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Exploring the Versatility of *Bacillus* spp. Secondary Metabolites: its mode of action and application in various field

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ABSTRACT

Bacillus spp. is a rich source of various secondary metabolites (SMs) that are used for diverse purposes. These include lipopeptides, non-ribosomal peptides, polyketides, and many more, which possess strong antimicrobial, antifungal, and insecticidal properties, thereby making them useful ingredients for biotechnology, agriculture, cosmetics, healthcare, and food preservation. This review provides an overview of the current understanding of *Bacillus* SMs' diversity that has been overlooked to some extent, as well as their mode of action and application prospects. Recent technological advancements—such as metagenomics, synthetic biology, CRISPR-Cas9, machine learning, microfluidics, and high-throughput screening—have accelerated discoveries and optimized SM production. The use of SMs as plant growth-promoting compounds in agriculture is controlling pathogens while they may also have pharmaceutical, nutraceutical, and cosmetic applications. Furthermore, they possess a broad range of industrial applications, including use in detergents and food preservatives, offering more versatility and environmental friendliness compared to chemical alternatives. Though there has been significant progress, there still exist major knowledge gaps in the regulation and ecological roles played by SMs that need to be addressed through research. As we uncover the secrets behind the production and various applications of SMs, microorganisms can become effective allies in addressing global challenges for a healthier, more sustainable planet.

1.Introduction

Bacillus spp., a ubiquitous Gram-positive bacterium, exhibits remarkable genetic adaptability and facilitates the colonization of a wide variety of environments. *B. subtilis* can withstand harsh conditions in space, including radiation, vacuum, and extreme temperatures, for six years [1]. It has served as a primary model organism in laboratories for decades, providing insights into the basic principles of spore-forming Gram-positive bacteria. In the field of biotechnology, it is an integral part and is used extensively for the synthesis of a wide range of products, ranging from enzymes to fine chemicals. Its suitability for genetic manipulation, due to natural competence, lack of an outer membrane, and well-characterized expression systems, makes it a preferred choice in numerous applications [2]. Additionally, it has proven to be a valuable biological control agent in agriculture, combating plant pathogens while promoting plant growth [3].

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Despite its role in the industrial production of biosurfactants, *Bacillus* spp. is still largely underestimated due to its innate biosynthetic capabilities. Wild-type strains from diverse origins have demonstrated the impressive diversity of biosynthetic pathways within this species. For example, an estimated 4-5% of the typical *B. subtilis* genome is dedicated exclusively to the biosynthesis of natural products [4]. While *B. subtilis* has long been known for the production of complex cyclic lipopeptides such as surfactins, iturins, and fengycins, which have attracted increasing attention in the biotechnology and pharmaceutical industries as biosurfactants and antibiotics, recent discoveries have revealed additional compounds. These include dihydroisocoumarins, polyketide-derived macrolides, and linear lipopeptides, all of which have potent antimicrobial properties, shedding further light on the biosynthetic potential of the bacterium [5].

The aim of this comprehensive review is to consolidate knowledge about biologically active SM of *Bacillus* spp. This review provides an updated perspective on current findings and applications in this field and highlights the often-overlooked diversity of natural compounds produced by this bacterium. In an era characterized by increasing antibiotic resistance and a growing demand for environmentally sustainable antimicrobial strategies, it is crucial not to ignore common microorganisms such as *Bacillus* spp. and their valuable natural products. Therefore, we anticipate that this review will rekindle interest in the utilization of *B. subtilis* for biological control systems and the production of antibiotics and biosurfactants.

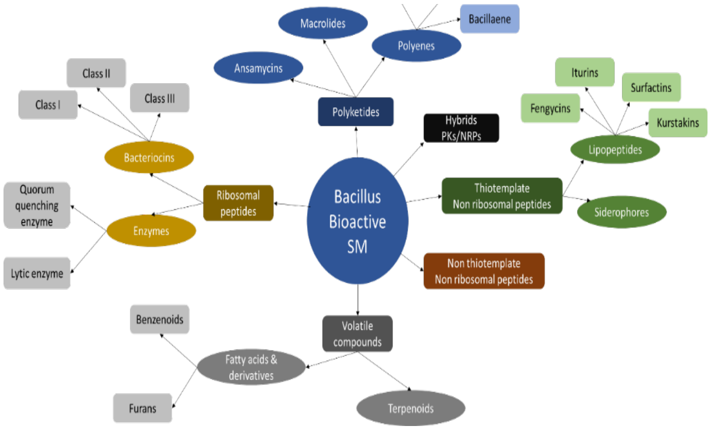

Figure 1. Various bioactive SMs produced by *Bacillus* spp.

2.Secondary metabolites from *Bacillus* spp.

