

Microbial Resistance as a Challenge: Therapeutic and Biotechnological Applications of Metabolites Produced by Bacteria of the *Bacillus* Genus. A descriptive review

La resistencia microbiana como desafío: aplicaciones terapéuticas y biotecnológicas de metabolitos producidos por bacterias del género *Bacillus*. Una revisión descriptiva

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RESUMEN

Los procariotas fueron las primeras formas de vida que existieron en la Tierra hace 3500 millones de años y se cree que son los últimos organismos que quedaron tras la extinción de los organismos multicelulares. Las bacterias han establecido una estrecha relación con los seres humanos, que albergan el microbioma, donde estas interacciones pueden adoptar varias formas. Algunas son mutualistas, protegiendo al organismo de especies nocivas, produciendo metabolitos beneficiosos o estimulando el sistema inmunitario, mientras que otras pueden causar daño. Los antibióticos son los metabolitos microbianos más estudiados y utilizados por los seres humanos. Dado que la resistencia a los antibióticos se ha convertido en una preocupación mundial, la búsqueda de microorganismos capaces de producir nuevos antibióticos con potencial clínico ha cobrado cada vez más importancia. Se ha informado de que las bacterias pueden biosintetizar dos tipos de sustancias antimicrobianas: las bacteriocinas, que son de síntesis ribosómica, y los lipopéptidos, que son de síntesis no ribosómica. Los miembros del género *Bacillus* pueden producir lipopéptidos y otras sustancias antimicrobianas, como sideróforos, enzimas líticas, toxinas e inductores de resistencia sistémica en las plantas, lo que hace que este género sea de especial interés en la búsqueda de estos metabolitos. Este trabajo describe algunas especies del género *Bacillus* que producen metabolitos antimicrobianos relevantes para la salud y la biotecnología.

Palabras clave: *Bacillus*, antimicrobiano, resistencia, antibiótico, metabolito.

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ABSTRACT

Prokaryotes were the first living forms to exist on Earth 3.5 billion years ago and are believed to be the last organisms to remain after the extinction of multicellular organisms. Bacteria have established a close relationship with humans, who host microbiomes, where these interactions can take several forms. Some are mutualistic, protecting the body from harmful species, producing beneficial metabolites, or stimulating the immune system, while others can cause harm. Antibiotics are the most studied and widely used microbial metabolites by humans. As antibiotic resistance has become a global concern, the search for microorganisms capable of producing novel antibiotics with clinical potential has become increasingly important. It has been reported that bacteria can biosynthesize two types of antimicrobial substances: bacteriocins, which are of ribosomal synthesis, and lipopeptides, which are of non-ribosomal synthesis. Members of the genus *Bacillus* can produce lipopeptides and other antimicrobial substances such as siderophores, lytic enzymes, toxins, and inducers of systemic plant resistance, making this genus of particular interest in the search for these metabolites. This work describes some species of the genus *Bacillus* that produce antimicrobial metabolites relevant to health and biotechnology.

Keywords: *Bacillus*, antimicrobial, resistance, antibiotic, metabolite.

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INTRODUCTION

The *Bacillus* genus has been widely reported in the literature for its importance as a producer of various metabolites with antimicrobial activity against pathogenic bacteria, as well as its ability to synthesize compounds of biotechnological significance. Given the relevance of this genus, the objective of this review was to analyze the available information on *Bacillus* genus that synthesize compounds used in health and biotechnology.

In this work, a documentary review of scientific articles was conducted using PubMed, Google Scholar, Scopus, and Scielo, which are specialized search engines. The search query included keywords such as *Bacillus*, antimicrobial, resistance, antibiotic, and *Bacillus* metabolites. The articles reviewed mainly include those published within the past 10 years, but some highly cited older articles were also included.

Antibiotic resistance: definition, brief history and global impact

Antibiotic resistance is the ability of bacteria to survive and multiply despite exposure to antimicrobial agents designed to inhibit or kill them. Although it is a natural, evolutionary phenomenon, its prevalence and clinical significance have increased rapidly in recent decades due to the combined effects of inappropriate prescribing, incomplete treatment courses, widespread use in livestock farming and environmental pollution. Natural reservoirs in soil, water, and wildlife serve as a source of resistance genes that can be transferred to human pathogens through horizontal gene transfer. In ancient permafrost samples, β -lactamase genes have been found to predate human antibiotic use and illustrate the deep

evolutionary roots of resistance (Kochetkov, 2024; Larson & Flach, 2022).

The modern era of antibiotics began in the early 20th century with Paul Ehrlich's synthesis of Salvarsan for the treatment of syphilis, followed by Alexander Fleming's discovery of penicillin in 1928 and its mass production in the 1940s. This period, often called the golden age of antibiotics, saw the introduction of most major classes still in use today. These discoveries revolutionized healthcare, enabling complex surgical procedures and drastically reducing mortality from bacterial infections (Hutchings *et al.*, 2019; Laborda *et al.*, 2024).

