Review Paper:

Bioprospecting Actinomycetes from the Rhizosphere of *Piper nigrum*: Potential and Challenges

Balaji Muralikrishnan, Senthil Kumar J. and Ashwini Malla*
Department of Biotechnology, PSG College of Arts and Science, Coimbatore 641014, Tamil Nadu, INDIA
*ashwini@psgcas.ac.in

Abstract
This prospective review explores the biotechnological potential of actinomycetes isolated from the rhizospheres of Piper nigrum L. which is commonly known as black pepper. Actinomycetes are Gram-positive filamentous bacteria that are well known for their ability to produce a plethora of bioactive compounds, including antibiotics. The rhizosphere, a nutrient-rich zone surrounding plant root, offers a unique microenvironment that fosters the growth of diverse actinomycetes with unique metabolic capabilities. This review underscores the significance of these microorganisms in soil ecosystems, highlighting their crucial role in the discovery of novel antibiotics in the context of rising antimicrobial resistance.

Furthermore, it delves into the challenges associated with bioprospecting actinomycetes including difficulties in isolation and cultivation, genetic manipulation and the complex, time-intensive pipeline required to develop and commercialize new antibiotics. Through addressing these challenges, this review provides insights into improving strategies for harnessing the full potential of rhizosphere actinomycetes in antibiotic discovery.

Keywords: Actinomycetes, Antibiotics, Bioprospecting, *Piper nigrum*, Rhizosphere.

Introduction

Actinomycetes, especially those belonging to the genus *Streptomyces*, have been instrumental in the development of antibiotics since the mid-20th century[34]. The rhizosphere, which is the soil region immediately surrounding plant roots, is a microbially rich environment[2]. *Piper nigrum* L., or black pepper, is not only a staple spice but a plant of significant economic and medicinal value[41]. This review explores the untapped potential of actinomycetes from the rhizosphere of *P. nigrum* L., for bioprospecting novel bioactive compounds with a focus on antibiotics.

Actinomycetes, particularly those from the genus *Streptomyces*, have played a pivotal role in antibiotic development since the discovery of streptomycin in 1943. This genus is responsible for producing over two-thirds of clinically useful antibiotics of natural origin including well-known drugs such as tetracycline, erythromycin and vancomycin[8]. These filamentous Gram-positive bacteria are prolific producers of secondary metabolites which include not only antibiotics but also antitumor agents, immunosuppressants and enzymes[20].

The rhizosphere, the narrow region of soil directly influenced by root secretions and associated soil microorganisms, is a hotspot for microbial diversity and activity. In this dynamic environment, plant roots exude various organic compounds, creating nutrient-rich niches that foster the growth of diverse microbial communities[36]. Microbial diversity is crucial for plant health and soil fertility, as rhizosphere microorganisms engage in nutrient cycling, disease suppression and plant growth promotion[10].

P. nigrum L., commonly known as black pepper, is a perennial climbing vine widely cultivated for its fruit, which is dried and used as a spice and seasoning. Beyond its culinary uses, black pepper has significant economic and medicinal value. It is rich in piperine, an alkaloid with anti-inflammatory, antioxidant and antimicrobial properties[30]. The rhizosphere of *P. nigrum* L. is of particular interest for bioprospecting because of its unique chemical environment which may be suitable for actinomycetes with novel metabolic pathways and bioactive compound production capabilities.

This review investigates the untapped potential of actinomycetes from the rhizosphere of *P. nigrum* L. for the discovery of new bioactive compounds, with a focus on antibiotics. The exploration of this niche is driven by the urgent need for new antibiotics despite rising antimicrobial resistance, which poses a significant threat to global health[46].

Significance of Actinomycetes: Actinomycetes are a group of Gram-positive bacteria that are pivotal in biotechnology due to their ability to produce various bioactive compounds. These microorganisms are renowned for their role in natural antibiotic production, making them essential for pharmaceutical development and bioprospecting efforts.

