Forest Industry Effluent

In general, the composting of organic waste, for example source-separated household biowaste, proceeds without any specific additives; only a bulking agent (e.g. wood chips) and proper aeration are required to maintain compost aerobic. Old compost can act as an inoculum (Rainisalo et al. 2011). However, the inoculum can be used in specific applications, such as in the decomposition of organic waste from paper and pulp industry, or in remediation of contaminated material. In the following example by Limaye et al. (2017), eight actinobacterial strains were selected toward the development of compost for enhanced degradation of organic waste from plant material, followed by the composting of effluent from paper and pulp industry.

The selected eight actinobacterial isolates were allowed to grow at a temperature of 40 °C, for about 96 h on ISP medium no. 4 to yield microbial spores. The spore suspension was cultivated for each strain using saline, and 0.01% Tween 80.

The viable counts of spore suspensions were adjusted at 10^9 spores/mL using cultivation on plate count agar. The combined spore suspension was prepared by adding 100 mL of spore suspension of individual strains.

In developing composting, a unit containing dimensions of 35 cm × 25 cm × 40 cm (length × width × height) was used. About 300 g of organic fruit and vegetable waste was added to individual composter unit. Fifty grams of dehydrated banana peels were poured into the unit, which improves the oxygen supply and circumvents raw material tamping. Three hundred milliliters of spore consortium mixture was added to the composter, and all supplements were mixed thoroughly into a uniform mixture. The negative control of the uninoculated compost was amended 300 mL of saline.

Composting units was kept under the shade. The compost was maintained for about 25 days, and composting could be extended for a longer period. The moisture/available water level was controlled by adding 20 mL water to the heap daily. Furthermore, the compost bin was mixed weekly to confirm the aerobic conditions. After 25 days, the physical and chemical properties of compost were within the desired range.

There are several standard norms laid by the National Centre of Organic Farming (NCOF), Department of Agriculture, Government of India to be followed for the systematic study.

14.3.1 Compsting Plant Material

The parameters include (1) moisture, (2) pH, (3) C:N ratio, (4) total nitrogen, (5) organic carbon, and (6) conductivity. In addition, temperature monitoring allows the evaluation of the stage of composting. In the European Union, legislation related to composting is included in the health rules for animal by-products not intended for human consumption, and rules on the making available on the market fertilizing products. A composting plant must be equipped with a closed composting reactor with temperature control without bypass possibility. The maximum particle size of compost is 12 mm and the minimum temperature of compost material is 70 □ for 60 min. Other standardized process parameters are permitted, when the applicant ensures that biological risks are minimized (European Parliament and Council 2009, 2019 and references therein).

As presented above, the potential actinobacterial strains were selected based on enzyme production capacity for the treatment of paper and pulp industry effluent. The selected microbial strains were allowed to grow on nutrient agar (pH about 7.5) in 10% paper and pulp mill effluent supplemented prior to sterilization. This ensures the capacity of strains to grow under rich environments. Especially the paper and pulp effluents can be treated through laccase synthesizing strains of actinobacteria, the specific inoculum being called strain R.

An effluent sample from paper and pulp industries was autoclaved at the beginning of the investigation, and diluted in the ratio of 1:2 using sterilized distilled water. The nitrogen source of 0.1% peptone and carbon source of 0.1% dextrose were supplemented, and the pH was adjuted to 7.5 using sodium hydroxide. To prepare the spore suspension of *Streptomyces* strain R, saline and 0.01% of Tween 80 were mixed with the spores scrapped from sporulation ISP4 agar medium. Five percent of the spore suspension having density of 10^5 spores/mL was utilized as the inoculum. The inoculated mixture was continuously aerated using an air pump, and incubated at room temperature. Optical density, chemical oxygen demand, and the degradation of effluent compounds were followed on days 0, 7, and 14. The results showed that the phenolic compounds were cleaved, and various degradation products were formed. The actinobacteria could have potential in the hazardous waste treatment.

14.4 Actinobacterial in Air and Soil

14.4.1 Airborne Actinobacteria

Actinobacterial spores are understood to be significant air contaminants under professional environments, for example, in biowaste composting facilities and agriculture (Nielsen et al. 1997; Lacey and Crook 1988). They have been found to be indicators of mold growth and indoor air problems in buildings. Actinobacteria do not belong to the usual indoor air microbial populations; however, they have been

observed in buildings, which suffer from mold and water damage problems (Nevalainen et al. 1991; Suutari et al. 2002; Samson et al. 1994). In particular, *Streptomyces griseus, Streptomyces albidoflavus,* and *Streptomyces coelicolor* have been detected in water-damaged buildings, and a real-time PCR test has been developed to detect then (Rintala et al. 2001; Suutari et al. 2002). The spores of numerous actinobacterial taxa, such as *Streptomyces albus, Thermoactinomyces vulgaris, Micropolyspora faeni,* and *Saccharomonospora viridis,* have been correlated with the occurrence of allergic alveolitis and other serious health illness (Lacey and Crook 1988; Lacey and Dutkiewichz 1976). Investigations have revealed that *Streptomyces* sp., and especially their spores, have the ability to induce lung macrophage reactions that can result in illnesses like tissue injury and inflammation (Hirvonen et al. 1997, 2001).

The spores of actinobacteria are generated via prevailing hyphae subdivision or swelling, or via endogenous spore production. Actinobacterial hyphae are subdivided into spores without or with sheath that are partially left under the spores even after disintegration (Williams et al. 1973). As a result, three major spore types are generated: Aleuriospores (sheathless hypha), arthrospores (sheathed hypha), and the endospores. These variabilities are likely to cause distinction among spore resilience and airborne characters (Williams et al. 1973). In the environment, actinobacterial spores can be converted to airborne by means of mechanical disturbances of the matter they are growing on, for instance, through operating the agricultural tools or by unveiling toward the blustery wind (Lloyd 1969). Laboratory investigations have been conducted using airborne actinobacterial spores, and the impact collection has been found to influence the recovery, injuries, and consequently the colony numbers (Stewart et al. 1995). Lacey and Dutkiewicz (1976) detected the actinobacterial spores in polluted hay by mechanical handling followed by Andersen sampler collection, while Madelin and Johnson (1992) detected these actinomycete spores in culture media through air currents. Due to their smaller size, actinobacterial spores are more difficult to aerosolize than fungal spores (Reponen et al. 1997).

The production of airborne actinomycete spores using general laboratory practices allows the measurement of spore aerodynamic and physical sizes, and the viability of actinobacterial spores. The aerodynamic spore size has been 1.28 μm for *Micromonospora halophytica* aleuriospores, 0.85 μm for *Streptomyces albus* arthrospores, and 0.57 μm for *Thermoactinomyces vulgaris* endospores with the largest size variation. As a result, the spore sizes were close to monodisperse. The physical sizes of the spores were 0.55×0.72 μm for *M. halophytica*, 0.68×0.84 μm for *S. albus*, and 0.66×0.79 μm for *T.vulgaris*. The variations among the spore sizes seem to be connected to the preparation methods for microscopic and aerosol observations. Water will be absorbed by the spores, resulting in extending their diameter. Consequently, the size measurements of dry spores seem to be more suitable for human health-associated studies (Madelin and Johnson 1992; Reponen et al. 1998).

Comparison of the viabilities of spores indicated that spores from *S. albus* had the maximum survival level of 35.3%, whereas that of *M. halophytica* spores was 7.4% and that of *T. vulgaris* spores was 4.6%. These differences seemed to be related to the spore wall structure. The spores of *S. albus* possess the protective outer sheath against physical injury and drying, which is lacking in the spores from *M. halophytica*. The spores from *T. vulgaris* have the dormant or inactive nature that require activation before their germination. In this study, the conventional heat activation was not possible due to the risk of agar melting, and the less effective cold activation at 20 °C for 24 h improved the recovery of spores 10-fold. The collection of bacterial spores in the filter would enable heat activation (Reponen et al. 1998).

14.4.2 Actinobacteria in Acidic Soil

The words acidophilic and acid-tolerant actinobacteria can be seen in the literature from the end of the last century (Khan and Williams 1975). The research of that time has surprised with the opinion that almost all actinobacteria belong to neutrophilic taxa, with the optimal growth pH range of 6.5-8.0 (Kutzner 1986). However, the particular ecological characters of acidophilic actinobacteria and their influence toward the activity in the soil microbial communities are persisted as unexplored. The most continuously described acidophilic actinobacteria belonged to the genus *Streptomyces*, partly because they are abundant in nearly all soils (Zakalyukina et al. 2002, Kim et al. 2004). Previous reports (Nioh et al. 1995) have shown that within the actinobacteria populations under the acidic soil in tea culture, the acid-tolerant actinobacteria especially belonged to *Streptomycetes,* and to actinobacteria generally associated to *Glycomyces* and *Actinoplanes* genera. It has subsequently been observed that the growth pH ranges of *Streptomyces* spp. are not constant species-specific properties, but depend on environmental nutrients. *Streptomyces* spp. were able to growth over a wide pH range in the presence of high organic matter content but not under nutritionally restricted conditions.

Williams et al. (Khan and Williams 1975) isolated acidophilic actinobacteria from pine forests, soils, and mine wastes, i.e. microbes that cannot survive within the neutral medium. Further investigations have indicated that the numbers of acidophilic actinobacteria recovered from various soils can be little or larger based on the soil, or acidity. Within the soils of pine forests, the existence of acidophilic actinobacteria was about 80% of all the recovered actinobacteria, 63% in broad-leaved coniferous forest soils, and 33% among the plowed soils (Kim et al. 2004). In later studies, the acidophilic actinobacteria were found to be widespread in acid soils, and an essential mycelial prokaryote component of the soil microbial communities in the soils of the main soil climatic zones, in the intrazonal alluvial soils,

and in the anthropogenic substrates in Mongolia and Russia (Zenova et al. 2004, 2006).

Although actinobacteria are clearly widespread in soils, the measured actinobacterial numbers can vary greatly depending on the cultivation conditions. For example, the actinobacterial counts recovered from acid soils using acid cultivation medium exceeded the counts of mycelial prokaryotes isolated on neutral cultivation medium, a phenomenon not observed in chernozems and slightly alkaline soils (Zakalyukina et al. 2002; Selyanin et al. 2005). The dominant actinobacteria recovered on the acid medium belonged to the genera *Micromonospora* and *Streptomyces,* and the quantity of *Micromonospora* spp. isolates on the acid medium was significantly higher than on the neutral medium. The acidophility constant was calculated as actinobacterial counts on acid isolation medium (pH 5.3) divided by the counts on neutral medium (pH 7.0). As a result, among the soils studied, the actinobacteria recovered on the acidic and neutral media varied based on the counts of mycelial prokaryotes, and also concerning to their taxonomic composition. The results revealed that commonly used cultivation on a neutral medium may be a selective factor that distorts actual numbers and may limit the availability of unique isolates (Zakalyukina et al. 2002).

The pH of the cultivation medium also affected the numbers and diversity of the acid soil *Streptomycetes* isolates. However, unlike *Micromonospora* species, the numbers and taxonomic diversity of *Streptomyces* spp. declined in the acid medium in comparison with the neutral medium, when *Streptomycetes* were isolated from acid soils. The taxa of the imperfectus section turn out to dominate within the acidophilic streptomycete complex. In contrast, among the various soil types, taxa from different sections and series prevailed on neutral medium (Zakalyukina et al. 2002). In chernozems with humic substances and buffer capacity, the streptomycete complex tolerated better the acid medium conditions and the numeric and species diversity of the *Streptomycetes* was not affected. The measured features, for example, the taxonomic arrangements and numbers of soil actinobacteria, and the effects of medium acidity are displaying the processes occurring in the soil. The practical evidence of this is the outcome realized in actinomycetes numbers during the microbial succession in peat soils after fire (Zenova et al. 2008).

The actinobacteria are one of the essential microbial groups in peat soils, and have particular characters discrete from those of mycelial bacteria within the zonal soils. This complex acts as an indicator of variations occurring within the microbial communities of the peatland subsequently to the exclusion of peat for fuel and fertilizer purposes, followed by reconditioning. Fires in peat soils may increase the biophilic element concentration in the burned surface layer, alter the acid-base balance, and modify the hydrothermal regime, followed by the development of particular phytocenoses simultaneously with the evolving actinobacterial succession. The ash consists of only calcium oxalate and calcium carbonate; and the carbon content will range from 0.68 to 0.71%, affecting actinobacterial composition (Zenova et al. 2008).

14.4.3 Actinobacteria in Saline Soil

The physiological adaptation to osmotic stress is of huge importance in the metabolic activities of microbes. Despite the involvement of microbial cells in metabolism, salinity supports the osmotic pressure continuing the dynamic activity of the organisms. The osmotic pressure within the actinobacterial cells is understood to be somewhat higher; it permits them to endure to be in soils within the moisture lagging and higher salt conditions.

Most actinobacterial isolates grow well in the medium having a salt concentration of about 4% (Tresner et al. 1968). The obligatory halophilic *Actinopolyspora halophila* gen. et sp. nov., with a minimum essential salt (NaCl) concentration of about 10% within the solid and 12% in the suspension medium, could be regarded as an extreme halophyte (Gochnauer et al. 1975). The presence of high counts of halotolerant actinobacteria in solonchaks proved the need for high salt tolerance in such extreme conditions (Selyanin et al. 2005). However, actinobacteria isolated from saline soils can vary in the intensity of halophilicity, and many of halophilic mycelial actinobacteria are denoted to rare genera, or they correspond to the novel taxon (Gochnauer et al. 1975). For example, *Microbispora coralline* actinobacteria that have an ability to grow in 3% NaCl under mesophilic conditions were recovered at saline soils (Nakajima et al. 1999). An unique actinobacterium *Actinopolymorpha singaporensis* gen. nov., sp. nov. (Nocardioidacease family) that can grow at 15% of NaCl concentration was recovered from the soil in the tropical forest of Singapore (Wang et al. 2001). Two novel species recovered from the alkaline soils in Korea, *Nocardioides lentus* sp. nov. and *Nocardioides debius* sp. nov., have the potential ability to grow at pH 8.0 in the presence of 0.5% of salts (NaCl) (Yoon et al. 2005, Yoon et al. 2006). The halophiles *Nocardiopsis xinjiagensis* sp. nov. and *Nocariopsis salina* sp. nov. were recovered from soil (Li et al. 2004), and the alkaliphile *Streptomyces sodiiphilus* sp. nov. was identified from the salt lake in China (Li et al. 2005). The novel *Salinispora* genus belonging to the family *Micromonosporaceae* denoted by novel species *Salinispora tropica* and *Salinispora arenicola* is among the group of marine actinobacteria based on investigating their phenotypic properties and genetic features (Maldonado et al. 2005). Saline soils are also often habitually alkaline, specifically, if their salinity is analyzed by the Ca^{2+}, CO_3^{2-}, and Na^{+}, ions. Consequently, the growth is not only limited to halophiles but also alkalophilic in such soils. The ability of the extent of alkalitolerant actinobacteria obtained from different locations is listed in Table 14.1.

14.4.4 Psychrotolerant Actinobacteria in Soil

The psychrotolerant microbes would prefer to grow under lower temperatures, which is associated with the higher specific growth rate (μ) than in mesophiles, the higher concentrations of the unsaturated fatty acids among the membrane lipids, and

Table 14.1 Extent of alkalitolerance of actinobacteria

Name of the actinobacteria	Location	Extent of pH
Actinopolyspora mortivallis	Saharan soil	5.0–9.0
N. metallicus	Alkaline slag dump	7.0–10.5
N. metallicus	Germany, alkaline conditions of slag dump	8.5–10.5
N. alkaliphila	Desert soil in Egypt	9.5–10.0
Streptomyces sp.	Coastal region of Gujrat	11.0
S. altiplanensis	Sediments from Soda Lake	8.4–10.6
S. deccanensis	Karnataka Province, India	8.0–10.5

by means of particular protein conformation supporting low temperature adaptation. The psychrotolerant bacteria play a beneficial role in the organic matter breakdown in the cold terrestrial ecosystems. Bacteria in the cryogenic peatland of the tundra are adjusted to the growth and functioning at low temperatures and do not grow properly at high temperatures (Lipson and Schmidt 2004; Thormann et al. 2004).

The actinobacteria are common within the bacterial communities in the cryogenic soils. The psychrotolerant actinobacteria have been identified from a variety of different environments, including stony rocks, moraine, oceanic water and ice, soil, boreal groundwater, and animal fur. For example, the psychrotolerant actinobacteria of the genera *Micromonospora*, *Promicromonospora*, *Nocardia*, and *Streptomyces* were recovered from the alpine meadow habitats (Wang et al. 2004). The predominant species corresponded to the *streptomycetes* in the cold climate region soils in China (Xu et al. 1996). The species of *Actinosynnema* were detected in the field and forest soils of southeastern Tibet mountains (He et al. 2006). Furthermore, new species have also been described within the psychrophilic actinobacteria. The actinobacterial genus *Frigoribacterium* was recovered from animal shed (Kämpfer et al. 2000). *Pseudonocardia antarctica* sp. nov. was isolated from the Antarctic moraine (Prabahar 2004). The psychrophilic bacteria, *Subtercola frigorans* sp. nov. and *Subtercola boreus* gen. nov., sp. nov., were found the cold boreal groundwater (Männistö et al. 2000). The soil isolate, *Modestobacter multiseptatus* gen. nov., sp. nov. was described from the Antarctic mountains (Mevs et al. 2000).

The occurrence of psychrotolerant actinobacteria in the peat soils of the southern taiga and tundra has been investigated. As the summer temperature in the top layers and litter of such peat soils does not surpass the temperature of 8–10 °C, the conditions are advantageous for the growth and development of the psychrotolerant actinobacteria (Dobrovol'skaya et al. 2014, Zenova et al. 2012). As a result, psychrotolerant and psychrophilic actinobacteria recovered from the soils extended from thousands to several hundred thousands of colony-forming units, depending on the soil horizon layer and type. The numbers of psychrotolerant actinobacterial isolates recovered from the soils were similar to those of mesophilic species. The psychrotolerant actinobacterial counts were highest of hundred thousand in the marshland moss and litter layers, while their number decreased within the deeper

deposits due to the shortage of oxygen. The actinobacteria of the genus *Streptomyces* were the most common species (Dobrovol'skaya et al. 2014, Zenova et al. 2012).

14.5 Environmental Actinobacteria in Unique Habitats and Applications

14.5.1 Actinobacterial Natural Products and Fungus-Farming Ants

Among the most widely characterized multiorganism ecosystems with actinobacteria are those associated with the leaf-cutter ants, mostly found in America. The biologically active components produced by actinomycetes regulate the fungal growing leaf-cutter ants, and have vital roles in determining the ecosystem interactions (Currie et al. 1999). The actinobacterial biomolecules are important in maintaining a balanced network especially between the leaf-cutter ants of the genera *Atta* and *Acromyrmex,* their fungal food source *Leucoagaricus gongylophorus*, and the fungal gardens against the attach of pathogens (Andersen et al. 2015). Disruption of this stability would result in the attack by the fungal pathogens of the genus *Escovopsis* ensuing the compact garden biomass and the ultimate demolition of total ant population (Currie et al. 2003). The natural products produced by the symbiotic actinomycetes are utmost important in the protection of the wellbeing of the leaf-cutter ant populations by preventing *L. gongylophorus* from pathogenic infection.

Actinobacteria, which belong mainly to the genera *Pseudonocardia* and *Streptomyces*, are in direct connection with the cuticles of leaf-cutter ants. These actinobacteria synthesize a set of antifungal compounds such as candicidin, antimycins, nystatin, and dentigerumycin variations that inhibit *Escovopsis* and additional potential pathogens by leaving *L. gongylophorus* intact (Dângelo et al. 2016). An antifungal associated to dentigerumycin and known as gerumycins was produced by *Pseudonocardia* spp. related to ants *Trachymyrmex cornetzi* and *Apterostigma dentigerum.* (Sit et al. 2015). As part of this complex ecological niche, actinobacteria that live in symbiosis with the leaf-cutter ant produce molecules that help to keep away closely related bacteria that can replace the inhabiting strain. The stronger this antagonism among the various species of ant-associated *Pseudonocardia* was, the greater the phylogenetic distance from the *Pseudonocardia* living in symbiosis with the leaf-cutter ant, suggesting that the differences originally developed from the same evolutionary origin. In fact, *Pseudonocardia* strain is involved in the synthesis of rebeccamycin analog, which inhibits the development of competing *Pseudonocardia* (Van Arnam et al. 2015).

14.5.2 Biomolecules Protecting Beewolf Wasps Offsprings

The streptomycete *Candidatus Streptomyces philanthi* lives in a symbiotic association within the antennal glands of the female beewolf digger wasps (*Philanthus* spp., *Hymenoptera*, *Crabronidae*) (Kaltenpoth et al. 2005). This bacterial symbiont is stored against the internal walls of the defensive burrows on which they lay eggs. The streptomycetes can be combined with silk while the larvae are spinning the cocoons, which remain fixed within the humid burrows. The streptomycetes protect cocoons by producing nine antimicrobial compounds to the outer surface to protect against the bacterial and fungal pathogens, which live in soil or are carried to the burrow by honeybees bringing food for the larvae.

Three of the important bioactive compounds in situ were piericidin A_1, piericidin B_1, and streptochlorin, which were uniformly distributed on the cocoon outer surface (Kroiss et al. 2010). The distribution of antimicrobials in higher concentrations outside than inside the cocoon suggests that the bioactive compounds protect from invasive pathogens present in outside environment. Four antimicrobials were inhibitory to the growth of ten soil microorganisms, including fungi *Metarhizium* and *Aspergillus*, which can cause an infection in the cocoons (Kroiss et al. 2010). In situ examination indicated that the insect related streptomycetes, and piericidin A_1 and B_1 were localized together (Kaltenpoth et al. 2016). The streptomycetes protect cocoons by producing nine antimicrobial compounds to the outer surface to protect the beewolf cocoons in situ from possible microbial infections, and ensure offspring viability. The unique system is prominent as it is one of the rare instances where the antimicrobials from actinobacteria have been imagined in situ. The chemical and genetic manipulation of the system could be one of the excellent opportunities to assess the direct effect of bioactive compounds produced by the actinobacteria at a natural environment in situ. It will be a challenge to predict what the likely impact of the actinomycetes antimicrobial compounds would be at the ecosystem level.

14.5.3 Specialized Metabolism in Rhizosphere

The remarkably high number of actinobacteria in the rhizosphere can have a huge impact on plant health, as soil actinobacteria synthesize various kinds of natural products that would bring about protection against the pathogenic microorganisms and supports plants in nutrient absorption (Harikrishnan et al. 2014, Hirsch and Mauchline 2012).

Streptomycetes colonizing plant roots are potential producers of antimicrobial compounds, such as 3-acetonylidene-7-prenylindolin-2one, antimycin A18, diastaphenazine, and staurosporine, the in situ investigations of which provide the observation of molecular interactions under natural rhizospheric conditions, as well as the development of plant health and disease resistance through their understanding (Li et al. 2015). Studies have revealed that rhizosphere actinobacteria have a

huge impact on health and disease tolerance abilities. For instance, when the endophytic actinobacteria, *Micromonospora chalcea*, *Streptomyces spiralis*, or *Actinoplanes campanulatus* were inoculated alone or in a mixture to cucumber (*Cucumis sativus*), the adverse effects of the soilborne fungal phytopathogen *Pythium aphanidermatum* were attenuated, such as crown and root rot, and the plant was exclusively healthier (El-Tarabily et al. 2009). In another case, the prodiginines produced by *Streptomyces lividans* protected *Arabidopsis thaliana* in the root rhizosphere from the deleterious effects of the pathogen *Verticillium dahliae* fungi (Meschke et al. 2012). Similar results have also been obtained in other different plant systems, where by inoculating actinobacteria into the roots, the plant was protected from the invasion of harmful phytopathogens. Such examples of the ability of rhizosphere actinomycetes to produce metabolites and provide protection against antagonistic phytopathogens emphasis the importance of actinobacteria for plant welfare in primary production.