However, resistance emerged soon after the introduction of each new antibiotic. Penicillin-resistant *Staphylococcus aureus* was reported within two years of clinical use; methicillin-resistant strains appeared in 1960, tetracycline resistance in *Shigella* in the late 1950s and vancomycin-resistant enterococci in the late 1980s. This recurring pattern demonstrates the remarkable adaptability of bacterial populations (Uddin *et al.*, 2021).

Since the 1980s, the discovery and approval of entirely new classes of antibiotics with a broad spectrum of activity have become rare. Most recent approvals have been for derivatives of existing agents, with combinations of β -lactam and β -lactamase inhibitors comprising a large proportion of candidates. The lack of new agents, particularly those effective against multidrug-resistant Gram-negative bacteria, is due in part to the scientific challenges of identifying new drug targets and economic disincentives that have led many pharmaceutical companies to withdraw from antibiotic research (Iskandar *et al.*, 2022).

The global impact of resistance on health is significant. In 2019, resistant bacterial infections were responsible for approximately 1.27 million deaths and were associated with nearly 4.95 million deaths worldwide, surpassing mortality from HIV/AIDS and malaria. If current trends continue, annual deaths could reach 10 million by 2050. The burden is particularly high in low- and middle-income countries, where limited access to diagnostics, weaker surveillance systems, and higher levels of inappropriate antibiotic use contribute to rapid spread. High-risk pathogens include the ESKAPE group: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, which cause multidrug-resistant hospital-acquired infections (Antimicrobial Resistance Collaborators, 2022; Church & McKillip, 2021).

The economic consequences are equally concerning. Drug-resistant infections are linked to longer hospital stays, more expensive treatments, higher mortality risk, and increased readmission rates. Poudel *et al.* (2023), reported that the additional cost per episode of resistant infection can be several times higher than for susceptible infections, with an average increase in hospitalization of 6–12 days. At the macroeconomic level, projections suggest that Antimicrobial Resistance (AMR) could reduce global Gross Domestic Product (GDP) by up to 3.8% by 2050, with low-income regions bearing the greatest economic burden.

Resistance is not confined to hospitals. Community-acquired resistant infections, driven by factors such as over-the-counter antibiotic sales and self-medication, are a growing concern. Environmental contamination from sewage, agricultural runoff, and pharmaceutical production can maintain selection pressure in natural ecosystems. In some cases, antibiotic concentrations in industrial effluents have been high enough to actively select for resistance (Larsson & Flach, 2022).

Addressing this crisis requires a coordinated One Health approach that integrates human, animal, and environmental health. This involves improving antibiotic stewardship, strengthening infection prevention, expanding surveillance, reducing environmental contamination, and investing in innovative therapeutic development. Among potential alternatives, bioactive metabolites from *Bacillus* species have attracted attention due to their structural diversity, unique mechanisms of action, and ability to bypass existing resistance pathways (Halawa *et al.*, 2024). Table 1 lists antimicrobial compounds produced by certain *Bacillus* species.

Characteristics of the genus *Bacillus*

Taxonomic Diversity

The genus *Bacillus* is a highly diverse group of rod-shaped, endospore-forming bacteria in the phylum Firmicutes (Turenne *et al.*, 2015). Historically, *Bacillus* served as a “catch-all” genus for aerobic spore-formers, but modern phylogenetic studies have redefined its scope. According

Table 1. Antibiotics produced by distinct species of the genus *Bacillus* are used in Medicine and Human Health.

Antibiotic produced	Used against	Species of the genus <i>Bacillus</i>	References
Bacitracin	<i>Staphylococcus aureus</i> , <i>Micrococcus luteus</i> , <i>Pantoea ananatis</i> , <i>Bacillus cereus</i> .	<i>Bacillus licheniformis</i> <i>Bacillus subtilis</i>	Rukimi <i>et al.</i> , (2015)
Gramicidin	Gram-positive bacteria and certain Gram-negative bacteria	<i>AneuriniBacillus migulanus</i> (previously known as <i>Bacillus migulanus</i>)	Berditsch <i>et al.</i> , (2007)
Tyrocidine	<i>Salmonella typhi</i> , <i>Staphylococcus aureus</i> , Gram-positive bacteria	<i>Bacillus aneurinolyticus</i>	Zhu <i>et al.</i> , (2021) Vosloo <i>et al.</i> , (2013)
Subtilin	<i>Listeria monocytogene</i> , Gram positive- and Gram-negative bacteria	<i>Bacillus.licheniformis</i> <i>Bacillus subtilis</i>	Halami (2019)
Polymyxin	Gram-negative bacteria	<i>PaeniBacillus polymyxa</i>	Stoica <i>et al.</i> , (2019)
Edeíne	<i>Conidia sp.</i> , Gram positive- and Gram-negative bacteria	<i>BreviBacillus brevis</i>	Yang <i>et al.</i> , (2023)
Butirosin	Gram-negative bacteria, molds, and yeast	<i>Bacillus circulans</i>	Kudo <i>et al.</i> , (2005)

