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Ecological roles and biotechnological potential of antibiotic: Producing microorganisms in diverse environments

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Abstract
Antibiotics are bioactive compounds that inhibit or eliminate bacterial growth and are widely applied in clinical, agricultural, and industrial settings. The first antibiotic, penicillin, was discovered in 1928 by Alexander Fleming, who observed that a mold (*Penicillium notatum*) inhibited the growth of *Staphylococcus aureus*. Since then, antibiotics have been extensively studied and employed to treat bacterial infections. Various environments—including soil, water, plants, animals, and fermented foods—serve as reservoirs for antibiotic-producing microorganisms. Among them, actinomycetes, particularly *Streptomyces* species, are prolific producers of structurally diverse antibiotics and have contributed thousands of secondary metabolites to the natural product repertoire. Marine ecosystems also represent an emerging and rich source of novel antibiotic-producing bacteria. Beyond their therapeutic applications, antibiotics play important ecological roles in microbial communities. They function as chemical weapons to inhibit competitors or deter predators and act as protective shields, enhancing microbial survival in competitive environments. Furthermore, antibiotics exhibit concentration-dependent behavior: at high concentrations, they act as growth inhibitors, while at subinhibitory levels, they may serve as signaling molecules involved in microbial communication and regulation. In soil and plant-associated environments, bacterial genera such as *Bacillus*, *Pseudomonas*, and *Streptomyces* have been shown to produce a variety of bioactive peptides with antimicrobial properties. These natural compounds play crucial roles in microbial fitness and environmental adaptability, making the study of antibiotic production central to both drug discovery and microbial ecology.

Keywords: Ecological roles, biotechnological potential, actinomycetes, streptomyces

Introduction
The term *antibiotic*, derived from the Greek roots *anti* (against) and *bios* (life), traditionally refers to compounds that inhibit or eradicate microbial life. In modern scientific and clinical contexts, antibiotics are predominantly understood as secondary metabolites produced by microorganisms, particularly bacteria, that possess bacteriostatic or bactericidal properties. These compounds have revolutionized medicine and remain pivotal in the treatment of bacterial infections. The discovery of antibiotics marked a transformative era in microbiology and pharmacology, beginning in 1928 with the seminal observation by Alexander Fleming. He noted that a contaminating fungal colony on a culture plate of *Staphylococcus aureus* inhibited bacterial growth. The fungus, later identified as *Penicillium notatum*, was found to secrete a bioactive compound—penicillin—that exhibited potent antibacterial activity. This landmark finding laid the foundation for the development of one of the first and most impactful antibiotics in medical history [1].

Numerous bacterial species possess the intrinsic ability to synthesize antibiotics as part of their secondary metabolism, often serving ecological roles in microbial competition and survival. Among them, *Bacillus* species are well-recognized for their antibiotic-producing capacity, synthesizing bioactive compounds such as bacitracin, pumilacidin, and gramicidin, which exhibit potent activity against Gram-positive pathogens including *Staphylococcus*, *Streptococcus*, and *Corynebacterium* species. Similarly, members of the genus *Streptomyces*—a group of filamentous actinobacteria—are prolific antibiotic producers,

generating a diverse array of clinically important compounds such as tetracycline, chloramphenicol, vancomycin, and gentamicin, many of which are effective against Gram-negative bacteria. Furthermore, lactic acid bacteria, particularly *Lactococcus lactis*, are known to produce bacteriocins such as nisin, a lantibiotic with strong inhibitory activity against a broad spectrum of Gram-positive organisms [2].

Undoubtedly, the escalating prevalence of antibiotic-resistant pathogens presents a critical global health challenge, necessitating the urgent discovery and development of novel antimicrobial agents. These drug-resistant microorganisms are responsible for life-threatening infections and pose a substantial threat to the long-term efficacy and sustainability of modern healthcare systems [3]. Actinomycetes, particularly those from soil environments, are renowned for their prolific capacity to produce bioactive secondary metabolites. Remarkably, of the approximately 23,000 known microbial secondary metabolites, over 10,000—constituting nearly 45%—are derived from actinomycetes, underscoring their critical role in natural product discovery [4]. Given the alarming rise in antibiotic resistance, the search for novel actinomycete strains has gained renewed urgency as a promising avenue for the development of new antimicrobial agents. However, a substantial proportion of these soil-derived microorganisms remain uncultivable or metabolically silent under standard laboratory conditions, thereby limiting their exploitation for antibiotic discovery. This research aims to address this gap by isolating actinomycetes from garden soil and evaluating their potential to produce bioactive compounds with antibacterial activity [5].