Production of Secondary Metabolites: Actinomycetes are known for their capacity to produce a vast array of secondary metabolites including antibiotics, antifungal agents and anticancer compounds. These microorganisms play a crucial role in decomposing organic material, thereby contributing to soil fertility and health[8]. The genus *Streptomyces* is particularly noteworthy as it is responsible for more than two-thirds of the clinically valuable antibiotics used in medicine today[9]. The biosynthesis of these metabolites is often regulated by complex gene clusters, which can be activated under specific environmental conditions or stress. Advances in genomics and metabolic engineering have facilitated the discovery and enhanced the production of valuable compounds, highlighting the potential of actinomycetes in drug discovery and development[7].

Additionally, the ability of actinomycetes to thrive in diverse and sometimes extreme environments underscores their potential as a source of novel compounds. The intricate interactions between plant roots and rhizosphere microorganisms stimulate the production of unique secondary metabolites. Specific root exudates released by plants can influence microbial gene expression and promote the synthesis of compounds[4]. This interaction-driven production of metabolites highlights the rhizosphere of *P. nigrum* as a promising target for bioprospecting. The diverse microbial community in the rhizosphere, influenced by plant exudates, can lead to the discovery of novel bioactive compounds with potential applications in agriculture and medicine[31].

The Role of Soil Ecosystems: Actinomycetes play a pivotal role in soil ecosystems by decomposing organic matter and cycling nutrients. Their filamentous structure allows them to penetrate complex substrates, breaking down resilient compounds such as cellulose, lignin and chitin. This process releases essential nutrients such as nitrogen, phosphorus and sulfur, vital for plant growth and soil fertility[6]. Additionally, actinomycetes produce secondary metabolites, including antibiotics, which suppress soil-borne pathogens and enhance plant health by reducing disease incidence[20]. These organisms contribute to bioremediation by detoxifying contaminated soil and enhancing soil health. They aid in the formation and stabilization of compost piles and the creation of stable humus.

By working alongside other soil microorganisms, actinomycetes help to break down tough plant residues like cellulose and animal remains, maintaining the biotic equilibrium of the soil through nutrient cycling. Additionally, actinomycetes improve soil structure by producing volatile organic compounds that enhance soil aggregation and aeration[11]. These diverse functions highlight the indispensable role of actinomycetes in maintaining soil ecosystem health and boosting agricultural productivity.

Importance of Antibiotic Production: The genus *Streptomyces* from actinomycetes, has been pivotal in antibiotic production and has contributed significantly to modern medicine. The discovery of streptomycin from *Streptomyces griseus* marked a breakthrough for treating tuberculosis and demonstrated the therapeutic potential of actinomycetes[37]. In addition to streptomycin, actinomycetes have produced other essential antibiotics such as erythromycin, tetracycline and chloramphenicol, each addressing various bacterial infections and saving countless lives[8]. The need for novel antibiotics is underscored by the escalating incidence of antibiotic-resistant pathogens, which threaten global health security[43].

Actinomycetes remain a primary focus in this research because of their rich metabolic diversity and ability to produce structurally unique and potent antimicrobial compounds. This continuous exploration highlights the enduring importance of actinomycetes in combating emerging infectious diseases and maintaining the efficacy of antimicrobial therapies.

Bioprospecting from the Rhizosphere: Bioprospecting actinomycetes in the rhizosphere of *P. nigrum* L. presents a promising avenue for enhancing plant health and productivity. Actinomycetes, a group of Gram-positive bacteria known for their ability to produce a wide array of bioactive compounds, play a significant role in the soil microbiome by promoting plant growth and suppressing pathogens. The rhizosphere of black pepper, which is rich in root exudates, creates a conducive environment for the proliferation of these beneficial microorganisms.