Bacillus spp., *a diverse genus of bacteria, are renowned for their ability to produce a wide array of SMs with various biological activities. These metabolites play crucial roles in microbial ecology, agriculture, medicine, and industry. Among the most notable SMs produced by Bacillus spp. are antimicrobial SMs such as bacitracin, polymyxin, and gramicidin, which have been extensively used in clinical settings for combating bacterial infections. Bacillus spp. also produce enzymes like proteases, amylases, and lipases, which find applications in various industrial processes, including food processing and detergent manufacturing. Furthermore, Bacillus spp.* are known for synthesizing bioactive compounds with potential pharmaceutical properties, such as surfactins, iturins, and fengycins, which exhibit antimicrobial, antifungal, and anticancer activities. These SMs underscore the immense biotechnological potential of *Bacillus* spp. and continue to inspire research aimed at harnessing their diverse metabolic capabilities for beneficial purposes. Different bioactive SMs produced by *Bacillus* spp. are shown in fig. 1 and fig. 2.

3.Antimicrobial Secondary Metabolites and Their Mechanism of Action

Antimicrobial SMs produced by *Bacillus* spp. exhibit strong activity against a broad spectrum of pathogens. These metabolites function through various mechanisms, such as disrupting microbial cell walls and cell membranes, inhibiting essential enzymes, interfering with DNA synthesis, and many more, thereby effectively neutralizing harmful microorganisms. Understanding the precise mechanisms of action of these antimicrobial compounds is crucial for optimizing their application in biotechnology, agriculture, and healthcare, offering sustainable alternatives to synthetic chemicals.

3.1 Secondary metabolites targeting cell wall

The bacterial cell wall consists of a peptidoglycan layer that provides integrity and protection to the cell. This layer consists of linear glycan strands alternating between N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues connected by β-1,4 bonds. The peptidoglycan pathway shown in Fig. 3 explains the crucial steps required to produce a proper peptidoglycan layer [6]. Several key blocking points have been identified, as specified in the image, detailing how they obstruct the synthesis of the cell wall:

Bacitracin: It hinders the dephosphorylation of undecaprenyl pyrophosphate (C55-PP) to undecaprenyl phosphate (C55-P), preventing the formation of lipid I/II and ultimately destroying the peptidoglycan layer [7].

Bacilysin: Glucosamine-6-phosphate synthetase (G6PS) is an enzyme that catalyzes the production of uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), a precursor for peptidoglycan synthesis. Bacilysin enters the cell by binding to a transmembrane transport protein and is subsequently hydrolyzed to anticapsin, a G6PS inhibitor [8].

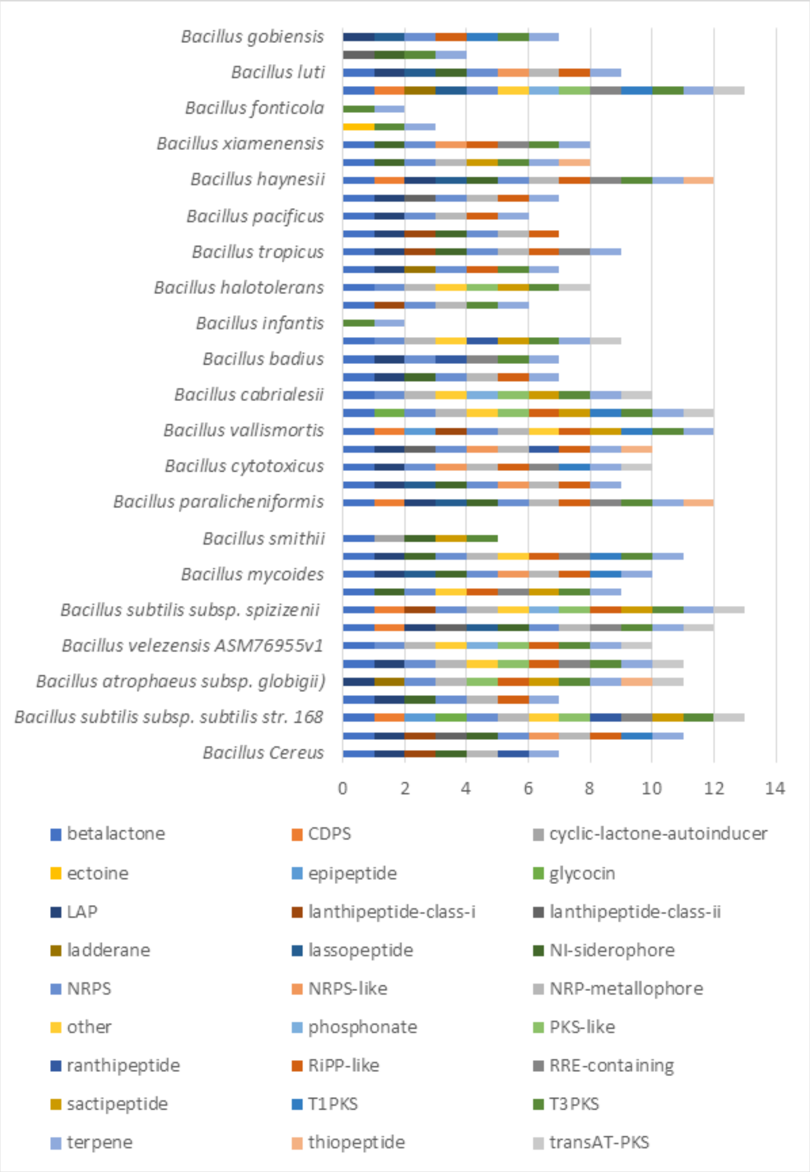

Figure 2. List of SMs class produced by different *Bacillus* spp. Data acquired using antiSMASH tool.