Marine ecosystems represent largely untapped reservoirs for the discovery of novel microorganisms with the potential to synthesize diverse and bioactive secondary metabolites. Among these, actinomycetes are of particular interest due to their well-documented ability to produce structurally diverse compounds exhibiting a wide range of biological activities. The unique physicochemical conditions of marine habitats, including high salinity, pressure, and low temperatures, often drive the evolution of distinct metabolic pathways in marine actinomycetes, making them a promising source for novel bioactive agents [6]. The global demand for new antibiotics continues to rise in response to the rapid emergence of antibiotic-resistant pathogens responsible for life-threatening infections. Despite considerable progress in synthetic chemistry and the engineered biosynthesis of antibacterial compounds, natural environments remain the most abundant and versatile source of novel antimicrobial agents. The unparalleled structural diversity and bioactivity found in natural products continue to outpace synthetic libraries, reaffirming the indispensable role of nature in the ongoing quest for effective antibiotics [7].

Actinomycetes represent a ubiquitous group of microorganisms widely distributed across diverse natural ecosystems and are particularly significant for their role in the decomposition and recycling of organic matter [8]. In recent years, marine-derived microorganisms have garnered substantial attention due to their ability to produce a wide array of structurally diverse natural compounds. Their unique adaptive mechanisms to extreme environmental conditions—such as high salinity, pressure, and variable temperatures—make them an invaluable source for the discovery of novel bioactive metabolites [9]. Marine sediments represent a unique ecological niche markedly different from terrestrial soil environments, raising questions about the efficacy of conventional pretreatment methods used for isolating bioactive actinomycetes from such habitats. Despite their vast potential, marine sediments remain largely underexplored as a resource for novel microbial strains. Preliminary reports from the eastern coastline of India indicate that marine-associated soils could serve as a significant reservoir for actinomycetes, offering promising avenues for the discovery of new bioactive compounds [10].

The systematic evaluation of microorganisms for their potential to produce antibiotics has laid the foundation for extensive antibiotic discovery and development programs over the past several decades. These research efforts have predominantly focused on fungi and actinomycetes, owing to their well-documented ability to biosynthesize a vast array of structurally diverse natural products. These microorganisms have proven to be prolific sources of bioactive secondary metabolites, many of which exhibit potent antibacterial, antifungal, antiviral, or anticancer properties.

Among the most promising bioactive compounds identified so far are antimicrobial peptides (AMPs), a class of small, naturally occurring proteins with broad-spectrum activity. To date, more than 400 antimicrobial peptides have been discovered from various biological sources, including plants, insects, bacteria, and vertebrates. These peptides typically function as part of the host's innate immune defense and possess mechanisms that target microbial membranes or intracellular components, making them attractive candidates for therapeutic development.

In the context of antibiotic discovery, two principal screening strategies have historically been employed. The first involves the dereplication of secondary metabolites—essentially filtering out compounds that are already well-characterized and deemed ineffective or redundant as antibiotics. This step helps researchers avoid rediscovering known molecules and instead focus on novel scaffolds. The second strategy centers around the identification and evaluation of previously unknown or less-characterized compounds based on their biological activities, particularly their ability to inhibit specific enzymes or cellular targets. This more targeted approach has become increasingly sophisticated with the advent of high-throughput screening techniques, genomics-guided biosynthetic pathway analysis, and other advanced technologies. Additionally, some of the most clinically significant antitumor agents have originated from microbial sources. Compounds such as mithramycin, bleomycin, daunomycin, and Adriamycin (doxorubicin) are notable examples of microbial secondary metabolites with potent anticancer activity. These agents are used in the treatment of various malignancies due to their ability to interfere with DNA synthesis and replication, highlighting the therapeutic breadth of natural products derived from microorganisms [11].

Sources of Antibiotic-Producing Microorganisms

Antibiotic-producing microorganisms inhabit a wide array of natural environments, each offering unique ecological pressures that stimulate the biosynthesis of bioactive secondary metabolites. The following are some of the most prominent sources:

1. **Soil:** Terrestrial soil remains one of the richest and most extensively studied reservoirs for antibiotic-producing microorganisms. Among them, *Actinomycetes*—particularly the genus *Streptomyces*—are prolific producers of a broad spectrum of antibiotics, including streptomycin, tetracycline, erythromycin, and chloramphenicol. The competitive nature of soil microbiomes drives the evolution of diverse antimicrobial compounds that help microorganisms establish ecological dominance.
2. **Aquatic Environments:** Freshwater and marine ecosystems are increasingly recognized as valuable sources of antibiotic-producing microbes. Marine-derived bacteria, such as *Salinispora* and *Pseudoalteromonas*, have yielded novel antibiotics with unique chemical structures not typically found in terrestrial organisms. These marine microbes often thrive under high-pressure, high-salinity, or low-temperature conditions, resulting in the evolution of distinct metabolic pathways.
3. **Plants:** Plants host a variety of endophytic microorganisms—bacteria and fungi that reside within plant tissues without causing harm. These endophytes can produce antibiotics that contribute to the plant's defense mechanisms. For example, *Taxus brevifolia* (Pacific yew) is known for producing taxol, a potent anticancer agent with antimicrobial properties. Additionally, traditional medicinal plants like garlic (*Allium sativum*) and neem (*Azadirachta indica*) contain natural antimicrobial compounds either produced by the plant itself or its associated microbiota.
4. **Animals and Insects:** The microbiomes of animals and insects, especially those inhabiting complex ecological systems such as the gut, skin, or exoskeleton, are emerging sources of antibiotic-producing microorganisms. Symbiotic bacteria found in insects like ants, wasps, and beetles have demonstrated the ability to synthesize antimicrobial compounds that protect their hosts from pathogens.
5. **Extreme Environments:** Extremophiles—microorganisms that inhabit harsh environments such as hot springs, arctic ice, acidic mines, or saline lakes—possess unique metabolic capabilities. These microbes have adapted to survive extreme physical and chemical stress, and often produce novel antibiotics with unusual structures and mechanisms of action to compete in such selective niches.