to the most recent classifications, *Bacillus* comprises approximately 435 recognized species and 12 subspecies, although this number continues to grow as new species are described (Xu, 2025). Phylogenetically, the genus is divided into two main clades with no close evolutionary relationship to each other. The first is the “*Subtilis* clade,” often termed *Bacillus sensu stricto*, which includes the type species *Bacillus subtilis* and closely related species such as *B. licheniformis*, *B. pumilus*, and *B. amyloliquefaciens*. The second is the “*Cereus* clade,” also known as *Bacillus cereus sensu lato*, which encompasses *B. cereus* and several pathogenic or environmentally significant species, including *B. anthracis* (the anthrax *Bacillus*), *B. thuringiensis* (an entomopathogen), *B. mycoides*, *B. cytotoxicus*, and others. Notably, these two clades are sufficiently distinct that the genus *Bacillus* is polyphyletic unless additional related genera are included (Blanco Crivelli et al., 2024). Over the past few decades, many species formerly classified as *Bacillus* have been reassigned to separate genera as molecular data revealing deep divisions. For example, *PaeniBacillus* (Latin *paene*, “almost” a *Bacillus*) was established to include certain nitrogen-fixing and plant-associated bacilli (e.g. *PaeniBacillus polymyxa*) that diverged from the *Bacillus sensu stricto* lineage (Ash et al., 1991; Shida et al., 1997). Similarly, genera such as *BreviBacillus*, *GeoBacillus*, *LysiniBacillus*, and others were separated from the original *Bacillus* group following 16S rRNA analyses that identified at least five distinct clusters within the former genus (Patel & Gupta, 2020). In practice, *Bacillus sensu stricto* now refers to members of the *Subtilis* and *Cereus* clades, whereas organisms in the related genera (*PaeniBacillus*, *BreviBacillus*, etc.) differ in certain phenotypic and chemotaxonomic features despite sharing the ability to form endospores (Carroll, et al., 2022). For example, *PaeniBacillus* species are often Gram-variable (some even appear Gram-negative in older cultures) and typically ferment a broader range of sugars, distinguishing them from the most strictly aerobic profile of true *Bacillus* (Shida et al., 1997; Grady et al., 2016). Ongoing revisions of *Bacillus* taxonomy highlight the genus heterogeneity: recent phylogenomic proposals have introduced many new genera (e.g. *AlkalihaloBacillus*, *CytoBacillus*, *MesoBacillus*, *NeoBacillus*, *PeriBacillus*, *MetaBacillus*, *Evansella*, *Gottfriedia*, etc.) to separate genetically distant clades (Li, et al., 2024). Nonetheless, in practice, the name *Bacillus* still conventionally includes the two major clades mentioned, which together comprise many important species in medicine, industry, and agriculture (Harichi, 2022).

Common Microbiological Characteristics

The genus *Bacillus* includes a wide variety of Gram-positive, rod-shaped bacteria (a characteristic that gives them their name) that can be either strictly aerobic or facultative anaerobic. Their spores are refractive, meaning they reflect light, and can withstand high tempera-

tures due to their protective covering (Logan & Vos, 2015). They are generally motile by means of peritrichous flagella, enabling active movement in liquids and across surfaces; an exception is *B. anthracis* (and a few strains of *B. cereus*), which lack motility and instead form encapsulated, non-flagellated cells (Wales et al., 2023).

The optimal growth temperature for mesophilic *Bacillus* species is often in the 30–37°C range, though growth can occur from about 10°C up to approximately 45°C for many species. There are also extremophilic members: certain lineages (often reclassified into other genera) include thermophiles (e.g. *GeoBacillus* species) that grow at 55–75°C, as well as psychrotolerant strains isolated from permafrost or refrigerated foods that are capable of growth at approximately 4°C (Hassan, 2022).

Most *Bacillus* species prefer neutral pH, but some are remarkably alkalitolerant or acidtolerant. For instance, *B. alcalophilus* (now *AlkalihaloBacillus*) grows at around pH 9–10, while *B. coagulans* can produce lactic acid and tolerate pH below 5. Salt tolerance also varies: *Bacillus* species are generally not extreme halophiles but can cope with moderate salinity, with several species growing in up to 7–10% NaCl (Ibarra-Villarreal et al., 2021).

During sporulation, the cell divides asymmetrically into two parts: a small forespore and a large mother cell. The mother cell wraps around the forespore in a process called engulfment, enclosing it in a double membrane. The forespore then matures into a resistant, dormant spore, which is eventually released into the environment (Checinska et al., 2015). Sporulation is a multi-stage process: in stage 1, the genes involved are induced. At this point, endospore development can be interrupted if nutrients are supplied; however, once stage 2 begins, the process becomes irreversible and continues until the spore is fully formed (Fan et al., 2024; Tan & Ramamurthi, 2013).