Soil actinobacteria are also recognized as plant health enhancers through synthesizing the plant phytohormones like IAA (indole-3-acetic acid) and IPYA (indole-3pyruvic acid), which regulate essential functions while maintaining coordinated cell growth and gene regulation at least in wheat, lettuce, rye and tomato (Subramaniam et al. 2016). These metabolites have been particularly indicated to be produced by endophytic acinomycetes from the genera *Actinoplanes*, *Micromonospora*, and *Nocardiopsis* to promote health plant growth and development (Toumatia et al. 2016). The distinction of microbially and plant produced phytohormones is theoretically challenging, and must be done in in vitro experiments. Both actinobacteria and plants can also produce siderophores, which are important in iron uptake. The siderophores produced by actinobacteria in the rhizosphere or directly in the roots can help the plant to collect iron from the surrounding soil, and thus improve the efficiency of iron-related metabolism. Soil *Streptomyces* species can also promote the growth of critical symbiotic nitrogen fixing bacteria, such as *Rhizobiales*, and aid in root nutrient uptake, thus contributing to the overall efficiency of plants nutrient scavenging activity. The symbiotic *Rhizobiales* clade bacteria can stimulate the production of leguminous plant root nodules, where the bacteria fix atmospheric nitrogen to plants as a nutrient. The inoculation of *Streptomyces* sp. into chickpea plant enhanced nodulation as well as increased nodule size, resulting in an inclusive stimulating effect on plant nitrogen uptake rate (Gopalakrishnan et al. 2015). In general, nitrogen is a nutrient limiting plant growth, emphasing the importance of actinobacteria in the overall efficiently of nutrient uptake.

14.5.4 Plant Growth Promoting Activity

Actinobacteria in symbiosis with plants and rhizosphre bacteria protect against microbial plant pathogens and improve nutrient availability, as described above. Besides these, actinomycetes are also recognized for their insecticidal activities,

thereby preventing plant pathogenesis, and improving plant growth. In addition to being involved in the binding of iron by siderophores, actinomycetes regulate the oxidation stage of iron, which is bioavailable to the plant growth in the reduced form of Fe^{2+}, whereas the oxidized form (Fe^{3+}) is common under alkaline soil conditions. The alkaliphilic actinomycetes can reduce the iron from Fe^{3+} to a soluble form of Fe^{2+} that is boavailable to the plant species and microbes (Francis et al. 2010; Valencia-Cantero et al. 2007). For example, *Kocuria rosea* HN01 can reduce Fe^{3+} to the soluble Fe^{2+} form, which is available for plants adapted to alkaline soils (Wu et al. 2014). In addition to iron, actinomycetes are also able to solubilize phosphorus under alkaline circumstances, the solubility of which reduces in alkaline or acidic soils (Palaniyandi et al. 2013).

14.5.5 Oxido-reduction of Humic Substances

The oxido-reduction of humic substances has a great impact throughout the biotransformation of organic and inorganic pollutants. In humic acids, the quinone moieties act as centers for oxidation and reduction reactions that have a great impact throughout the anaerobic biotransformation of organic and inorganic pollutants. The oxidized humic acids accept electrons released from the organic pollutant mineralization, while the reduced humic acid is involved in biotransformation by reducing insoluble, oxidized pollutants to the soluble, reduced form. The reduced form of humic acid can also reduce the insoluble Fe^{3+} to the soluble Fe^{2+} form that is bioavailable for plant assimilation. *Corynebacterium humireducens* is an alkaliphilic actinobacterium known for its ability to biotransform humic acids into a reduced form, as well as the reduction of quinones to hydroquinones, which improve pollutant mineralization, like that of 2,4-dichlorophenoxy acetic acid (Wu et al. 2011; Wang et al. 2009).

14.5.6 Bioweathering

The process of bioweathering involves the microbially mediated fragmentation of the rock constituents into smaller fragments during decay, decomposition or erosion. These substances are additionally broken into a mobilized form of the elements (e.g., Na, K, Mg, Ca, Mn, Fe, Cu, Zn, Co, and Ni) and essential nutrients (e.g., P and S).

The microbial communities of especially actinobacteria and bacteria survive in the rocks under desiccation, radiation, and nutritional depletion conditions, and particularly the filamentous microorganisms can promote the bioweathering process through invading the rocks by producing mycelia. The *Streptomyces* species with the filamentous structure and anthrospore formation ability can grow under oligotrophic conditions in rocks, utilize recalcitrant organic materials, and release rock

contituents for trasport to fields through water or wind. Other sites where actinobacteria have been associated with the bioweathering phenomenon include volcanic rocks, Mediterranean stones and monuments, and soil. *Knoellia*, *Rhodococcus*, *Arthrobacter*, *Kribbella*, and *Brevibacterium* species have been isolated from volcanic rocks in Iceland (Cockell et al. 2013). The species from the genera *Blastococcus*, *Modestobacter*, and *Geodermatophilus* (*Geodermatophilaceae* family) have been involved in the bioweathering of Mediterranean area stones and monuments (Urzì et al. 2001). Other actinomycete species associated with the acceleration of bioweathering are from the genera *Nocardioides*, *Kibdelosporangium* (Abdulla 2009), *Arthrobacter*, and *Leifsonia* (Frey et al. 2010). Besides these, alkalitolerant actinobacterial soil isolates from Nanjing (China), *Arthrobacter nanjingensis* A33T and *Isoptericola nanjingensis* H17T, have potential to carry out biowethering of rocks (Huang et al. 2015, Huang et al. 2012).

14.5.7 Gold Nanoparticle Synthesis

In the gold nanoparticle synthesis, Au^{3+} is reduced in incubation with gold chloride and microorganisms, either intracellularly of extracellularly (Beveridge and Murray 1980). The prokaryotes are preferred in the nanoparticle sythesis as they tolerate high metal concentrations in producing large quantities of nanopartilces. The synthesis of nanoparticles by actinomycetes has the extra advantage of polydispersity properties that prevents the self-aggregation. Of the actinobacteria at least *Thermomonospora* sp. (Ahmad et al. 2003a) and alkali-resistant *Rhodococcus* sp. (Ahmad et al. 2003b) have been used in investigations on the gold nanoparticle synthesis. Nonetheless, prokaryotic bacteria and actinobacteria, as well as eukaryotic yeasts, fungi, and algae have all been explored for nanoparticle synthesis. The gold nanoparticles play a vital role in various applications for diagnostic, therapeutic, and catalytic applications. The exploration of actinobacteria is much required for considering their role in creating the sustainable environment (Ahmad et al. 2003b; Prabhu et al. 2015; Khieu et al. 2015; Manikprabhu et al. 2016; Wang et al. 2017).

14.6 Conclusion

In this chapter, environmental actinobacteria were examined in air and in different types of soils, including acidic, saline, and cold soils, as well as in the composting of plant biomass, forest industry waste, and hazardous material. Further, the importance of actinobacterial bioactive compounds in protecting fungus-farming ants, beewolf wasps offspring, and plants in rhizosphere was specifically examined. Actinobacteria with mycelial growth were found to be important in promoting plant growth by enabling nutrient availability, oxidation and reduction reactions of humic substances, dissolution of minerals in bioweathering, and synthesis of gold

nanoparticles. Phylogenetic and metagenomic studies have revealed that there are a huge number of poorly characterized environmental actinobacteria with completely unknown sequences in their genomes, indicating that a huge number of completely untapped biological resources are still available. The environmental applications presented are only a small part of the broad potential functions that actinobacteria have as producers of various enzymes and biologically active compounds, as well as biological activity that is beneficial to other living organisms in the environment.

References

Abdulla H (2009) Bioweathering and biotransformation of granitic rock minerals by actinomycetes. Microbiol Ecol 58:753–761. https://doi.org/10.1007/s00248-0099549-1

Aguelmous A, El Fels L, Souabi S, Zamama M, Hafidi M (2019) The fate of total petroleum hydrocarbons during oily sludge composting: a critical review. Rev Environ Sci Biotechnol 18:473–493. https://doi.org/10.1007/s11157-019-09509-w

Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003a) Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycetes *Thermomonospora* spp. Langmuir 19:3550–3553. https://doi.org/10.1021/la026772l

Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003b) Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete *Rhodococcus* species. Nanotechnology 14:824–828. https://doi.org/10.1088/0957-4484/14/7/323

Andersen SB, Yek SH, Nash DR, Boomsma JJ (2015) Interaction specificity between leaf-cutting ants and vertically transmitted *Pseudonocardia* bacteria. BMC Evol Biol 15:27. https://doi.org/10.1186/s12862-015-0308-2

Atchley S, Clark JB (1979) Variability of temperature, pH and moisture in an aerobic composting process. Appl Environ Microbiol 38:1040–1044

Barthod J, Rumpel C, Dignac M-F (2018) Composting with additives to improve organic amendments. A review. Agron Sustain Dev 38:17. https://doi.org/10.1007/s13593-018-0491-9

Beveridge TJ, Murray RGE (1980) Site of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol 141:876–887

Cockell CS, Kelly LC, Marteinsson V (2013) Actinobacteria – an ancient phylum active in volcanic rock weathering. Geomicrobiol J 30:706–720. https://doi.org/10.1080/01490451.2012.758196

Currie CR, Scott JA, Summerbell RC, Malloch D (1999) Fungus growing ants use antibiotic-producing bacteria to control garden parasites. Nature 398:701–704. https://doi.org/10.1038/19519

Currie CR, Wong B, Stuart AE, Schultz TR, Rehner SA, Mueller UG et al (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. Science 299:386–388. https://doi.org/10.1126/science.1078155

Dângelo RAC, de Souza DJ, Mendes TD, Couceiro J, da C, Lucia TMCD (2016) Actinomycetes inhibit filamentous fungi from the cuticle of *Acromyrmex* leafcutter ants. J Basic Microbiol 56:229–237. https://doi.org/10.1002/jobm.201500593

Dobrovol'skaya TG, Golovchenko AV, Zvyagintsev DG (2014) Analysis of ecological factors limiting the destruction of high-moor peat. Eurasian Soil Science 47:182–193

El-Tarabily KA, Nassar AH, Hardy GESJ, Sivasithamparam K (2009) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J Appl Microbiol 106:13–26. https://doi.org/10.1111/j.1365-2672.2008.03926.x

European Parliament and Council (2009) Health rules as regards animal by-products and derived products not intended for human. European Parliament and Council consumption 1069/2009

European Parliament and Council (2019) Rules on the making available on the market of EU fertilising products and amending. European Parliament and Council regulations 1069/2009

Francis I, Holsters M, Vereecke D (2010) The gram-positive side of plant microbe interactions. Environ Microbiol 12:1–12. https://doi.org/10.1111/j.14622920.2009.01989.x

Frey B, Rieder SR, Brunner I, Plötze M, Koetzsch S, Lapanje A et al (2010) Weathering-associated bacteria from the Damma Glacier Forefield: physiological capabilities and impact on granite dissolution. Appl Environ Microbiol 76:4788–4796. https://doi.org/10.1128/AEM.00657-10

Gochnauer MB, Leppard GG, Komaratat P et al (1975) Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. J Microbiol 21:1500–1511

Gopalakrishnan S, Srinivas V, Alekhya G, Prakash B, Kudapa H, Rathore A et al (2015) The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea. SpringerPlus 4:31. https://doi.org/10.1186/s40064-015-0811-3

Harikrishnan H, Shanmugaiah V, Balasubramanian N (2014) Optimization for production on indole acetic acid (IAA) by plant growth promoting *Streptomyces* sp. VSMGT1014 isolated from rice rhizosphere. Int J Curr Microbiol Appl Sci 3:158–171

Haq I, Mazumder P, Kalamdhad AS (2020) Recent advances in removal of lignin from paper industry wastewater and its industrial applications – a review. Bioresour Technol 312:123636. https://doi.org/10.1016/j.biortech.2020.123636

He JQ, Wu YF, Zhang GJ (2006) Activity and ecological distribution of actinomycetes from soil in the southeastern of Tibet. Wei Sheng Wu Xue Bao 46(5):773–780

Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? Nat Biotechnol 30:961–962. https://doi.org/10.1038/nbt.2387

Hirvonen M-R, Nevalainen A, Makkonen M, Mönkkönen J, Savolainen K (1997) Streptomyces spores from mouldy houses induce nitric oxide, TNF_x and IL-6 secretion from RAW264.7 macrophage cell line without causing subsequent cell death. Environ Toxicol Pharmacol 3:57–63

Hirvonen M-R, Suutari M, Ruotsalainen M, Lignell U, Nevalainen A (2001) Effect of growth medium on potential of Streptomyces anulatus spores to induce inflammatory responses and cytotoxicity in RAW264-7 macrophages. Inhal Toxicol 13:55–68

Huang Z, Sheng XF, Zhao F, He LY, Wang HJ (2012) *Isoptericola nanjingensis sp.* nov., a mineral-weathering bacterium. Int J Syst Evol Microbiol 62:971–976. https://doi.org/10.1099/ijs.0.033043-0

Huang Z, Bao YY, Yuan TT, Wang GX, He LY, Sheng XF (2015) *Arthrobacter nanjingensis* sp. nov., a mineral-weathering bacterium isolated from forest soil. Int J Syst Evol Microbiol 65:365–369. https://doi.org/10.1099/ijs.0.069492-0

Imam A, Kanaujia PK, Ray A, Suman SK (2021) Removal of petroleum contaminants through bioremediation with integrated concepts of resource recovery: a review. Indian J Microbiol 61:250–261. https://doi.org/10.1007/s12088-021-00928-4

Jayachandra SY, Parameshwar AB, Mohan Reddy K, Sulochana MB (2012a) Characterization of extracellular hydrolytic enzymes producing extremely halophilic bacterium *Virgibacillus* sp. JS5. World J Sci Technol 2(2):23–26

Jayachandra SY, Anil Kumar S, Merley DP, Sulochana MB (2012b) Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. Recent Res Sci Technol 4(4):46–49

Kaltenpoth M, Göttler W, Herzner G, Strohm E (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. Curr Biol 15:475–479. https://doi.org/10.1016/j.cub.2004.12.084

Kaltenpoth M, Strupat K, Svatoš A (2016) Linking metabolite production to taxonomic identity in environmental samples by (MA)LDI-FISH. ISME J 10:527–531. https://doi.org/10.1038/ismej.2015.122

Kämpfer P, Rainey FA, Andersson MA et al (2000) *Frigoribacterium Faeni* gen. nov., sp. nov., a novel psychrophilic genus of the family Micromonosporaceae. Int J Syst Evol Microbiol 1:355–363

Karimi K (2015) Biofuel and biorefinery technologies, vol. 1—Lignocellulose based bioproducts. Springer, Berlin

Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr Microbiol 57:503–507. https://doi.org/10.1007/s00284-008-9276-8

Khan MR, Williams ST (1975) Studies on the ecology of actinomycetes in soil: VIII. Distribution and characteristics of acidophilic actinomycetes. Soil Biol Biochem 7:345–349

Khieu T-N, Liu M-J, Nimaichand S, Quach N-T, Chu-Ky S, Phi Q-T, Thu-Trang V, Nguyen T-D, Xiong Z, Manikprabhu D, Jun-Li W (2015) Characterization and evaluation of antimicrobial and cytotoxic effects of *Streptomyces* sp. HUST012 isolated from medicinal plant *Dracaena cochinchinensis* Lour. Front Microbiol 6:574. https://doi.org/10.3389/fmicb.2015.00574

Kiiskinen L-L, Rättö M, Kruus K (2004) Screening for novel laccase-producing microbes. J Appl Microbiol 97:640–646. https://doi.org/10.1111/j.1365-2672.2004.02348.x

Kim SB, Seong CN, Jeon SJ et al (2004) Taxonomic study of neutro tolerant acidophilic actinomycetes isolated from soil and description of *Streptomyces yeochonensis* sp. nov. Int J Syst Evol Microbiol 54:211–214

Kroiss J, Kaltenpoth M, Schneider B, Schwinger M-G, Hertweck C, Maddula RK et al (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. Nat Chem Biol 6:261–263. https://doi.org/10.1038/nchembio.331

Kumar A, Srivastava NK, Gera P (2021) Removal of color from pulp and paper mill wastewater – methods and techniques – a review. J Environ Manag 298:113527. https://doi.org/10.1016/j.jenvman.2021.113527

Kutzner HJ (1986) The family Streptomycetaceae. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. A handbook on habitats, isolation, and identification of bacteria, vol 2. Springer-Verlag, New York, pp 2028–2090

Lacey J, Crook B (1988) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. Ann Occup Hyg 32:515–533

Lacey J, Dutkiewicz J (1976) Isolation of actinomycetes and fungi from mouldy hay using a sedimentation chamber. J Appl Bacteriol 41:315–319

Li WJ, Park SK, Tank SK et al (2004) *Nocardiopsis salina* sp. nov., a novel halophilic actinomycete isolated from saline soil in China. Int J Syst Evol Microbiol 54(5):1805–1809

Li WJ, Zhang YG, Zhang YQ et al (2005) *Streptomycetes sodiiphilus* sp. nov., a novel alkaliphilic actinomycete. Int J Syst Evol Microbiol 55:1329–1333

Li Y, Han L, Rong H, Li L, Zhao L, Wu L et al (2015) Diastaphenazine, a new dimeric phenazine from an endophytic *Streptomyces diastaticus* subsp. *ardesiacus*. J Antibiot (Tokyo) 68:210–212. https://doi.org/10.1038/ja.2014.124

Limaye L, Patil R, Ranadive P, Kamath G (2017) Application of potent actinomycete strains for bio-degradation of domestic agro-waste by composting and treatment of pulp-paper mill effluent. Adv Microbiol 7:94–108. https://doi.org/10.4236/aim.2017.71008

Lipson DA, Schmidt SK (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky mountains. Appl Environ Microbiol 70:2867–2879. https://doi.org/10.1128/AEM.70.5.2867–2879.2004

Lloyd AB (1969) Dispersal of streptomyces in air. J Gen Microbiol 57:35–40

Ma C, Lo PK, Xu J, Li M, Jiang Z, Li G, Zhu Q, Li X, Leong SY, Li Q (2020) Molecular mechanisms underlying lignocellulose degradation and antibiotic resistance genes removal revealed via metagenomics analysis during different agricultural wastes composting. Bioresour Technol 314:123731

Madelin TM, Johnson HE (1992) Fungal and actinomycete spore aerosols measured at different humidities with an aerodynamic particle sizer. J Appl Bacteriol 72:400–409

Maldonado LA, Fenical W, Jensen PR, Kauffman CA, Mincer TJ, Ward AC, Bull AT, Goodfellow M (2005) Salinispora arenicola gen. nov., Sp. nov. and Salinispora tropica sp. nov., obligate marine actinomycetes belonging to the family Micromonosporacease. Int J Syst Evol Microbiol 55:1759–1766. https://doi.org/10.1099/ijs.0.63625-0

Manikprabhu D, Cheng J, Chen W, Sunkara AK, Mane SB, Kumar R, Hozzein WN, Duan Y-Q, Li W-J (2016) Sunlight mediated synthesis of silver nanoparticles by a novel actinobacterium (*Sinomonas mesophila* MPKL 26) and its antimicrobial activity against multi drug resistant *Staphylococcus aureus*. J Photochem Photobiol B Biol 158:202–205. https://doi.org/10.1016/j.jphotobiol.2016.01.018

Männistö MK, Schumann P, Rainey FA, Kämpfer P, Tsitko I, Tiirola MA, Salkinoja-Salonen MS (2000) *Subtercola boreus* gen. nov., sp. nov. and *Subtercola frigoramans* sp. nov., two new psychrophilic actinobacteria isolated from boreal groundwater. International Journal of Systematic and Evolutionary Microbiology 50:1731–1739

Meddeb-Mouelhi F, Moisan JK, Beauregrd M (2014) A comparison of plate assay methods for detecting extracellular cellulose and xylanase activity. Enzym Microb Technol 66:16–19. https://doi.org/10.1016/j.enzmictec.2014.07.004

Meschke H, Walter S, Schrempf H (2012) Characterization and localization of prodiginines from *Streptomyces* lividans suppressing *Verticillium dahliae* in the absence or presence of *Arabidopsis thaliana*. Environ Microbiol 14:940–952. https://doi.org/10.1111/j.1462-2920.2011.02665.x

Mevs U, Stackebrant E, Schuman P et al (2000) *Modestobacter multifepatus* gen. nov., sp. nov., a budding actinomycete from soil of the Asgard Range (Transantarctic Mountains). Int J Syst Evol Microbiol 50(1):337–373

Mohan Reddy K, Siva Deepthi S, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867

Nagar S, Mittal A, Gupta VK (2012) A cost effective method for screening and isolation of xylan degrading bacteria using agro waste material. Asian J Biol Sci. https://doi.org/10.3923/ajbs.2012

Nakajima Y, Kitpreechevanich V, Suzuki K, Kudo T (1999) *Microbispora coralline* sp. nov., a new species of genus microbispora isolated from Thai soil. Int J Syst Evol Microbiol 49:1761–1767

Nevalainen A, Pasanen A-L, Niininen M, Reponen T, Jantunen MJ, Kalliokoski P (1991) The indoor air quality in Finnish homes with mold problems. Environ Int 17:299–1302

Nielsen EM, Breum NO, Nielsen BH, Würtz H, Poulsen OM, Midtgaard U (1997) Bioaerosol exposure in waste collection: a comparative study on the significance of collection equipment, type of waste and seasonal variation. Ann Occup Hyg 41:325–344

Nioh I, Osada M, Yamamura T, Muramatsu K (1995) Acidophilic and acidotolerant actinomycetes in an acid tea field soil. J Gen Appl Microbiol 41(2):175–180

Palaniyandi SA, Yang SH, Zhang L, Suh JW (2013) Effects of actinobacteria on plant disease suppression and growth promotion. Appl Microbiol Biotechnol 97:9621–9636. https://doi.org/10.1007/s00253-0135206-1

Partanen P, Hultman J, Paulin L, Auvinen P, Romantschuk M (2010) Bacterial diversity at different stages of the composting process. BMC Microbiol 10:94. https://doi.org/10.1186/1471-2180-10-94

Prabahar V (2004) *Pseudonocardia antarctica* sp. nov., an actinomycete from McMurdo Dry Valleys, Antarctica. Syst Appl Microbiol 27:66–71

Prabhu DM, Quadri SR, Cheng J, Liu L, Chen W, Yang Y, Hozzein WN, Lingappa K, Li W-J (2015) *Sinomonas mesophila* sp. nov., isolated from ancient fort soil. J Antibiot 68:318–321

Rainisalo A, Romantschuk M, Kontro MH (2011) Evolution of clostridia and streptomycetes in full-scale composting facilities and pilot drums equipped with on-line temperature monitoring and aeration. Bioresour Technol 102:7975–7983. https://doi.org/10.1016/j.biortech.2011.05.087

Rasul F, Afroz A, Rashid U, Mehmood S, Sughra K, Zeeshan N (2015) Screening and characterization of cellulose producing bacteria from soil and waste (molasses) or sugar industry. Int J Biosci 6:230–238. https://doi.org/10.12692/ijb/6.3.230-238

Reponen TA, Gazenko SV, Grinshpun SA, Willeke K, Cole EC (1998) Characteristics of airborne actinomycete spores. Appl Environ Microbiol 64:3807–3812

Reponen T, Willeke K, Ulevicius V, Grinshpun SA, Donnelly J (1997) Techniques for dispersion of microorganisms into air. Aerosol Sci Technol 27:405–421

Reyes-Torres M, Oviedo-Ocaña ER, Dominquez I, Komilis D, Sánchez A (2018) A systematic review on the composting of green waste: feedstock quality and optimization strategies. Waste Manag 77:486–499. https://doi.org/10.1016/j.wasman.2018.04.037

Rintala H, Nevalainen A, Rönkä E, Suutari M (2001) PCR primers targeting the 16S rRNA gene for the specific detection of streptomycetes. Mol Cell Probes 15:337–347. https://doi.org/10.1006/mcpr.2001.0379

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349–410

Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG, Hoekstra ES (eds) (1994) Health implications of fungi in indoor environments. Air quality monographs, vol. 2. Elsevier, Amsterdam

Sayara T, Sánchez A (2020) Bioremediation of PAH-contaminated soils: process enhancement through composting/compost. Appl Sci 10:3684

Selyanin VV, Oborotov GV, Zenova GM, Zvyagintsev DG (2005) Soil alkalophilic actinomycetes. Mikrobiologiya 74(6):838–844