Clausin: Lipid II, a peptidoglycan intermediate, is formed when the glycosyltransferase MurG adds N-acetylglucosamine (GlcNAc) to lipid I. Clausin interacts with both lipid I/II and GlcNAc, forming stable complexes that hinder its role in peptidoglycan biosynthesis [9].

Amylolysin A: Lipid II migrates across the plasma membrane and transfers MurNAc and GlcNAc to the peptidoglycan layer. Amylolysin A interacts with lipid II to impede peptidoglycan biosynthesis [10].

3.2 Metabolites targeting cell membrane

The plasma membrane consists of a phospholipid bilayer that serves as a barrier between the intracellular and extracellular compartments while facilitating the selective transport of metabolites across the membrane. SMs challenge the integrity of the cell membrane in different ways, as shown in Fig. 4.

Lipopeptides: The lipid bilayer plays a crucial role in controlling the permeability and shape of the plasma membrane. The negative charge of the outer phospholipid layer influences its properties. Any change in the lipid composition or the phospholipid layer can disrupt the function of the membrane as a barrier and lead to the release of essential ions from the cell and ultimately cell death. Lipopeptides show antimicrobial action by disruption of the plasma membrane [11].

Bacteriocins: Certain SMs have the ability to aggregate and form pores in the plasma membrane. These pores lead to the leakage of nucleic acids, essential ions, and ATP from the cell, ultimately leading to necrosis. Bacteriocins can

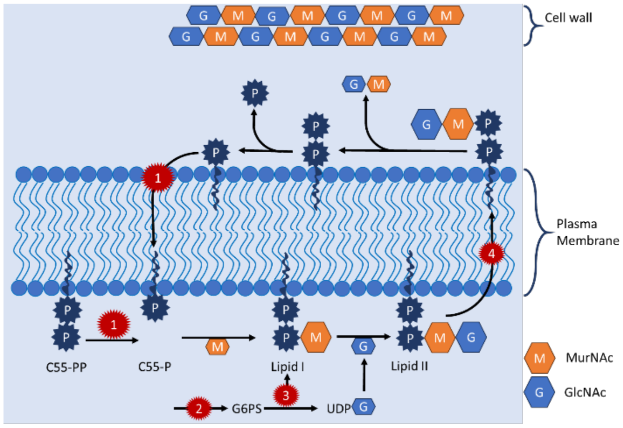

Figure 3. Interaction of SMs in the cell wall synthesis pathway, showing how 1 (Bacitracin), 2 (Bacilysin), 3 (Clausin), and 4 (Amylolysin A) interfere with the process. Key components involved include C55-PP (undecaprenyl pyrophosphate), C55-P (undecaprenyl phosphate), MurNAc (N-acetylmuramic acid), GlcNAc (N-acetylglucosamine), and G6PS (glucosamine-6-phosphate synthetase).

disrupt the plasma membrane of target bacteria by forming pores, which disrupt membrane potential and ion gradients, leading to cell death [12].

Peptides and lipopeptides: *Bacillus* SMs can employ diverse mechanisms to interact with the plasma membrane of target cells. In addition to forming pores, as previously discussed, these metabolites can also act by forming ion channels or anchoring to membrane receptors, as well as binding electrostatically to the plasma membrane. Some *Bacillus* SMs, including various peptides and lipopeptides, may also interact with specific membrane receptors on target cells. By binding to these receptors, these metabolites can trigger downstream signaling pathways or disrupt normal receptor function, leading to cellular dysfunction and potentially cell death [13].

3.3 Metabolites Targeting Intracellular Processes

In addition to their effects on the plasma membrane, *Bacillus* SMs can also target intracellular processes within target cells, shown in fig. 5. These metabolites often interfere with essential cellular functions, ultimately leading to cell death or inhibition of growth.

Gramicidin: Damage to the nucleic material leads to the dysfunction of cell activities. For example, gramicidin S can also induce the formation of reactive oxygen species (ROS), which damages intracellular DNA [14]. It also binds to the DNA and inhibits transcription and cell growth.

Sublancin: Sublancin hinders DNA replication, transcription, and translation, while leaving cell wall biosynthesis unaffected [15].

