Chapter

Bacillus subtilis: A Promising Bacterial Candidate for One Health Applications

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Abstract

Bacillus subtilis is widely recognized as a beneficial and safe microbe for both living species and the environment due to its broad spectrum of bioactive properties. Used for decades as a probiotic, *B. subtilis* produces diverse bioactive metabolites with antimicrobial, antitumor, antioxidant, and immunomodulatory activities. It forms resilient biofilms, sporulates under stress, promotes plant growth, enhances nutrient uptake, controls pathogens, and contributes to the bioremediation of environmental pollutants such as heavy metals and hydrocarbons. Its long-standing use in food fermentation supports its safety profile and recognition as a safe organism in various applications. Consequently, *B. subtilis* stands out as a promising candidate for achieving optimal "One Health" outcomes for humans, animals, plants, and their interconnected ecosystems. This chapter provides a biochemical classification of the metabolites and derivatives produced by B. *subtilis*, highlighting their properties and functions that offer health benefits to both living organisms and the environment.

Keywords: metabolites, probiotics, peptides, enzymes, antimicrobe, nutraceuticals, safety

1. Introduction

Faced with recent global disease outbreaks such as COVID-19, there has been a growing adoption of a One Health approach that considers how environmental changes influence the risks of infectious and chronic diseases for humans, animals, and plants. Therefore, designing adaptive, forward-looking, and multidisciplinary solutions to these challenges is necessary, with more preventive actions by addressing the root causes and drivers of infectious diseases, particularly at the animal-human-ecosystem interface. One Health approaches, such as reducing synthetic agricultural inputs and increasing biodiversity, present a global strategic framework to reduce the risks of new emerging infectious diseases at the animal-human-ecosystem interface. By focusing on preventive solutions, One Health contributes to better living species health and a more sustainable environment in the future [1].

The exploration of beneficial microbes appears to be an interesting route in One Health initiatives, bridging the realms of human, animal, plant, and environmental health throughout the world [2]. *B. subtilis*, a Gram-positive and spore-forming bacterium, is recognized among these beneficial microbes to the living species and their environment for its multiple properties and functions, as well as its broad versatility in various sectors such as medicine, agriculture, and environmental sciences [3]. This bacterial species acts in promoting health in direct and indirect manners by preventing and treating many diseases in humans and animals by producing and converting organic substrates into a wide range of bioactive metabolites, including antimicrobial peptides, enzymes, vitamins, exopolysaccharides, and volatile organic compounds [4]. It is also capable of forming complex and robust biofilms involved, for instance, in the protection of nerve cells and in long and healthy human longevity [5]. *B. subtilis* sporulates and survives in hostile conditions, high temperature, and acidic environment conditions, while being harmless to bacteria, making them effective food and feed ingredients [6, 7]. It also promotes plant growth and controls pathogens and pests responsible for plant diseases and damage [8], helps nutrient absorption from soil [9], and remediates the environment from toxic compounds [10]. One of its emerging and innovative applications is the capacity to synthesize nanoparticles [11], which have numerous applications [12]. Moreover, *B. subtilis* has been widely used as a cell factory for the industrial production of bioactive compounds, owing to a highly efficient protein secretion system and adaptable metabolism [13]. Consequently, *B. subtilis* has many assets to be a suitable bacterial candidate to reach optimal "One Health" for humans, animals, plants, and the related ecosystem.

This chapter overviews (1) the bioactive metabolites of *B. subtilis* and their roles in One Health, (2) the properties and applications of *B. subtilis* that are relevant to One Health, and finally (3) the safety and regulation aspects of its applications.