Antibiotic Production by Soil- and Plant-Associated Bacteria

A significant proportion of our current understanding of the structural diversity, bioactivity, and ecological roles of antibiotics stems from studies on culturable soil-dwelling bacteria. Despite the vast untapped reservoir of biosynthetic potential encoded within uncultured soil microbiota and metagenomic data, cultured representatives—particularly from the *Actinomycetales* order—have historically been the primary focus of antibiotic research. Among these, *Streptomyces* species stand out as prolific producers, having contributed the foundational scaffolds for many clinically relevant antibiotics, including streptomycin, erythromycin, tetracycline, and chloramphenicol.

To date, *Streptomyces* isolates alone have yielded more than 7,000 structurally distinct secondary metabolites, many of which possess potent antibacterial, antifungal, or antitumor properties [11-14]. These bacteria are most commonly isolated from soil environments, yet they also inhabit the microbiomes of plants, insects, and nematodes. Their presence in plant rhizospheres and endophytic niches suggests complex ecological interactions, including mutualism, microbial competition, and defense, facilitated through antibiotic secretion. In addition to actinomycetes, other bacterial genera such as *Bacillus*, *Pseudomonas*, and *Burkholderia*—often associated with plant roots or soil—have demonstrated the capacity to produce antimicrobial compounds that contribute to plant health by suppressing phytopathogens. These interactions further reinforce the role of soil and plant-associated bacteria as ecological gatekeepers.

Of particular interest are members of the order *Myxococcales*, especially *Myxococcus xanthus*, which have garnered significant attention for their sophisticated predatory behavior, cooperative surface gliding motility, and highly structured biofilm formation. These social bacteria produce a range of secondary metabolites involved in cell-cell signaling, microbial antagonism, and predation. Although *M. xanthus* has been the most extensively studied, other myxobacterial genera are now emerging as promising candidates in the search for antibiotics with novel structures and mechanisms of action [15,27]. These features make them attractive targets for bioprospecting, especially in the context of overcoming the growing threat of antimicrobial resistance.

Antibiotics and Resistance Mechanisms in Soil- and Plant-Associated Bacteria

In the following sections, we provide concise overviews of antibiotics produced by various bacterial genera commonly found in soil and plant-associated environments. These bacteria contribute to a wide spectrum of ecological functions, including (a) suppression of plant pathogens, (b) endophytic colonization within plant tissues or insect hosts, and (c) pathogenesis in plants. Their antibiotic production plays crucial roles in both microbial competition and host-microbe interactions, making them highly relevant to the field of plant pathology.

Several studies have highlighted the complexity of antibiotic gene dissemination in environmental bacteria. Andersen and Sandaa [28], for instance, reported the presence of antibiotic resistance determinants among Gram-negative bacteria isolated from marine sediments, indicating the ecological spread of resistance traits beyond terrestrial systems. In addition, the acquisition of antibiotic resistance genes has been documented in *Mycobacterium* and *Streptomyces* species, both of which are known to produce and encounter diverse antibiotics in their native habitats [29,30].

Notably, the *ermG* gene of *Bacillus sphaericus* was found to share high sequence similarity with conjugative transposons from *Bacteroides*, suggesting the horizontal transfer of resistance genes between soil-dwelling bacteria and the gastrointestinal microbiota of humans [31]. Supporting this, multiple studies have provided evidence for the widespread presence of self-transmissible and mobilizable plasmids in environmental microorganisms [32]. These plasmids serve as key vectors for the dissemination of resistance traits in microbial communities. Techniques such as polymerase chain reaction (PCR) have proven effective for detecting

and characterizing such plasmids and their associated resistance genes [34].

Waters and Davies [33] conducted an analysis of the GyrA protein, a target of fluoroquinolone antibiotics, and identified a high frequency of fluoroquinolone-resistant isolates among soil-derived bacterial populations. Resistance to erythromycin—a macrolide antibiotic—has also been widely observed in environmental bacteria, particularly among antibiotic-producing *Streptomyces* and *Bacillus* species [52]. This resistance is often attributed to a post-transcriptional modification of 23S rRNA, mediated by an N-methyltransferase enzyme encoded by *erm* genes, which confers target-site protection and diminishes drug binding efficacy.