Shanmugapriya K, Saravana PS, Krishnapriya MM, Mythili A, Joseph S (2012) Isolation, screening and partial purification of cellulose from cellulose producing bacteria. Int J Adv Biotechnol Res 3:509–514

Sit CS, Ruzzini AC, Arnam EBV, Ramadhar TR, Currie CR, Clardy J (2015) Variable genetic architectures produce virtually identical molecules in bacterial symbionts of fungus-growing ants. Proc Natl Acad Sci U S A 112:13150–13154. https://doi.org/10.1073/pnas.1515348112

Stewart SL, Grinshpun SA, Willeke K, Terzieva S, Ulevicius V, Donnelly J (1995) Effect of impact stress on microbial recovery on an agar surface. Appl Environ Microbiol 61:1232–1239

Subramaniam G, Arumugam S, Rajendran V (2016) Plant growth promoting actinobacteria: a new avenue for enhancing the productivity and soil fertility of grain legumes. Springer, Singapore

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54 (5):418–424. https://doi.org/10.1002/jobm.201200770

Sundberg C, Yu D, Franke-Whittle I, Kauppi S, Smårs S, Insam H, Romantschuk M, Jönsson H (2013) Effects of pH and microbial composition on odour in food waste composting. Waste Manag 33:204–211. https://doi.org/10.1016/j.wasman.2012.09.017

Suutari M, Rönkä E, Lignell U, Rintala H, Nevalainen A (2002) Characterisation of Streptomyces spp. isolated from water-damaged buildings. FEMS Microbiol Ecol 39:77–84

Thormann MN, Bayley SE, Currah RS (2004) Microcosm tests of the effect of temperature and microbial species number on the decomposition of *Carexaquatilis* and *Sphagnum fuscum* litter from southern boreal peatlands. Can J Microbiol 50:793–802

Toumatia O, Compant S, Yekkour A, Goudjal Y, Sabaou N, Mathieu F et al (2016) Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. South Afr J Bot 105:234–239. https://doi.org/10.1016/j.sajb.2016.03.020

Tresner HD, Yayes JS, Backus EY (1968) Differential tolerance of streptomycetes to sodium chloride as a taxonomic tool. Appl Microbiol 16:1134–1136

Tuomela M, Vikman M, Hatakka A, Itavaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183. https://doi.org/10.1016/S0960-8524(99)00104-2

Unuofin JO, Okoh AI, Nwodo UU (2019) Recovery of laccase-producing gammaproteobacteria from wastewater. Biotechnol Rep 21:e00320. https://doi.org/10.1016/j.btre.2019.e00320

Urzì C, Brusetti L, Salamone P, Sorlini C, Stackebrandt E, Daffonchio D (2001) Biodiversity of *Geodermatophilaceae* isolated from altered stones and monuments in the Mediterranean basin. Environ Microbiol 3:471–479. https://doi.org/10.1046/j.1462-2920.2001.00217.x

Valencia-Cantero E, Hernandez-Calderon E, Velazquez-Becerra C, Lopez-Meza JE, Alfaro-Cuevas R, Lopez-Bucio J (2007) Role of dissimilatory fermentative iron-reducing bacteria in Fe uptake by common bean (*Phaseolus vulgaris* L.) plants grown in alkaline soil. Plant Soil 291:263–273. https://doi.org/10.1007/s11104-007-9191-y

Van Arnam EB, Ruzzini AC, Sit CS, Currie CR, Clardy J (2015) A rebeccamycin analog provides plasmid-encoded niche defense. J Am Chem Soc 137:14272–14274. https://doi.org/10.1021/jacs.5b09794

Velasco MI, Campitelli PA, Ceppi SB, Havel J (2004) Analysis of humic acid from compost of urban wastes and soil by fluorescence spectroscopy. AgriScientia 21:31–38

Ventorino V, Pascale A, Fagnano M, Adamo P, Faraco V, Rocco C, Fiorentino N, Pepe O (2019) soil tillage and compost amendment promote bioremediation and biofertility of polluted area. J Clean Prod 239:118087. https://doi.org/10.1016/j.jclepro.2019.118087

Viswanath B, Rajesh B, Janardhan A, Kumar A, Narasimha G (2014) Fungal laccases and their applications in bioremediation. Enzyme Res 2014:163242. https://doi.org/10.1155/2014/163242

Wang YM, Zhang YS, Xu XL et al (2001) *Actinopolymorpha singaporensis* gen. nov., sp., nov., a novel actinomycete from the tropical rainforest of Singapore. Int J Syst Evol Microbiol 51:467–473

Wang YM, Cao GM, Jiang WB, Zhang YS (2004) Study on actinomycetes population of alpine meadow soil in Qinghai. Wei Sheng Wu Xue Bao 44(6):733–736

Wang YB, Wu CY, Wang XJ, Zhou SG (2009) The role of humic substances in the anaerobic reductive dechlorination of 2,4-dichlorophenoxyacetic acid by *Comamonas koreensis* strain CY01. J Hazard Mater 164:941–947. https://doi.org/10.1016/j.jhazmat.2008.08.097

Wang H-F, Li Q-L, Xiao M, Zhang Y-G, Zhou X-K, Rao MPN, Duan Y-Q, Li W-J (2017) *Streptomyces capparidis* sp. nov., a novel endophytic actinobacterium isolated from fruits of *Capparis spinosa* L. Int J Syst Evol Microbiol 67:133–137. https://doi.org/10.1099/ijsem.0.001586

Wei Y, Li J, Shi D, Liu G, Zhao Y, Shimaoka T (2017) Environmental challenges impending the composting of biodegradable municipal solid waste: a critical review. Resour Conserv Recycl 122:51–65. https://doi.org/10.1016/j.resconrec.2017.01.024

Williams ST, Sharples GP, Bradshaw RM (1973) The fine structure of the actinomycetales. In: Sykes G, Skinner FA (eds) Actinomycetales: characteristics and practical importance. Academic Press, New York, pp 113–130

Wu C-Y, Zhuang L, Zhou S-G, Li F-B, He J (2011) *Corynebacterium humireducens* sp. nov., an alkaliphilic, humic acid-reducing bacterium isolated from a microbial fuel cell. Int J Syst Evol Microbiol 61:882–887

Wu CY, Chen N, Li H, Li QF (2014) *Kocuria rosea* HN01, a newly alkaliphilic humus-reducing bacterium isolated from cassava dreg compost. J Soils Sediments 14:423–431. https://doi.org/10.1007/s11368-013-0679-1

Xu L-H, Li Q-R, Jiang C-L (1996) Diversity of soil actinomycetes in Yunnan, China. Appl Environ Microbiol 62:244–248

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi J, Shoba H, Banapurmath N, Hunashyal A, Sulochana M, Shettar A, Ganachari S (2018) Alternative and renewable bio-based and biodegradable plastics. In: Martínez LMT et al (eds)

Handbook of ecomaterials. Springer International Publishing AG, Cham. https://doi.org/10.1007/978-3-319-68255-6_150

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Yoon J-H, Lee C-H, Oh T-K (2005) Nocardioides dubius sp. nov., isolated from an alkaline soil. Int J Syst Evol Microbiol 52:2209–2212. https://doi.org/10.1099/ijs.0.63748-0

Yoon J-H, Lee C-H, Oh T-K (2006) *Nocardioides lentus* sp. nov., isolated from an alkaline soil. Int J Syst Evol Microbiol 56:271–275. https://doi.org/10.1099/ijs.0.63993-0

Yu H, Zeng G, Huang H, Xi X, Wang R, Huang D, Huang G, Li J (2007) Microbial community succession and lignocellulose degradation during agricultural waste composting. Biodegradation 18:793–802. https://doi.org/10.1007/s10532-007-9108-8

Zakalyukina YV, Zenova GM, Zvyagintsev DG (2002) Acidophilic soil actinomycetes. Microbiology 71:342–345

Zenova GM, Zakalyukina YV, Selyanin VV, Zvyagintsev DG (2004) Isolation and growth of acidophilic soil actinomycetes from the *Micromonospora* genus. Pochvovedenie 7:847–852. [Eur. Soil Sci. 37 (7), 737–742 (2007)]

Zenova GM, Glushkova NA, Bannikov MV, Pozdnyakov AI (2006) Actinomycete complex as an index of peat soil transformation under the effect of agricultural development and degradation processes. Vestn Mosk Univ Ser. 17 Pochvoved 4:25–28

Zenova GM, Glushkova NA, Bannikov MV et al (2008) Actinomycetal complexes in drained peat soils of the Taiga Zone upon pyrogenic succession. Pochvovedenie 4:441–447. [Eur. Soil Sci. 41 (4), 394–399 (2008)]

Zenova GM, Kozhevin PA, Manucharova NA, Dubrova MS, Zvyagintsev DG (2012) Temperature as a factor of development of psychrotolerant mycelial bacteria complexes in soils of north regions. Biol Bull 39:416–422

Chapter 15
Biotechnological Importance of Actinomycetes

Merja H. Kontro, Jayachandra S. Yaradoddi, Nagaraj R. Banapurmath, Sharanabasava V. Ganachari, and Basavaraj S. Hungund

Abstract Microorganisms, mainly bacteria and fungi, contributed to the majority of the enzymes produced by industries. Among enzymes, production of hydrolases itself shares around 75% of the commercially available enzymes. Hence, they are in high demand. Actinomycetes are important groups of organisms, and they can be primarily harnessed for commercial production of nutraceuticals, pharmaceuticals, antitumor agents, enzymes, enzyme inhibitors, and chemotherapeutic agents. At present, the market value of enzymes in the food and beverage industries is 5.6 billion USD, and one of the reports estimated that by 2024 the global enzyme market possibly exceeds up to 9.63 billion USD. Enzymes carry extremely interesting thermostability properties and good activity in a wide range of pH, and their solvent tolerance potentials made it a clear choice for industrial processes. These special properties of the enzymes have turned beneficial in terms of medical and biotechnological perceptions. The enzymes such as kinases, nucleases, and polymerases produced by *Streptomyces*

M. H. Kontro (✉)
Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
e-mail: merja.kontro@helsinki.fi

J. S. Yaradoddi
Biomaterials Laboratory, Center of Material Science, KLE Technological University, Hubballi, Karnataka, India

Faculty of Biological and Environmental Sciences, Department – Ecology, and Environment Research Program, University of Helsinki, Lahti, Finland

N. R. Banapurmath
Biomaterials Laboratory, Centre for Material Science, School of Mechanical Engineering, KLE Technological University (Formerly Known as B.V. Bhoomaraddi College of Engineering and Technology), Hubballi, Karnataka, India

S. V. Ganachari
Center for Material Science, Advanced Research in Nanoscience & Nanotechnology, KLE Technological University, Hubballi, Karnataka, India

B. S. Hungund
Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India

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thermoviolaceus can retain their properties in adverse conditions like the presence of high detergent concentrations. Cellulase recovered using *Thermomonospora fusca* was used for degradation of cotton and Avicel. Their applications are not only limited for biotechnological perspective but also inevitable for economical production due to the use of cheap substrates like fruit peels, wheat straw, etc.

Keywords Biotechnology · Enzymes · Medicine · Nutraceuticals · Antitumor agents · Enzyme inhibitors

15.1 Introduction

The biocatalysis provides environmentally friendly solutions over the chemical processes, grows rapidly, and produces challenging outcomes as the most valued substitutes for chemical technologies. The biochemical processes are generally carried out using bioactive enzymes via biocatalysis; they are essential constituents among the biological systems. The occurrence of enzymes in typical fermentation led to the manufacturing of wine, beer, vinegar and bread, all of which were practiced even thousands of years ago. Yet, the functions of enzymes were not clear; they were considered a fundamental part of human lives since the ancient period. In the year 1914, people started using enzymes as detergents. Nevertheless, a profitable breakthrough was not achieved until 1959 onwards through description of the large-scale production of protease enzymes by the industrial giant Novozymes. Since then, because of advancements in enzymology, the enzyme industries has not only observed a huge progression, but also bettering along with the novel technology prospective (Eggleston and Vercelotti 2007).

The occurring commercial enzymes are generally derived from microbial, plant, and animal sources. The enzymes obtained from plants comprise of bromelain, papain, lipoxygenase, and ficin. Among the animal-derived enzymes are pepsin and renin. Still, the largest part of commercially sustainable enzymes can be extracted using microorganisms because of their rapid development, limited nutritional requirements, and feasible recovery processes (downstream processing). A major concern is to reach the daily requirements; there is a need of robust technology, high throughput, and economical and readily accessible biocatalyst. The research is constantly progressing for novel enzymes or their sources, and for upgrading presently available enzymes through manipulation at gene and protein level. The hunt for new enzymes from unusual environments could be an interesting opportunity that can result in high-throughput screening methods (Jayachandra et al. 2012a, b; Yaradoddi and Sulochana 2020; Yaradoddi et al. 2020a, b). Several industrially important enzymes produced by the actinomycetes are listed along with their applications (Table 15.1).

The development of novel enzymes and their physiological and chemical features, such as higher productivity; stability in extreme conditions including osmolarity, pH, and temperature; specificity; and other associated properties including

Table 15.1 Commercially important enzymes produced by actinobacteria

Name of the enzyme	Industrial applications
Amylase	• Baking
	• Detergent preparation
	• Starch industries
	• Textile industries
	• Paper and pulp
Protease	• Food industry
	• Brewing
	• Leather industries
	• Medical applications
	• Detergents
Cellulase	• Textile industry
	• Paper and pulp
	• Detergents
Xylanase	• Preparation of animal feed
	• Paper and pulp
	• Baking
Pectinase	• Textile industries
	• Beverages
Glucose oxidase	• Baking purposes
Hemicellulase	• Baking
	• Food preservation
	• Beverages
Ligninase	• Chemical
	• Food and feed
	• Fuel
Peroxidases	• Textile industries

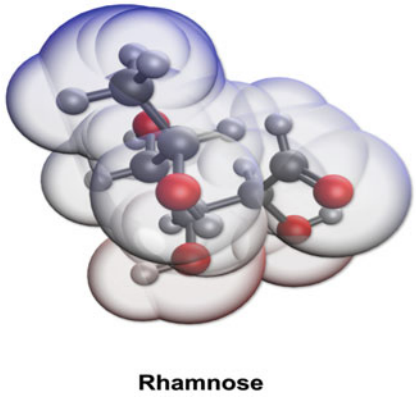

Fig. 15.1 Three-dimensional structure of rhamnose

lower production expenditure and resistance toward inhibitors are fascinating for the industrial perception. Actinobacteria utilize a broad range of compounds including polysaccharides, like rhamnose (Fig. 15.1) as substrates for their activities.

A vital measure in examining enzymes obtained using microbial origin remains distinguished by GRAS (generally regarded as safe) status (Sewalt et al. 2016). Several microorganisms, specifically fungi and bacteria, are presently used to produce different kinds of industrial enzymes. The class of hydrolases alone contributes over 75% of the commercially available enzymes and, thus, regularly has an enormous demand. They are mainly utilized as a crude form in order to construct the process sustainable so as to reach the demand of enzyme on a larger scale. The protease enzymes acquire the largest portion among the hydrolase class with a market share of about 60%, due to their broad applications in detergent industries. The enzymes required by starch industries contribute the second largest share, and are used in bakery, food, textile, and feeder industries (Ravindran et al. 2018; Hamza 2017). Commercial applications of enzymes produced by actinobacteria were highlighted in Table 15.1. This chapter covers the global as well as Indian enzyme market situation and the biotechnological potentials of actinobacteria as a major foundation for significant industrial enzymes such as protease, pectinases, cellulase, and chitinases. They may be used in the recovery of several value-added products concerning waste management and biomedicine.

15.2 Indian as well as International Status of Enzyme Markets

The global industrial enzyme market will be anticipated to increase annually in successive development by about 5% towards about $7 billion in the year 2021, or about 12 billion in 2024 (Ravindran et al. 2018; Datta et al. 2020). Western Europe and North America are the largest producers of enzymes and were also forecasted to grow strongly, whereas the maximum expansion is possible in developing nations such as Africa, Asia, and Middle East, besides Eastern Europe and Latin America. China is evolving as the imperative basis, including a market for commercial enzymes because of numerous research and development activities established by numerous industrial pioneers that account for 10% among the global market. The requirement for therapeutic and diagnostic enzymes can be expected to grow due to advancements in medical care facilities in developing nations and global healthcare improvements. There are limited production industries in the globe, which include Advanced Enzyme Technologies Ltd., AB Enzymes GmbH, Asahi Kasei Pharma Corporation, DuPont, Roche, BASF, Cargill Texturizing Solutions, Amano Enzyme Inc., Genencor International Inc., Nexgen Biotechnologies Inc., Hayashibara Company, Novozymes, DSM Food Specialties, and Maps Enzymes Ltd. The international commercial enzyme market has changed continuously because of several mergers and attainments. The giants in the enzyme industry, DuPont, Roche, and Novozymes have acquired the market shares of about 75%. The technical enzymes were worth over $1 billion in 2010, which is anticipated to increase up to $1.5 billion in 2015 along with the maximum sales projected on bioethanol and leather markets

(Li et al. 2012). In a similar way, the food and beverage enzyme segments were anticipated to achieve about $1.3 billion by 2015 (Binod et al. 2013). The enzyme markets for industry have been growing about 7% a year, reaching $10.5 billion in 2024 (Cavalcante et al. 2021). This is well connected to the patent number filed over several years that indicated the increasing trends. As per the literature from 2000 to date, there has been considerable increment in the number of patents filed or granted on different enzymes. The highest number of patents is especially on proteases followed by amylases and cellulases, which could be due to the wide use of these bioactive molecules. The process of application for different enzyme patents and the patents granted are predicted to multiply because of advancements in green technologies. Especially enzymes with exceptional properties such as specific activity, thermostability, and activity at a broad range of pH will be most desirous in the upcoming years because of their high tolerance. These characteristic features of enzymes could be useful in medicine and biotechnology, for instance, polymerases, kinases, and nucleases. Apart from this, their application in broad range of personal care products will reform the cosmetic industries as well (Binod et al. 2013; Li et al. 2012).

15.3 Actinobacteria as a Source for Commercial Enzymes

Actinobacteria can be explored toward the production of nutraceuticals, pharmaceuticals, enzymes, enzyme inhibitors, antitumor agents, etc. (Zhao et al. 2016; Suriya et al. 2016; Fernandes et al. 2014; Shivlata and Satyanarayana 2015). These secondary metabolites are of industrial value, and therefore the actinomycetes are frequently screened for the synthesis of new bioactive molecules. A broad range of enzymes and their products employed in biotechnological industries and in biomedical fields have been described from different kinds of actinobacteria genera. Subsequently, there is vast information available with the advent of the genome as well as protein sequencing data; these bacteria have been constantly employed for the production of amylase, protease, chitinase, xylanases, and others—the typical example of commercially important enzymes from actinobacteria. The below diagram explains the methods involved in the extraction of bioactive metabolites from actinomycetes species (Fig. 15.2).

15.3.1 Cellulases

The enzyme group cellulases (endogulcanase, exoglucanase, β-glucosidase) can convert cellulose into smaller polysaccharides called cellodextrins, or completely into glucose units suitable for human consumption. The largest microbial producer group of cellulases belongs to the genus *Streptomyces* (Bettache et al. 2018; Anderson et al. 2012). The cellulases derived using *Streptomyces* spp. have

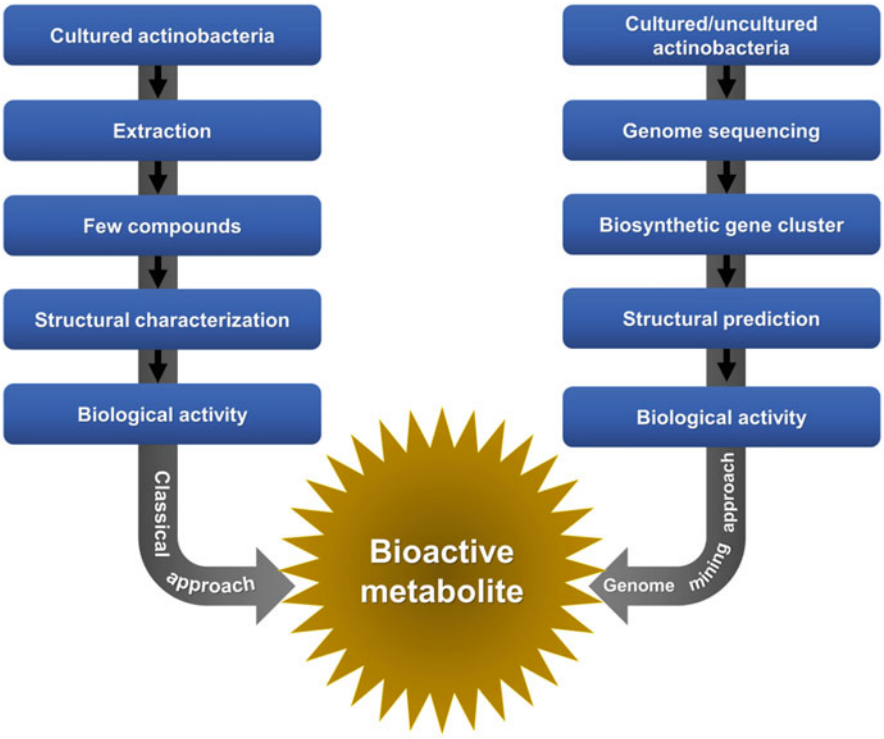

Fig. 15.2 Schematic indicating the methods involved in extraction of bioactive metabolite synthesis using actinobacteria

been reported to require an acid to alkaline pH optimum of 5–10 with premium thermal stability (Saratale et al. 2012; George et al. 2001). The enzymes have been utilized as additives in detergents for softening, cleaning, and restoring the color of the textiles. Cellulases have also been explored in textile, and paper and pulp processings, and as animal feed supplements (Sing et al. 2016). Apart from *Streptomyces*, actinobacteria from other genera, such as *Micromonospora* and *Thermobifida* have also been able to produce cellulases of some commercial value (Chen et al. 2020; Liu et al. 2020). Recombinant cellulase with a high pH and thermal stability has been described from *Streptomyces* sp. The modified enzyme was stable and also active in the presence of commercial detergents, emphasizing its superiority over preexisting cellulases, and the potential to improve the properties of enzymes using genetic engineering (Juturu and Wu 2014). *Thermomonospora fusca* cellulase has been utilized in the degradation of Avicel cellulose gel and cotton (Bothwell et al. 1997). The potential of these enzymes is not only restricted to biotechnological applications, but they can also be economically viable in many other applications as a result of their relatively low production costs. Their microbial biosynthesis can be carried out using inexpensive substrates such as wheat straw, rice, and fruit peels (Juturu and Wu 2014; Chen et al. 2020; Ozatay 2020; Bettache et al. 2018).

15.3.2 Amylases

The enzyme amylase is also an important class of hydrolytic enzymes that can be utilized in starch industries, especially in converting starch to high fructose syrups, which belongs to the major focused sectors much associated with starch industries. Their main objective has been to modify the length of malt oligosaccharides that are generally produced using amylases through the specific mode of action. The thermophilic, as well as acidophilic, amylases play a critical role in alcohol, brewing, and baking industries (Li et al. 2012; Ozatay 2020). They have been studied exclusively from *Streptomyces erumpents* (Kar et al. 2009). The thermostable amylases from the actinobacterium *Nocardiopsis* sp. have vital applications in paper and bakery industrial sectors (Bennur et al. 2015; Rigoldi et al. 2018). The enzyme amylase recovered from *Thermobifida* sp. can produce maltotriose as the main end product from raw sago starch and refined starch (Yang and Liu 2004). Such types of amylases are beneficial in nutrition and healthcare sectors. Apart from this, the final product-specific amylases may be utilized in the production of maltooligosaccharides derived from cheap starch substrates. The majority of the reported actinomycetes can be used in the synthesis of cold-active α-amylases which could be used in detergent-, textile-, and bioethanol-producing industries (Kuddus et al. 2011; Paul 2016).

15.3.3 Pectinases

In food and textile industries, the use of pectinases includes the fruit juice clarification, winemaking, fiber degummings, and bast fiber rettings. Pectinases have also been derived from *Streptomyces* sp., though the literature suggests that the rest of the actinobacterial pectinases are minimally studied. There is a huge demand for the cold-active pectate lyases because of their capacity to maintain the sweetness and the nutritional features of several food ingredients. The existence of pectin digesting genes among few actinobacteria proposes that their characterization can certainly yield pectinases having new properties (Rebello et al. 2017; Borah and Thakur 2020; Bharadwaj and Udupa 2019).