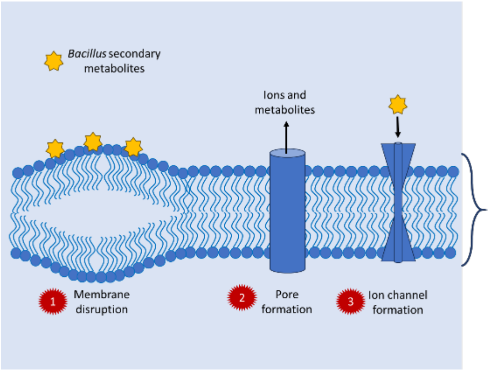

Figure 4. Interaction of SMs with cell membrane, showing how 1 (Lipopetide), 2 (Bacteriocins), 3 (peptides and lipopetides)

Amicoumacin: Amicoumacin inhibits bacterial growth by targeting the ribosome during translation. The Ami binding site is situated near the E-site codon of mRNA. Interestingly, Ami does not interfere with mRNA but rather stabilizes it, indicating a unique mechanism of translation inhibition [16].

Fengycins: Fengycins able to disrupt multiple intracellular components of the cell, especially interfere with the functionality of various membrane-bound proteins and enzymes, further compromising cellular metabolism and homeostasis [17].

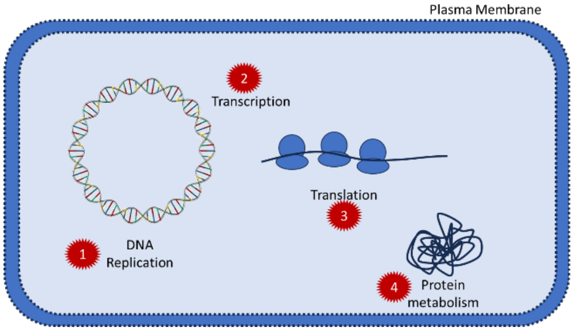

Figure 5. Interaction of SMs with intracellular activities

Additionally, *Bacillus* SMs can target nucleic acid synthesis and replication within bacterial cells. Compounds such as gramicidin and polymyxin, produced by *Bacillus* spp., can disrupt DNA or RNA synthesis by interfering with the function of enzymes involved in these processes [18]. This disruption of nucleic acid synthesis prevents bacterial cells from replicating and dividing, leading to their eventual demise.

Furthermore, *Bacillus* SMs may interfere with cellular metabolism by inhibiting key metabolic enzymes or pathways. For example, fengycins can directly inhibit specific enzymes involved in cellular processes such as glycolysis or the tricarboxylic acid cycle [19]. Surfactin can disrupt the function of ATP synthase, an essential enzyme in the cellular energy production pathway [20]. Mycosubtilin inhibits the activity of essential enzymes in the fatty acid synthesis pathway, which is crucial for maintaining cell membrane structure and function [21].

3.4 Metabolites Aiming at Different Targets

Certain *Bacillus* SMs can interfere with bacterial communication systems known as quorum sensing. Quorum sensing regulates gene expression in response to cell population density and plays a role in various bacterial processes such as biofilm formation and virulence. Compounds like surfactin and fengycin produced by *Bacillus amyloliquefaciens* have been shown to interfere with quorum sensing in various pathogens. For instance, surfactin can inhibit the quorum sensing of *Pseudomonas aeruginosa*, thereby disrupting its ability to form biofilms and coordinate virulence factors [22]. Fengycin has been observed to inhibit quorum sensing in several Gram-positive bacteria, including *Staphylococcus aureus* and *Listeria monocytogenes* [23].

Bacillus SMs can interfere with the formation and stability of bacterial biofilms. Biofilms are complex communities of bacteria embedded within a matrix of extracellular polymeric substances (EPS) [24]. For example, **surfactin** produced by Bacillus subtilis has been shown to prevent the initial attachment of Pseudomonas aeruginosa to surfaces by reducing surface tension, thereby inhibiting biofilm formation [25]. Additionally, **fengycin** from Bacillus amyloliquefaciens disrupts the EPS matrix of biofilms of Staphylococcus aureus, leading to destabilization and dispersal of the biofilm structure [26]. This disruption can make bacteria more susceptible to antibiotics and other antimicrobial agents.

Some *Bacillus* SMs possess the ability to neutralize or inhibit the activity of bacterial toxins. For instance, mycosubtilin produced by *Bacillus subtilis* can bind to and neutralize toxins produced by pathogenic bacteria like *Listeria monocytogenes* [27]. This neutralization prevents the toxins from exerting their harmful effects on host cells and tissues, providing a potential therapeutic strategy against bacterial infections.

Certain *Bacillus* spp. secrete specific SMs that serve to obtain specific nutrients from their environment. These nutrients are often critical for microbial growth, and utilization of these metabolites can provide a competitive advantage over neighboring microorganisms. Siderophores, small molecules secreted by microorganisms, play a key role in the absorption of iron (Fe2+) from the environment. Iron is essential for microbial growth, and strategies have been developed to deprive pathogenic microorganisms of this essential nutrient through the use of siderophores [28]. For example, *Bacillus subtilis* produces the siderophore **bacillibactin,** which effectively scavenges iron from the environment. This deprives pathogenic microorganisms such as *Pseudomonas aeruginosa* of this essential nutrient, thereby inhibiting their growth [29]. This strategy helps *Bacillus subtilis* outcompete other microbes for vital resources, supporting its survival and proliferation in various environments.