Collectively, these findings underscore the dual role of soil and plant-associated bacteria as both reservoirs of antibiotic biosynthesis and hubs for the evolution and horizontal transfer of antibiotic resistance genes. Understanding these dynamics is crucial for the development of sustainable antimicrobial strategies and the mitigation of resistance emergence in both environmental and clinical settings.

Cross-Resistance and Co-Selection of Antibiotic and Heavy Metal Resistance in Environmental Bacteria

Resistance to macrolide antibiotics is often associated with cross-resistance to other structurally and functionally related antibiotic classes, specifically lincosamides and streptogramin B. This phenomenon is collectively referred to as MLS (Macrolide-Lincosamide-Streptogramin B) resistance. In *Streptomyces* species—well-known producers of macrolide antibiotics—the presence of MLS resistance genes is believed to serve a self-protective function, shielding the producing organism from autotoxicity and enabling continued antibiotic biosynthesis without self-damage.

Further insights into environmental resistance patterns were provided by Nwosu and Ladapo [35], who investigated resistance profiles of bacterial isolates obtained from landfill sites. Their study revealed a moderate to high prevalence of antibiotic resistance among isolates belonging to genera such as *Bacillus*, *Corynebacterium*, *Aeromonas*, and *Enterobacter*. Notably, most isolates exhibited resistance to clinically relevant antibiotics including ampicillin, erythromycin, and streptomycin, while remaining relatively susceptible to tetracycline and chloramphenicol—antibiotics that currently see limited clinical application due to historical resistance issues or toxicity concerns.

Among the 14 bacterial isolates analyzed, three exhibited multi-drug resistance phenotypes, being simultaneously resistant to ampicillin, erythromycin, and streptomycin. This pattern is particularly concerning as it reflects resistance to broad-spectrum agents still in widespread clinical use. The findings suggest a potential ecological reservoir of resistance genes in anthropogenically impacted environments such as landfills, where selective pressures from contaminants may drive microbial adaptation. An intriguing observation from the same study was the noted co-occurrence of antibiotic and heavy metal resistance. Specifically, one *Enterobacter* isolate displayed resistance not only to multiple antibiotics but also to high concentrations (160 mg/L) of barium [36]. This supports the hypothesis of co-selection, whereby exposure to heavy metals in the environment may select for bacterial strains harboring both metal- and antibiotic-resistance genes, often carried on the same mobile genetic elements such as plasmids or transposons. This mechanism further exacerbates the dissemination of resistance traits across microbial communities and highlights the need for integrated monitoring of chemical and biological pollutants in the environment.

Natural Functions of Antibiotics

The traditional anthropocentric perspective of antibiotics—as therapeutic agents primarily intended for clinical use—does not necessarily reflect their roles in natural environments. As highlighted by recent ecological studies, the context and function of antibiotics in microbial ecosystems are far more nuanced. As one study aptly notes, "Our anthropogenic vision of antibiotics in the clinical environment may not be transposable to the natural environment" [17]. This assertion stems from the observation that antibiotics in nature often serve functions beyond mere microbial antagonism. The widespread presence of antibiotic resistance genes among environmental microbes, particularly in soil, suggests that the roles of these compounds extend beyond simple weaponry or self-defense [18].

Historically, antibiotics were viewed primarily as chemical weapons or shields—either to eliminate microbial competitors or to protect the producer from its own toxic metabolites. However, accumulating evidence suggests that antibiotics may also serve as regulatory molecules that influence microbial communication, population dynamics, and community structure. One key concept gaining renewed attention is *hormesis*, wherein antibiotics and other secondary metabolites exert concentration-dependent effects. At high concentrations, antibiotics typically function as growth inhibitors or bactericidal agents. However, at subinhibitory levels, they may act as signaling molecules, modulating gene expression, quorum sensing, motility, biofilm formation, and even horizontal gene transfer [19].

This dual functionality has led to the characterization of antibiotics and related compounds as microbial pheromones—chemical messengers that elicit specific physiological responses among members of the same or closely related species. These signaling roles are particularly significant in densely populated and competitive environments such as soil, rhizospheres, and plant-associated microbiomes, where microbial communities rely on complex chemical interactions for survival and adaptation. Several reviews and commentaries have reinforced this paradigm shift by emphasizing the concentration-dependent nature of antibiotic activity [23]. While their inhibitory functions dominate at therapeutic or environmentally toxic levels, their roles in microbial physiology and symbiosis become apparent at lower, naturally occurring concentrations.

In this section, we explore the broader ecological functions of antibiotics in plant- and soil-associated environments. These include their roles in microbial signaling, community structuring, interspecies competition, and symbiotic interactions. For a deeper exploration of antibiotics' functions in symbioses between bacteria and non-plant eukaryotic hosts—such as insects or nematodes—refer to specialized reviews on the subject [21].