15.3.4 Proteases

Surveys for the new proteolytic enzymes that are proteases and products made from them for various industries, such as animal feed, breweries and detergents have been reported from past several decades. The majority of actinobacterial protease enzyme producers are related to *Streptomyces* sp. The enzymes can be alkali resistant, and few of them can be salinity tolerant, many of them are produced by bacteria

belonging to the genus *Streptomyces* (Horikoshi 1999). Apart from the *Streptomyces*, the proteases produced by the genus *Nocardiopsis* spp. can be used in detergent additives (Moreira et al. 2002), and also skin hair removal most common in leather industries. The process of dehairing of goatskin by a protease from *Streptomyces* sp. can turn the whole process more environmentally friendly and economical. The waste rich in keratin, such as hairs, feathers, nails, and horn is generated in agro-industrial processes. *Streptomyces* spp. have produced protease mixtures that can degrade the keratin even at higher temperature of 50 °C. Several *Streptomyces* have been able to hydrolyze keratin through pronases, as seen for *Streptomyces griseus* (Mitra and Chakrabartty 2005; Brandelli 2008; Jaouadi et al. 2010). Proteases recovered from other sources could be used in conjunction with actinobacterial enzymes to obtain antioxidants from shellfish waste. Protease can be produced by growing *Microbispora* sp. on shellfish waste. The end products of the proteolysis will be rich in peptides and amino acids; they can be served as low-cost animal feeds. Antioxidants were also possible to extract after marine waste hydrolysis using proteases and chitinases (Nawani et al. 2010).

15.3.5 Chitinases

Chitinase enzymes are an interesting class of hydrolases that have received astounding importance in recent decades. These enzymes belong to glycosyl hydrolases, which catalyze the biodegradation of chitin, which is an insoluble linear β-1,4 linked polymer of *N*-acetylglucosamine (GlcNAc). Chitin is one of the main constituents in the shells of crustaceans, in the cell walls of the various fungi, and in the exoskeleton of insects. Chitinases enable a protoplast preparation using a fungal source; they possess biocontrol activities against the phytopathogenic fungi, nematodes, etc. They can be used in the extraction of biomedically important chitin oligomers. Chitinases are present in many of the actinobacteria, and they have unique characters pertaining to the thermostability and their activities under wide range of pH, making them appropriate for industrial process applications (Bhattacharya et al. 2007; Kawase et al. 2004).

One of the major advantages of chitinases is the synthesis of chitin oligosaccharides. These oligosaccharides have amazing antimicrobial, anticoagulant, anticancer, anticholesteremic, wound healing, and antioxidant activities, so they proved to be an important choice for biomedical applications. Chitin oligosaccharides can be obtained using cheap substrates such as crab, shrimp, and squid pen waste. Chitinase from *Microbispora* sp. has been used for the production of chitobiose, the repeating disaccharide unit of chitin. It has potent antioxidant properties that are utilized primarily in the manufacture of food additives and in biomedical applications (Gomes et al. 2001; Nawani et al. 2002). Chitin is a renewable source that can be used for the cultivation and enrichment of the many chitinolytic microbes, and also for the efficient recovery of chitin oligosaccharides on a large

scale. The process of waste recycling can also be efficiently carried out through the biological utilization of actinobacteria (Nawani et al. 2010).

15.3.6 Other Important Enzymes from Actinobacteria

The selection of actinobacterial enzymes for the other specific industrial applications has been reported. They consist of laccases, tyrosinases, and lignin peroxidases that are capable of treating the textile dyes, and found suitable in most prominent applications among the waste treatment for recycling purposes. Furthermore, enzymes, such as amidases and esterases recovered from *Nocardia* sp., could be suitable for enhancing the hydrophilicity of polyamide fibers and polyethylene terephthalate. The environmentally friendly and affordable technique would be suitable for the textile industry (Bettache et al. 2018; Fernandes et al. 2014; Roy et al. 2014; Heumann et al. 2006).

The high-throughput screening programs have been adopted for the selection of rare actinobacteria as sources of new compounds, to obtain enzymes with novel functionalities and properties. An example of a successful case consists of L-asparaginase and thrombinase from the marine *Streptomyces* sp. that can be used as therapeutic enzymes for the treatment associated with myocardial infarction as well as leukemia. *Streptomyces* sp. recovered from sponge produces enzymatically phytoene, a carotenoid with potential as a food additive by means of high antioxidant activity (Jayaprakashvel 2012). The advent of the latest technologies has made it promising to obtain the unexplored microorganisms having new properties (Sulochana et al. 2014a; b; Mohan Reddy et al. 2015a, b; Yaradoddi et al. 2018), and actinobacteria which are considered to be natural reservoirs for exceptional enzymes. The current HTS processes are useful in selecting the industrially important bacteria, however, they are not specifically targeted to actinobacteria. Still there are also numerous HTS techniques that are adopted in the exploration of novel enzymes from actinobacteria (Amin et al. 2020). The FACS (fluorescence-activated cell sorting) has been fruitfully adapted for categorizing required clones among the genomic library using a fluorescent substrate to specifically reveal a particular type of enzyme. Biocatalytic activity is shown by the positive fluorescence. Gel microdrop is a method to detect clones which are positive for particular enzymes by catching the fluorescence emitted because of catalytic degradation of biotinylated substrate (Becker et al. 2004; Baret et al. 2009). Progression in metagenomics allows the use of fast screening methods wherever the bioactivities by uncultured microorganisms could be analyzed. The DNA or mRNA sequence data library is constructed by using metagenomics in extreme habitats, such as ocean beds, arid regions, stratospheres, and other environments from which the microorganisms cannot be cultivated within the laboratory conditions, preventing the bioactive potential of these non-cultured microbes from being studied. The contemporary advancements can help in determining the functional properties of the microenvironments (Jayaprakashvel 2012; Garlapati et al. 2019).

Simultaneously, the metagenomic sequencing has numerous benefits and suffers from a common problem of reduced or no expression of the genes of interest. Several shift amplifications have allowed scientists to combat this issue of reduction or absence of expression. The whole-genome amplification can be performed from single cells that can unlock the full biochemical potentials of an uncultured microbial community from a complex niche. Other groundbreaking approaches such as SIGEX (substrate-induced gene expression screening), PAIPCR (preamplification inverse-PCR), and the metagenomic DNA shuffle will provide the necessary insights on the functional metagenomics of the specific niche or habitat (Uchiyama et al. 2004; Uchiyama and Watanabe 2006). Actinobacteria can be able to secrete bioactive molecules that are in a wide range of capacities.

About two-thirds of the antibiotics are recovered from the actinobacteria; most of them belong to the genera *Micromonospora* and *Streptomyces* (Barka et al. 2016). The antimicrobial drug resistance concerning to the infectious diseases is among the foremost cause of death around the globe. Antibiotics are very important in treating infections in daily life. Due to the present increase in microbial resistance, there is a huge requirement for novel antimicrobials to fight against the pathogenic microorganisms resistant to current antibiotics available in the market. Therefore, antibiotic resistance has become one of the most important public health issues, and there is a huge need for novel effective antibiotics (United Nations 2019). Numerous investigations have been carried out to recover actinobacteria from various locations, which include mountains, soil, marine environments, estuaries, and swamps (Jeffrey 2008; Guo et al. 2015; Kumar et al. 2013; Gurung et al. 2009; Rao et al. 2012; Olano et al. 2009). They have produced excellent results where the antibiotics against various Gram-negative and Gram-positive bacteria as well as fungi have been isolated. Moreover, some among bioactive compounds extracted from actinobacteria have indicated antitumor activities. The latest research is going to find novel antibiotics. Therefore, the current chapter also uncovers the bioprospecting of several new antibiotic-producing actinobacteria.

15.4 Screening for Antimicrobial Compounds

Numerous review articles have surveyed screening methods for new antimicrobial compounds. A few to mention, Balouiri et al. (2016) present various diffusion and dilution methods, thin-layer chromatography-bioautography, time-kill test, ATP bioluminescence assay, and flow cytofluorometric method in susceptibility testing. De Castro et al. (2014) present insights into the use of metagenomics in antibacterial research. Lei et al. (2020) have used high-throughput screening for antimicrobials in the droplet-microarray. Yu et al. (2010) describe the rationale for studying antimicrobial metabolites produced by endophytes, including actinobacteria. Li et al. (2021) have evaluated methods for screening antimicrobials against plant pathogens from marine organisms, including actinobacteria, while Indraningrat et al. (2016) have reviewed sponge-associated microbes as producers of antimicrobials. Among

the most commonly used antimicrobial susceptibility tests is Kirby-Bauer disk diffusion method (Bauer et al. 1966), which will be presented. Screening of antimicrobial metabolites can be done by reviving the actinobacterial cultures using e.g. nutrient agar plates, followed by cultivation in the liquid broth. The strains are further screened by following the disc diffusion method. As per the studies, sterilized paper discs (Whatman No. 1) with a diameter of about 6 mm should be saturated with 15 μL of the culture broth, and then placed on Mueller Hinton agar containing the test microbe(s). These plates are incubated for yeasts at 30 °C temperature for 48 h, for bacteria at 37 °C for 24 h, and for fungi at 30 °C for about 96 h. The isolates producing bioactive compounds can be determined by measuring the inhibition zone diameter (millimeters, mm). The commonly used antibiotics can be considered as a positive control, for example for fungi itraconazole (2 μg/mL) and for bacteria chloramphenicol (30 μg), whereas the disc saturated with physiological saline can be used as a negative control. The isolates positive for antimicrobial activity can be further screened for the antimicrobial potential against, for example, the MRSA (methicillin-resistant *Staphylococcus aureus*) by following the same technique.

15.4.1 Morphological and Biochemical Characterization

The morphological and biochemical analyses can be performed for the selected antimicrobial potential actinobacteria species. For the studies, the cultures are inoculated, for example, on the starch casein agar, and the plates are incubated at 30 °C until colonies are visible, typically for 7–10 days. Apart from this, a modified coverslip culture method can also be used to cultivate actinobacteria for the characteriszation of hyphae and their spore arrangement at higher magnification (Kandasamy et al. 2012). Various media, such as cornmeal agar can be used in culturing microbes. Subsequently, the color of the aerial mycelium, pigments, and the morphology of the colonies can be recorded. The distinguishing characteristic features of the *Streptomyces* spp. have been observed to be, e.g. white-grayish aerial mycelium or brownish substrate mycelium (Rana and Salam 2014; Kontro et al. 2005, 2007). A previous study has revealed that most of the actinobacteria isolated from soil had morphological features related to *Streptomyces* spp. (Taddei et al. 2006). Various biochemical tests, such as citrate utilization, casein hydrolysis, catalase test, and urea hydrolysis can be conducted to help in the possible identification of actinobacteria. The above tests can be carried out according to Cappuccino and Sherman (2002), and several easy-to-use biochemical tests are also commercially available. The biochemical tests have indicated that a significant proportion of the isolates have the potential to hydrolyze casein, and many of the isolates showed urease and catalase enzyme activity, which may be due to similar metabolism (Rana and Salam 2014; Sharma 2014). Most of the isolates from the soil have indicated positive tests for citrate, which mainly served as a carbon source, and they were also catalase positive. Based on the characterization, the strains recovered in India were reported to be similar to the actinobacteria, three of which were

Streptomyces sp. (Dileep et al. 2013). The bioactive potentials of the actinobacteria concerning the extracellular hydrolytic enzyme production is of ultimate interest. Generally, the environmental sources by which the actinobacteria are recovered can influence the characters of enzymes they produce (Saadoun et al. 2007; Sharmin et al. 2005). The dominant *Streptomyces* species have been described from the soil (Priyadharsini and Dhanasekaran 2015; Han et al. 2013). This genus has also been reported to be the most promising bacteria in the soil as it can perform various ecological functions such as the biodegradation of organic matter and support in the formation of compost (Adegboye and Babalola 2012). Thus, this dominates about 90% of the actinobacteria among soil diversity (Xu et al. 1996).

The sequencing of actinobacterial isolates 16S rRNA gene using actinobacteria-specific primers has shown that many of the actinobacteria are closely related, while only about one-third of the isolates could be amplified by using the group-specific primers. This may indicate that the isolates that could not be amplified may be related to the rare actinobacteria. In the future perspective, more general primers, or more primer pairs, including genus-specific primers should be utilized, or shotgun sequencing should be used. Interestingly, the actinobacteria that were not amplified consisted of strains that were active against *Staphylococcus aureus* MRSA. The 16S rDNA sequence similarity of 98% and higher revealed that most of the isolates belonged to the genus *Streptomyces*. *Rhodococcus opacus* like isolate had the antimicrobial activity against *Trichophyton mentagrophyte,* while one retrieved in Lake of Magadi indicated antibiotic activity against *Pseudomonas aeruginosa* and *Escherichia coli* (Rotich et al. 2017; Nonoh et al. 2010).

15.4.2 Hemicellulose Bioconversion

Hemicellulose belongs to the major components of plant cell walls and is the second most abundant renewable biopolymer resource and a common component in numerous agricultural wastes from plants, accounting for 7–35% of the dry weight (Dhiman et al. 2008; Prajapati et al. 2018). Hemicellulose heteropolymer is composed of diverse sugars, hexoses (glucose, mannose, galactose, rhamnose), pentoses (xylose, arabinose), sugar acids, and xylan (Hamdi et al. 2019). In the hemicellulose bioconversion, hydrolytic enzymes act on different heteropolymers present in lignocellulose biomass, including the enzymes β-xylosidase (exoglycosidase), endoxylanase, galactosidase, acetyl xylan esterase, xylanase, α-glucuronidase, and α-arabinofuranosidase (Houfani et al. 2020). In the microbial consortium enriched in manure compost using rice-straw substrate, actinobacteria dominated. The endoxylanase of the phylum Chloroflexi bacterium *Thermobaculum terrenum* mainly hydrolysed the glycosidic bonds of the heteroxylan, while the phylum Bacteroidetes bacterium *Rhodothermus marinus* degraded shorter oligosaccharides, and also galactolipids. The actinobacteria producing β-xylosidase and endoxylanase enzymes were less common, and more diverse, including species *Thermobispora bispora*, *Actinoplanes missouriensis*, *Thermomonospora curvata*, *Streptomyces*

scabiei, *Streptosporangium roseum*, *Beutenbergia cavernae*, *Micromonospora lupini*, *Stackebrandtia nassauensis*, *Verrucosispora maris*, and *Conexibacter woesei* (Wang et al. 2016). These reports reveal that the strains from the phyla Bacteroidetes and Chloroflexi likely have a vital role in the biodegradation of hemicellulose in the compost under thermophilic conditions. The significant role of *Bacteroidetes* in the degradation of hemicellulose was also established in the recent report concerning the mesophilic fermentation platform community, though Actinobacteria were more common (Hollister et al. 2012).

The greatest part of the actinobacterial species is reportedly known to be potential producers of numerous hemicellulose hydrolyzing enzymes. For example, endoxylanases have been produced by *Streptomyces* spp. and *Thermomonospora* spp. Similarly, side-chain cleaving acetyl xylan esterases, α-glucuronidases and arabinofuranosidases have also been detected in actinobacteria, including *Streptomyces* spp. and *Thermomonospora* spp. (Sunna and Antranikian 1997). Xylanase is an enzyme that breaks down xylan into xylose. The xylanase activity has been reported to occur in *Nonomuraea flexuosa* or *Thermopolyspora flexuosa* (previously *Actinomadura flexuosa*) actinobacterium. Xylanases from Actinobacteria and fungi, fused by molecular methods, have been utilized in paper and pulp industries, because of their higher pH and temperature stability (Leskinen et al. 2005; Dhiman et al. 2008). As pure xylan is generally expensive, it can be substituted by inexpensive, e. g. agricultural waste-derived substrates, which can serve as important nutrient sources for industrial processes. *Streptomyces* sp. has been capable of producing significant quantities of xylanases while utilizing the crude rice straw as a substrate with the outcome of substantial bio-bleaching activities. Likewise, the *Streptomyces* sp. has decomposed varieties of agro-based wastes such as straw waste and oil cake, which has led to improved biogas production (Sinjaroonsak et al. 2020; Prajapati et al. 2018; Rifaat et al. 2005; Biely et al. 1986). In this perception, the synergism among the xylanases and arabinofuranosidases/esterases corresponding to the enzymatic degradation of xylan can greatly impact the effective and complete the xylan breakdown process during the bioconversion of hemicellulose.

15.4.3 Lignin Bioconversion

Lignin is one of the major structural components of the plant cell wall, which is a highly complex aromatic heterogeneous polymeric constituent covalently cross-linked with the polysaccharide fibers. Lignocellulose consists of about 10–25% of lignin (Hamdi et al. 2019). Degradation of lignin involves the oxidative mechanism that requires a group of oxidative enzymes, such as manganese peroxidases, lignin peroxidases, laccases, and versatile peroxidases (Welinder 1992). In the manure compost enrichment using rice-straw substrate, the genes encoding for the putative ligninases from the AA2 family accounted about 8.7% of all auxiliary activities (AAs). The phylogenetic distribution of the genes within the family AA2 revealed that the major members of lignin degrading bacteria were *Symbiobacterium*

thermophilum of the *Firmicutes* phylum, and a variety of actinobacterial species, that includes *Mycobacterium xenopi, Thermomonospora curvata, Mycobacterium thermoresistibile* and *Amycolicicoccus sub flavus*. The biodiversity of previously unidentified members highly increases in the account of ligninases; meanwhile, the actinobacteria identified by in vitro assay for lignin biodegradation were associated within the limited number of genera, which includes *Rhodococcus*, *Nocardia*, and *Streptomyces* (Wang et al. 2016). The result is consistent with previous reports that soil bacteria that degrade aromatic substances also degrade lignin (Bugg et al. 2011). In addition, actinobacteria from the genera *Nocardia* and *Nonomuraea* are reported to breakdown lignin using peroxidases (Hamdi et al. 2019).

15.5 Conclusion

The major biotechnologically important species that belong to actinomycetes are listed (Table 15.2). The present chapter emphasizes the synergistic activity of the predominant actinomycetes along with other bacteria, including phyla *Bacteroidetes*, *Chloroflexi*, and *Firmicutes,* associated with the hydrolysis of lignocellulose, and variety of polysaccharides, stach, chitin as well as proteins in the compost and variety of different habitats. This report also much emphasizes, based on required supportive genetic proofs, that the microbiomes in different contexts consists of a wide range of novel microbes with unexplored genes. The majority of them are from actinobacteria with unknown features, such as the members of acetyl xylan esterases, β-glucosidases, pectin lyases, ligninases, and arabinofuranosidase activity. These data uncover a great variety of functional ecological diversity of mesophilic and thermophilic actinomycetes involved in carbohydrate metabolism

Table 15.2 List of potential biotechnological applications of the actinomycetes

Actinobacterial strains	Biotechnological potentials
Streptomyces sp.	Chitinase enzyme
Thermomonospora sp., *Actinoplanes*, *Streptomyces* sp., etc.	Cellulase
Nocardia spp.	Protease
Thermomonospora curvata	Amylase
Nocardia spp.	Peptidase
Nocardia autotrophica	Lignases
Streptomyces sp.	Lipases
Streptomyces sp.	B 1,3-Glucanase
Streptomyces sp. and *Micromonospora* sp.	Phenazines
Streptomyces sp.	Siderophores
Streptomyces sp., *Nocardia* spp., and *Saccharopolyspora*	Antifungal agents
Micromonospora sp.	Phosphate solubilization
Frankia spp.	Nitrogen fixation
Micromonospora sp.	Plant hormones

within distinct habitats, including the compost, plant, and soil conditions, greater than earlier expected (Li et al. 2020; Rao et al. 2020a, b; Manikprabhu et al. 2016; Wang et al. 2017). There is yet a wide array of actinobacteria to be discovered with biotechnological potential in the field of bioactive compounds, biomass hydrolysis to replace fossil raw materials, novel products, and in the biofuel factories. It is also distinguished that organic matter and lignocellulose degradation in the different fields of biotechnology, ranging from composting, mining using bioleaching, and food processing to the manufacturing of new products to replace fossile carbon based materials is a huge field of science. In the processes, the dynamic interactions among the complex microbial communities and complex recalcitrant substances are still insufficiently understood. It is also essential to perform a broad range of sampling and implicate wide varieties of approaches, comprising of proteomics, metabolomics, and metatranscriptomics, to recognize and quantify the expression of genes and metabolic pathways related to the bioconversion of main constituents in transformation of the plant biomass, such as hemicellulose, cellulose, lignin, pectin, as well as in developing novel bioactive products.