Research has demonstrated that actinomycetes isolated from the rhizosphere of black pepper exhibit strong antagonistic activity against phytopathogens such as *Phytophthora capsici* and *Fusarium* spp. which are responsible for diseases like quick wilt and slow decline[33]. These microorganisms produce various secondary metabolites including antibiotics, enzymes and growth-promoting substances, which enhance plant resilience against diseases and environmental stresses [38]. For instance, the application of actinomycetes, such as *Streptomyces* species, has been shown to significantly reduce disease incidence and improve the overall health of black pepper plants. These bacteria not only inhibit pathogen growth through the production of antimicrobial compounds but also contribute to soil fertility by decomposing organic matter and cycling nutrients[33,38].

The strategic use of actinomycetes in the rhizospheres of black pepper underscores the potential for sustainable agriculture. By leveraging the natural capabilities of these microorganisms, it is possible to develop bio-products that reduce the reliance on chemical pesticides and fertilizers, thus promoting an eco-friendly approach to black pepper cultivation[33].

Nutrient-Rich Environment: Plant roots exude various organic compounds into the soil including sugars, amino acids and organic acids. These root exudates create a nutrient-rich microenvironment that attracts diverse microorganisms including actinomycetes. Rich microbial diversity in the rhizosphere can lead to unique microbial interactions and metabolic processes, potentially resulting in the production of novel bioactive compounds. Plant roots secrete an array of organic compounds into the soil such as sugars, amino acids and organic acids, forming a nutrient-rich microenvironment known as the rhizosphere[3]. Nutrient availability attracts a diverse community of microorganisms including actinomycetes[36]. The microbial diversity in the rhizosphere fosters unique interactions and metabolic processes leading to the synthesis of novel bioactive compounds[10]. These bioactive compounds have significant implications for plant health and soil ecology, demonstrating the intricate relationships between plant roots and soil microorganisms[28].

Example Studies and Findings: Research has shown that actinomycetes isolated from the rhizospheres of various plants can produce a wide range of bioactive compounds. For example, studies on the rhizospheres of medicinal plants such as *Azadirachta indica* and *Ocimum sanctum* have led to the discovery of actinomycetes with potent antimicrobial and anticancer activities. Similarly, exploring the rhizosphere of *P. nigrum* is a promising approach for discovering novel compounds with unique biological activities.

Recent research underscores the pivotal role of actinomycetes isolated from the rhizospheres of various plants in the production of bioactive compounds. Investigations into the rhizosphere of medicinal plants such as *A. indica* and *O. sanctum*, have yielded actinomycetes with significant antimicrobial and anticancer properties[26,44]. These findings suggest that the unique microenvironment of plant rhizospheres fosters microbial communities capable of synthesizing potent secondary metabolites. For instance, *A. indica*, which is known for its medicinal properties, harbors actinomycetes that produce novel antibiotics effective against multidrug-resistant pathogens[22]. Similarly, actinomycetes isolated from *O. sanctum* have exhibited promising anticancer activities, including the inhibition of tumor cell proliferation and induction of apoptosis[18].

Extending this research to the rhizosphere of *P. nigrum* could unveil new actinomycetes species with unique biological activities, potentially leading to the discovery of novel compounds with significant therapeutic applications[42]. These studies highlight the immense potential of plant-associated actinomycetes as a source of new bioactive molecules, advancing the frontier of natural product discovery and drug development.

Challenges in Bioprospecting: Bioprospecting actinomycetes from the rhizosphere holds significant promise, but is fraught with substantial challenges. Key difficulties include the isolation and culturing of specific strains in the complex soil microbiome and the extensive time and resources necessary to advance new antibiotics from discovery to market[5,9]. These obstacles underscore the need for innovative methodologies and sustained investments in natural product research.

Isolation and Cultivation: The isolation and cultivation of actinomycetes from soil samples present significant challenges in bioprospecting because of the unique growth requirements and high microbial diversity within soil environments. Actinomycetes are slow-growing and require specific nutrients and environmental conditions that are challenging to replicate in laboratory settings. The complexity is further compounded by the presence of diverse microbial populations in the soil samples, which complicates the isolation of individual strains[5].