2. Metabolites of *B. subtilis* and their roles in One Health

B. subtilis has been well-known for the last decades as a producer of a variety of bioactive compounds, either primary metabolites, which are essential for proper bacterial growth, or secondary metabolites produced during the stationary phase of growth. Secondary metabolites are not essential for cell growth but provide competitive or adaptive advantages for both experimental and industrial uses [14]. *Bacillus subtilis* offers several technological advantages, such as a rapid growth rate, an ease of genetic manipulation, and a wealth of accumulated experimental data. It can serve as a tool for producing bioactive compounds for which the applications cover agriculture, medicine, biomaterials, and many industrial sectors such as cosmetics, food, textile, paint, and detergent, and can therefore provide benefits to humans, animals, plants, and the environment. It is not surprising that *B. subtilis* is appearing as a potential candidate for playing a pivotal role in One Health. Bioactive metabolites from *B. subtilis* can be classified according to several criteria, among others, their molecular structures, biosynthetic pathways, or based on their beneficial functionalities to the host. Chemical structure classification has its importance in terms of rational design through the structure-activity relationship investigations for searching for the most efficient biological activities or for increasing the knowledge on bioactive metabolite action mechanisms. According to their biosynthetic pathways, *B. subtilis* secondary metabolites can be categorized into non-ribosomal and ribosomal peptides, polyketides, and hybrid and volatile metabolites, which are further classified into different subclasses, as reviewed in detail in Ref. [15].

In this section, a classification based on the chemical structure, biochemical traits (proteins, carbohydrates, lipids, small organic and inorganic compounds, nanoparticles, and vesicles), and functionalities of the different categories of bioactive compounds is described. Some of the representative biomolecules for each class are illustrated in **Figure 1**.

2.1 Peptides and enzymes

Among the most important bioactive compounds from *B. subtilis* are those composed of amino acids linked by peptide bonds. These include cyclic and linear lipopeptides, peptides, and polypeptides, as well as lytic and quorum quenching enzymes and amino acids as monomers. Cyclic lipopeptides mainly belong to three families, surfactins, iturins, and fengycins, which contain 7–10 amino acid residues cyclized by β-hydroxy or β-amino fatty acid chains with a lactone or amide bond, respectively. These biomolecules exist in homologous series varying in fatty acid chain length (12–18 carbon atoms) and configuration (linear, iso, or anteiso) and in isoforms with various amino acid residues with L and D configurations [16]. For linear lipopeptides, we can find siderophores (e.g., bacillibactin) [17], gageopeptides from a marine *B. subtilis* strain [18], and subtilin, and other peptides such as dihyroisocoumarins, bacilysin, chlorotetain, and rhizocticins, which contain two or more amino acid residues, with or without other compounds in their structure. Polypeptides consist of polymers containing one amino acid residue, such as poly-γ-glutamic acid [19].

Enzymes secreted by *B. subtilis* can be categorized into lytic enzymes such as proteases, chitinases, glucanases, and amylases, and those involved in quorum quenching, that is, those that have the capacity to inactivate the production of signal or quorum sensing molecules (e.g., N-acyl-homoserine lactones) responsible for the growth of deleterious microbial pests. These enzymes are, for instance, lactonase, decarboxylase, acylase, and deaminase [4]. A particular lytic enzyme produced by

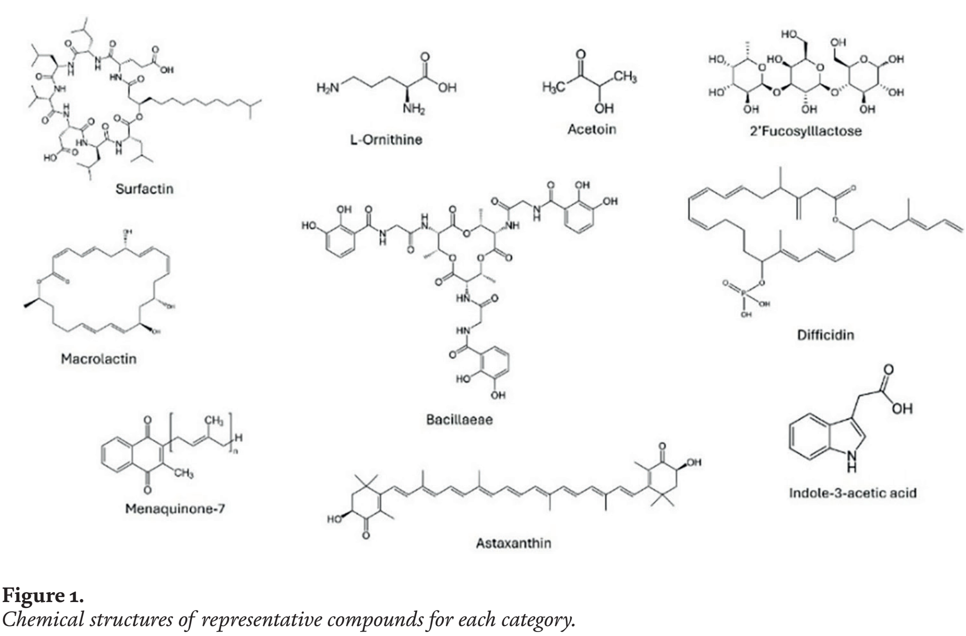

Figure 1.
Chemical structures of representative compounds for each category.