Bacillus owes much of its ecological success to a rich array of biochemical capabilities shared among many of its species (Stülke et al., 2023). A prominent feature is the secretion of numerous hydrolytic enzymes into the environment. Soil-dwelling *Bacillus* use exoenzymes to degrade complex organic polymers; for example, *B. subtilis* and its relatives secrete proteases, amylases, cellulases, chitinases, lipases, and other enzymes that break down proteins, starch, plant cell walls, insect cuticle, and oils (Akinsemolu, 2024). This enzymatic versatility enables them to utilize diverse nutrients and positions certain *Bacillus* species as key industrial enzyme producers. Many *Bacillus* species are also known for producing secondary metabolites with bioactive properties. Genomic analyses show that *B. subtilis* devotes approximately 4–5% of its genome to genes involved in secondary me-

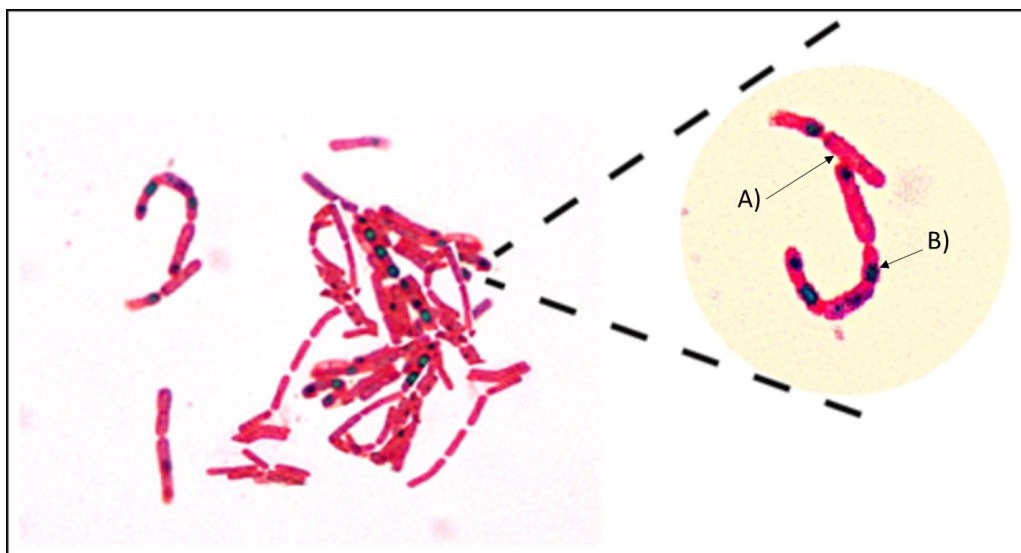


Figure 1. Illustrates the preparation of a *Bacillus subtilis* culture undergoing sporulation. The sample was stained using malachite green, whereby vegetative cells were stained red, and spores exhibited a green coloration. Malachite green staining of a sporulating *Bacillus subtilis* culture. A) Vegetative cell; B) Spore. Image by authors.

tabolite biosynthesis, reflecting dozens of different natural products (Blanco Crivelli *et al.*, 2024). Pigmentation in *Bacillus* is generally less conspicuous than in some other genera, but some species do form colony pigments. For example, *Bacillus subtilis* can produce yellow-brown carotenoids under certain conditions (Stannius *et al.*, 2025), *B. atrophaeus* (formerly *B. globigii*) forms a brown-black pigment (Kocak *et al.*, 2025), and *B. circulans* often shows orange colonies (Kao *et al.*, 2022). These pigments may play roles in protection against oxidative stress. More universally, *Bacillus* cells have robust stress responses: even in the vegetative (non-sporulated) state, they encode multiple DNA repair systems and antioxidant enzymes (catalases, superoxide dismutases) that confer tolerance to UV radiation and reactive oxygen species (Shrestha, 2025). Many *Bacillus* species also produce osmoprotectant compounds or adjust their membrane composition to withstand osmotic and temperature stresses (Valencia-Marin *et al.*, 2024).

Mobile genetic elements in *Bacillus* genomes – plasmids, transposons, insertion sequences, and bacteriophages – contribute significantly to strain-to-strain variation. Horizontal gene transfer is frequent among soil bacilli, which exchange plasmids and even chromosomal segments, rapidly spreading traits such as antibiotic production or resistance across species boundaries (Hinnekens *et al.*, 2008).

At the broader genus level, comparative genomics reveal an open pan-genome for *Bacillus*, especially when considering the full diversity of the genus. For example, in the *B. cereus* group (*sensu lato*), a recent pan-genomic

analysis of 114 genomes identified approximately 60,000 distinct genes in the pan-genome, of which only about 600 were core genes present in at least 99% of strains (Bazin, 2017).