Traditional isolation techniques, such as serial dilution and plating on selective media, are commonly employed; however, they exhibit a bias toward fast-growing and easily culturable microorganisms, thereby potentially overlooking slow-growing or fastidious actinomycetes[40]. To mitigate this limitation, advanced isolation techniques have been developed. These include the use of selective media supplemented with antibiotics or antifungal agents to suppress the growth of competing microorganisms, thereby enhancing the isolation of actinomycetes[23].

Cultivation conditions are critical for the successful growth of actinomycetes. Optimal growth requires specific environmental parameters including pH, temperature, moisture content and nutrient availability[25]. Innovative cultivation techniques replicate the natural rhizosphere environment, which can significantly improve the recovery and growth of actinomycetes. Co-cultivation with other rhizosphere microorganisms has also been shown to stimulate the growth of actinomycetes and to enhance the production of secondary metabolites[45]. These approaches underscore the importance of creating conducive conditions that mimic natural habitats to unlock the full potential of actinomycetes to produce valuable bioactive compounds.

Complexity of Soil Microbiomes: The soil ecosystem is an intricate and dynamic matrix in which microbial communities are shaped by several biotic and abiotic factors including pH, temperature, moisture content and interactions with plant roots[1]. This intricate network complicates the isolation and identification of specific microorganisms such as actinomycetes, that are responsible for the synthesis of bioactive compounds[16]. The shear diversity and interdependence of soil microbes often necessitate the use of advanced molecular techniques to decode complex interactions within these communities. For instance, metagenomics and next-generation sequencing (NGS) have emerged as pivotal tools for unraveling the microbial diversity present in soil samples, allowing for a more precise identification of potential bioprospecting targets[27,32].

Metagenomics, High-Throughput Sequencing and Functional Genomics: The pursuit of novel antibiotics has become increasingly critical because of the rise of antibiotic-resistant pathogens. However, traditional methods of culturing microorganisms in the laboratory often fall short of tapping into the full spectrum of microbial diversity, especially in complex environments like soil. Actinomycetes, soil-dwelling microorganisms, are known for their prolific production of antibiotics, yet many remain unexplored due to the limitations of standard culturing techniques[16].

https://doi.org/10.25303/2011rjbt3950401

Metagenomics has emerged as a powerful tool to overcome these encounters. By enabling the direct analysis of genetic material from environmental samples, metagenomics bypasses the need for culturing organisms and allows, thereby allowing researchers to explore the genetic potential of unculturable microbes. The advent of High-Throughput Sequencing (HTS) technologies has further revolutionized this field, enabling the comprehensive characterization of microbial diversity in soil, particularly in the rhizosphere. Through HTS, researchers can identify biosynthetic gene clusters (BGCs) responsible for secondary metabolite production, which is crucial for discovering new antibiotics[29]. However, the shear complexity and vast diversity of microbial communities present a significant challenge in identifying and isolating the most promising BGCs for antibiotic development[39].

Functional metagenomics offers a solution by going beyond the mere identification of genetic material to include the functional expression of environmental DNA in heterologous hosts. This approach allows the discovery of novel bioactive compounds from microorganisms that are difficult or impossible to culture under standard laboratory conditions[32].

By constructing and screening metagenomic libraries, researchers can identify antimicrobial activities, thereby accelerating the discovery of new antibiotics[13]. However, this process is not without challenges. Successful expression of environmental genes in heterologous hosts is often limited by differences in codon usage, regulatory elements and protein folding requirements. In addition, the vast diversity of soil actinomycetes makes it difficult to capture and express all potential bioactive compounds[16].

Challenges in metagenomics and functional metagenomics include the complexity of microbial communities, making it difficult to accurately identify and classify organisms[21]. High diversity and the presence of rare species complicate data analysis. Current sequencing technologies may also struggle to assemble complete genomes from mixed communities, leading to incomplete datasets. Additionally, the functional annotation of genes remains challenging because of limited databases and the need for better computational tools to link genes to their functions[17]. Continued research and technological advancements are essential to overcome these hurdles.