B. subtilis natto is nattokinase, which is a fibrinolytic serine protease found in Korean and Japanese foods [20].

Amino acid monomers such as L-Ornithine [21] and L-tryptophane [22] can be overproduced by *B. subtilis* strains for use in human health.

2.2 Polyketides

Polyketides (PKs) represent bioactive metabolites with diverse structures that contain an alternative methylene and carbonyl group [23]. This class of biomolecules has a multitude of activities encompassing antimicrobial, antitumor, and immunosuppressive effects [4]. Typical PKs produced by *B. subtilis* are difficidins and macrolactins, which are macrocyclic polyenes, and bacillaene, which is largely a linear compound. Difficidins are known for their broad-spectrum antibacterial activities, whereas macrolactins have activities against methicillin-resistant *Staphylococcus aureus* [24], and bacillaene can inhibit various fungi such as *Myxococcus xanthus* and *Trichoderma spp*. [25].

2.3 Carbohydrate-based compounds

B. subtilis produces carbohydrate-based metabolites such as exopolysaccharides (EPS) and oligosaccharides, which are substrate-dependent production. EPS are carbohydrate-based biopolymers with multiple structures and functions, which are excreted either in bound form around the cell surface (capsular EPSs) or in free form into the medium of cell growth (slime EPSs) [26]. *B. subtilis* has been reported to produce EPS (e.g., levan and hyaluronic acid) and prebiotic oligosaccharides such as fructooligosaccharides [27] and 2′-fucosyllactose [28]. This category of *B. subtilis* metabolites can exert anticancer, antioxidant, antibacterial, anti-inflammatory, and immunomodulatory activities [29].

2.4 Terpenes and terpenoid derivatives

B. subtilis is known for its ability to produce isoprene (C5H8 units), the simple form of a terpenoid molecule, and other important terpenoid compounds with particular importance for human and animal health, such as carotenoids, amorphadiene, taxadiene, and menaquinone-7 (MK-7), by means of engineered strains [30]. Terpenoids are mostly lipophilic compounds, considered volatile organic metabolites with a low boiling point and high vapor pressure, and exist in a diverse structural class of linear, cyclic, or polycyclic compounds, which generate multiple biological activities [31].

2.5 Small organic and inorganic compounds (<500 Da)

These bioactive metabolites produced by *B. subtilis* include volatile organic and inorganic compounds having a low molecular mass (< 500 Da). One of the most important categories is represented by organic acids, fatty acids, and derivatives, and other categories of small organic compounds [4]. The distribution of volatile secondary metabolites from *B. subtilis* has been reviewed [32]. The most abundant volatile secondary metabolites produced by *B. subtilis* can be classified according to their functional group as follows: ketones 15%, nitrogen-containing compounds 14%, hydrocarbons 14%, aromatic compounds 14%, and alcohols 11%.

Volatile inorganic compounds produced by *B. subtilis* are mainly gaseous by-products of primary metabolism, such as carbonated (CO_2, CO), hydrogenated (H_2), sulfur (H_2S), or nitrogen (N_2, NH_3, NO)-containing compounds [4]. Particular attention is addressed to nitric oxide (NO) for its role in human longevity [33].

2.6 Nanoparticles

B. subtilis is also able to synthesize nanoparticles during incubation time after cultivation through metal ion reductases, such as iron oxide, for reducing arsenic toxicity in rice plants [11] and silver nanoparticles for depolluting water [34].