Biotechnological and Ecological Importance

Bacillus species play diverse roles in biotechnology and ecology, making them among the most utilized and studied bacterial groups outside of medical pathogens. Ecologically, *Bacillus* functions as both beneficial symbionts and aggressive antagonists in various environments. Many *Bacillus* strains are prominent plant growth-promoting rhizobacteria (PGPR). In soil and root environments, they benefit plants through several mechanisms (Miljaković *et al.*, 2020). *Bacillus* can enhance nutrient availability; for example, certain species fix atmospheric nitrogen symbiotically or solubilize insoluble phosphates, making these nutrients accessible to plants (Miljaković *et al.*, 2020). They also produce phytohormones such as indole-3-acetic acid (auxin) and cytokinins, which stimulate root growth. Additionally, *Bacillus*-colonized roots often exhibit induced systemic resistance (ISR), where the plant's innate defenses are primed by bacillar elicitors (siderophores, flagellin, lipopeptides, etc.), leading to enhanced resistance against pathogens (Linette Naveena, 2024). Because of these traits, formulations of *Bacillus* spp. are used as bio-fertilizers and biostimulants in sustainable agriculture. For instance, *B. velezensis* (formerly *B. amyloliquefaciens* FZB42) is a commercial inoculant known to improve crop vigor and suppress soil-borne diseases through its antimicrobial metabolites (Yang *et al.*, 2023).

Some notable members of the genus *Bacillus*

The genus *Bacillus* is one of the most widely distributed groups of bacteria in the biosphere. Gram-positive bacteria, including *Bacillus* species, have been isolated from nearly every environment studied by microbiologists (Zeigler & Perkins, 2021).

In medicine, species of the genus *Bacillus* gained prominence when Pasteur and Koch identified *B. anthracis* as the microorganism that causes anthrax at the end of the 19th century (Zeigler & Perkins, 2021). One of the *Bacillus* species with the greatest use and interest in fields such as industry, basic science, and medicine is *Bacillus subtilis*.

B. subtilis is a model organism frequently studied to gain insights into key metabolic processes, including cell division, sporulation, protein secretion, biofilm formation, and the synthesis of various secondary metabolites (Kovács, 2019).

B. subtilis was the first Gram-positive bacterium whose genome was sequenced. Its genome comprises 4,214,810 base pairs, including 4,100 protein-encoding genes (Kunst *et al.*, 1997); however, only 253 of these genes are required in vitro culture. The antimicrobial and antifungal properties are due to the bioactivity of secondary metabolites such as surfactin and plipastin (Kovács, 2019; Kunst *et al.*, 1997).

Another bacterium of the *Bacillus* genus important for the biological control of insect pests and vectors is *Bacillus thuringiensis*. This bacterium was first identified in 1901, when a microorganism infecting silkworms was reported

and initially named *Bacillus sotto*. Later, in 1915, it was isolated from diseased larvae of the moth *Ephesia kuehniella*, in the city of Thuringia, Germany, after which it was named *B. thuringiensis* (Sansinenea, 2012).

Antimicrobial peptides of the genus *Bacillus*.

A study published in The Lancet in 2022 analyzed 33 pathogenic bacteria associated with 11 types of infections across 204 countries for the year 2019. The results showed that these pathogens were responsible for 7.7 million deaths, representing 13.6% of all deaths worldwide, making them the second leading cause of mortality globally (Ikuta *et al.*, 2022). Many infectious pathogens, especially Gram-negative ones, have developed resistance to most conventional antibiotics; in some rare cases, they have even developed resistance to all available antibiotics (Davies & Davies, 2010; De Kraker *et al.*, 2016; World Health Organization: WHO, 2017). Among the options to address "superbugs" are antimicrobial peptides (AMPs), which are natural antibiotics encoded in the genome of some species of the genus *Bacillus* (Sumi *et al.*, 2015; Puan *et al.*, 2023). The synthesis of antimicrobial peptides by members of the genus *Bacillus* can be classified into two types: those synthesized by ribosomes and those produced independently of ribosomal activity (Stoica *et al.*, 2019; Sansinenea, 2012; Puan *et al.*, 2023).

B. subtilis produces three ribosomal antibiotics (TasA, subtilisin, and sublancin) and four non-ribosomal antibiotics (bacitracin, bacilisin, plipastatin, and surfactin) (Ikuta *et al.*, 2022; Stoica *et al.*, 2019; Sumi *et al.*, 2015).

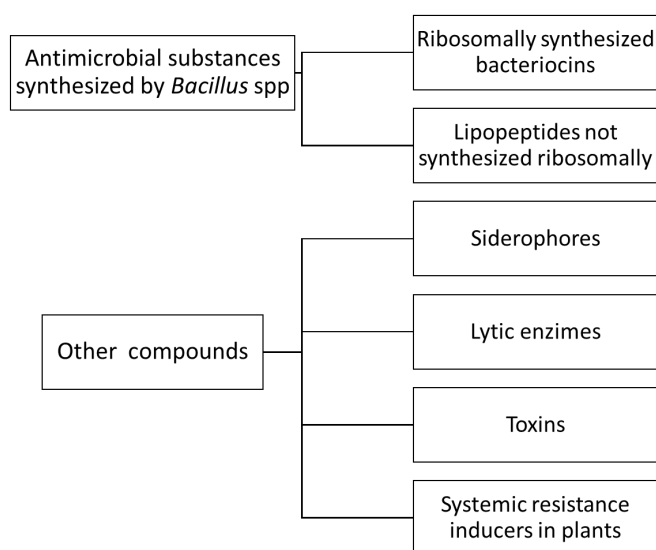


Figure 2. Metabolites are produced by the genus *Bacillus*. Image by authors

Members of the genus *Bacillus* also synthesize other compounds such as siderophores, lytic enzymes, toxins, and inducers of systemic plant resistance (Ikuta et al., 2022), Figure 2.