4. Recent Technological Advances in *Bacillus* SMs Research

4.1Metagenomics and Synthetic Biology

Metagenomics and synthetic biology have revolutionized the discovery and application of *Bacillus* secondary metabolites (SMs). These advanced techniques enable researchers to explore the vast genetic diversity of *Bacillus* species across various environments, revealing the extensive metabolic potential these bacteria offer.

Metagenomics involves directly extracting and analyzing genetic material from environmental samples, bypassing traditional cultivation methods. This approach has led to the identification of numerous novel *Bacillus* species and their associated biosynthetic gene clusters (BGCs), many of which remain undetected through standard culturing techniques.

Metagenomic sequencing has unearthed a wide array of unique BGCs in diverse environments such as soil, marine sediments, and plant rhizospheres. For example, research using metagenomic analysis on soil samples has revealed new antimicrobial compounds, significantly expanding the repertoire of *Bacillus*-derived secondary metabolites. [30].

One notable study exemplifies the potential of metagenomic sequencing to discover antimicrobial compounds. By applying these techniques to soil samples, researchers identified previously uncharacterized BGCs responsible for novel metabolite production in *Bacillus* species. Such discoveries are vital as they enhance our understanding of *Bacillus* metabolic capabilities and broaden the spectrum of *Bacillus* SMs available for biotechnological and therapeutic applications.

4.2 CRISPR-Cas9

The CRISPR-Cas9 gene editing tool has become invaluable for precise manipulation of *Bacillus* genes, enabling researchers to selectively knock out, activate, or modify genes involved in secondary metabolite production. This technique allows for targeted genetic modifications, which can optimize *Bacillus* strains to enhance the yield and functionality of specific metabolites [31].

CRISPR-Cas9 in *Bacillus* research involved creating a non-mucoid strain of *Bacillus licheniformis* by deleting the pgsBCAE operon, a genetic cluster responsible for poly-γ-glutamic acid synthesis. This modification reduced the production of mucoid substances and enabled further genetic edits targeting pathways associated with lactate, glycerol, and ethanol production. By modifying these genes, researchers successfully enhanced the production of 2,3-butanediol (BDO), an industrially valuable compound used in applications ranging from biofuels to polymers [32].

CRISPR-Cas9 was used to enhance heme production in *Bacillus amyloliquefaciens* [33]. In this case, genes associated with the synthesis of volatile compounds linked to undesirable odors were deleted, a change that not only boosted heme production but also improved the strain's suitability for biotechnological applications where odor can impact product acceptability.

4.3 Application of Machine Learning and AI

Machine learning (ML) and artificial intelligence (AI) have become essential tools in unlocking the full potential of biotechnology. These advanced computational approaches allow researchers to analyze vast datasets from genomic, transcriptomic, and metabolomic studies, offering powerful insights into the genes and regulatory networks involved in SM biosynthesis [34].

By using machine learning, scientists can now accurately predict novel biosynthetic gene clusters (BGCs) and evaluate their bioactivity potential. This predictive ability has transformed the research landscape, enabling targeted exploration of *Bacillus* secondary metabolites and dramatically accelerating the discovery process. Additionally, ML reveals the complex relationships between the structures and functions of *Bacillus* metabolites, making it easier to identify promising compounds for specific applications in medicine, agriculture, and industry.

An example of this powerful application is the use of machine learning algorithms to optimize surfactin production. Researchers applied ML to streamline the production process, ultimately achieving a 160% increase in surfactin yield compared to the wild strain. This breakthrough not only underscores the potential of AI in enhancing metabolite production but also opens doors to more sustainable and cost-effective industrial processes [35].

Machine learning continues to redefine *Bacillus* SM research, offering innovative pathways for increased productivity, efficiency, and targeted applications across diverse fields.

4.4 Microfluidics and High-Throughput Screening

Microfluidic systems and high-throughput screening (HTS) have become game-changing technologies for rapidly identifying *Bacillus* strains and secondary metabolites (SMs) with desired bioactivities [36]. These tools allow researchers to efficiently screen hundreds or even thousands of *Bacillus* strains and SM variants simultaneously, all while using minimal sample volumes. This high efficiency is essential for identifying high-yield or high-potency producers, significantly speeding up the discovery and selection of promising strains.

Microfluidic systems, in particular, offer additional advantages in studying real-time interactions between *Bacillus* metabolites and target pathogens or host cells. By enabling precise control over conditions at microscale levels, microfluidics allow researchers to observe these interactions dynamically, providing valuable insights into the mechanisms of action of *Bacillus* SMs against specific pathogens.

This combination of HTS and microfluidics is reshaping *Bacillus* SM research, making it faster, more efficient, and more accurate in identifying metabolites and understanding their bioactivity [37]. Through these advanced screening methods, researchers can quickly pinpoint *Bacillus* strains with enhanced properties for applications in medicine, agriculture, and other industries.

These technological advances have collectively transformed *Bacillus* SMs research, enabling faster discovery, enhanced production, and novel applications in medicine, agriculture, and industry. By leveraging these tools, researchers can unlock the full potential of *Bacillus* SMs as natural and sustainable solutions in various fields.