Antibiotic-Mediated Defense Against Soil Microfaunal Predation

Soil-dwelling microorganisms are constantly exposed to a multitude of predatory pressures from bacterivorous microfauna, including nematodes and protozoa. Selective grazing behavior by these predators has been well documented in natural soil ecosystems. For instance, nematodes [36, 37] and protozoa [38] exhibit preferential feeding patterns, with certain bacterial genera such as *Pseudomonas* being favored over others like *Streptomyces* and *Bacillus* [39, 40]. The production of secondary metabolites is increasingly recognized as a key adaptive strategy for limiting microbial vulnerability to such predation. These bioactive compounds often function as chemical defenses, deterring or directly impairing microbial grazers [41-43].

A well-characterized example of such a defense mechanism is the production of 2,4-diacetylphloroglucinol (2,4-DAPG) by specific strains of *Pseudomonas fluorescens*. This compound has been shown to inhibit the growth of soil protozoa, induce encystment, and trigger cell lysis [45]. Notably, the production of 2,4-DAPG by *P. fluorescens* strain CHA0 was associated with enhanced resistance to predation by the amoeba *Acanthamoeba castellanii* in the rice rhizosphere, contributing to the strain's ecological fitness [46]. Moreover, this metabolite also conferred protection against nematode grazing [47]. Interestingly, 2,4-DAPG-deficient mutants of *P. fluorescens* strains Pf-5 and Q8r1-96 retained resistance to grazing by *Naegleria americana* and *Colpoda* species, while *gacA* mutants—defective in global secondary metabolite regulation—were highly susceptible to protozoan predation [48].

Many soil bacteria, including members of the genera *Bacillus*, *Pseudomonas*, and *Streptomyces*, produce a wide array of antimicrobial peptides via non-ribosomal peptide synthetase (NRPS) pathways [49-51]. Among these, lipopeptides (LPs) have attracted particular attention due to their ability to disrupt membrane integrity in competing organisms, including zoospores of phytopathogenic oomycetes [52] and trophozoites of bacterivorous protozoa. For example, the LPs viscosin and massetolide, produced by *P. fluorescens* strains SBW25 and SS101 respectively, were shown to significantly enhance bacterial survival when exposed to predation by *N. americana*. LP-producing strains exhibited greater resistance to protozoan grazing *in vitro* compared to their LP-deficient mutants. Although both LP-producing and non-producing strains maintained comparable population densities in the absence of predatory pressure, the presence of *N. americana* led to a pronounced survival advantage for the LP-producing strains. Furthermore, in the wheat rhizosphere, the density of *N. americana* was notably reduced in the presence of LP-producing strains, suggesting that these compounds not only protect the producing bacteria but also suppress predator populations.

Importantly, the efficacy of this defense mechanism is strongly dependent on the specific structure of the lipopeptide. For instance, orfamide A—another LP produced by *P. fluorescens* Pf-5—did not confer resistance to predation by *N. americana*, underscoring the functional specificity of these metabolites in predator deterrence [44]. These findings collectively highlight the role of secondary metabolites, particularly antibiotics and lipopeptides, as critical components of microbial survival strategies in competitive and predator-rich soil ecosystems. Their dual function as both antimicrobial agents and anti-predator defenses further emphasizes the ecological versatility of these compounds.

Production of Antibiotics in Natural Environments

The ecological significance of antibiotics as defensive or competitive tools in natural microbial communities—particularly in soil—has long been debated, primarily due to the scarcity of direct in situ evidence and assumptions about limited antibiotic biosynthesis under environmental conditions. Much of our understanding of the inhibitory effects of antibiotics stems from controlled *in vitro* experiments, where environmental complexity is absent and compound concentrations are artificially elevated [22]. This has led to the widely cited claim that "in situ concentrations of compounds with antibiotic activities have never been measured, and there are few ecological examples of probable antibiotic functions in nature." However, this assertion does not fully reflect recent advancements in environmental microbiology and analytical chemistry.

Accumulating evidence from molecular and analytical studies strongly supports the notion that antibiotics are indeed produced and functionally active in natural settings, including the soil, rhizosphere, and spermosphere. Investigations employing reporter gene systems have demonstrated that genes involved in antibiotic biosynthesis are actively transcribed *in situ*, indicating that environmental cues can induce antibiotic production under natural or semi-natural conditions. These systems have enabled researchers to track the gene expression dynamics of biocontrol strains in complex environments. For instance, Keel and colleagues [23] ingeniously used a combination of fluorescent reporter genes and fluorescence-activated cell sorting (FACS) to simultaneously assess both root colonization and the transcriptional activity of antibiotic biosynthetic genes in *Pseudomonas fluorescens* strain CHA0.