Antimicrobial compounds produced by ribosomal synthesis: Bacteriocins from *Bacillus* spp.

Bacteriocins are peptide toxins synthesized by ribosomes that inhibit bacterial growth. In Gram-positive bacteria such as *Bacillus* spp., they are encoded to release the toxin using specific bacteriocin transport systems, such as ABC transporters. They also have specific regulation of toxin production, preventing their metabolism from being affected by the excretion of this substance (Riley & Wertz, 2002).

Bacteriocins can be divided into two groups: those produced by ribosomal synthesis and bacteriocin-type inhibitory substances (SITB), which refer to antimicrobial compounds whose peptide nature has not been confirmed or that are produced by non-ribosomal synthesis (Abriouel et al., 2011). Although the latter have not been characterized, they can be quantified and replicated, which would allow the use of new substances produced by *Bacillus*.

One potential application of bacteriocins produced by *B. thuringiensis* is their use as additives in food preservation, due to their ability to inhibit the growth of both Gram-positive and Gram-negative bacteria (Arenas & Melo, 2018).

Bacteriocins are polypeptide antibiotics, some of which are characterized by an amino acid chain that is sometimes circular, as in the case of polymyxin B, which is produced by *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*). The structure of polymyxin is shown in Figure 3.

Due to the toxicity of polymyxins, they are applied topically (Zeigler & Perkins, 2021). Some advantages of topically administered antibiotics are that they can be used on exposed wounds, maintain moisture at the injury site, and, by being administered directly at the site of infection, deliver a large amount of the active component directly to the pathogens, making the probability of generating drug resistance very low (Kovács, 2019).

The mechanism of action, depending on the drug concentration, is either bacteriostatic or bactericidal. It inhibits the incorporation of amino acids and nucleotides into the cell wall, blocks bacterial cell wall synthesis, inhibits the regeneration of phospholipid receptors involved in peptidoglycan synthesis, and can also damage already formed membranes, causing lysis and bacterial death (Sidek et al., 2018).

Antimicrobial compounds produced by non-ribosomal synthesis: Lipopeptides

Lipopeptides are cyclic or linear peptides with a lipophilic hydrocarbon tail at the N-terminus. *Bacillus subtilis* produces lipopeptides that exhibit antibiotic properties due to their toxic effects on organisms (Ajuna, 2024). Based on their length, these lipopeptides are classified as polypeptides. According to their structural characteristics, they can be further divided into categories, including polymyxins, polypeptides, and octapeptides.

Polymyxins exhibit strong antimicrobial activity against Gram-negative bacteria. They exert their bactericidal effect by binding to the lipid A component of lipopolysaccharide (LPS), leading to disruption of the bacterial outer membrane (Ayoub, 2020).

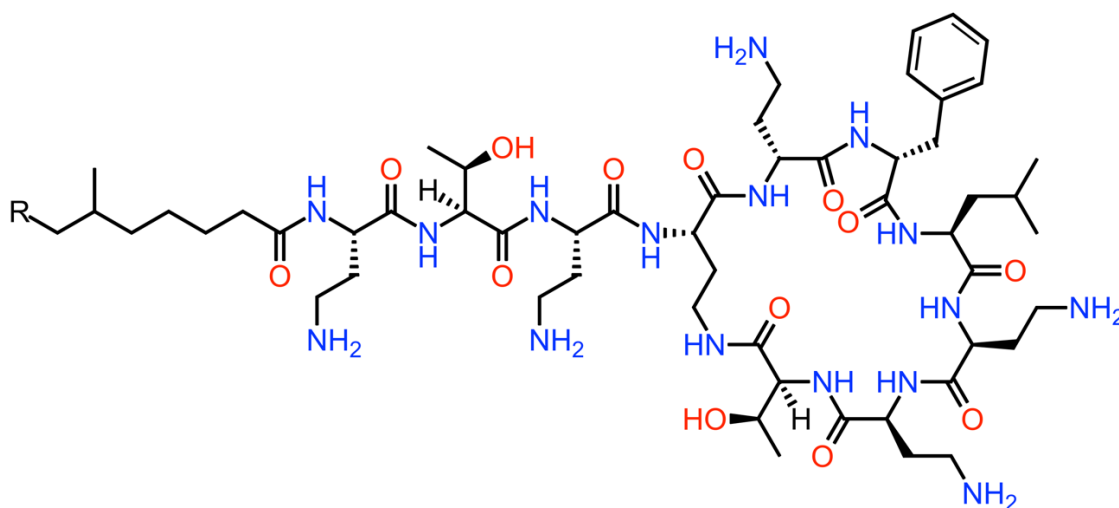


Figure 3. Polymyxin B structure. Image by authors.

Polypeptides demonstrate broad-spectrum antimicrobial activity, effective against Gram-negative, Gram-positive, and anaerobic bacteria (Bleam, 2012).