Discovery-to-Commercialization Pipeline: The pathway from discovery to commercialization of novel antibiotics is both protracted and financially burdensome, involving a succession of intricate stages that encompass initial screening, preclinical evaluation, clinical trials and regulatory authorization. Each phase is fraught with distinct challenges and inherent risks, contributing to the significant attrition rate of promising candidates throughout the pipeline. The substantial costs and extended timelines associated with antibiotic development pose formidable obstacles to the successful introduction of new therapeutics into the market.

Initial Screening and Characterization: The initial phase of antibiotic discovery involves meticulous screening actinomycetes to assess their antimicrobial efficacy against a spectrum of pathogenic microorganisms. Compounds exhibiting promising activities are further characterized by delineating their chemical nature using advanced analytical methodologies. Techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are employed to identify and elucidate the structural composition of bioactive molecules.

Preclinical and Clinical Testing: Compounds that demonstrate potential in the initial screening are subjected to preclinical testing, a critical phase in which their efficacy, toxicity and pharmacokinetic profiles are rigorously evaluated. These studies, conducted both *in vitro* and *in vivo*, provide preliminary insights into the safety and therapeutic viability of the compounds. The transition from preclinical testing to clinical development requires comprehensive documentation and regulatory approval[14].

The clinical evaluation of new antibiotics is conducted in a phased manner to determine their safety, efficacy and optimal dosage in human subjects. Phase I trials will be initiated with a small cohort of healthy volunteers to evaluate safety and pharmacokinetic parameters. Subsequently, phase II and III trials will be expanded to larger patient populations to assess therapeutic efficacy and monitor for adverse effects. The successful culmination of these trials is imperative for securing regulatory approval and advancing to commercialization[15].

Regulatory Approval: The final hurdle in the antibiotic development pipeline is the attainment of regulatory approval, a process overseen by authoritative bodies such as the United States Food and Drug Administration (USFDA) and the European Medicines Agency (EMA). These agencies meticulously review preclinical and clinical data to ensure the safety and efficacy of new antibiotics. The approval process comprehends stringent scrutiny of manufacturing processes, quality control protocols and clinical data. Post-approval monitoring is mandatory to detect any long-term adverse effects and to maintain ongoing safety standards[43].

Prospects for Actinomycetes Research- A Genomics and Metabolomics-Driven Approach: The convergence of advances in genomics and metabolomics presents a transformative opportunity to unlock the biosynthetic potential of actinomycetes, promising a new era in the discovery of therapeutic agents. By integrating these cutting-edge technologies with traditional microbiological methods, researchers can significantly accelerate the identification of novel bioactive compounds. The successful realization of these prospects hinges on robust collaboration among academic institutions, industry and regulatory bodies which together can overcome existing challenges and fully harness the potential of actinomycetes in drug discovery.

Integrating Genomics and Metabolomics Data: Genomic and metabolomic technologies enable an unprecedented exploration of the genetic and metabolic diversity of actinomycetes. Whole-genome sequencing has become a powerful tool for identifying BGCs responsible for producing secondary metabolites, which are often the source of valuable therapeutic agents. Through comparative genomics, researchers can elucidate the diversity and distribution of these BGCs across different actinomycete strains, thereby shedding light on their biosynthetic capacities[47]. Genome mining tools like antiSMASH further enhance this process by predicting the presence of novel BGCs, guiding researchers toward the discovery of previously unknown bioactive compounds[12].

Metabolomic Profiling: Integrating metabolomics data with genomic data provides a comprehensive view of the metabolites produced by actinomycetes. Techniques such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are instrumental in identifying and quantifying these metabolites, enabling researchers to link specific BGCs with the production of particular compounds[35]. This holistic approach not only streamlines the discovery process but also facilitates a deeper understanding of the biosynthetic pathways and regulatory mechanisms that govern metabolite production.