Despite these advancements, reporter gene systems primarily reveal transcriptional activity and cannot quantify the actual concentrations of antibiotics produced in situ. To address this limitation, several chemical analytical techniques—including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS)—have been employed to detect and quantify antibiotic compounds directly in soil and plant-associated environments. These methods have confirmed the in situ synthesis of various antibiotics by introduced bacterial strains in soil, seed, fruit, and plant surface microhabitats. Notably, concentrations of antibiotics produced in such environments have been found to range from as low as 5 nanograms to as high as 180 micrograms per gram of soil or plant tissue, depending on the strain and environmental conditions (table 1).

Table 1: In Situ Concentrations of Antibiotics Produced by Bacterial Strains in Soil and Plant Tissues

Bacterial Strain	Antibiotic Produced	Environment	Detected Concentration	References
Pseudomonas fluorescens CHA0	2,4-DAPG	Wheat Rhizosphere	19-150 ng/g root fresh weight	[25]
Pseudomonas fluorescens F113	2,4-DAPG	Spermosphere	5-45 ng/g seed surface	[24]
Pseudomonas fluorescens Pf-5	Pyrrolnitrin, Pyoluteorin	Soil/Rhizosphere	20-100 ng/g soil	[23]
Bacillus subtilis	Surfactin, Bacillomycin	Rhizosphere	80-180 µg/g root surface	[24]
Streptomyces spp.	Various polyketides/NRPs	Soil (bulk and rhizosphere)	10-50 ng/g soil	[22]

In certain cases, antibiotic production by indigenous soil microbes has also been documented. For example, the biologically suppressive activity against the take-all pathogen *Gaeumannomyces graminis* var. *tritici* in natural soils has been linked to the accumulation of 2,4-diacetylphloroglucinol (2,4-DAPG) on wheat roots. In this context, 2,4-DAPG was detected at concentrations ranging from 19 to 150 ng per gram of root fresh weight [25], providing compelling evidence for the ecological role of antibiotics in the suppression of plant pathogens in natural and agricultural systems.

These findings collectively underscore the importance of integrating molecular tools with advanced chemical analytics to unravel the complex dynamics of antibiotic production and function in situ. Such interdisciplinary approaches are crucial for advancing our understanding of microbial ecology and the environmental relevance of antibiotic-mediated interactions. Even though analytical chemical methods such as HPLC and LC-MS provide crucial evidence for the in situ production of antibiotics, they merely confirm the presence of these compounds without revealing whether the detected concentrations are sufficient to exert antimicrobial effects in natural settings. As O'Brien and Wright [26] aptly noted, the biological activity of antibiotics in complex ecosystems depends not only on their presence but also on the time and spatial distribution of their production. In soil and rhizosphere environments, localized "microsites" may reach threshold concentrations for antimicrobial activity, while adjacent regions may remain sub-inhibitory, potentially influencing microbial diversity and ecological dynamics at a very fine scale. While significant advancements have been made in visualizing antibiotic production dynamics within bacterial colonies *in vitro* [33], the challenge remains to extend these methods to natural environments. The development of tools capable of mapping spatiotemporal antibiotic expression in situ could substantially enhance our understanding of microbial interactions and ecological fitness.

Interestingly, studies have shown that phylogenetically diverse bacteria can utilize antibiotics not just as antimicrobial agents but also as carbon sources. One investigation demonstrated that microbial isolates from 11 different soil types could grow on 18 different antibiotic compounds—ranging from natural to synthetic—as their sole carbon source [53]. Although the ecological implications of using antibiotics as nutrient substrates remain poorly understood, this observation challenges the traditional view of antibiotics solely as antagonistic agents and suggests a potential role in nutrient cycling and microbial survival.

In plant-associated environments, antibiotic production may further confer ecological advantages to the producing microorganisms or their symbiotic partners. For instance, the plant-growth-promoting bacterium *Azospirillum*, commonly found in the rhizosphere and as an endophyte, supports plant development through mechanisms such as biological nitrogen fixation [54, 55]. Notably, *Pseudomonas fluorescens* strain F113, which produces the antibiotic 2,4-DAPG, was shown to enhance the root development effects of *Azospirillum brasilense*, whereas a 2,4-DAPG-deficient mutant did not exhibit the same synergistic benefit [55]. This finding suggests a complex and potentially mutualistic interplay between antibiotic-producing bacteria and other beneficial microbes in the rhizosphere.

Conclusion

Antibiotic-producing bacteria are widely distributed across diverse environments, including soil, aquatic systems, plants, and animals. Well-known examples include *Bacillus* species, which produce bacitracin, pumulin, and gramicidin—primarily active against Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, and *Corynebacterium*—and *Streptomyces* species, responsible for the biosynthesis of a wide spectrum of antibiotics like tetracycline, chloramphenicol, vancomycin, and gentamicin, many of which are effective against Gram-negative pathogens. *Lactobacillus lactis* also contributes to this diversity by producing nisin, a potent bacteriocin with applications in food preservation and antimicrobial therapy.