2.7 Extracellular vesicles

B. subtilis produces extracellular cell-derived membrane-surrounded vesicles (EVs) carrying bioactive molecules such as proteins and nucleic acids. Their average sizes range from 20 to 100 nm in diameter. EVs find applications as immunostimulants, adjuvants, or even vaccine vehicles, particularly in the aquaculture sector [35].

In terms of functionality, these metabolites can play essential roles in One Health approaches (**Figure 2**). They contribute to improved health outcomes across humans, animals, and plants, while also promoting a safer environment, either directly or indirectly. Their multiple activities and functionalities include antimicrobial, antioxidant, antitumor, immunostimulatory, and anti-inflammatory effects for preventing and treating various human infections and diseases. Additionally, they enhance nutrient availability, support plant growth and stress resistance, and aid in the degradation or immobilization of soil contaminants such as heavy metals and hydrocarbons.

Recognized as probiotics, *B. subtilis* finds many applications in food and feed as a supplement ingredient for promoting human and animal health, including both

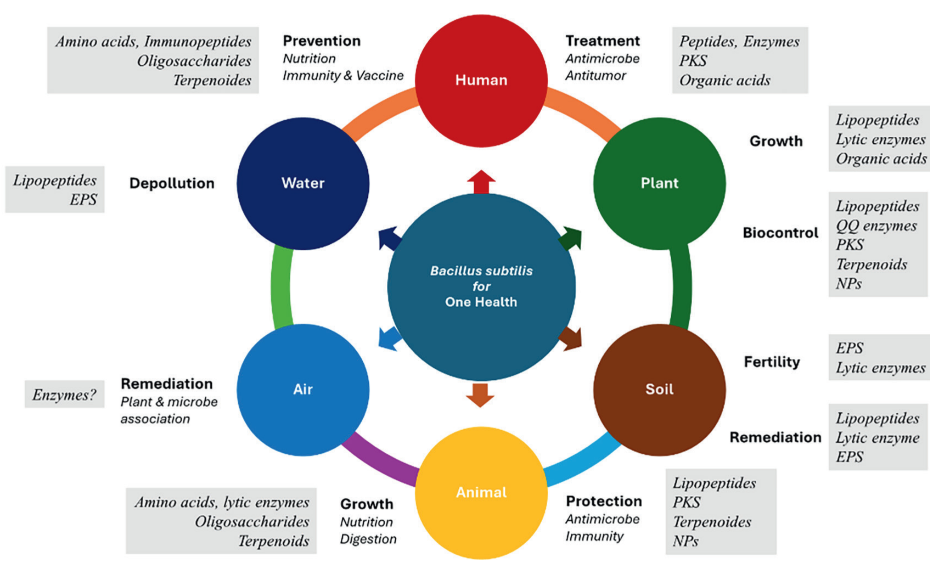

Figure 2.
B. subtilis *metabolites in One Health – EPS: Exopolysaccharides; QQ: quorum quenching; PKS: Polyketides; NPs: Nanoparticles.*

husbandry and aquatic animals [36]. Besides their use as experimental models and microbial cell factories by humans, the species *B. subtilis* var. natto is the main functional ingredient for fermented soybean foods such as Japanese natto [37], Korean cheonggukjang [35], and Chinese douche [38].

For humans and animals, most metabolites from *B. subtilis* can be used as nutraceuticals, providing both nutritional and pharmaceutical functions, and as therapeutic/antibiotic agents. Bioactive peptides such as cyclic lipopeptides (e.g., surfactin) are active against microbial infections (virus, bacteria, and fungi), especially against drug-resistant pathogens and cancer cells, inducing apoptosis and inhibiting tumor cell migration and invasion [39]. Immunomodulator peptides can stimulate human responses and reduce inflammation [40]. In addition, nattokinase, a lytic enzyme produced by *B. subtilis* natto, may play an important role in cardiovascular health through its fibrinolytic properties [7].

Polyketides have a wide spectrum of bioactivity, such as antimicrobial, antitumoral, immunosuppressive, and many antagonistic abilities, and can consequently be used in the human health sector. For instance, dihydroisocoumarins exhibit strong activity against *Helicobacter pylori* [41].

EPS possesses multiple biological functions, such as anticancer, antimicrobial, anti-inflammatory, and immunostimulant activities, and shows prebiotic effects in enhancing probiotic capacity in the gut by acting as protective agents for intestinal health [29].