Synergy with Traditional Methods: Although advanced molecular techniques offer substantial advantages, traditional microbiological methods are still indispensable. The cultivation and screening of actinomycetes are crucial for confirming the production of bioactive compounds and evaluating their pharmaceutical potential[19].

Cultivation techniques that mimic the natural rhizosphere environment, including co-cultivation with other microorganisms, can stimulate secondary metabolite production, leading to the discovery of novel antibiotics. High-throughput screening assays further aid in assessing the antimicrobial activity of actinomycete extracts, with promising candidates undergoing detailed structural and functional characterization[24].

Collaboration and Regulatory Support: The transition from scientific discovery to commercial production requires a concerted effort across academia, industry and regulatory bodies. Academic-industry partnerships are vital for translating basic research into practical applications because academic researchers can leverage industry resources to advance drug development. Regulatory agencies play a critical role by providing guidelines and support throughout the drug approval process, ensuring that new antibiotics reach the market efficiently and safely[43]. Early engagement with regulatory bodies can expedite this process, helping to address the urgent need for new antimicrobial agents in the face of rising antibiotic resistance.

Conclusion

The rhizosphere of *P. nigrum* represents a promising yet underexplored niche for bioprospecting actinomycetes. These microorganisms can produce a diverse bioactive compounds that can address the global challenge of antibiotic resistance. However, realizing this potential requires overcoming significant challenges related to isolation, cultivation and the lengthy discovery-to-commercialization pipeline.

By leveraging advances in genomics and metabolomics, integrating traditional and molecular methods and fostering collaboration between academia, industry and regulatory bodies, researchers can unlock the full potential of actinomycetes from the rhizosphere of *P. nigrum* and can contribute to the discovery of new therapeutic agents.

Acknowledgement

The authors are grateful to the Department of Science and Technology for the FIST grant.

References

1. Abdul Rahman N.S.N., Abdul Hamid N.W. and Nadarajah K., Effects of Abiotic Stress on Soil Microbiome, *International Journal of Molecular Sciences*, **22**, 9036 **(2021)**

2. Anusree T., Suseela Bhai R., Ahammed Shabeer T.P. and Oulkar D., *Streptomyces spp* from Black Pepper Rhizosphere: A Boundless Reservoir of Antimicrobial and Growth Promoting Metabolites, *Journal of Biologically Active Products from Nature,* **9**, 1-23 **(2019)**

3. Bais H.P., Weir T.L., Perry L.G., Gilroy S. and Vivanco J.M., The role of root exudates in rhizosphere interactions with plants and other organisms, *Annual Review of Plant Biology*, **57**, 233-266 **(2006)**

4. Bakker P., Pieterse C.M.J., De Jonge R. and Berendsen R.L., The Soil-Borne Legacy, *Cell*, **172**, 1178-1180 **(2018)**

5. Baltz R.H., Gifted microbes for genome mining and natural product discovery, *Journal of Industrial Microbiology and Biotechnology*, **44**, 573-588 **(2017)**

6. Barka E.A., Vatsa P., Sanchez L., Gaveau-Vaillant N., Jacquard C., Meier-Kolthoff J.P., Klenk H.P., Clément C., Ouhdouch Y. and Van Wezel G.P., Taxonomy, Physiology and Natural Products of Actinobacteria, *Microbiology and Molecular Biology Reviews*, **80**, 1-43 **(2016)**

7. Bentley S.D. et al, Complete genome sequence of the model *actinomycete Streptomyces coelicolor* A3(2), *Nature,* **417**, 141-147 **(2002)**

8. Bérdy J., Bioactive Microbial Metabolites, *J of Antibiotics*, **58**, 1-26 **(2005)**

9. Bérdy J., Thoughts and facts about antibiotics: Where we are now and where we are heading?, *Journal of Antibiotics*, **65**, 385-395 **(2007)**

10. Berendsen R.L., Pieterse C.M. and Bakker P.A., The rhizosphere microbiome and plant health, *Trends in Plant Science*, **17**, 478-486 **(2012)**