References

Adegboye MF, Babalola OO (2012) Taxonomy and ecology of antibiotic producing actinomycetes. Afr J Agric Res 7:2255–2261

Amin EH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EMH (2020) Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. 44:5. https://doi.org/10.1186/s42269-019-0266-8

Anderson I, Abt B, Lykidis A, Klenk H-P, Kyrpides N, Ivanova N (2012) Genomics of aerobic cellulose utilization systems in actinobacteria. PLoS One 7(6):e39331. https://doi.org/10.1371/journal.pone.0039331

Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: a review. J Pharmaceutical Anal 6:71–79

Baret J-C, Miller OJ, Taly V, Ryckelynck M, El-Harrak A, Frenz L, Rick C, Samuels ML, Hutchison JB, Agresti JJ, Link DR, Weitz DA, Griffiths AD (2009) Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. Lab Chip 9:1950–1858. https://doi.org/10.1039/b902504a

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, van Wezel GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496

Becker S, Schmoldt H-U, Adams TM, Wilhelm S, Kolmar H (2004) Ultra-high-throughput screening based on cell-surface display and fluorescence-activated cell sorting for the identification of novel biocatalysts. Curr Opin Biotechnol 15:323–329. https://doi.org/10.1016/j.copbio.2004.06.001

Bennur T, Kumar AR, Zinjarde S, Javdekar V (2015) Nocardiopsis species: incidence, ecological roles and adaptations. Microbiol Res 174:33–47. https://doi.org/10.1016/j.micres.2015.03.010

Bettache A, Azzouz Z, Boucherba N, Bouiche C, Hamma S, Maibeche R, Gahfif O, Benallaoua S (2018) Lignocellulosic biomass and cellulolytic enzymes of actinobacteria. SAJ Biotechnol 5:203

Bharadwaj PS, Udupa PM (2019) Isolation, purification and characterization of pectinase enzyme from Streptomyces thermocarboxydus. J Clin Microbiol Biochem Technol 5:1–6

Bhattacharya D, Nagpure A, Gupta RK (2007) Bacterial chitinases: properties and potential. Crit Rev Biotechnol 27:21–28. https://doi.org/10.1080/07388550601168223

Biely P, MacKenzie C, Puls J, Schneider H (1986) Cooperativity of esterases and xylanases in the enzymatic degradation of acetyl xylan. Nat Biotechnol 4(8):731–733

Binod P, Palkhiwala P, Gaikaiwari R, Nampoothiri KM, Duggal A, Dey K, Pandey A (2013) Industrial enzymes – present status and future perspectives for India. J Sci Ind Res 72:271–286

Borah A, Thakur D (2020) Phylogenetic and functional characterization of culturable endophytic actinobacteria assocated with Camellia spp. for growth promotion in commercial tea cultivars. Front Microbiol 11:318. https://doi.org/10.3389/fmicb.2020.00318

Bothwell MK, Daughhetee SD, Chaua GY, Wilson DB, Walker LP (1997) Binding capacities for Thermomonospora fusca E3, E4 and E5, the E3 binding domain, and Trichoderma Reesei CBHI on Avicel and bacterial microcrystalline cellulose. Bioresour Technol 60:169–178

Brandelli A (2008) Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. Food Bioprocess Technol 1:105–116. https://doi.org/10.1007/s11947-007-0025-y

Bugg TD, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22:394–400

Cappuccino JG, Sherman N (2002) Microbiology: a laboratory manual, 6th edn. Pearson Education, San Francisco, pp 15–224

Cavalcante FTT, Cavalcante ALG, de Sousa IG, Neto FS, dos Santos JCS (2021) Current status and future perspectives of supports and protocols for enzyme immobilization. Catalysts 11:1222. https://doi.org/10.3390/catal11101222

Chen SH, Lam MQ, Thevarajoo S, Manan FA, Yahya A, Chong CS (2020) Genome analysis of cellulose and hemicellulose degrading Micromonospora sp. CP22. 3 Biotech 10:160

Datta S, Rajnish KN, Samuel MS, Pugazlendhi A, Selvarajan E (2020) Metagenomic applications in microbial diversity, bioremediation, pollution monitoring, enzyme and drug discovery. A review. Environ Chem Lett 18:1229–1241. https://doi.org/10.1007/s10311-020-01010-z

de Castro AP, Fernandes GDR, Franco OL (2014) Insights into novel antimicrobial compounds and antibiotic resistance genes from soil metagenomes. Front Microbiol 5:489. https://doi.org/10.3389/fmicb.2014.00489

Dhiman SS, Sharma J, Battan B (2008) Industrial applications and future prospects of microbial xylanases: a review. Bioresources 3:1377–1402

Dileep N, Junaid S, Rakesh KN, Kekuda P, Onkaraa R (2013) Antibacterial activity of three Strepyomyces species isolated from soils of Shikaripura, Karnataka, India. J Biol Sci Opin 1:173–177. https://doi.org/10.7897/2321-6328.01307

Eggleston G, Vercelotti JR (2007) Industrial application of enzymes on carbohydrate-based material. ACS symposium series, vol 972. American Chemical Society, Washington, DC. https://doi.org/10.1021/bk-2007-0972.pr001

Fernandes TAR, da Silveira WB, Passos FML, Zucchi TD (2014) Laccases from Actinobacteria – what we have and what to expect. Adv Microbiol 4:285–296. https://doi.org/10.4236/aim.2014.46035

Garlapati D, Charankumar B, Ramu K, Madeswaran P, Murthy MVR (2019) A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Rev Environ Sci Biotechnol 18:389–411. https://doi.org/10.1007/s11157-019-09501-4

George SP, Ahmad A, Rao MB (2001) Studies on carboxymethyl cellulose produced by an alkalothermophilic actinomycete. Bioresour Technol 77:171–175

Gomes RC, Semedo LTAS, Soares RMA et al (2001) Purification of a thermostable endochitinase from *Streptomyces* RC1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. J Appl Microbiol 90(4):653–661

Guo X, Liu N, Li X, Ding Y, Shang F, Gao Y, Ruan J, Huang Y (2015) Red soils harbor diverse culturable actinomycetes that are promising sources of novel secondary metabolites. Appl Environ Microbiol 81:3086–3103. https://doi.org/10.1128/AEM.03859-14

Gurung TD, Sherpa C, Agrawal VP, Lekhak B (2009) Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapatthar, Mount Everest Region, Nepal. J Sci Technol 10:173–182

Hamdi C, Arous F, Jaouani A (2019) Actinobacteria: a promising source of enzymes involved in lignocellulosic biomass conversion. Adv Biotech & Micro 13:555874

Hamza TA (2017) Bacterial protease enzyme: safe and good alternative for industrial and commercial use. Int J Chem Biomol Sci 3:1–10

Han NY, Yi TJ, Chia YT (2013) Antimicrobial activity of actinomycetes isolated from Paya Maga, Sarawak. Jurnal Teknologi 62:17–19. https://doi.org/10.11113/jt.v62.1872

Heumann S, Eberl A, Pobeheim H, Liebminger S, Fischer-Colbrie G, Almansa E, Cavaco-Paulo A, Gübitz GM (2006) New model substrates for enzymes hydrolyzing polyethyleneterephthalate and polyamide fibers. J Biochem Biophys Methods 39:89–99. https://doi.org/10.1016/j.jbbm.2006.02.005

Hollister EB, Forrest AK, Wilkinson HH, Ebbole DJ, Tringe SG, Malfatti SA, Holtzapple MT, Gentry TJ (2012) Mesophilic and thermophilic conditions select for unique but highly parallel microbial communities to perform carboxylate platform biomass conversion. PLoS One 7(6): e39689. https://doi.org/10.1371/journal.pone.0039689

Horikoshi K (1999) Alkaliphiles: some applications of their products for biotechnology. Microbiol Mol Biol Rev 63:735–750

Houfani AA, Anders N, Spiess AC, Baldrian P, Benallaoua S (2020) Insights from enzymatic degradation of celluloses and hemicellulose to fermentable sugars – a review. Biomass Bioenergy 134:105481. https://doi.org/10.1016/j.biombioe.2020.105481

Indraningrat AAG, Smidt H, Sipkema D (2016) Bioprospecting sponge-associated microbes for antimicrobial compounds. Mar Drugs 14:87

Jaouadi B, Abdelmalek B, Fodil D, Ferradji FZ, Rekik H, Zaraî N, Bejar S (2010) Purification and characterization of a thermostable keratinolytic serine alkaline proteinase from Streptomyces sp. strain AB1 with high stability in organic solvents. Bioresour Technol 101:8361–8369

Jayachandra SY, Parameshwar AB, Mohan Reddy K, Sulochana MB (2012a) Characterization of extracellular hydrolytic enzymes producing extremely halophilic bacterium *Virgibacillus* sp. JS5. World J Sci Technol 2(2):23–26

Jayachandra SY, Anil Kumar S, Merley DP, Sulochana MB (2012b) Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. Recent Res Sci Technol 4(4):46–49

Jayaprakashvel M (2012) Therapeutically active biomolecules from marine actinomycetes. J Mod Biotechnol 1:1–7

Jeffrey LSH (2008) Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. Afr J Biotechnol 7:3697–3702

Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Renew Sust Energ Rev 33:188–203

Kandasamy S, Muthusamy G, Thangaswamy S, Senthilkumar B (2012) Screening and identification of antibiotic producing actinomycetes and their antagonistic activity against common pathogens. World Res J Antimicrob Agents 1:7–10

Kar S, Swain MR, Ray RC (2009) Statistical optimization of alpha-amylase production with immobilized cells of Streptomyces erumpens MTCC 7317 in Luffa cylindrical L. sponge discs. Appl Biochem Biotechnol 152:177–188. https://doi.org/10.1007/s12010-008-8248-6

Kawase T, Saito A, Sato T, Kanai R, Fujii T, Nikaidou N, Miyashita K, Watanabe T (2004) Distribution and phylogenetic analysis of family 10 chitinases in Actinobacteria. Appl Environ Microbiol 70:1135–1144. https://doi.org/10.1128/AEM.70.2.1135–1144.2004

Kontro M, Lignell U, Hirvonen M-R, Nevalainen A (2005) pH effects on 10 Streptomyces spp. growth and sporulation depends on nutrients. Lett Appl Microbiol 41:32–38

Kuddus M, Roohi AJM, Ramteke PW (2011) An overview of cold-active microbial α-amylase: adaptation strategies and biotechnological potentials. Biotechnology 10:246–258

Kumar V, Gajraj SB, Omprakash G (2013) Terrestrial actinomycetes from diverse locations of Uttarakhnad, India: isolation and screening for their antibacterial activity. Iran J Microbiol 5:299–308

Lei W, Demir K, Overhage J, Grunze M, Schwartz T, Levkin PA (2020) Droplet-microarray: miniaturized platform for high-throughput screening of antimicrobial compounds. Adv Biosys 4:2000073

Leskinen S, Mäntylä A, Fagerström R, Vehmaanperä J, Lantto R, Paloheimo M, Suominen P (2005) Thermostable xylanases, Xin10A and Xin11A, from actinomycete Nonomuraea flexuosa: isolation of the genes and characterization of recombinant Xyn11A polypetides produced in Trichoderma reesei. Appl Microbiol Biotechnol 67:495–505. https://doi.org/10.1007/s00253-33-1797-x

Li S, Yang X, Yang S, Zhu M, Wang X (2012) Technology prospecting on enzymes: application, marketing and engineering. Comput Struct Biotechnol J 2:e201209017

Li YQ, Zhang H, Xiao M, Dong ZY, Zhang JY, Rao MPN, Li WJ (2020) *Cellulomonas endophytica* sp. nov., isolated from *Gastrodia elata* Blume. Int J Syst Evol Microbiol 70:3091–3095. https://doi.org/10.1099/ijsem.0.004133

Li X, Zhao H, Chen X (2021) Screening of marine bioactive antimicrobial compounds for plant pathogens. Mar Drugs 19:69

Liu Y, Nemmary B, Chundawat SPS (2020) Thermobifida fusca cellulases exhibit increased endo-exo synergistic activity, but lower exocellulase activity, on cellulose-III. ACS Sustain Chem Eng 8:5028–5039

Manikprabhu D, Cheng J, Chen W, Sunkara AK, Mane SB, Kumar R, Hozzein WN, Duan Y-Q, Li W-J (2016) Sunlight mediated synthesis of silver nanoparticles by a novel actinobacterium (*Sinomonas mesophila* MPKL 26) and its antimicrobial activity against multi drug resistant *Staphylococcus aureus*. J Photochem Photobiol B Biol 158:202–205. https://doi.org/10.1016/j.jphotobiol.2016.01.018

Mitra P, Chakrabartty PK (2005) An extracellular protease with depilation activity from Streptomyces nogalator. J Sci Ind Res 64:978–983

Mohan Reddy K, Siva DS, Parameshwar AB, Jayachandra SY, Sulochana MB (2015a) Thermo and alkali tolerant exo-inulinase produced by *Streptomyces* sp. isolated from unexplored terrestrial habitat. Int J Curr Res Acad Rev 3(10):354–363

Mohan Reddy K, Siva Deepthi S, Jayachandra SY, Parameshwar AB, Dayanand A, Bikshapathi E, Sulochana MB (2015b) In *silico* structural analysis for exo-inulinases in proteomes of *Streptomyces* sp. using PDB structures as templates. Int J Curr Microbiol Appl Sci 4(11):858–867

Moreira KA, Albuquerque BF, Teixeira MFS, Porto ALF, Lima Filho JL (2002) Application of protease from Nocardiopsis sp. as a laundry detergent additive. World J Microbiol Biotechnol 18:307–312

Nawani NN, Kapadnis BP, Das AD, Rao AS, Mahajan SK (2002) Purification and characterization of a thermophilic and acidophilic chitinase from Microbispora sp. V2. J Appl Microbiol 93:965–975

Nawani NN, Prakash D, Kapadnis BP (2010) Extraction, purification and characterization of an antioxidant from marine waste using protease and chitinase cocktail. World J Microbiol Biotechnol 26:1509–1517. https://doi.org/10.1007/s11274-010-0327-5

Nonoh JO, Lwande W, Masiga D, Herrmann R, Presnail JK, Schepers E et al (2010) Isolation and characterization of *Streptomyces* species with antifungal activity from selected National Parks in Kenya. Afr J Microbiol Res 4:856–864. http://www.academicjournals.org/journal/AJMR/article-abstract/1AEDE3B13179

Olano C, Carmen M, José AS (2009) Antitumor compounds from marine actinomycetes. Mar Drugs 7:210–248. https://doi.org/10.3390/md7020210

Ozatay S (2020) Recent applications of enzymes in food industry. J Curr Res Eng Sci Technol 6 (1):17–30

Paul D (2016) Microorganisms and α-amylase: a concise review. Innov J Sci 4:1–5

Prajapati AS, Panchal KJ, Pawar VA, Noronha MJ, Patel DH, Subramanian RB (2018) Review on cellulose and xylanase engineering for biofuel production. Ind Biotechnol 14:38–44. https://doi.org/10.1089/ind.2017.0027

Priyadharsini P, Dhanasekaran D (2015) Diversity of soil allelopathic actinobacteria in Tiruchirappalli District, Tamilnadu, India. J Saudi Soc Agric Sci 14:54–60. https://doi.org/10.1016/j.jssas.2013.07.001

Rana S, Salam MD (2014) Antimicrobial potential of actinomycetes isolated from soil samples of Punjab, India. J Microbiol Exp 1(2):1–6

Rao KVR, Rao TR, Kumar KS, Rao DB (2012) Isolation and screening of antagonistic actinomycetes from mangrove soil. J Biochem Technol 3:361–365

Rao MPN, Li Y-Q, Zhang H, Dong Z-Y, Dhulappa A, Xiao M, Li W-J (2020a) *Amycolatopsis alkalitolerans* sp. nov., isolated from *Gastrodia elata* Blume. J Antibiot (Tokyo) 73:35–39. https://doi.org/10.1038/s41429-019-0222-8

Rao MPN, Dong ZY, Jiao JY, Zhou Y, Zhao J, Xiao M, Li WJ (2020b) Genome sequence and comparative analysis of DRQ-2, the type strain of *Nonomuraea indica*. Genomics 112:2842–2844. https://doi.org/10.1016/j.ygeno.2020.03.023

Ravindran R, Hassan SS, Williams GA, Jaiswal AK (2018) A review on bioconversion of agro-industrial wastes to industrially important enzymes. Bioengineering 5:93. https://doi.org/10.3390/bioengineering5040093

Rebello S, Anju M, Aneesh EM, Sindhu R, Binod P, Pandey A (2017) Recent advancements in the production and application of microbial pectinases: an overview. Rev Environ Sci Biotechnol 16:381–394. https://doi.org/10.1007/s11157-017-9437-y

Rifaat HM, Nagieb ZA, Ahmed YM (2005) Production of xylanases by Streptomyces species and their bleaching effect on rice straw pulp. Appl Ecol Environ Res 4:151–160

Rigoldi F, Donini S, Redaelli A, Parsini E, Gautieri A (2018) Review: engineering of thermostable enzymes for industrial applications. APL Bioengineering 2:011501

Rotich MC, Magiri E, Bii C, Maina N (2017) Bio-prospecting for broad spectrum antibiotic producing actinomycetes isolated from virgin soils in Kericho country, Kenya. Adv Microbiol 7:56–70

Roy S, Das I, Munjal M, Karthik L, Kumar G, Kumar S, Rao KVB (2014) Isolation and characterization of tyrosinase produced by marine actinobacteria and its application in the removal of phenol from aqueous environment. Front Biol 9(4):306–316. https://doi.org/10.1007/s11515-014-1324-0

Saadoun I, Rawashdeh R, Dayeh T, Ababneh Q, Mahasneh A (2007) Isolation, characterization and screening for fiber hydrolytic enzymes-producing Streptomycetes of Jordanian Forest soils. Biotechnology 6:120–128. https://doi.org/10.3923/biotech.2007.120.128

Saratale GD, Saratale RG, Oh SE (2012) Production and characterization of multiple cellulolyti enzymes by isolated Streptomyces sp. MDS. Biomass Bioenergy 47:302–315

Sewalt V, Shanahan D, Gregg L, La Marta J, Carillo R (2016) The generally recognized as safe (GRAS) process for industrial microbial enzymes. Ind Biotechnol 12:295–302

Sharma M (2014) Original research article actinomycetes: source, identification, and their applications. Int J Curr Microbiol Appl Sci 3:801–832

Sharmin S, Hossain TM, Anwar MN (2005) Isolation and characterization of protease producing bacteria Bacillus amovivorus and optimization of some factors of culture conditions for protease production. J Biol Sci 5:358–362. https://doi.org/10.3923/jbs.2005.358.362

Shivlata L, Satyanarayana T (2015) Thermophilic and alkaliphilic Actinobacteria: biology and potential applications. Front Microbiol 6:1014. https://doi.org/10.3389/fmicb.2015.01014

Sing R, Kumar M, Mittal A, Mehta PK (2016) Microbial enzymes: industrial progress in 21st century. 3 Biotech 6:174

Sinjaroonsak S, Chalyaso T, Kittikun AH (2020) Optimization of cellulose and xylanase production by Streptomyces thermocoprophilus TC13W using low cost pretreated oil palm empty fruit bunch. Waste Biomass Valoriz 11:3925–3936. https://doi.org/10.1007/s12649-019-00720-y

Sulochana MB, Jayachandra SY, Anil Kumar S, Dayanand A (2014a) Siderophore as a potential plant growth promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Appl Biochem Biotechnol 174(1):297–308. https://doi.org/10.1007/s12010-014-1039-3

Sulochana MB, Jayachandra SY, Anil Kumar SK, Dayanand A (2014b) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54 (5):418–424. https://doi.org/10.1002/jobm.201200770

Sunna A, Antranikian G (1997) Xylanolytic enzymes from fungi and bacteria. Crit Rev Biotechnol 17(1):39–67

Suriya J, Bharathiraja S, Manivasagan P, Kim S-K (2016) Enzymes from rare actinobacterial strains. Adv Food Nutr Res 79:67–98. https://doi.org/10.1016/bs.afnr.2016.08.002

Taddei A, Rodriguez MJ, Marquez-Vilchez E, Castelli C (2006) Isolation and identification of Streptomyces spp. from Venezuelan soils: morphological and biochemical studies. I. Microbiol Res 161:222–231. https://doi.org/10.1016/j.micres.2005.08.004

Uchiyama T, Watanabe K (2006) Improved inverse PCR scheme for metagenome walking. BioTechniques 41:183–188. https://doi.org/10.2144/000112210

Uchiyama T, Abe T, Ikemura T, Watanabe K (2004) Substrate-induced gene-expression screening of environmental metagenome libraries for isolation of catabolic genes. Nat Biotechnol 23:88–93. https://doi.org/10.1038/nbt1048

United Nations (2019) No time to wait: securing the future from drug-resistant infections. Interagency Coordination Group on Antimicrobial Resistance

Wang C, Dong D, Wang H, Müller K, Qin Y, Wang H, Wu W (2016) Metagenomic analysis of microbial consortia enriched from compost: new insights into the role of Actinobacteria in lignocellulose decomposition. Biotechnol Biofuels 9:22. https://doi.org/10.1186/s13068-016-0440-2

Wang H-F, Li Q-L, Xiao M, Zhang Y-G, Zhou X-K, Rao MPN, Duan Y-Q, Li W-J (2017) *Streptomyces capparidis* sp. nov., a novel endophytic actinobacterium isolated from fruits of *Capparis spinosa* L. Int J Syst Evol Microbiol 67:133–137. https://doi.org/10.1099/ijsem.0.001586

Welinder KE (1992) Superfamily of plant, fungal and bacterial peroxidases. Curr Opin Struct Biol 2 (3):388–393

Xu L, Li Q, Jiang C (1996) Diversity of soil actinomycetes in Yunnan, China. Appl Environ Microbiol 62:244–248

Yang C-H, Liu W-H (2004) Purification and properties of a maltotriose-producing α-amylase from Thermobifica fusca. Enzym Microb Technol 35:254–260

Yaradoddi JS, Sulochana MB (2020) Screening and characterization of bioactive compounds produced by the moderate halophile *Halobacillus* sp. JS6. Res J Biotechnol 15(12):131–136

Yaradoddi J, Shoba H, Banapurmath N, Hunashyal A, Sulochana M, Shettar A, Ganachari S (2018) Alternative and renewable bio-based and biodegradable plastics. In: Martínez LMT et al (eds) Handbook of ecomaterials. Springer International Publishing AG, Cham. https://doi.org/10.1007/978-3-319-68255-6_150

Yaradoddi JS, Sulochana MB, Kontro MH, Parameshwar AB, Dayanand A (2020a) The occurrence of potential and novel isolates of *Oceanobacillus* sp. JAS12 and *Salinicoccus* sp. JS20 recovered from West Coast of Arabian Sea, India. Res J Biotechnol 15(9):133–140

Yaradoddi JS, Banapurmath NR, Ganachari SV et al (2020b) Biodegradable carboxymethyl cellulose based material for sustainable packaging application. Sci Rep 10:21960. https://doi.org/10.1038/s41598-020-78912-z

Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, Sun P, Qin L (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165:437–449

Zhao XQ, Xu XN, Chen LY (2016) Production of enzymes from marine actinobacteria. Adv Food Nutr Res 78:137–151

Chapter 16
Actinomycetes in Medical and Pharmaceutical Industries

Ajay Kumar Oli, Nagaveni Shivshetty, Chandrakanth R Kelmani, and Parameshwar A Biradar

Abstract Actinomycetes are critical sources of medically important pharmaceutical metabolites. Among the identified actinomycetes, the genera *Streptomyces* has been investigated for several biomedically important antibiotics, mainly aminoglycosides, chloramphenicol, tetracycline, ivermectin, macrolides rifamycins, and other non-beta lactam antibiotics. Actinomycetes are used in the large-scale production of antibiotics; thus, they have a vast application in the biopharmaceutical industry and can be utilized in the production of antibacterial, antifungal, anti-inflammatory, and anticancer drugs. Many ecological niches remain underexplored as evidenced by the dearth of studies and reports. Therefore, it is always critical to recognize the unexplored environments or niches for obtaining novel secondary metabolites or drugs and diverse actinomycetes species. Different groups of actinobacteria produce a variety of bioactive compounds. However, extensive research has to be carried out to identify and recover new bacterial communities with a broad range of secondary metabolites. Recently, profound research work was conducted toward harnessing the rare actinomycetes diversity, and they are usually difficult to isolate. Many scientists believe that these types of rare actinobacteria have the potential to encompass distinctive bioactive compounds, which are prerequisites in the production of novel drugs.

A. K. Oli (✉)
Department of Biomedical Science, SDM Research Institute for Biomedical Sciences, A Constituent Unit (Shri Dharmasthala Manjunatheshwara University), Dharwad, Karnataka, India
e-mail: ajaykumar@sdmuniversity.edu.in

N. Shivshetty
Department of Microbiology and Food Science and Technology, GITAM Institute of Science, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India

C. R. Kelmani
Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India

P. A. Biradar
Faculty Biotechnology Skill Enhancement Program (BiSEP), Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India

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16.1 Introduction

Actinomycetes morphologically can be considered fungi and bacteria. However, their molecular organization is closely related to prokaryotes, indicating they belong to bacteria. They belong to the order Actinomycetales and are generally aerobic, Gram-positive, and spore-forming bacteria. A typical actinomycetes species can form 0.5–1.0 μm hyphae and appears similar to mycelium (Williams and Wellington 1982). Their cellular G + C content of DNA is greater than a 55-mole percent, and phylogenetic relationships can be determined using a 16S rRNA gene sequence. They are considered to be in the bacteria domain and considered as one of the largest taxonomic among the major 18 lineages. The name "Actinomycetes" comes from the Greek "atkis" (a ray) and "mykes" (fungus) (Pasindu 2016). They replicate by producing chains of spores and on their tips (through spores), while other filamentous species fragment into new cells. Hyphal development is charted by fragmentation and release of spores (Williams and Wellington 1982).

Actinomycetes are found in freshwater, seawater, cold and warm-blooded animals, and compost. The soil habitat is the main precursor for the availability of these species. Over 20 genera were isolated from the soil samples; a sustainable sums of several million per gram are common. Statistics in waterlogged, anaerobic soils and acidic soils are often originated to below 10^2–10^3 per grams of dry weight soil (Casida 1965; Williams et al. 1971; Hagedorn 1976). Actinomycetes are important microbe well exploited for secondary metabolites. For the past several years, a group of researchers has been involved in finding a prominent strain that can produce potential secondary metabolites. Among the main genera of actinomycetes species, a few examples are *Streptomyces*, *Actinobacteria*, *Nocardioforms*, *Actinoplanetes*, *Thermonospores*, and *Maduromycetes*. These are suprageneric, and are classified as irregular, non-sporing, Gram-negative rods (genus Actinomyces), Nocardio, from actinomycetes (genera *Nocardia* and *Rhodococcus*), and as actinomycetes with multi-ocular sporangia (genus *Dermatophilus*) (Goodfellow et al. 2012). These species are widely used in biotechnological applications for producing commercially important biomolecules.

Natural products play a pivotal role in various sectors of daily life. They are of major importance in industries, curing human disease, and pharmacological and biotechnology applications such as bacterial disease or infection and cancer (Girao et al. 2019). The natural products produced from actinobacteria include antibacterial, antitumor, anticancer, antifungal, antiviral, anti-inflammatory, immunosuppressive, and cytotoxic studies (Girao et al. 2019). Actinomycetes species have led the exploitation in the discovery of new compounds from conservative environments and reawakening of known compounds (Magad et al. 2019). The present review in

this chapter reveals the application of actinomycetes in medical and pharmacological industries.