5. Exploring the Application

Bacillus SMs offer sustainable, cost-effective, and environmentally friendly solutions across various fields, including agriculture, cosmetics, laundry, food, healthcare, and other industries, presenting clear advantages over traditional methods in terms of efficacy, safety, and environmental impact. Their diverse biological activities make them valuable resources for addressing societal challenges and advancing scientific knowledge.

5.1 Agriculture

Bacillus spp. are extensively used in agriculture due to their ability to produce various SMs that offer benefits ranging from pest control to plant growth promotion. Here are some common uses of *Bacillus* SMs in agriculture.

Biocontrol Agents: *Bacillus* spp. produce SMs such as antimicrobial compounds that inhibit the growth of plant pathogens. These biocontrol agents can be applied to crops to protect them from diseases caused by fungi, bacteria, and other harmful microorganisms [38]. Bacillus amyloliquefaciens produces the SM **iturin A** and bacillomycin D, which has been shown to inhibit the growth of the fungal pathogen Botrytis cinerea, a common cause of grey mold in various crops [39].

Bacillus thuringiensis (Bt) is perhaps the most well-known example of a *Bacillus* spp. used in agriculture. Bt produces insecticidal toxins known as crystal proteins (Cry toxins) during sporulation [40]. These toxins are highly specific to certain insect pests and can be incorporated into bioinsecticides. Bt-based insecticides are widely used to control caterpillars, beetles, and other insect pests in crops such as corn, cotton, and vegetables. When the fermented broth of *B. subtilis* YZ-1 was applied to *Tenebrio molitor*, a remarkable mortality rate of 90-95% was observed, with surfactins identified as the active compounds. This insecticidal activity was substantiated when a study with a surfactin deletion mutant of YZ-1 revealed a loss of effectiveness, underscoring the importance of surfactins [41].

Plant Growth Promoters: Some *Bacillus* spp. produce SMs that stimulate plant growth and enhance crop yields [42]. These metabolites include compounds like auxins, gibberellins, and cytokinins, which can promote root development, increase nutrient uptake, and improve stress tolerance in plants [43].

Systemic Resistance Inducer: Certain *Bacillus* spp. can trigger a plant's natural defense mechanisms, leading to enhanced resistance against pathogens and pests. This phenomenon, known as induced systemic resistance (ISR), involves the production of SMs such as lipopeptides and volatile organic compounds by *Bacillus* spp. These metabolites prime the plant's immune system, making it more effective at combating infections [44].

Biostimulants: *Bacillus*-based biostimulants are used to improve plant health and vigor by enhancing nutrient uptake, stimulating root growth, and increasing stress tolerance [45]. These biostimulants contain SMs can be applied to seeds, soil, or foliage to promote plant growth and productivity. Bacillus amyloliquefaciens produces **indole-3-acetic acid (IAA),** a plant hormone that stimulates root growth and enhances nutrient uptake, leading to improved plant growth and increased crop yields [46].

Bioremediation: Some *Bacillus* spp. possess the ability to degrade organic pollutants and toxins in soil and water environments. SMs produced by these bacteria, such as lipopeptides and biosurfactants, aid in the breakdown and metabolism of contaminants, thereby improving soil and water quality [47]. Bacillus subtilis produces the biosurfactant **surfactin**, which has been shown to enhance the degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils, effectively reducing pollutant levels and promoting a healthier environment [48]. Surfactin's mode of action involves reducing surface and interfacial tension between water and hydrophobic contaminants, increasing the bioavailability of PAHs. This facilitates the access of degrading microorganisms to the PAHs, enhancing their breakdown and subsequent removal from the environment. Additionally, surfactin can disrupt the structure of PAH aggregates, further aiding in their degradation and contributing to soil remediation.

5.2 Cosmetics

SMs exhibit antimicrobial, antioxidant, and skin rejuvenating properties, making them valuable ingredients in cosmetic formulations.

Biosurfactant, surfactin from *Bacillus subtilis* are used as an alternate to chemical surfactants for application in cosmetics industries which help to hydrate the skin, improve skin texture, and enhance overall skin health. They are often included in serums, creams, and masks [49, 50].

LactoSporin, produced by Bacillus coagulans, demonstrates significant efficacy as a natural ingredient for reducing fine lines and wrinkles in human subjects, making it a valuable addition to cosmetic formulations. Its proven absence of adverse skin reactions further establishes LactoSporin as a safe component for skincare products. LactoSporin mitigates fine lines and wrinkles primarily through its antioxidant [51] and anti-inflammatory properties [52]. By neutralizing free radicals that cause oxidative stress and damage to skin cells, it helps prevent premature aging. Additionally, LactoSporin promotes collagen synthesis and skin cell regeneration, enhancing skin elasticity and firmness [53]. This combined action reduces the appearance of fine lines and wrinkles, making it an effective ingredient in anti-aging skincare formulations.