Octapeptides are cationic cyclic peptides produced by *Bacillus* spp. They also display a broad spectrum of activity against both Gram-negative and Gram-positive bacteria (Velkov *et al.*, 2017).

Some important lipopeptides

Bacitracin is the most important cyclic polypeptide antibiotic produced by *B. subtilis* and is active against Gram-positive bacteria. It is used as an anti-infective agent in various pharmaceutical preparations, including sprays, topical lotions, ointments, and skin creams. The bactericidal activity of bacitracin results from its ability to inhibit cell wall synthesis (Setlow, 2014).

Bacitracin was discovered in 1943 and is produced by the sporulated bacterium *B. subtilis*. This microorganism was isolated from an exposed tibial fracture suffered by a girl named Margaret Tracey. The name of bacitracin comes from combining "Baci," referring to the bacterium, and Tracey's last name. *B. subtilis* was found to produce an antibiotic substance that acted as a bacteriostat against various microorganisms. Experimental tests on mice inoculated with minimally lethal doses of bacterial agents (hemolytic *Streptococcus*) demonstrated that bacitracin inhibited the growth of these pathogens (Tan & Ramamurthi, 2013). The structure of bacitracin is shown in Figure 4.

Considering these findings and its low toxicity in animals, including humans, the development of bacitracin for production was initiated. Although the process faced several setbacks, large-scale manufacturing was eventually achieved. However, due to its nephrotoxic properties, this drug should not be applied to deep wounds, as there is a risk of systemic spread through the circulatory system (Saha *et al.*, 2015).

Bacillus siderophores

Siderophores are low molecular weight molecules (400–1000 kDa) secreted by microorganisms under iron-deficient conditions. They can sequester Fe^{3+} ions from their environment, forming ferri-siderophore metal complexes. This process is driven from the cytoplasm by the TonB transport protein system, which transduces the necessary energy from the cytosol to the outer membrane (Saha *et al.*, 2015).

Microorganisms can utilize siderophores depending on their membrane receptors, either by a) using the Fe^{3+} siderophore complex at the membrane or b) reducing Fe^{3+} complexes extracellularly. Based on this function, siderophores are classified into three main categories: hydroxamates, catecholates, and carboxylates (Mohamed *et al.*, 2018).

Bacillus lytic enzymes

It has been reported that species of the genus *Bacillus* are prolific producers of enzymes, and, due to their broad environmental distribution and ease of cultivation, they represent a potential source for the isolation of vari-

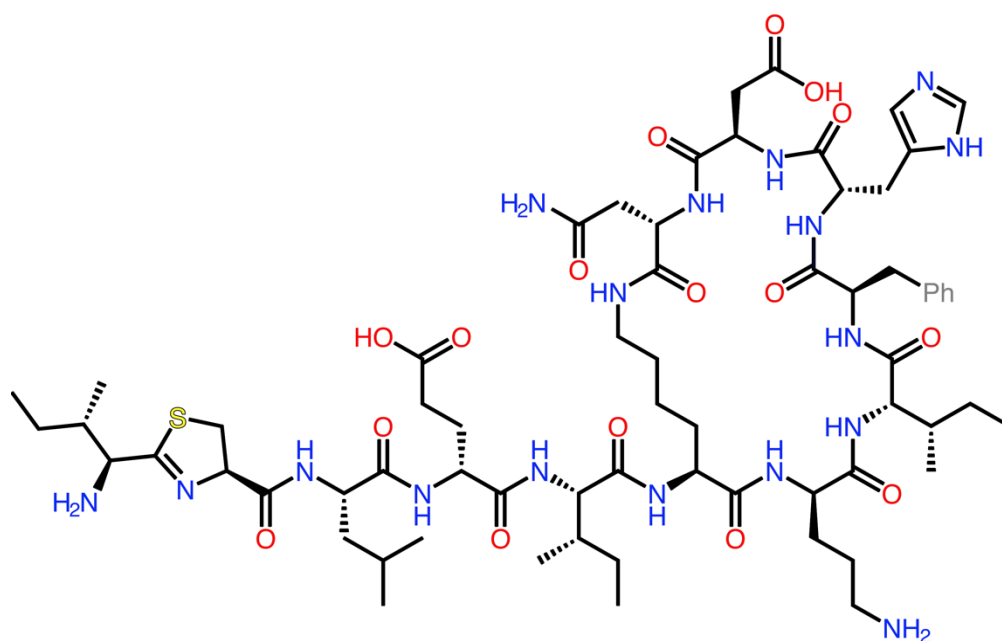


Figure 4. Structure of Bacitracin A. Image by authors.