16.1.1 Bioactivity of Actinomycetes

Among the actinomycetes species, *Streptomyces* isolates produce the most natural bioactive substances, approximately 70–80% of which are widely used in pharmaceutical and agrochemical applications (Berdy 2005; Ganachari et al. 2019; Manteca et al. 2008). Since 1955, the genus *Streptomyces* have been the most important strain used in the production of antibiotics (Watve et al. 2001). The first most crucial product produced from *Streptomyces* was antibiotics (Hwang et al. 2001). The other areas are antibacterial, antitumor, anti-parasitic, and antifungal (Kurtboke 2012; Dietera et al. 2003; Hopwood 1999). It can even be used to produce antivirals, herbicides, insecticides, and pesticides. They are used as a pharmacology substance as immune-modulators, immune-suppressive, and immune-stimulatory. In addition, they act as neurological agents and vasoactive substances (Petrosyan et al. 2003).

16.2 Production of Antibiotics

16.2.1 Tetracycline

In the 1940s, the first members of the tetracycline group were reported as chlortetracycline and oxytetracycline. These molecules were produced from *Streptomyces aureofaciens* and *Streptomyces rimosus* (Finch 1997). The structure is represented in Fig. 16.1a. In the strugecture, the functional groups are attached to the linear fused tetracyclic nucleus. The antibacterial activity was detected in 6-deoxy-6-demethyltetracycline, and it is called the minimum pharmacophore (Mitscher 1978).

16.2.1.1 Mode of Action

The antibiotic will prevent the overload of aminoacyl-tRNA with bacterial ribosome by inhibiting bacterial protein production (Chopra 1994; Schnappinger and Hillen 1996). Depending upon the susceptibility of Gram-positive or Gram-negative organisms, these molecules will transverse from one or more membrane systems to interact with their targets. The mechanism is primarily the uptake and ribosomal binding process. It has dual anti-bacterial and anti-protozoal properties, and the microbial selectively of the class as a whole (Ian and Marilyn 2001).

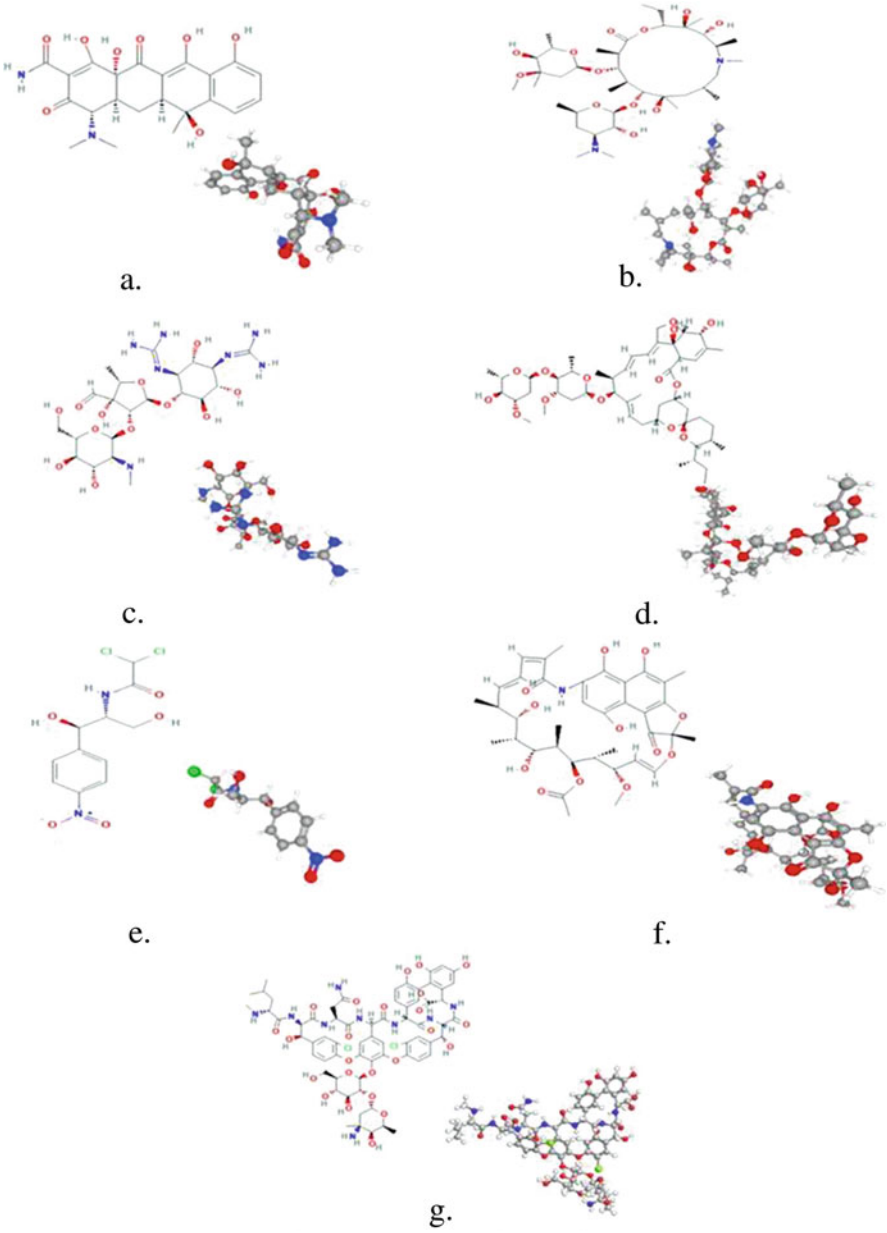

Fig. 16.1 Different types of antibiotic structures produced from the actinomycetes species. (**a**) Structure of tetracycline $C_{22}H_{24}N_2O_8$ 2D and 3D structure. (**b**) Structure of azithromycin (macrolide) $C_{38}H_{72}N_2O_{12}$ 2D and 3D structure. (**c**) Structure of streptomycin (aminoglycoside) $C_{21}H_{39}N_7O_{12}$ 2D and 3D structure. (**d**) Structure of ivermectin $C_{48}H_{74}O_{14}$ 2D and 3D structure. (**e**) Structure of chloramphenicol $C_{11}H_{12}Cl_2N_2O_2$ 2D and 3D structure. (**f**) Structure of rifamycin $C_{37}H_{47}NO_{12}$ 2D and 3D structure. (**g**) Structure of vancomycin (non-beta lactam antibiotic) $C_{66}H_{75}Cl_2N_9O_{24}$ 2D and 3D structure

16.2.1.2 Applications of Tetracycline

Pharmacokinetic Behavior and Administration in Humans

Tetracycline's mode of administration is generally carried out orally, and sometimes it is also available as parenteral products such as Rolitetracycline. The ability to use either oral or parenteral formulations of doxycycline provides the significant benefit of switching programs from intravenous to oral administration (Cunha 1999). It plays a part in the treatment of respiratory tract infections. It penetrates the sebum and is expelled in sweat, which contributes to its effectiveness in managing acne (Ian and Marilyn 2001).

Prophylactics and Human Therapy

For diseases associated with *Helicobacter pylori*, tetracycline is used as triple therapy management for gastritis and peptic ulcers (Van der Hulst et al. 1996). Due to the rapid increase in Mefloquine-resistant *P. falciparum* strains, tetracycline is used as a prophylaxis and malaria treatment (Bunnag et al. 1996; Pradines et al. 2000; Schwarz and Regev 1999). The combination of ofloxacin and minocycline is used in the treatment of leprosy (Ji et al. 1998).

Veterinary Medicine

It is used for the management of infection in cattle, sheep, swine, and poultry (Chopra et al. 1992; Gustafson and Kiser 1985). The drugs were supplemented directly in the form of feed or soluble, and can be directed in aerosols to animals. It is also used in the treatment of domestic pets (Kordick et al. 1997; Levy 1992).

Animal Growth Promoters

To improve the growth and feed translation proficiency of the animals, antibiotics are used in the foods both therapeutically and sub-therapeutically. Generally, low concentrations are auxiliary in the feed to promote the growth of the animals such as young chickens, for example, using chlortetracycline and oxytetracycline (Levy et al. 1999).

Other Uses

It is used to control the infection of aquacultures such as catfish, lobster, and salmon. Furthermore, it is used in treating fruit trees and other plants infected by *Erwiniaamulovara* and mycoplasma infection. It can even be used in seeds infected by the strain *Xanthomonascampestis* and foulbrood disease found in honeybees infected either by *Bacillus larvae* or *Streptococcus pluton* (Levy 1992).

16.3 Aminoglycoside

Aminoglycosides were first reported in 1944 produced from *Streptomyces griseus,* for example as Streptomycin. Others include Neomycin (*S. fradiae*), Kanamycin (*S. kanamyceticus*), Gentamicin (*M. purpurea*), Netilmicin (*Sisomicin*), Tobramycin (*S. tenebrarius*), and amikacin (resultant from kanamycin) (Kevin et al. 2016). The structure of aminoglycoside antibiotic, for example, streptomycin, is represented in Fig. 16.1b. It consists of glycoside linkages to a dibasic aminocyclitol, called 2-deoxystreptamin. It is also connected with residues of amino sugars (Mingeot-Leclercq et al. 1999). From the identity of the animocyclitol moiety, the drug has been categorized into four sub-classes as follows: (a) no deoxystreptamine (for example, carrying a streptidine ring called streptomycin); (b) a mono-substituted deoxystreptamine ring (for example, aparmycin); (c) a 4,5-di-substituted deoxystreptamine ring (for example, neomycin and ribostamycin); or (d) a 4,6-di substituted deoxystreptamine ring (for example, amikacin, gentamycin, tobramycin, and plazomicin) (Magnet and Blanchard 2005).

16.3.1 Mode of Action

Generally, aminoglycosides antibiotics are attracted to and bind the A site on the 16S ribosomal RNA of the 30S ribosome and inhibit the protein synthesis (Wachino and Arakawa 2012). It carries adverse specificity for different areas on the A site and alters its structure. Because of this interface, the antibiotic endorses mistranslation by prompting codon misreading on the aminoacyl transfer RNA delivery. Being prone to errors in protein production leads to the incorrect amassing in the polypeptide chain leading to cell membrane damage (Kotra et al. 2000; Mingeot-Leclercq et al. 1999; Davis et al. 1986; Ramirez and Tolmasky 2010). Some aminoglycoside groups can interact with protein synthesis by hindering elongation or directly inhibiting the origination process (Davis 1987; Wachino and Arakawa 2012; Ramirez and Tolmasky 2010).

16.3.2 Applications of Aminoglycosides

- For treatment, both empirical and definitive therapies for a broad range of these antibiotics are used in a single form of agent and in combination with other antibiotics (Avent et al. 2011; Jackson et al. 2013).
- In high-risk patients, or when the causative pathogen is resistant to commonly used agents for severe sepsis and nosocomial infections, these antibiotics are used in synergy with a beta-lactam for the empirical treatments (American Thoracic Society; Infectious Diseases Society of America 2005; Dellinger et al. 2013).
- A patient who suffers from the MDR strain of carbapenem-resistant *Enterobacteriaceae* (CRE) will be given the option of aminoglycoside antibiotic therapy.
- Combination therapies are used in treating multi-drug resistant tuberculosis (MDR-TB) and non-tuberculosis (NTM) infection. Aminoglycoside possesses potent bactericidal activity against *M. tuberculosis* (Ho et al. 1997).
- The antibiotic therapy is used for patients with fibro cavity, severe nodular/bronchiectasis, or macrolide-resistant lung disease caused by a strain known as *M. avium* complex infection with antibiotics such as amikacin and streptomycin (Griffith et al. 2007). It is also used in treating certain zoonotic infection such as plague and tularemia.

16.4 Macrolides Antibiotic (Azithromycin)

16.4.1 Azithromycin

Macrocyclic is an exciting molecule that possesses high specificity for treating different class of diseases. Macrocyclic drugs are primarily used to cure of infectious diseases (Dubravko and Roberto 2016). Azithromycin is derived from erythromycin, consisting of 14 membered macrolides, erythromycin A, isolated from the *Streptomyces erythreus* (*Saccharo-polysporaerythraea*), and has been used in humans since 1952. Azithromycin (9-dexo-9a-aza-9a-methyl-9a-homoerythromycin) results architecturally from erythromycin A by substituting the 9a carbonyl in the aglycone ring with a methyl-substituted nitrogen in addition to expansion of the ring to 15 members (Bright et al. 1988). The change in the structure blocks the internal response to form the hemiketal, resulting in acid hydrolysis of the ester bond to the neutral cladinose sugar as the main breakdown pathway (Fiese and Steffen 1990). The structure is represented in Fig. 16.1c.

16.4.1.1 Mode of Action

The antibacterial effects caused by azithromycin, such as erythromycin, mainly affect the ability to attach to the 50S ribosomal subunit of an organism by impeding bacterial protein synthesis (Retsema et al. 1987). Macrolides generally inhibit the RNA-dependent protein synthesis by the reverse binding process depending on the domain of the bacterial ribosome (Sturgill and Rapp 1992; Hansen et al. 1999).

16.4.1.2 Application

Upper Respiratory Tract Infections

Azithromycin is commonly used for the treatment of pharyngitis, otitis media, and sinusitis, which are frequently caused by bacteria (Jerry et al. 2011).

Lower Respiratory Tract Infections

Azithromycin has proved to be effective in the treatment of acute bronchitis, acute exacerbation of chronic bronchitis (ACEB), and community-acquired pneumonia, which are generally categorized as respiratory infections (Langtry and Brogden 1997).

Sexually Transmitted Diseases

Azithromycin has shown to be efficacious against *C. trachomatis,* which causes uncomplicated urethritis or cervicitis infection (Lau and Qureshi 2002). It is also recommended to cure genital ulcer diseases triggered by *H. ducreyi* (*chancroid*) (Workowski and Berman 2010). It can be used for the treatment of uncomplicated gonorrhea (Handsfield et al. 1994).

Helicobacter pylori Infections

Azithromycin has shown promise for treatment of *H. pylori* associated peptic ulcer disease, by decreasing ulcer recurrence and promoting healing (Jerry et al. 2011).

Other Diseases

It has shown good effectiveness in avoiding and handling the spread of *Mycobacterium aviumintracellulare* (MAC) disease in patients with human immunodeficiency virus (HIV) (Jerry et al. 2011).

16.5 Ivermectin

Ivermectin is a derivative of a broad spectrum of anti-parasitic macrocyclic lactones called ivermectins. It is isolated and extracted from the species *Streptomyces avermitilis*. Ivermectin (Mk-0933, 22,23-dihydro derivatives of avermectin B1) has arrangements similar to macrolide antibiotics (Sunita et al. 2012). It comprises a blend of two homologous compounds, 22,23-dihydro avermectin B_{1a} (>80%) and 22,23-dihydro avermectins B_{1b} (>20%), as shown in Fig. 16.1d (Campbell 1992).

16.5.1 Mode of Action

Ivermectin brings high-affinity efficacy binding to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells of the microfilaria. This process causes a surge in the absorptivity of the cell membrane to chloride ions and leads to hyperpolarization of the cell, which are important for paralysis and death of the parasite. It is also alleged that it acts as an antagonist of the neurotransmitter gamma-aminobutyric acid (GABA), thereby distracting GABA mediated central nervous system (CNS) neuro synaptic transmission (DRUGBANK Online n.d.).

16.5.2 Application

16.5.2.1 Onchocerciasis (African River Blindness)

Onchocerciasis is used against *Onchocerca volvulus*, characterized by subcutaneous nodules (*onchocercomas*), hard, thickened skin (lichenification), and disseminated prurigo on the trunk and extremities (Okulicz et al. 2004). It is extensively used in humans to control endemic *oncohocerciasis* (De Sol et al. 1990).

16.5.2.2 Filariasis

Filariasis is an infection by filarial worms (*Filariabancrofti, Wuchereriamalayi*) that are key vectors for mosquitoes (*Culex, Anopheles, Aedes, and Manonia*), and it is

cured with oral ivermectin in patients; it disappears, but the efficacy of the antibiotic depends on how often it is administered (Ottesen et al. 1990; Bockarie et al. 1998).

16.5.2.3 *Lymphatic filariasis*

It is a potential therapeutic to control *Bancroftianfilariasis* with or without combination with diethylcarbamazine (Bockarie et al. 1998).

16.5.2.4 Cutaneous Larva Migrans

The disease is mainly caused by *Ancylostomabraziliense*, and it generally occurs because of the cutaneous penetration of hookworm larvae from animals and is treated with a single oral dose of ivermectin (Caumes et al. 1992).

16.5.2.5 Cutaneous Larva Currens

This cutaneous manifestation is associated with *Strongyloidiasis* (Stonglyloidesstercoralis) and treated with ivermectin; patients usually recover within 3 months (Tarlow et al. 2002).

16.5.2.6 Demodicosis

Demodex flliculorum is commonly found on human skin, and it causes demodicosis. It is especially prevalent in immune-suppressed patients, and leads to demodicosis affecting the face, which is challenging to treat. This can be effectively treated with ivermectin; another example is papulopustular rosacea-like facial demodicosis cured by ivermectin and 5% permethrin (Dourmishev et al. 2005).

16.5.2.7 Myiasis

In humans, infection is passed on by fly larvae carrying *Cochilomyia hominivorax,* and the disease is treated with 1% propylene glycol and ivermectin (Victoria et al. 1999). Cutaneous myiasis is caused by *H. lineatum,* and it is cured by ivermectin antibiotic, leading to the impulsive passage of the maggots (Dourmishev et al. 2005).

16.5.2.8 Loiasis

The horsefly (Chyrsobs) is a vector for Loa loa. Clinical manifestation includes a transient prurigo nodularis-like bulge (Calabar distension) on the upper extremities.

Ivermectin has shown excellent results in the treatment of loiasis (Kombila et al. 1998).

16.5.2.9 Gnathostomiasis

Gnathostomiasis is also known as larva migrans profundus and infects humans through the nematode (roundworm) *Gnathostoma spinigerum* and *Gnathostoma hispidum*. Ivermectin has shown effective treatment of gnathostomiasis (Chappuis et al. 2001; Nontasut et al. 2000).

16.5.2.10 Crusted Scabies and Immune-Compromised Patients

Single doses of ivermectin or frequent doses or in union with keratolytic agents or topical scabicidal agents are used in the management of crusted scabies (Del Giudice et al. 1996). It is especially challenging to treat patients with HIV infection; ivermectin has been noted to bring relief in scabies-infected and HIV-infected patients (Meinking et al. 1995).

16.6 Chloramphenicol

Chloramphenicol was introduced in 1949 as the first broad-spectrum antibiotic, and it quickly gained acceptance. The synthesis process is easy, inexpensive, and does not have any significant toxicity, and it can be administered orally or parenterally (Henry et al. 1981). Chloramphenicol can be extracted through a synthetic process and isolated from soil and compost bacteria known as the genera *Streptomyces venzuelae*. This antibiotic can be used in treating meningitides caused by *Heamophilus influenza, Streptococcus pneumonia*, and *Neisseria meningitides* because of its bactericidal action alongside these organisms, and the facility to achieve great concentration in the cerebrospinal fluid (Howard 2004). The structure constitutes a nitrobenzene ring bonded with non-ionic chlorine. It consists of two unusual components, one-nitro ($–NO_2$) group and a dichloroacetyl ($–COCHCl_2$) group. The molecule possesses two asymmetric carbon atoms (shown in Fig. 16.1e). As an outcome, four optical isomers of chloramphenicol are possible. Out of these isomers, only the D(−) thero isomer carries a high efficacy active site for an antibiotic.

16.6.1 Mode of Action

Generally, mRNA's mechanism will bind to the ribosomes and form peptide bonds and inhibit protein synthesis. It will bind reversibly to the 50s subunit of the 70s ribosome, which prevents the attachment of an amino acid to the end of the aminoacyl-RNA (its binding region); hence, it inhibits the activity of the peptidyl transferase enzyme (Howard 2004).

16.6.2 Application

16.6.2.1 *H. influenzae*

Chloramphenicol antibiotic is the choice drug for dealing with severe infection caused by the ampicillin-resistant *H. influenzae* (McGowan et al. 1976). A combination of chloramphenicol and ampicillin was used in therapy for pediatric patients with meningitis caused by *H. influenzae* (American Academy of Pediatrics, Committee on Infectious Disease 1976).

Typhoid and Enteric

Chloramphenicol antibiotics are the prime drug to treat typhoid and enteric (Robertson et al. 1968; The choice of antimicrobial drugs 1978; Gleckman 1975) caused by the bacterium *Salmonella enterica* subsp. *Enterica serovar typhi* growing in the intestines and blood.

Brain Abscess

Some organisms, especially *B. fragilis,* are able to penetrate the blood–brain barrier and spread their toxicity, leading to a brain abscess. Chloramphenicol is the choice of drug for treatment (The choice of antimicrobial drugs 1978; Heineman and Braude 1963; Brewer et al. 1975; Finegold et al. 1975; Unsigned editorial 1978).

Rickettsial Infection

Anaplasmosis, ehrlichisois, and Q fever are types of infection mainly caused by bacteria and have the ability to grow inside cells of another organism. Most of these types of infections are spread through ticks, mites, fleas, or lice. Chloramphenicol or tetracycline can be used as treatment against rickettsia infections (Vianna and Hinman 1960; Peterson 1960; Haynes et al. 1970; Rose et al. 1950).

16.7 Rifamycins

The antibiotic rifamycins were discovered in 1957 and are commonly produced from the fermentation process by *Streptomyces mediteeranei* species (Sensi et al. 1960). The antibiotic for treatment belongs to a family known as Ansamycin antibiotics (Rinehart and Shields 1976; Wehrli 1977). It is so named because it has a basket-like molecular structural design encompassing an aromatic moiety linked at non-adjacent positions by an aliphatic chain (Prelog and Oppolzer 1973). It consists of a heterocyclic structure encompassing a naphthoquinone core spanned by an aliphatic Ansa chain. The napthoquinoic chromophore substance emits rifampicin as a red-orange crystalline color. It consists of four critical hydroxyl groups bound to the Ansa bridge and naphthol rings, which form hydrogen bonds with amino acid residues on the protein. It inhibits the activity of bacterial RNA polymerase (Campbell et al. 2001). Rifampicin 3-(4-methyl-1-piperazinyl)-iminomethyl is derived from the rifamycin SV precursor antibiotic (Bennett 2015), as shown in Fig. 16.1f.

16.7.1 Mode of Action

Rifamycins and other forms of this group of antibiotics have antibacterial activity by inhibiting RNA synthesis. The antibiotic will form strong bonds with the DNA-dependent RNA polymerase of prokaryotes and interfere with the RNA synthesis initially, which takes interfaces between the naphthalene rings and the aromatic moiety in the polymerase. RNA polymerase consists of zinc atoms that allow an obligatory phenolic–OH group to join the naphthalene ring (Keer 2013).

16.7.2 Applications

16.7.2.1 *M. tuberculosis*

Rifamycin has a bactericidal activity for both Gram-positive and Gram-negative bacteria. It is broadly used in infection triggered by *Mycobacterium* sp. (especially *M. tuberculosis*). It can be used in combination with another bactericidal agent to overcome the antibiotic drug resistance scenario.

16.7.2.2 *E. coli* and *C. difficile*

Rifamycin showed inhibition activity of the pathogenic strains of *E. coli* (enterohaemorrhagic, enterotoxigenic ETEC, enteropathogenic, and enteroaggregative EAEC strains). The *Clostridium difficile* strain has been treated with rifamycin SV (Farrell et al. 2011).

16.7.2.3 Other Infections

Rifamycin is commonly used to eliminate the infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) through synergetic antibiotics. It is also used in osteomyelitis and prosthetic joint infections (Perlroth et al. 2008). This drug can be used against *Neisseria meningitidis* (meningococcal) infections and as a substitute treatment for toxicities that occurred through tick-borne pathogens such as *Borrelia burgdorferi* and *Anaplasma phagocytophilum* (Wormser et al. 2006; Thomas et al. 2009).