11. Bhatti A.A., Haq S. and Bhat R.A., Actinomycetes benefaction role in soil and plant health, *Microbial Pathogenesis*, **111**, 458-467 **(2017)**

12. Blin K. et al, antiSMASH 6.0: improving cluster detection and comparison capabilities, *Nucleic Acids Research*, **49**, w29-w35 **(2021)**

13. Brady S.F., Construction of soil environmental DNA cosmid libraries and screening for clones that produce biologically active small molecules, *Nature Protocols*, **2**, 1297-1305 **(2007)**

14. Coates A.R., Halls G. and H Y., Novel classes of antibiotics or more of the same?, *British Journal of Pharmacology*, **163**, 184-194 **(2011)**

15. Doron S. and Davidson L.E., Antimicrobial stewardship, Mayo Clinic Proceedings, **86**, 1113-1123 **(2011)**

16. Farda B., Djebaili R., Vaccarelli I., Del Gallo M. and Pellegrini M., Actinomycetes from Caves: An Overview of Their Diversity, Biotechnological Properties and Insights for Their Use in Soil Environments, *Microorganisms*, **10**, 453 **(2022)**

17. Felczykowska A., Krajewska A., Zielinska S., Łoś J., Bloch S. and Nejman-Faleńczyk B., The most widespread problems in the function-based microbial metagenomics, *Acta Biochimica Polonica*, **62**, 161-166 **(2015)**

18. Gangwar M. et al, Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India, *African Journal of Microbiology Research*, **8**, 184-191 **(2014)**

19. Genilloud O., Actinomycetes: still a source of novel antibiotics, *Natural Product Reports*, **34**, 1203-1232 **(2017)**

20. Goodfellow M. and Fiedler H.P., A guide to successful bioprospecting: informed by actinobacterial systematics, *Antonie Van Leeuwenhoek*, **98**, 119-142 **(2010)**

21. Handelsman J., Metagenomics: application of genomics to uncultured microorganisms, *Microbiology and Molecular Biology Reviews*, **68**, 669-685 **(2004)**

22. Hassan A. and Sajid I., Inhibitory Effects of Plant Extracts and Putative Endophytic Actinomycetes from the Selected Members of Meliaceae Family against MDR Pseudomonas aeruginosa, *European Journal of Medicinal Plants*, **10**, 1-14 **(2015)**

23. Kapur M.K., Solanki R. and Lal R., Selective isolation of rare actinomycetes producing novel antimicrobial compounds, *International Journal of Advanced Biotechnology and Research,* **2**, 357-375 **(2011)**

24. Katz L. and Baltz R.H., Natural product discovery: past, present and future, *Journal of Industrial Microbiology and Biotechnology*, **43**, 155-176 **(2016)**

25. Kumar P., Dubey R.C. and Maheshwari D., Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens, *Microbiological Research*, **167**, 493-499 **(2012)**

26. Kumari C.A.U. and Kumar P., Effect of Actinomycetes on growth of Ocimum sanctum, *International Journal of Current Pharmaceutical Research*, **11(3)**, 45-47 **(2019)**

27. Lahlali R., Ibrahim D.S.S., Belabess Z., Kadir Roni M.Z., Radouane N., Vicente C.S.L., Menéndez E., Mokrini F., Barka E.A., Galvão De Melo E.M.M. and Peng G., High-throughput molecular technologies for unraveling the mystery of soil microbial community: challenges and future prospects, *Heliyon*, 7, e08142 **(2021)**

28. Lugtenberg B. and Kamilova F., Plant-Growth-Promoting Rhizobacteria, *Abiotic Stress and Legumes*, **63**, 541-556 **(2009)**

29. Medema M.H. et al, Minimum Information about a Biosynthetic Gene cluster, *Nature Chemical Biology*, **11**, 625-631 **(2015)**

30. Meghwal M. and Goswam T.K., Piper nigrum and piperine: an update, *Phytotherapy Research*, **27**, 1121-1130 **(2013)**