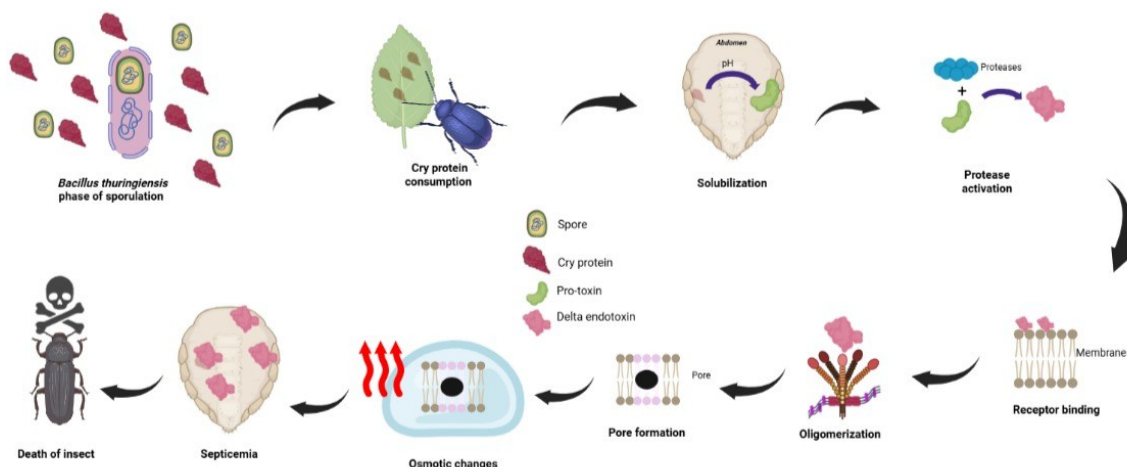


Figure 5. Process of toxins from *B. thuringiensis* against insects. Image by authors.

ous compounds of interest. Within the broad spectrum of enzymes, a group of special interest in the agronomic area is the lytic enzymes, which constitutes one of the most diverse groups of proteins in terms of possessing antimicrobial properties (Danilova & Sharipova, 2020).

Lytic enzymes such as chitinases, β -1,3-glucanases, peroxidases, proteases, and lipases are natural antimicrobials that depolymerize the peptidoglycan of the cell wall of target bacterial cells and cause rapid cell lysis, in fungi they deteriorate their cell wall affecting the glycosidic bonds of its glycoproteins and polysaccharides, thus causing cell lysis and death, at the same time this action allows the release of chitin and glucan oligomers, which function as elicitors of plant defenses (Jha *et al.*, 2014)

Toxins

Bacillus cereus is an important microorganism in the food industry, as it is considered one of the main causative agents of food poisoning. It produces two types of toxins: enterotoxins and emetics (Jovanovic *et al.*, 2021).

B. thuringiensis is a bacterium commonly used as a biological control agent due to its ability to produce toxic protein crystals during the stationary growth phase and sporulation. These crystals, known as Cry proteins, are highly specific and effective against insect pests and vectors. Although primarily insecticidal, some Cry proteins have also been reported to exhibit activity against other organisms, including nematodes, gastropods, and even cancer cells, as illustrated in Figure 5. In the insect digestive tract, these proteins must first be solubilized; the resulting protoxins are then activated by proteases, producing the active form of the toxin (delta endotoxin),

which binds to specific receptors on the intestinal membrane. This interaction leads to pore formation, disrupts the osmotic balance, and ultimately causes cell lysis. *B. thuringiensis* also produces other toxins, such as cytolytic proteins (Cyt), which are synthesized during the stationary phase, and vegetative insecticidal proteins (Vip), which, unlike the former, are associated with the vegetative phase of the cell and are secreted in soluble form; therefore, they do not require proteolytic activation (Gupta & Kumar, 2021).

Plant resistance inducers

The induction of plant growth and resistance to pathogens by *Bacillus* species can occur directly or indirectly. The direct mechanism involves bacteria capable of biological nitrogen fixation, solubilization of essential minerals for plant development, production of regulatory hormones, and activation of plant defense mechanisms. These functions are performed by bacterial species found in the plant's rhizosphere. The indirect mechanism involves the production of endotoxins that act as pathogen antagonists, such as the Cry proteins produced by *B. thuringiensis* (Tejera-Hernández *et al.*, 2011).

Bacillus spp. is considered a plant growth-promoting bacteria due to its production of plant hormones such as gibberellic acid and indole-3-acetic acid, which are closely related to nutrient availability for plants. *B. subtilis* is known for inducing the synthesis of jasmonic acid, ethylene, and non-expressor of pathogenesis-related genes 1 (NRP1), which promotes defense and restricts cell death by forming condensates that target specific substrates to degrade them (Hashem & Tabassum, 2019; Zavaliev *et al.*, 2020).

CONCLUSION

Currently, the search for new drugs that effectively address antimicrobial resistance is of the utmost importance.

Members of the genus *Bacillus* are an important group of microorganisms that produce a wide range of bioactive metabolites, including several with antibiotic properties, such as bacitracin and polymyxin B. Other metabolites provide a competitive advantage to the microorganisms that synthesize them, as seen with siderophores, lytic enzymes, toxins, and resistance inducers in plants. Due to the broad diversity of metabolites produced by species of this genus and their widespread distribution in nature, they represent a promising alternative in the search for new antibiotics.

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Conflicts of Interest

The authors declare no conflict of interest.

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