16.8 Vancomycin

Vancomycin is a tricyclic glycopeptide antibiotic, initially reported by the scientist of Eli Lily company in 1956. A new actinomycete species was discovered, known as *Streptomyces orientalis* (now *Nocardisorientalis*), isolated from the soil samples at Borneo (Jerome 1987). Another strain used in the production of vancomycin was reported in 1958, which is *Amycolatopsis orientalis,* and has been introduced in clinical practice (Rossolini et al. 2014). Vancomycin is prescribed to combat severe infection caused by Gram-positive pathogens and for the organisms that are resistant to the added antimicrobial agents. Vancomycin is recommended for patients allergic to penicillin and cephalosporin antibiotics (Gupta et al. 2011; Hicks and Hernandez 2011). It consists of a glycosylated hexapeptide chain connected with unusual amino acids. Vancomycin is rigid due its aromatic rings, which are halogenated and cross-linked with aryl ether bonds. It carriers a seven-member peptide chain with two sugar moieties, vancosamine and glucose. The vancomycin chemical structure is shown in Fig. 16.1g.

16.8.1 Mode of Action

Vancomycin can act upon the invading bacterial pathogens by directly inhibiting the cell wall synthesis, and more precisely, it will inhibit the peptidoglycan biosynthesis (Hicks and Hernandez 2011). In Gram-positive bacterium, this element is also considerable, which forms massive and insoluble layers on the outer membrane, totaling up to 40 layers comprising multiple skeletons of amino sugars *N*-acetyl-glucosamine and *N*-acetyl muramic (Chambers 2010). The latter encloses lateral short peptide filtrates with cross-links, which are a type of high glassy resistant polymeric chain (Chambers 2010). The drug constrains this polymerization or the transglycosylase reaction once it binds with high affinity to the C-terminal D-alanyl D-alanine residues of lipid-linked cell wall precursors and bridges the linkage to the glycopeptide polymer (Hicks and Hernandez 2011). As an outcome, it hinders the

cross-links of peptides from binding to tetrapeptide side chains; namely, it averts its linkage to the growing tip of the peptidoglycan (Chambers 2010).

16.8.2 Application

16.8.2.1 *Staphylococcus aureus*

Vancomycin is the most effective drug for treating severe penicillin-resistant staphylococcal infections, including pneumonia, osteomyelitis, endocarditis, sepsis, and wound infections (Geraci et al. 1956, 1985). It is an efficient drug for the treatment of MRSA (Craven et al. 1983; Thompson et al. 1982).

16.8.2.2 *Staphylococcus epidermidis*

The increased prevalence of *S. epidermidis*as nosocomial infections, and particularly patients suffering from granulocytopenia (MacCulloch 1981; Wade et al. 1982) and *S. epidermidis,* is most frequently associated with catheter infection (Tchekmedyian et al. 1986; Schoenbaum et al. 1975). Vancomycin can be used as therapy.

16.8.2.3 Non-enterococcal Streptococci

In patients infected with streptococcal and allergic to the beta-lactam antibiotic, vancomycin acts as an effective agent against these infections. It also has a synergistic effect with aminoglycoside treated *S. viridans* or *S. bovis,* which causes endocarditis on native valves (Geraci and Wilson 1981).

16.8.2.4 Enterococcus

The antibiotic vancomycin is generally used to treat penicillin-allergic patients. Its role is very significant in controlling enterococcal infections, together with endocarditis (Hande et al. 1976).

16.8.2.5 Diphtheroid

Vancomycin can be used as an empiric therapy against JK-diphtheroids. This strain has a crucial role in controlling the main pathogen in the immunocompromised host (Schoch and Cunha 1986). *C. diphtheria* is collectively known as diphtheroids (Hande et al. 1976).

16.8.2.6 *Pseudomembranous colitis*

PMC or antibiotic-associated *C. difficile* colitis is an ailment described by profuse watery diarrhea, including abdominal pain, blood stool, and fever. Vancomycin is effective in treating PMC and *C. difficile* colitis (Silva et al. 1981).

16.8.2.7 Antibacterial Activity

An agent or substance that hinders bacterial growth or destroys bacteria is known as antibacterial activity. Many antibacterial products were produced from actinomycetes species and are widely used in the present status. A bonactin compound extracted from *Streptomyces* sp. BD21–2 showed biological function over Gram-positive and Gram-negative organisms (Schumacher et al. 2003). A novel class of a gucyclinone antibiotic was extracted from the *Streptomyces griseus NTK* 97 strain, known as frigocyclinone (Fig. 16.2). The drug consists of the moiety terramycin to which a c-glycoside linkage is attached with amino-de-oxysugar ossamine. It has exhibited bactericidal tendency for Gram-positive bacteria (Bruntner et al. 2005). Benzooxzle antibiotics such as carboxamyci produced from *Streptomyces* sp. NTK 937 showed inhibition action for Gram-positive bacteria (Hohmann et al. 2009). An

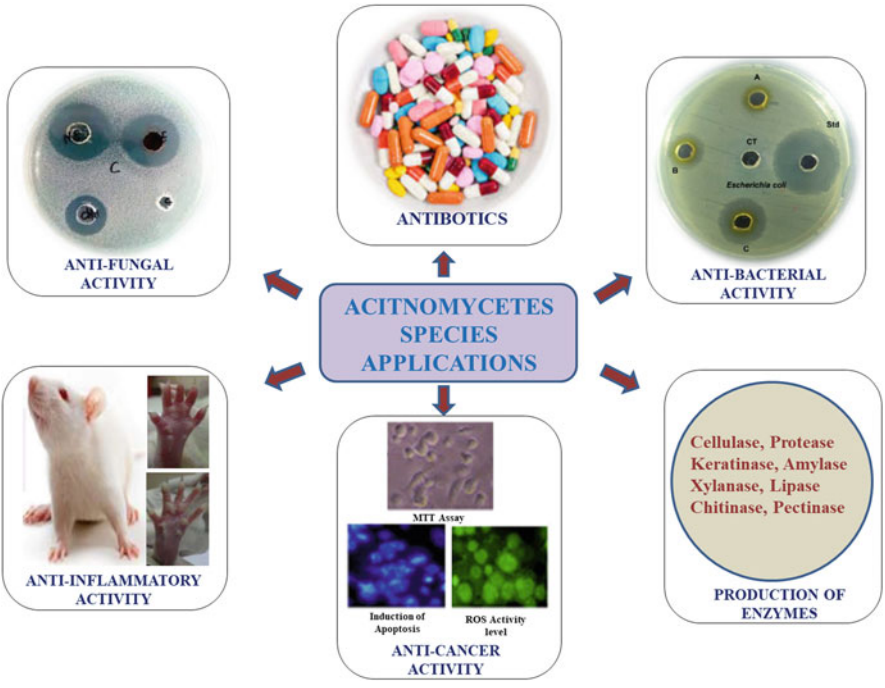

Fig. 16.2 Application of the products produced from actinomycetes species

antibiotic known as bis-anthraquinone extracted from *Streptomyces* sp. showed biotic activities against VRE, MSSA, and TRSA, respectively (Socha et al. 2006), as shown in Tables 16.1 and 16.2.

16.8.2.8 Antifungal Activity

A substance or agent that acts to inhibit or kill fungi is known as having antifungal activity. Numerous antibiotics have been isolated from different microorganisms and showed effective fungicidal activity against pathogenic fungi. Among *Streptomyces* species, the strain *Streptomyces* sp. DA11 isolated from marine samples can produce the enzyme chitinase and has fungicidal activity against *Aspergillus niger* and *Candida albicans* (Han et al. 2009). The chitinase enzyme has wide application in the biomedical field because it shows biocompatible quality and fungicidal activity. It is also used in wound healing, cartilage tissue engineering, drug delivery, and nerve generation (Shi et al. 2006; Yan et al. 2006). Candihexin is a compound produced from *Streptomyces viridoflavus* showing antifungal activity (Martin and McDaniel 1974). Similarly, another compound nanomycin, produced from the *Streptomyces rosa,* had antifungal activity (Omura et al. 1974).

16.8.2.9 Anti-inflammatory Activity

Anti-inflammatory drugs can be used for the treatment of symptoms like swelling and redness. Inflammation means consumed flames, and nowadays, it represents a type of soreness somewhere on your body that is red, feels hot, and swells up. Cyclomarin, a type of new cyclic heptapeptide compound extracted from *Streptomyces* sp., exhibited anti-inflammatory activity in both in vivo and in vitro assays (Renner et al. 1999). A strain called *Streptomyces* sp. CNB-091, isolated from the jellyfish *Cassiopeia xamachana*, produced bicyclic depsipeptides known as salinamides A and B; these metabolites showed anti-inflammatory activity (Moore et al. 1999). A bioactive compound extracted from the *streptomyces* sp. VITPSA strain from marine samples has shown 70% hemolysis, indicating it has a moderate anti-inflammatory activity (Pooja et al. 2017), as shown in Table 16.1.

16.8.2.10 Anticancer Activity

Cancer is a major problem affecting human wellbeing. Among the different kinds of cancer, breast cancer ranks second in universal and causes the highest mortality in women (Ravikumar et al. 2010). Chinikomycin is a compound extracted from *Streptomyces* sp. and has shown anti-tumor efficacy toward human cancer cell lines (Li et al. 2005). The *Streptomyces aueroverticillatus* NPS001583 strain was isolated from marine sediments, and it produces a 22 membered macrocyclic lactam known as *Aureoverticillactam.* It has shown anti-tumor activity for human colorectal

Table 16.1 Natural products derived from actinomycetes and their antimicrobial therapeutic

Source of organism	Natural product (NP) of derivative	Chemical class	Drug name	Application	References
Non-marine sources					
Saccharopolyspora erythraea	Erythromycin (NP-derivative)	Macrolide	Telithromycin	Antibacterial for both Gram-positive and Gram-negative	Butler et al. (2017)
Streptomyces cattelya	Thienamycin (NP-derivative)	Carbapenem	Biapenem	Antibacterial for both Gram-positive and Gram-negative	Butler et al. (2017)
Streptomyces cattelya	Thienamycin (NP-derivative)	Carbapenem	Ertapenem	Antibacterial for Gram-positive and Gram-negative	Butler et al. (2017)
Streptomyces roseosporus	Natural product	Lipopeptide	Daptomycin	Antibacterial for Gram-positive	Butler et al. (2017)
Stretomyces sp.	Thienamycin (NP-derivative)	Carbapenem	Doripenen	Antibacterial for both Gram-positive and Gram-negative	Butler et al. (2017)
Streptomyces aureofaciens	Tetracycline (NP-derivative)	Tetracycline	Tigecycline	Antibacterial for both Gram-positive and Gram-negative	Butler et al. (2017)
Stretomyces sp.	Thienamycin (NP-derivative)	Carbapenem	Tebipenem pivoxil	Antibacterial for Gram-positive	Butler et al. (2017)
Amycolatopis orientalis	Vancomycin (NP-derivative)	Glycopeptide	Tealvacin	Antibacterial for Gram-positive	Butler et al. (2017)
Dactylosporangium aurantiacum	Natural product	Tiacumicin	Fidaxomicin	Antibacterial for Gram-positive	Butler et al. (2017)
Nonomuria sp.	Teicoplanin (NP-derivative)	Glycopeptide	Dalbavancin	Antibacterial for Gram-positive	Butler et al. (2017)
Amylcolastopsis orientalis	Chloroeremomycin (NP-derivative)	Glycopeptide	Oritavancin	Antibacterial for Gram-positive	Butler et al. (2017)
Actinomycete Strain	NP-derivative	Penicillanic acid sulfone derivative and β-lactamase inhibitor	Tazobactam	Antibacterial for Gram-negative	Butler et al. (2017)

Marine sources					
Salinispora tropica	Natural product	Beta-lactone-gamma	Salinosporamide A (Marizomib)	Multiple cancer	Feling et al. (2003)
Salinispora sp.	Natural product	Lactam	Arenamides A and B	Inflammation	Asolkar et al. (2009)
Streptomyces sp.	Natural product	Polyketide	Anthracimycin	Anthrax	Jnag et al. (2013)

Table 16.2 Sreening of actinomycetes strains by physicochemical to discover new compound up to March 2018

Source of organism	Producing microorganisms	Name of the compound	Application	References
[a]KML, Irumamycin producing strain, [b]36 years	*Streptomyces sulbflavus* subsp. *Irumaenis AM-3603*	Bisoxazolomycin	Antibacterial	Koomsiri et al. (2017)
[a]KML, Streptomycin producing strain, [b]43 years	*Streptomyces griseus OS-3601*	Iminimycin A and B	Antibacterial	Nakashima et al. (2016a, b)
[a]KML, Nanomycin producing strain, [b]36 years	*Streptomyces rosa* subsp. *Notoensis OS-3966*	Nanomycin F–H	Inhibitor of epithelial–mesenchymal transition induced cells	Nakashima et al. (2015a, 2017)
Roots of Capsicum frutescents in Thailand	*Actinoallomu rus fulvus MK 10-036*	Actinoallolide A–E	Antitrypanosomal	Inahashi et al. (2015)
Roots of mondo grass in Saitama Pref, Japan	*A.fulvus K09-0307*	Actinoallolide A–E	Antitrypanosomal	Inahashi et al. (2015)
Roots of fern in Hamura city, Tokyo, Japan	*Allostreptomyces* sp. *K12-0794*	Hamuramicin A and B	Antibacterial	Suga et al. (2018)
Roots of orchid in Iriomote Island, Japan	*Streptosporangium oxazolinicym K07-0460* ^{T}	Sproxazomicin A–C	Antitrypanosomal	Inahashi et al. (2011a, b)
Roots of orchid in Iriomote Island, Japan	*Polymorphospora rubra K07-0510*	Trehangelin A–C	Anti-lipid peroxidation	Nakashima et al. (2013), Inahashi et al. (2016)
Roots of orchid in Iriomote Island, Japan	*Polymorphospora rubra K07-0510*	Trehangelin A–C	Enhanced production of collagen	Nakashima et al. (2013), Inahashi et al. (2016)
Sediment from mangrove forest in Iriomote Island, Japan	*Lechevalieria aerocolonigenes K10-0216*	Mangromicin A–I	Antitrypanosomal	Nakashima et al. (2014a, b, 2015b)
Sediment from mangrove forest in Iriomote island, Japan	*Lechevalieria aerocolonigenes K10-0216*	Mangromicin A–I	Antioxidative	Nakashima et al. (2014a, b, 2015b)
Sediment from mangrove forest in Iriomote Island, Japan	*Lechevalieria aerocolonigenes K10-0216*	K10-0216 KA and KB	Inhibitory effect on the lipid accumulation	Nakashima et al. (2015c)
Sediment from mangrove forest	*Lechevalieria aerocolonigenes K10-0216*	Pyrizomicin A and B	Antimicrobial	Kimura et al. (2018a)

(continued)

Table 16.2 (continued)

Source of organism	Producing microorganisms	Name of the compound	Application	References
in Iriomote Island, Japan				
Sea sediment, Namako Pond in Kagoshima Pre, Japan	*Mumia* sp. *YSP-2-79*	Mumiamicin	Antibacterial	Kimura et al. (2018b)
Sea sediment, Namako Pond in Kagoshima Pre, Japan	*Mumia* sp. *YSP-2-79*	Mumiamicin	Antioxidative	Kimura et al. (2018b)
Soil, Kangawa a Pref, Japan	*Actinomadura* sp. *K13-0306*	Sagamilactam	Cytotoxicity	Kimura et al. (2016)
Soil, Kangawa a Pref, Japan	*Actinomadura* sp. *K13-0306*	Sagamilactam	Antitrypanosomal	Kimura et al. (2016)
Soil, Okinawa a Pref, Japan	*Amycolatopsis* sp. *K16-0194*	Dipyrimicin A and B	Antibacterial	Izuta et al. (2018)

[a]*KML* Kitasato Microbial Library
[b]Length of preservation by Lyophilization**; No. 1–3,** Compounds from the KML**; No. 4–13,** Compounds from fresh isolates **(No. 4–7, Roots of plants; No. 8–10, Sediment of mangrove forest; No. 11, Marine sediment; Nos. 12 and 13, Soil)**

adenocarcinoma HT-29, Jurkat leukemia, and mouse melanoma B16F10 cell lines (Mitchell et al. 2004). *Streptomyces chinaensis* AUBN1/7 produced the polyketide structural compound called 1-hydroxyl-1-norresistomycin from marine samples and has shown antitumor activity (Gorajana et al. 2005). A butenolides structure compound extracted from the species *Streptoverticillium luteoverticillantum* 11,014 has shown antitumor activity (Li et al. 2006) (Table 16.1).

16.8.2.11 Antitrypanosomal Activity

The first antitrypanosomal drug was discovered in 1922 by scientist Paul Ehrlich. He discovered two drugs known as suramin and pentamidine. These two are highly effective against cerebral stages of African trypanosomiasis, of both *T. brucei gambiense* and *T. brycei rhodesiense* type (Stefan and Walter 2008). The source, organism, and its application are shown in Table 16.2.

16.8.2.12 Industrially Important Enzyme Production from Actinomycetes

Actinomycetes secrete the enzyme called amylases from cells to indicate its extracellular digestion activity is ready, for example, alpha-amylase. Starch degrading

amylolytic enzymes have wide applications in the food industry, fermentation, textile and paper industries, and other biotechnological applications (Pandey et al. 2000). Another important enzyme, lipase, is also produced from actinomycetes species. These enzymes are extensively used in detergent industries, foodstuff, oleochemical diagnostic settings, and pharmaceuticals (Schmid and Verger 1998). L-Asparagine produced from *S. albidoflavus, S. griseus, S. Karnatakensis,* and *Nocardia* sp. has therapeutic use in managing human cancers, especially acute lymphoblastic leukemia (Gallagher et al. 1989; Verma et al. 2007).

16.8.2.13 Other Applications

- Ecological importance: They are used as degraders of toxic materials and in bioremediation.
- Volatile organic compounds: Actinomycetes are used in the production of geosmin.
- Extracellular peroxidase activity: Actinomycetes species produce the extracellular components that can be used to prepare diagnostic kits.
- Agro active compounds: They are used in the production of fungicides such as kasugamycin and polyoxin B and D.
- Plant growth-promoting agents: They are widely used in producing bio-control agents and plant growth-promoting products for controlling *Fusarium* and *Verticillium wilts* as a seed coating and hormone-like auxins, gibberellins, and cytokinins, respectively.

16.9 Conclusion

Actinomycetes species have been known for the past 50 years as the prolific manufacturer of novel bioactive compounds widely used in different applications. Hence, these actinomycetes can be explored further to produce different bioactive compounds that would significantly improve health conditions among the population and develop humankind's socio-economic status.

References

American Academy of Pediatrics, Committee on Infectious Disease (1976) Current status of ampicillin-resistant *Hemophilus influenzae* type b. Pediatrics 57:417

American Thoracic Society; Infectious Diseases Society of America (2005) Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 171:388–416

Asolkar RN, Freel KC, Jensen PR, Fenical W, Kondratyuk TP, Park PJM (2009) Arenamides A-C, cytotoxic NFκB inhibitors from the marine actinomycete *Salinispora arenicola*. J Nat Prod 72: 396–402

Avent ML, Rogers BA, Cheng AC, Paterson DL (2011) Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. Intern Med J 41:441–449

Bennett J (2015) Principles and practice of infectious diseases. Elsevier Health Sciences, Amsterdam, p 339

Berdy J (2005) Bioactive microbial metabolites and antibiotics. J Antibiot 58:1–26

Bockarie MJ, Alexander ND, Hyun P et al (1998) Randomized community based of annual single dose diethylcarbazamine with or without ivermectin against *Wucheria bancrofti* infection in human beings and mosquitoes. Lancet 351:162–168

Brewer NS, MacCarty CS, Wellman WE (1975) Brain abscess: a review of recent experience. Ann Intern Med 82:571–576

Bright GM, Nagel AA, Bordner J, Desai KA, Dibrino JN, Nowakowska J et al (1988) Synthesis, in vitro and in vivo activity of novel 9-deoxo-9a-AZA-9a-homoerythromycin A derivatives: a new class of macrolide antibiotics, the azalides. J Antibiot 41:1029–1047

Bruntner C, Binder T, Pathom-aree W, Goodfellow M, Bull AT, Potterat O et al (2005) Frigocyclinone, a novel angucyclinone antibiotic produced by a *Streptomyces griseus* strain from Antarctica. J Antibiot 58(5):346–349

Bunnag D, Karbwang J, Na-Bangchang K, Thanavibul A, Chittamas S, Harinasuta T (1996) Quinine-tetracycline for multidrug resistant falciparum malaria. Southeast Asia J Trop Med Public Health 27:15–18

Butler MS, Blaskovich MA, Cooper MA (2017) Antibiotics in the clinical pipeline at the end of 2015. JAntibiot 70:3–24

Campbell WC (1992) The genesis of the antiparasitic drug, ivermectin. In: Weber RJ, Perkins DN (eds) Inventive minds: creativity in technology. Oxford University Press, Oxford, pp 194–214

Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104(6): 901–912

Casida LE (1965) An abundant microorganism in soil. Appl Microbiol 13:327–334

Caumes E, Darty A, Paris L et al (1992) Efficacy of ivermectin in the therapy of cutaneous larva migrans. Arch Dermatol 128:994–995

Chambers HF (2010) Antimicrobial agents: protein synthesis inhibitors and miscellaneous antibacterial agents. In: Hardman JG, Limbird LE (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 11th edn. McGraw-Hill, New York, pp 1074–1077

Chappuis F, Farinelli T, Loutan L (2001) Ivermectin treatment of a traveler who returned from Peru with cutaneous gnathostomiasis. Clin Infect Dis 33:e17–e19

Chopra I (1994) Tetracycline analogs whose primary target is not the bacterial ribosome. Antimicrob Agents Chemother 38:637–640

Chopra I, Hawkey PM, Hinton M (1992) Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother 29:245–227

Craven DE, Kollisch NR, Hsieh CR et al (1983) Vancomycin treatment of bacteremia caused by oxacillin-resistant *Staphylococcus aureus*; comparison with 13-lactam antibiotic treatment of bacteremia caused by oxacillin-sensitive *Staphylococcus aureus*. J Infect Dis 147:137

Cunha BA (1999) Doxycycline re-visited. Arch Intern Med 159:1006–1007

Davis BD (1987) Mechanism of bactericidal action of aminoglycosides. Microbiol Rev 51:341–350

Davis BD, Chen LL, Tai PC (1986) Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. Proc Natl Acad Sci 83:6164–6168

De Sol G, Dadzie KY, Giese J et al (1990) Lack of adverse reactions in ivermectin treatment for onchocerciasis. Lancet 335:1106–1107

Del Giudice P, Carles M, Couppie P, Bernard E, Lacour JP, Marty P, Pradinaud R, Ortonne JP, Dellamonica P, Le Fichoux Y (1996) Successful treatment of crusted (Norwegian) scabies with ivermectin in two patients with human immunodeficiency virus infection. Br J Dermatol 135: 494–495

Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R et al (2013) Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 41:580–637

Dietera A, Hamm A, Fiedler HP, Goodfellow M, Muller WE, Brun R et al (2003) Pyrocoll, an antibiotic, anti-parasitic and antitumor compound produced by a novel alkaliphilic *Streptomyces* strain. J Antibiot 56:639–646

Dourmishev AL, Dourmishev LA, Schwartz RA (2005) Ivermectin: pharmacology and application in dermatology. Int J Dermatol 44:981–988

DRUGBANK Online (n.d.). https://go.drugbank.com

Dubravko J, Roberto A (2016) Review: from erythromycin to azithromycin and new potential ribosome-binding antimicrobials. Antibiotics 5(29):21–13

Farrell DJ, Putnam SD, Biedenbach DJ, Moro L, Bozzella R, Celasco G, Jones RN (2011) In vitro activity and single step mutational analysis of rifamycin SV tested against enteropathogens associated with traveler's diarrhea and *Clostridium difficile*. Antimicrob Agents Chemother 55(3):992–996

Feling RH, Buchanan GO, Mince TJ, Kauffman CA, Jensen PR, Fenical W (2003) Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. Angew Chem Int Ed Engl 42:355–357

Fiese EF, Steffen SH (1990) Comparison of the acid stability of azithromycin and erythromycin A. J Antimicrob Chemother 25(Suppl A):39–47

Finch RG (1997) Tetracyclines. In: O'Grady F, Lambert HP, Finch RG, Greenwood D (eds) Antibiotic and chemotherapy, 7th edn. Churchill Livingstone Ltd, New York, pp 469–484

Finegold SM, Bartlett JG, Chow AW, Flora DJ, Gorbach SL, Harder EJ, Tally FP (1975) Management of anaerobic infections. Ann Intern Med 83:375–389