31. Mendes R., Garbeva P. and Raaijmakers J.M., The rhizosphere microbiome: significance of plant beneficial, plant pathogenic and human pathogenic microorganisms, *FEMS Microbiology Reviews*, **37**, 634-663 **(2013)**

32. Nam N.N., Do H.D.K., Loan Trinh K.T. and Lee N.Y., Metagenomics: An Effective Approach for Exploring Microbial Diversity and Functions, *Foods*, **12**, 2140 **(2023)**

33. Nguyen S.D., Trinh T.H.T., Tran T.D., Nguyen T.V., Chuyen H.V., Ngo V.A. and Nguyen A.D., Combined Application of Rhizosphere Bacteria with Endophytic Bacteria Suppresses Root Diseases and Increases Productivity of Black Pepper (Piper nigrum L.), *Agriculture*, **11(1)**, 15 **(2021)**

34. Olanrewaj O.S. and Babalola O.O., Streptomyces: implications and interactions in plant growth promotion, *Applied Microbiology and Biotechnology*, **103**, 1179-1188 **(2019)**

35. Patti G.J., Yane O. and Siuzdak G., Metabolomics: the apogee of the omics trilogy, *Nature Reviews Molecular Cell Biology,* **13**, 263-269 **(2012)**

36. Philippot L., Raaijmakers J.M., Lemanceau P. and Van Der Putten W.H., Going back to the roots: the microbial ecology of the rhizosphere, *Nature Reviews Microbiology*, **11**, 789-799 **(2013)**

37. Quinn G.A., Banat A.M., Abdelhameed A.M. and Banat I.M., Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery, *Journal of Medical Microbiology*, **69**, 1040-1048 **(2020)**

38. Rizvi A. et al, Bioprospecting Plant Growth Promoting Rhizobacteria for Enhancing the Biological Properties and Phytochemical Composition of Medicinally Important Crops, *Molecules*, **27**, 1407 **(2022)**

39. Sharma V., Malla M., Kori R., Yada R. and Azam Z., Applications of Metagenomics for Unrevealing the Extended

Horizons of Microbiota Prevalence from Soil to Human Health, *The Open Microbiolgy Journal*, **15**, 177-187 **(2021)**

40. Shirling E.B. and Gottlieb D., Methods for characterization of *Streptomyces species* 1, *International Journal of Systematic and Evolutionary Biology*, **16**, 313-340 **(1966)**

41. Takooree H., Aumeeruddy M.Z., Rengasamy K.R.R., Venugopala K.N., Jeewon R., Zengin G. and Mahomoodally M.F., A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications, *Critical Reviews in Food Science and Nutrition*, **59**, s210-s243 **(2019)**

42. Thampi A. and Bhai R.S., Rhizosphere actinobacteria for combating Phytophthora capsici and Sclerotium rolfsii, the major soil borne pathogens of black pepper (Piper nigrum L.), *Biological Control*, **109**, 1-13 **(2017)**

43. Ventola C.L., The antibiotic resistance crisis: part 1: causes and threats, *Pharmacy & Therapeutics*, **40**, 277-283 **(2015)**

44. Verma V., Gond S., Kumar A., Mishra A., Kharwar R. and Gange A., Endophytic Actinomycetes from Azadirachta indica A. Juss.: Isolation, Diversity and Anti-microbial Activity, *Microbial Ecology*, **57**, 749-756 **(2008)**

45. Vetsigian K., Jajoo R. and Kishony R., Structure and Evolution of Streptomyces Interaction Networks in Soil and In Silico, *PLOS Biology*, **9**, e1001184 **(2011)**

46. WHO, Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline **(2020)**

47. Ziemert N., Alanjary M. and Weber T., The evolution of genome mining in microbes - a review, *Natural Product Reports*, **33**, 988-1005 **(2016)**.

(Received 22nd August 2024, accepted 23rd October 2024)