The potential antioxidant properties were investigated using extracts of *Bacillus* cereus. In particular, the rhamno-galactan-EPS fraction from *Bacillus* cereus showed remarkable scavenging activity against 2,2′-diphenyl-1-picrylhydrazyl (Inhibition Concentration, IC50: 0.6 mg/ml), superoxide (IC50: 2.6 mg/ml) and hydroxyl radicals (IC50: 3.1 mg/ml) [54]. Such extracts show promise in protecting skin from free radical damage and environmental stressors such as UV radiation and pollution. They are therefore often integrated into formulations for anti-aging and protective skin care.

SMs produced by *Bacillus* spp. can enhance skin exfoliation, providing various benefits for skin health and appearance. These metabolites promote the removal of dead skin cells, leading to smoother and brighter skin. **Subtilisin p**roduced by Bacillus subtilis has a proteolytic that helps break down proteins in dead skin cells, facilitating their removal and promoting skin exfoliation [55].

5.3 Laundry

Bacillus SMs play a crucial role in improving the effectiveness, sustainability, and environmental friendliness of laundry products. Their enzymatic activities and other beneficial properties make them valuable ingredients for achieving clean, fresh-smelling, and well-maintained fabrics.

Bacillus SMs have significant commercial value in the laundry industry due to their effectiveness in enhancing cleaning efficiency and fabric care. Key examples include **subtilisin**, a protease enzyme produced by Bacillus licheniformis, which breaks down protein-based stains like blood, sweat, and grass. **Lipase** from Bacillus subtilis targets and degrades lipid-based stains such as oils and greases, while **amylase** from Bacillus amyloliquefaciens breaks down carbohydrate-based stains from starches and food residues. **Cellulase** from Bacillus agaradhaerens is used to break down cellulose fibers, softening fabrics, reducing pilling, and maintaining the brightness of clothes by removing microfibrils. These *Bacillus*-derived SMs not only improve stain removal and fabric care but also provide an environmentally friendly laundry aid.

Odor Control: Certain *Bacillus* spp. produce enzymes and metabolites that help degrade organic compounds responsible for unpleasant odors in laundry, such as sweat, body oils, and food residues. These odor-neutralizing properties make *Bacillus*-derived products particularly useful in laundry detergents and fabric softeners. For example, **subtilisin**, a protease produced by Bacillus licheniformis, breaks down protein-based odor-causing compounds, while **lipase** from Bacillus subtilis targets and degrades fats and oils. Additionally, **amylase** from Bacillus amyloliquefaciens breaks down starches and food residues, all contributing to a fresher and cleaner scent in laundered fabrics.

Bacillus enzymes can aid in maintaining the vibrancy and brightness of colored fabrics by preventing the accumulation of dirt and grime that can dull colors over time. Additionally, *Bacillus* SMs may help to minimize the fading of dyes during the washing process, preserving the appearance of colored garments [56].

Bacillus-derived enzymes and metabolites can contribute to the overall care and maintenance of fabrics by gently removing stains and soils without causing damage or excessive wear to fibers. This can help extend the lifespan of clothing and textiles, reducing the need for frequent replacement [57].

Bacillus enzymes are compatible with septic systems and can help to break down organic matter, preventing the buildup of sludge and maintaining the efficiency of septic tanks and drain fields [58]. Laundry detergents containing *Bacillus*-derived enzymes are suitable for use in households with septic systems, promoting proper waste treatment and disposal.

Crude cyclic lipopeptide (CLP) biosurfactants from *Bacillus* subtilis strains were studied for their compatibility and stability with some locally available commercial laundry detergents [59]. CLP biosurfactants from *B. subtilis* strains act additively with other components of the detergents to further improve the wash quality of the detergents. The thermal resistance and extreme alkaline pH stability of *B. subtilis* CLP biosurfactants favor their inclusion in laundry detergent formulations. Similarly, lipopeptide biosurfactants produced by *Bacillus* subtilis SPB1 in the formulation of a washing powder were investigated [60]. It was observed that the biosurfactant acts additively with a commercial detergent and enhances its performance from 33 to 45% in removing oil stains and from 57 to 64% in removing tea stains [60].

5.4 Healthcare

Bacillus SMs offer promising opportunities for addressing various healthcare challenges, including infectious diseases, wound management, and gastrointestinal disorders. Further research and development in this field may lead to the discovery of novel therapeutic agents and diagnostic tools derived from *Bacillus* spp.

Bacillus species are renowned for their ability to produce a variety of antibiotics, including bacitracin and polymyxin, which are particularly effective in treating bacterial infections. Bacitracin [61] and polymyxin [62] are among these antibiotics. Bacitracin, produced by *Bacillus licheniformis* [63] and *Bacillus subtilis* [64], inhibits cell wall synthesis in Gram-positive bacteria and is commonly used in topical ointments to prevent infection in minor wounds. A study on the use of topical bacitracin to prevent sternal wound infections after cardiac surgery demonstrated that the routine application of bacitracin ointment significantly reduced the incidence of deep sternal wound infections. Over a nine-year period, no deep sternal wound infections were observed among 1,495 patients, highlighting the potential of bacitracin as an effective prophylactic measure against such infections [65]. On the other hand, polymyxin, produced by *Bacillus polymyxa*, disrupts cell membranes of Gram-negative bacteria. Polymyxin B is often used in topical formulations for skin infections, while polymyxin E (colistin) is reserved for severe, multidrug-resistant infections, such as those caused by *Pseudomonas aeruginosa,* and is administered intravenously or by inhalation for lung infections. These antibiotics underscore the significant therapeutic contributions of *Bacillus* species in modern medicine [66].