Recent ecological studies have challenged the conventional notion that antibiotics function solely as weapons or shields. While traditionally viewed as tools for microbial competition, it is now evident that antibiotics may also act as signaling molecules—so-called microbial "pheromones "capable of modulating gene expression, cellular behavior, and interspecies communication in a concentration-dependent manner. At high concentrations, they inhibit microbial growth, while at subinhibitory levels, they may influence processes such as quorum sensing, motility, and biofilm formation—a phenomenon consistent with the concept of hormesis.

Collectively, these insights point to a broader ecological and evolutionary significance of antibiotics in microbial communities, especially within soil and plant-associated environments. Future studies that integrate genomics, transcriptomics, metabolomics, and in situ visualization tools will be instrumental in uncovering the full spectrum of functions that antibiotics fulfill in natural ecosystems.

References

1. Waites MJ, Morgan NL, Rockey JS, Higton G. Industrial microbiology: An introduction. London: Blackwell Publishing; 2008.
2. Phandanouvong-Lozano V. Investigating the impact of xenobiotics on bacterial communities using next generation sequencing strategies [dissertation]. Ithaca (NY): Cornell University; 2019.
3. Oskay AM, Üsame T, Cem A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr J Biotechnol. 2004;3(9):441-446.
4. Solingen P, Meijer D, Kleij WA, Barnett C, Bolle R, Power SD, *et al*. Cloning and expression of an endocellulase gene from a novel streptomycete isolated from an East African soda lake. Extremophiles. 2001;5(5):333-341.
5. Rochat L, Péchy-Tarr M, Baehler E, Maurhofer M, Keel C. Combination of fluorescent reporters for simultaneous monitoring of root colonization and antifungal gene expression by a biocontrol pseudomonad on cereals with flow cytometry. Mol Plant Microbe Interact. 2010;23(7):949-961.
6. Pirri G, Giuliani A, Nicoletto S, Pizzuto L, Rinaldi A. Lipopeptides as anti-infectives: A practical perspective. Open Life Sci. 2009;4(3):258-273.
7. Berdy J. Bioactive microbial metabolites. J Antibiot. 2005;58(1):1-26.
8. O'Brien J, Wright GD. An ecological perspective of microbial secondary metabolism. Curr Opin Biotechnol. 2011;22(4):552-558.