Gallagher MP et al (1989) Asparaginase drug for treatment of acute lymphoblastic leukemia. Essays Biochem 24:1–40

Ganachari S, Yaradoddi J, Somappa S, Mogre P, Tapaskar R, Salimath B, Venkataraman A, Viswanath V (2019) Green nanotechnology for biomedical, food and agricultural applications. In: Martínez LMT, et al (ed) Handbook of ecomaterials. Springer. https://doi.org/10.1007/978-3-319-68255-6_184

Geraci JE, Wilson WR (1981) Vancomycin therapy for infective endocarditis. Rev Infect Dis 3 (Suppl):250

Geraci JE, Heilman FB, Nichols OR et al (1956) Some laboratory and clinical experience with a new antibiotic, vancomycin. Proc Staff Meet Mayo Clin 31:564

Geraci JE, Heilman FR, Nichols OR et al (1985) Antibiotic therapy of bacterial endocarditis: VII. Vancomycin for acute micrococcal endocarditis: preliminary report. Proc Staff Meet Mayo Clin 33:172

Girao M, Ribeiro I, Riberio T, Azevdo IC, Pereira F, Urbatzka R, Leao PN, Caravlho MF (2019) Actinobacteria isolated from *Laminariao chroleuca*: a source of new bioactive compounds. Front Microbiol 8:1535

Gleckman RA (1975) Warning-chloramphenicol may be good for your health. Arch Intern Med 135:1125–1126

Goodfellow M, Kampher P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB (eds) (2012) Bergey's manual of systematic bacteriology, part A, vol 5, 2nd edn. Springer, London

Gorajana A, Kurada BV, Peela S, Jangam P, Vinjamuri S, Poluri E, Zeeck A (2005) 1-Hydroxy-1-norresistomycin, a new cytotoxic compound from a marine actinomycete, *Streptomyces chibaensis* AUBN1/7. J Antibiot 58:526–529

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF et al (2007) An official ATS/IDSA statement: diagnosis, treatment, and prevention of non-tuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–416

Gupta A, Biyani M, Khaira A (2011) Vancomycin nephrotoxicity: myths and facts. Neth J Med 69: 379–383

Gustafson RH, Kiser JS (1985) Nonmedical uses of the tetracyclines. In: Hlavka JJ, Boothe JH (eds) Handbook of experimental pharmacology, vol 78. Springer-Verlag KG, Berlin, pp 405–446

Hagedorn C (1976) Influences of soil acidity on *Streptomyces* populations inhabiting forest soils. Appl Environ Microbiol 32:368–375

Han Y, Yang B, Zhang F, Miao X, Li Z (2009) Characterization of antifungal chitinase from marine *Streptomyces sp*., DA11 associated with South China Sea sponge *Craniella australiensis*. Mar Biotechnol 11(1):132–140

Hande KR, Witebsky FG, Brown MS et al (1976) Sepsis with a new species of *Corynebacterium*. Ann Intern Med 85:423

Handsfield HH, Dalu ZA, Martin DH et al (1994) Multicenter trial of single-dose azithromycin vs. ceftriaxone in the treatment of uncomplicated gonorrhea: Azithromycin Gonorrhea Study Group. Sex Transm Dis 21(2):107–111

Hansen LH, Mauvais P, Douthwaite S (1999) The macrolide-ketolide antibiotic binding site is formed by structures in domains II and V of 23S ribosomal RNA. Mol Microbiol 31(2):623–631

Haynes RE, Sanders DY, CramblettHG (1970) Rocky Mountain spotted fever in children. J Pediatr 76:685–693

Heineman HS, Braude AI (1963) Anaerobic infection of the brain. Am J Med 35:682–697

Henry MF, Osier C Jr, Eufronio GM (1981) Chloramphenicol: review of its use in clinical practice. Rev Infect Dis 3(3):479–491

Hicks RW, Hernandez J (2011) Perioperative pharmacology: a focus on vancomycin. AORN J 93: 593–599

Ho YI, Chan CY, Cheng AF (1997) In-vitro activities of aminoglycoside-aminocyclitols against mycobacteria. J Antimicrob Chemother 40:27–32

Hohmann C, Schneider K, Bruntner C, Irran E, Nicholson G, Bull AT et al (2009) Caboxamycin, a new antibiotic of the benzoxazole family produced by the deep-sea strain *Streptomyces sp* NTK 937. J Antibiot (Tokyo) 62(2):99–104

Hopwood DA (1999) Forty years of genetics with *Streptomyces*: from in vivo through in vitro to *in-silico* (review article). Microbiology 145:2183–2202

Howard JB (2004) Chloramphenicaol: a review. Pediatr Rev 25(8):284–288

Hwang BK, Lim SW, Kim BS, Lee JY, Moon SS (2001) Isolation and in vivo and in vitro antifungal activity of phenyl acetic acid and sodium phenyl acetate from *Streptomyces humidus*. Appl Environ Microbiol 67:3730–3745

Ian C, Marilyn R (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65(2):232–260

Inahashi Y, Matsumoto A, Omura S, Takahashi Y (2011a) *Streptosporangium oxazolinicum sp*. nov., a novelendophytic actinomycete producing new antitrypanosomal antibiotics, spoxazomicins. J Antibiot 64:297–302

Inahashi Y, Iwatsuki M, Ishiyama A, Namatame M, Nishihara-Tsukashima A, Matsumoto A, Hirose T, Sunazuka T, Yamada H, Otoguro K et al (2011b) Spoxazomicins A-C, novel antitrypanosomal alkaloids produced by an endophytic actinomycete, *Streptosporangium oxazolinicum* K07-0460T. J Antibiot 64:303–307

Inahashi Y, Iwatsuki M, Ishiyama A, Matsumoto A, Hirose T, Oshita J, Sunazuka T, Watanalai PW, Takahashi Y, Kaiser M et al (2015) Actinoallolides A-E, new anti-trypanosomal macrolides, produced by an endophytic actinomycete, *Actinoallomurus fulvus* MK10-036. Org Lett 17: 864–867

Inahashi Y, Shiraishi T, Palm K, Takahashi Y, Omura S, Kuzuyama T, Nakashima T (2016) Biosynthesis of Trehangelin in *Polymorphospora rubra* K07-0510: identification of metabolic pathway to angelyl-CoA. ChemBioChem 17:1442–1447

Izuta S, Kosaka S, Kawai M, Miyano R, Matsuo H, Matsumoto A, Nonaka K, Takahashi Y, Omura S, Natashima T (2018) Dipyrimicins A and B, microbial compounds interacted with ergosterolresin, from *Amycolatopsis* sp. K16-0194. J Antibiot 71:535–537

Jackson J, Chen C, Buising K (2013) Aminoglycosides: how should we use them in the 21st century? Curr Opin Infect Dis 26:516–525

Jerome FL (1987) Vancomycin: a review. Med Clin North Am 71(6):1135–1145
Jerry MZ, Fozia Q, Bartholomew RB (2011) Review of macrolides (azithromycin, clarithromycin), ketolids (telithromycin) and glycylcyclines (tigecycline). Med Clin N Am 95:761–791
Ji B, Sow S, Perani E, Lienhardt C, Diderot V, Grosset J (1998) Bactericidal activity of a single-dose combination of ofloxacin plus minocycline, with or without rifampin, against *Mycobacterium leprae* in mice and in lepromatous patients. Antimicrob Agents Chemother 42:1115–1120
Jnag KH, Nam SJ, Locke JB, Kauffamn CA, Beatty DS, Paul LA, Fenical W (2013) Anthracimycin, a potent anthrax antibiotic from a marine-derived actinomycetes. Angew Chem Int Ed Engl 52:7822–7824
Keer P (2013) Fighting multidrug resistance with herbal extracts, essential oils and their components. Academic Press, New York
Kevin MK, Alisa WS, Timothy RK, Lynn EC (2016) Aminoglycosides: an overview. Cold Spring Harb Perspect Med 6(a27029):1–18
Kimura T, Iwatsuki M, Asami Y, Ishiyama A, Hokari R, Otoguro K, Matsumoto A, Sato N, Shiomi K, Takahashi Y et al (2016) Anti-trypanosoma compound, sagamilactam, a new polyene macrocyclic lactam from *Actinomadura sp*. K13-0306. J Antibiot 69:818–824
Kimura T, Inahashi Y, Matsuo H, Suga T, Iwatsuki M, Shiomi K, Takahashi Y, Omura S, Nakashima T (2018a) Pyrizomicin A and B: structure and bioactivity of new thiazolyl pyridines from *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 71:606–608
Kimura T, Tajima A, Inahashi Y, Iwatsuki M, Kasai H, Mokudai T, Niwano Y, Shiomi K, Takahashi Y, Omura S et al (2018b) Mumiamicin: structure and bioactivity of a new furan fatty acid from Mumia sp. YSP-2-79. J Gen Appl Microbiol 64:62–67
Kombila M, Duong TH, Ferrer A, Perret JC, Marion MC, Nguiri C et al (1998) Short and long-term action of multiple doses of ivermectin on *loiasis microfilaremia*. Am J Trop Med Hyg 58: 458–460
Koomsiri W, Inahashi Y, Kimura T, Shiomi K, Takahashi Y, Omura S, Thamchaipenet A, Nakashima T (2017) Bisoxazolomycin A: a new natural product from '*Streptomyces subflavus shbsp. Irumaensis*' AM-3603. J Antibiot 70:1142–1145
Kordick DL, Papich MG, Breitschwerdt EB (1997) Efficacy of enrofloxacin or doxycycline for treatment of *Bartonella henselae*or *Bartonella clarridgeiae* infection in cats. Antimicrob Agents Chemother 41:2448–2455
Kotra LP, Haddad J, Mobashery S (2000) Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother 44: 3249–3256
Kurtboke DI (2012) Bio discovery from rare actinomycetes: an eco-taxonomical perspective. Appl Microbiol Biotechnol 93(5):1843–1852
Langtry HD, Brogden RN (1997) Clarithromycin. A review of its efficacy in the treatment of respiratory tract infections in immunocompetent patients. Drugs 53(6):973–1004
Lau CY, Qureshi AK (2002) Azithromycin versus doxycycline for genital chlamydial infections: a meta-analysis of randomized clinical trials. Sex Transm Dis 29(9):497–502
Levy SB (1992) The antibiotic paradox: how miracle drugs are destroying the miracle. Plenum Press, New York
Levy SB, McMurry LM, Barbosa TM, Burdett V, Courvalin P, Hillen W, Roberts MC, Rood JI, Taylor DE (1999) Nomenclature for new tetracycline resistance determinants. Antimicrob Agents Chemother 43:1523–1524
Li F, Maskey RP, Qin S, Sattler I, Fiebig HH, Maier A et al (2005) Chinikomycins A and B: isolation, structure elucidation, and biological activity of novel antibiotics from a marine *Streptomyces sp*. isolate M045. J Nat Prod 68(3):349–353
Li DH, Zhu TJ, Liu HB, Fang YC, Gu QQ, Zhu WM (2006) Four butenolides are novel cytotoxic compounds isolated from the marine-derived bacterium, *Streptoverticillium luteoverticillatum* 11014. Arch Pharm Res 29:624–626
MacCulloch O (1981) Vancomycin in pregnancy. N Z Med J 93:9

Magad MA, Lina AB, Salah AA (2019) Unexplored extreme habitats as sources of novel and rare actinomycetes with enzyme and antimicrobial activities. IOSR J Pharm Biol Sci 14(6):45–54

Magnet S, Blanchard JS (2005) Molecular insights into aminoglycoside action and resistance. Chem Rev 105:477–498

Manteca A, Alvarez R, Salazar N, Yague P, Sanchez J (2008) Mycelium differentiation and antibiotic production in submerged culture of *Streptomyces coelicolor*. Appl Environ Microbiol 74:3877–3886

Martin JF, McDaniel LE (1974) Isolation, purification and properties of the hexaene macrolides candihexin I and candihexin II. J Antibiot 27:610–619

McGowan JE, Terry PM, Nahmias AJ (1976) Susceptibility of *Haemophilus influenzae* isolates from blood and cerebrospinal fluid to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Antimicrob Agents Chemother 9:137–139

Meinking TL, Taplin D, Jorge L et al (1995) The treatment of scabies with ivermectin. N Engl Med J 333:26–30

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43:727–737

Mitchell SS, Nicholson B, Teisan S, Lam KS, Barbara C (2004) Aureoverticillactam, a novel22-atom macrocyclic lactam from the marine actinomycete *Streptomyces aureoverticillatus*. J Nat Prod 67(8):1400–1402

Mitscher LA (1978) The chemistry of the tetracycline antibiotics. Marcel Dekker, New York

Moore BS, Trischman JA, Seng D, Kho D, Jensen PR, Fenical W (1999) Salinamides, anti-inflammatory depsipeptides from a marine *Streptomycete*. J Org Chem 64(4):1145–1150

Nakashima T, Okuyama R, Kamiya Y, Matsumoto A, Iwatsuki M, Inahashi Y, Yamaji K, Takahashi Y, Omura S (2013) Trehangelins A, B and C, novel photo-oxidative hemolysis inhibitors produced by an endophyticactinomycete, *Polymorphospora rubra* K07-0510. J Antibiot 66:311–317

Nakashima T, Iwatsuki M, Ochiai J, Kamiya Y, Nagai K, Matsumoto A, Ishiyama A, Otoguro K, Shiomi K, Takahashi Y et al (2014a) Mangromicins A and B: structure and antitrypanosomal activity of two new cyclopentadecane compounds from *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 67:253–260

Nakashima T, Kamiya Y, Iwatsuki M, Takahashi Y, Omura S (2014b) Mangromicins, six new anti-oxidative agents isolated from a culture broth of the actinomycete, *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 67:533–539

Nakashima T, Boonsnongcheep P, Kimura T, Iwatsuki M, Sato N, Nonaka K, Prathanturarug S, Takahashi Y, Omura S (2015a) New compounds, nanaomycin F and G, discovered by physicochemical screening from a culture broth of *Streptomyces rosa subsp. notoensis* OS-3966. J Biosci Bioeng 120:596–600

Nakashima T, Kamiya Y, Iwatsuki M, Sato N, Takahashi Y, Omura S (2015b) Mangromicin C, a new analog of mangromicin. J Antibiot 68:220–222

Nakashima T, Kamiya Y, Yamaji K, Iwatsuki M, Sato N, Takahashi Y, Omura S (2015c) New steroidal compounds from an actinomycete strain, *Lechevalieria aerocolonigenes* K10-0216. J Antibiot 68:348–350

Nakashima T, Miyano R, Iwatsuki M, Shirahata T, Kimura T, Asami Y, Kobayashi Y, Shiomi K, Petersson GA, Takahashi Y, Omura S (2016a) Iminimycin A, the new iminium metabolite produced by *Streptomyces griseus* OS-3601. J Antibiot 69:611–615

Nakashima T, Miyano R, Matsuo H, Iwatsuki M, Shirahata T, Kobayashi Y, Shiomi K, Petersson GA, Takahashi Y, Omura S (2016b) Absolute configuration of iminimycin B, the new indolizidine alkaloid from *Streptomyces griseus* OS-3601. Tetrahedron Lett 57:3284–3286

Nakashima T, Kimura T, Miyano R, Matsuo H, Hirose T, Kimishima A, Nonaka K, Iwatsuki M, Nakanishi J, Takahashi Y et al (2017) Nanaomycin H: a new nanaomycin analog. J Biosci Bioeng 123:765–770

Nontasut P, Bussaratid V, Chullawichit S, Charoensook N, Visetsuk K (2000) Comparison of ivermectin and albendazole treatment for gnathostomiasis. Southeast Asian J Trop Med Public Health 31:374–377

Okulicz JF, Stibich AS, Schwartz RA et al (2004) African river blindness: onchocercoma. Int J Dermatol 43:170–172

Omura S, Tanaka H, Koyama Y, Oiwa R, Katagiri M (1974) Letter: nanaomycins A and B, new antibiotics produced by a strain of Streptomyces. J Antibiot 27:363–365

Ottesen EA, Vijayesekaran V, Kumaraswami V et al (1990) A controlled trial of ivermectin and diethylcarbazamine in lymphatic filariasis. N Engl J Med 322:1113–1117

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial analysis. Biotechnol Appl Biochem 31:135–152

Pasindu CHJM (2016) Advanced study on selected taxonomic groups of bacteria and Archea. A text for B.Sc (UG) Microbiology (sp). University of Kelaniya, Kelaniya

Perlroth J, Kuo M, Tan J, Bayer AS, Miller LG (2008) Adjunctive use of rifampicin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. Arch Intern Med 168(8):805–819

Peterson JC (1960) Rickettsial infections. Pediatr Clin N Am 7:1003–1014

Petrosyan P, Gartia-Varela M, Luz-Madrigal A, Huitron C, Flores ME (2003) *Streptomyces mexicanus* a xylanolytic microorganism isolated from soil. Int J Syst Evol Microbiol 53: 269–273

Pooja S, Aditi T, Jemimah Naine S, Subathra Devi C (2017) Bioactive compounds from marine *Streptomyces sp* VITPSA as therapeutics. Front Biol 12(4):280–289

Pradines B, Spiegel A, Rogier C, Tall A, Mosnier J, Fusai T, Trape JF, Parzy D (2000) Antibiotics for prophylaxis of *Plasmodium falciparum* infections: in vitro activity of doxycycline against Senegalese isolates. Am J Trop Med Hyg 62:82–85

Prelog V, Oppolzer W (1973) Ansamyine, eine neuartige Klasse von mikrobiellen stoffwechselprodukten. Helv Chim Acta 56(7):2279–2287

Ramirez MS, Tolmasky ME (2010) Aminoglycoside modifying enzymes. Drug Resist Updat 13: 151–171

Ravikumar S, Gnanadesigan M, Thajuddin N, Chakkaravarthi V, Banerjee B (2010) Anti-cancer property of sponge associated actinomycetes along Palk Strait. J Pharm Res 3(10):2415–2417

Renner MK, Shen YC, Cheng XC, Jensen PR, Frankmoelle W, Kauffman CA et al (1999) Newanti-inflammatory cyclic peptides produced by a marine bacterium (*Streptomyces sp.*). J Am Chem Soc 121(49):11273–11276

Retsema J, Girard A, Schelkly W et al (1987) Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob Agents Chemother 31:1939–1947

Rinehart KL, Shields LS (1976) Chemistry of the ansamycin antibiotics. Chem Org Nat 33: 231–307

Robertson RP, Wahab MFA, Raasch FO (1968) Evaluation of chloramphenicol and ampicillin in *Salmonella enteric* fever. N Engl J Med 278:171–176

Rose HM, Kneeland YJ, Gibson CD (1950) Treatment of rickettsialpox with aureomycin. Am J Med 9:300–307

Rossolini GM, Arena F, Pollini S (2014) Novel infectious diseases and emerging gram-positive multi-resistant pathogens in hospital and community acquired infections. In: Marinelli F, Genilloud O (eds) Antimicrobials, 1st edn. Springer Verlag, Berlin

Schmid RD, Verger R (1998) Lipases: interfacial enzymes with attractive applications. Angew Chem Int Ed 37:1608–1633

Schnappinger D, Hillen W (1996) Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol 165:359–369

Schoch PE, Cunha BA (1986) The JK diphtheroids. Infect Control 7:466

Schoenbaum SC, Gardner P, Shillito J (1975) Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. J Infect Dis 131:543

Schumacher RW, Talmage SC, Miller SA, Sarris KE, Davidson BS, Goldberg A (2003) Isolationand structure determination of an antimicrobial ester from a marine sediment-derived bacterium. J Nat Prod 66(9):1291–1293

Schwarz E, Regev YG (1999) Primaquine as prophylaxis for malaria for non-immune travelers: a comparison with mefloquine and doxycycline. Clin Infect Dis 29:1502–1506
Sensi P, Greco AM, Ballotta R (1960) Rifomycins. I. Isolation and properties of rifomycin B and rifomycin complex. Antibiot Annu 1959–60:262–270
Shi C, Zhu Y, Ran X, Wang M, Su Y, Cheng T (2006) Therapeutic potential of chitosan and its derivatives in regenerative medicine. J Surg Res 133(2):185–192
Silva J Jr, Batts DH, Fekety R et al (1981) Treatment of *Clostridium difficile* colitis and diarrhea with vancomycin. Am J Med 71:815
Socha AM, Laplante KL, Rowley DC (2006) New bisanthraquinone antibiotics and semi-synthetic derivatives with potent activity against clinical *Staphylococcus aureus* and *Enterococcus faecium* isolates. Bioorg Med Chem 14(24):8446–8454
Stefan O, Walter R (2008) Antitrypanosomal drugs. Encyclopedia of molecular pharmacology. Springer-Verlag, Berlin
Sturgill MG, Rapp RP (1992) Clarithromycin: review of a new macrolide antibiotic with improved microbiologic spectrum and favorable pharmacokinetic and adverse effect profiles. Ann Pharmacother 26(9):1099–1108
Suga T, Kimura T, Inahashi Y, Iwatsuki M, Nonaka K, Take A, Matsumoto A, Takahashi Y, Omura S, Nakashima T (2018) Hamuramicins A and B, 22-membered macrolides, produced by an endophytic actinomycetes *Allostreptomyces sp*. K12-0794. J Antibiot 71:619–625
Sunita BC, Dimple SM, Bhaven CK (2012) Review article: ivermectin: pharmacology and therapeutic applications. Int J Basic Clin Pharmacol 1(3):132–139
Tarlow MM, Piela S, Schwartz RA (2002) Gram –negative folliculitis: a clinical challenge. Dermatol Klin 4:7–9
Tchekmedyian NS, Newman KA, Moody MR et al (1986) Special studies of the hickman catheter in a patient with recurrent bacteremia and candidemia. Am J Med Sci 291:419
The choice of antimicrobial drugs (1978) Med Lett Drugs Ther 20:1–8
Thomas RJ, Dumler JS, Carlyon JA (2009) Current management of human granulocytic anaplasmosis, human *monocytic ehrlichiosis* and *Ehrlichia ewingii ehrlichiosis*. Expert Rev Anti-Infect Ther 7(6):709–722
Thompson HL, Cabezudo I, Wenzel RP (1982) Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann Intern Med 97:309
Unsigned editorial (1978) Chemotherapy of brain abscess. Lancet 2:1081–1082
Van der Hulst RWM, Keller JJ, Rauws EAJ, Tytgat GNJ (1996) Treatment of *Helicobacter pylori* infection: a review of the world literature. Helicobacter 1:6–19
Verma N et al (2007) L-asparaginase: a promising chemotherapeutic agent. Crit Rev Biotechnol 27(1):45–62
Vianna NJ, Hinman AR (1960) Rocky Mountain spotted fever on Long Island. Am J Med 51: 725–730
Victoria Y, Trujillo R, Barreto M (1999) Myiasis: a successful treatment with topical ivermectin. Int J Dermatol 38:142–144
Wachino J, Arakawa Y (2012) Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: an update. Drug Resist Updat 15:133–148
Wade JC, SchimpfF SC, Newman KA et al (1982) *Staphylococcus epidermidis*: an increasing cause of infection in patients with granulocytopenia. Ann Intern Med 97:503
Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 176:386–390
Wehrli W (1977) Ansamycins chemistry, biosynthesis and biological activity. Top Curr Chem 72: 21–49
Williams ST, Wellington EMH (1982) Methods of soil analysis, part 2. Chemical and microbiological properties—agronomy monograph no. 9, 2nd edn. Wiley, New York

Williams ST, Davies FL, Mayfield CI, Khan MR (1971) Studies on the ecology of actinomycetes in soil-II. The pH requirements of *Streptomycetes* from two acid soils. Soils Biol Biochem 3: 187–195

Workowski KA, Berman SM (2010) Centers for disease control and prevention. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 59(RR-12):1–110

Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, Krause PJ, Bakken JS, Strle F, Stanek G, Bockenstedt L, Fish D, Dumler JS, Nadelman RB (2006) The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 43(9):1089–1134

Yan J, Li X, Liu L, Wang F, Zhu TW, Zhang Q (2006) Potential use of collagen chitosan hyaluronan tri-copolymer scaffold for cartilage tissue engineering. Artif Cells Blood Substitutes Biotechnol 34(1):27–39