Bacillus spp. produce enzymes and SMs that can disrupt bacterial biofilms, which are implicated in chronic infections and medical device-associated infections [67]. Surfactin is a potent biosurfactant with notable antibiofilm activity, which is crucial for wound healing applications. Biofilms, which are structured communities of bacteria encased in a self-produced matrix, often pose a major challenge in chronic wound management due to their resistance to conventional treatments. Surfactin A from *B. subtilis* disrupts biofilm formation by inhibiting the adhesion and aggregation of pathogenic bacteria, thereby enhancing the efficacy of antimicrobial agents and promoting faster wound healing [68]. Additionally, other Bacillus-derived SMs such as bacitracin and iturin further contribute to this process by directly targeting and eliminating pathogenic microbes. The combined action of these compounds not only helps in preventing infection but also accelerates the wound healing process [69].

Bacillus-based biocontrol agents are being increasingly investigated as alternatives to chemical disinfectants and antibiotics for combating healthcare-associated pathogens. Various *Bacillus* spp. can produce potent antimicrobial peptides, bacteriocins, macrolactin, and other SMs that effectively inhibit the growth of multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) [70]. Bacitracin is particularly effective against Gram-positive bacteria, including MRSA and VRE, by interfering with cell wall synthesis. Its mechanism of action involves inhibiting the dephosphorylation of lipid carriers involved in peptidoglycan synthesis, thereby disrupting cell wall formation and leading to bacterial death. These compounds demonstrate lower toxicity compared to traditional antimicrobials. For instance, Bacitracin exhibits low toxicity levels, with LD50 values greater than 3750 mg/kg body weight when orally administered to mice [71]. This safety profile underscores the potential of *Bacillus*-based biocontrol agents in providing effective and safer alternatives for managing healthcare-associated infections.

5.5 Food Industry

Bacillus spp. produce a variety of SMs with significant applications in the food industry due to their antimicrobial properties. These natural compounds inhibit foodborne pathogens and spoilage bacteria, making them valuable as natural preservatives. *Bacillus amyloliquefaciens* ALB65 has shown effectiveness in inhibiting *Listeria monocytogenes* on cantaloupe melons, highlighting its potential in biocontrol and food safety applications [72].

Reuterin and nisin are widely recognized for their potent antibacterial properties and are extensively used as natural preservatives in various food products. In dairy products such as cheese, nisin prevents the growth of spoilage organisms and pathogenic bacteria during fermentation and aging processes, ensuring the safety and quality of the final product. It is particularly effective in processed cheese, where it inhibits the growth of Clostridium tyrobutyricum, a bacterium that can cause late blowing defect and lead to spoilage [73].

These SMs not only enhance the safety and quality of food products but also contribute to sustainable food production practices by reducing the reliance on synthetic preservatives and antibiotics

6. Unveiling the Secrets

Despite significant advancements, numerous mysteries surrounding *Bacillus* SMs remain. Understanding the intricate regulatory networks governing their production, exploring the full spectrum of undiscovered compounds, and elucidating their precise ecological roles are crucial areas for future research.

7. Conclusion

In summary, the SMs produced by soil-dwelling *Bacillus* spp. embody the ingenuity and effectiveness of microbial life. By delving into their intricacies, we uncover a wealth of potential solutions to address agricultural, medical, and environmental challenges. As we embark on a journey to explore their hidden arsenal, we imagine a future in which these tiny warriors of the soil become formidable allies in shaping a healthier, more sustainable world.

These *Bacillus* SMs, with their remarkable adaptability and versatility, enable *Bacillus* spp. to thrive in diverse environments and perform important ecological functions. From nutrient uptake to interaction with hosts to defense against pathogens, these bioactive compounds play a critical role in ecosystem dynamics. Furthermore, *Bacillus* spp. SMs offer promising opportunities to improve agriculture by serving as effective biocontrol agents against pests and pathogens while promoting sustainable agricultural practices. In the pharmaceutical sector, they hold potential for the development of novel antibiotics, biofertilizers, and nutraceuticals, with applications spanning cosmetics, detergents, healthcare products, and food preservation.

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Despite advances in understanding *Bacillus* SMs, many aspects of their biology remain enigmatic. Future research efforts should primarily focus on elucidating the regulatory mechanisms of their production, elucidating their ecological functions, and discovering new compounds with therapeutic and industrial applications. By continuing to unlock the mysteries of *Bacillus* metabolites, we can harness their full potential to advance human health, agriculture, and environmental sustainability.

8. Conflict of Interest

The authors of this review article declare that there is no conflict of interest associated with the publication

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