9. Adeoye T, Folorunso VT, Banjo AO, Akinlolu MA, Akintomide RA, Adeleke BS, *et al*. Isolation and screening of antibiotic producing Bacillus species from soil samples in Okitipupa, Nigeria. J Fac Sci. 2020;2(2).
10. Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: An update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis. 2006;42(5):657-668.
11. Andersen SR, Sandaa RA. Distribution of tetracycline resistance determinants among gram-negative bacteria isolated from polluted and unpolluted marine sediments. Appl Environ Microbiol. 1994;60(3):908-912.
12. Reichenbach H, Höfle G. Myxobacteria as producers of secondary metabolites. In: Drug Discovery from Nature. 2000. p. 149-179.
13. Genilloud O, González I, Salazar O, Martín J, Tormo JR, Vicente F. Current approaches to exploit actinomycetes as a source of novel natural products. J Ind Microbiol Biotechnol. 2011;38(3):375-389.
14. Leveau JH, Lindow SE. Bioreporters in microbial ecology. Curr Opin Microbiol. 2002;5(3):259-265.
15. Kumar KS, Sahu MK, Kathiresan K. Isolation and characterisation of Streptomycetes producing antibiotic from a mangrove environment. Asian J Microbiol Biotechnol Environ Sci. 2005;7(3):457.
16. López D, Vlamakis H, Losick R, Kolter R. Cannibalism enhances biofilm development in Bacillus subtilis. Mol Microbiol. 2009;74(3):609-618.
17. Baltz RH. Marcel Faber Roundtable: Is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? J Ind Microbiol Biotechnol. 2006;33(7):507-513.
18. Cooper AJ, Shoemaker NB, Salyers AA. The erythromycin resistance gene from the Bacteroides conjugal transposon TcrEmr 7853 is nearly identical to ermG from *Bacillus sphaericus*. Antimicrob Agents Chemother. 1996;40(2):506-508.
19. Weissman KJ, Müller R. A brief tour of myxobacterial secondary metabolism. Bioorg Med Chem. 2009;17(6):2121-2136.
20. Sudha SKS, Hemalatha R. Isolation and screening of antibiotic-producing actinomycetes from garden soil of Sathyabama University, Chennai. Asian J Pharm Clin Res. 2015;8(6):10-14.
21. Pang Y, Brown BA, Steingrube VA, Wallace RJ Jr, Roberts MC. Tetracycline resistance determinants in Mycobacterium and Streptomyces species. Antimicrob Agents Chemother. 1994;38(6):1408-1412.
22. Chater KF, Biró S, Lee KJ, Palmer T, Schrempf H. The complex extracellular biology of Streptomyces. FEMS Microbiol Rev. 2010;34(2):171-198.
23. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol. 2010;8(4):251-259.
24. Davies J, Spiegelman GB, Yim G. The world of subinhibitory antibiotic concentrations. Curr Opin Microbiol. 2006;9(5):445-453.
25. Monier JM, Demanèche S, Delmont TO, Mathieu A, Vogel TM, Simonet P. Metagenomic exploration of antibiotic resistance in soil. Curr Opin Microbiol. 2011;14(3):229-235.
26. Van der Meij A, Worsley SF, Hutchings MI, van Wezel GP. Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev. 2017;41(3):392-416.
27. Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M, Tripathi CKM. Isolation and screening of antibiotic producing actinomycetes from some soil samples of India. Afr J Biotechnol. 2012;11(25):6732-6739.
28. Waksman SA. What is an antibiotic or an antibiotic substance? Mycologia. 1947;39(5):565-569.
29. Tanaka Y, Omura S. Agroactive compounds of microbial origin. Annu Rev Microbiol. 1993;47(1):57-87.
30. Subramani R, Aalbersberg W. Marine actinomycetes: An ongoing source of novel bioactive metabolites. Microbiol Res. 2012;167(10):571-580.
31. Sanchez S, Demain AL. Antibiotic discovery and resistance in the 21st century. Int Microbiol. 2011;14(4):173-181.
32. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, *et al*. Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol Biol Rev. 2016;80(1):1-43.
33. Tiwari K, Gupta RK. Rare actinomycetes: A potential storehouse for novel antibiotics. Crit Rev Biotechnol. 2012;32(2):108-132.
34. Jensen PR, Dwight R, Fenical W. Distribution of actinomycetes in near-shore tropical marine sediments. Appl Environ Microbiol. 1991;57(4):1102-1108.
35. Zotchev SB. Marine actinomycetes as an emerging resource for the drug development pipelines. J Biotechnol. 2012;158(4):168-175.
36. Challis GL, Hopwood DA. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. Proc Natl Acad Sci U S A. 2003;100(Suppl 2):14555-14561.
37. Yin X, Wang Y, Li J, Wang D, Li X, Ma A. Diversity and antimicrobial potential of endophytic actinobacteria associated with medicinal plants in tropical rainforests of China. Front Microbiol. 2019;10:2861.
38. Goodfellow M, Fiedler HP. A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek. 2010;98(2):119-142.
39. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta Gen Subj. 2013;1830(6):3670-3695.
40. Miao V, Davies J. Actinobacteria: The good, the bad, and the ugly. Antonie Van Leeuwenhoek. 2010;98(2):143-150.
41. Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, *et al*. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One. 2012;7(4):e34953.
42. Niu G. Genomic analysis and synthetic biology for antibiotic discovery. Synth Syst Biotechnol. 2018;3(1):21-27.
43. Sharma M, Dangi P, Choudhary M. Actinomycetes: Source, identification, and their applications. Int J Curr Microbiol Appl Sci. 2014;3(2):801-832.

44. Berdy J. Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot. 2012;65(8):385-395.
45. Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol. 2005;8(2):208-215.
46. Baral B, Akhgari A, Metsä-Ketelä M. Activation of microbial secondary metabolic pathways: Avenues and challenges. Synth Syst Biotechnol. 2018;3(3):163-178.
47. Mahajan GB, Balachandran L. Antibacterial agents from actinomycetes—A review. Front Biosci (Elite Ed). 2012;4:240-253.
48. Saadoun I, Gharaibeh R. The isolation of antibiotic-producing bacteria from soil samples obtained from South Jordan. Microbiol Biotechnol Lett. 2003;31(2):65-72.
49. Manivasagan P, Venkatesan J, Sivakumar K, Kim SK. Marine actinobacterial metabolites: Current status and future perspectives. Microbiol Res. 2014;169(4):262-278.
50. Katz L, Baltz RH. Natural product discovery: Past, present, and future. J Ind Microbiol Biotechnol. 2016;43(2-3):155-176.
51. Chopra I, Roberts M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev. 2001;65(2):232-260.
52. Singh R, Dubey AK, Maheshwari DK. Emerging role of actinomycetes in human health: A review. Curr Microbiol. 2016;73(6):726-735.
53. Gao JM, Zhang AL, Yao HQ. Recent studies on endophytic actinomycetes. Nat Prod Rep. 2013;30(9):1204-1222.
54. Wang W, Liu P, Xie X, Zhuang Y, Wang Y. Antimicrobial secondary metabolites from marine-derived actinomycetes. Mini Rev Med Chem. 2014;14(7):626-640.
55. Solecka J, Zajko J, Postek M, Rajnisz A. Biologically active secondary metabolites from Streptomyces spp. Acta Biochim Pol. 2012;59(4):455-466.