Organic acids such as dipicolinic acid and L-lactic acid are beneficial for human health by playing an anti-blood coagulation role [42] and an antimicrobe role [43], respectively. Terpenoids, also known as isoprenoids, are represented by some vitamins, such as metaquinone-7 or vitamin K2, and antioxidants (e.g., carotenoids) are, for instance, potential nutraceuticals, while isopentenol may be a potential therapeutic agent in malaria and cancer treatment [31].

Volatile organic and inorganic metabolites have a wide spectrum of antimicrobial activities against several human and plant pathogens in both prevention and treatment actions, for instance, against Candida albicans, one of the most common deep pathogenic fungi [44]. Interestingly, nitric oxide compounds can play a role in human longevity and stress resistance enhancement [5].

B. subtilis is also recognized as a plant growth promoter [45] and biocontrol agent, as well as a biofertilizer, through nitrogen fixation, phosphate solubilization, mitigation of ammonia emission, and improvement of nutrient availability in the soil. As plant growth promotor rhizobacteria (PGPR), *B. subtilis* produces phytohormones (e.g., indole-3-acetic acid), fixing atmospheric N via nitrogenase activity and solubilizing phosphorus [8]. Owing to metabolite production, including antimicrobial peptides (AMPs), cellulolytic and chitinase activity enzymes, VOCs, EPS, and quorum quenching compounds, *B. subtilis* is recognized as a biocontrol agent via direct and indirect mechanisms such as plant growth-promoting metabolites, inducing systemic resistance (ISR), forming biofilm, and inhibiting deleterious phytopathogens.

Finally, *B. subtilis* is an excellent bioremediating agent of contaminated soil, wastewater [46], and even air when associated with plants [47]. It can be used as a detoxifying and degrading agent of heavy metals [48, 49] and contaminant hydrocarbons [50] thanks to enzymes, biosurfactants, and biodegradative compounds. These compounds convert complex organic pollutants into less or non-toxic compounds. **Table 1** lists some examples of important biomolecules produced by *B. subtilis* and their functionalities, as well as the possible roles in One Health.

Health applications	Category	Compounds	Roles	Ref.
Human health prevention and treatment	Enzyme Vitamin Organic acid Amino acid Oligosaccharide Inorganic compound	*Nattokinase* *Menaquinone-7* *Dipicolinic acid* *L-ornithine* *2′-fucosyllactose* *Nitric oxide*	*Fibrinolytic activity* *Anti-arterial calcification* *Anti-blood coagulation* *Anti-obesity* *Prebiotic activity* *Longevity and stress resistance enhancer*	[20] [51] [42] [21] [28] [33]
Animal growth and protection	Enzyme Lipopeptide EPS	*Protease* *Surfactin* *ß-glucan*	*Shrimp growth performance* *Antimicrobe* *Immunostimulant in tilapia*	[52] [53] [54]
Plant growth and protection	Enzyme Organic acid	*Cellulases* *Indole-3-acetic acid*	*Plant defense against pathogens* *Phytohormone (PGPR)*	[8] [55]
Soil and environmental health	Enzymes Polyamino acid Lipopeptide	*Chitinase* *Poly-Υ- glutamic acid* *Surfactin*	*Bioremediation* *Biofertilizer, biochelator* *Bioremediation*	[8] [48] [53]

EPS: Exopolysaccharides; PGPR: Plant Growth-Promoting Rhizobacteria.

Table 1.
Representative biomolecules excreted by B. subtilis *and their potent roles in One Health.*

3. Properties of *B. subtilis* relevant to One Health

B. subtilis is a Gram-positive, rod-shaped bacterium known for its remarkable resilience and adaptability. Its ability to form endospores allows it to endure extreme environmental conditions, making it a common inhabitant of soil, as well as the gastrointestinal tracts of both humans and animals. This resilience, combined with its diverse metabolic capabilities, positions *B. subtilis* as a key player in the concept of One Health, which underscores the interconnectedness of human, animal, plant, and environmental health [56]. As a highly adaptable organism, *B. subtilis* can thrive in a variety of environments, including soil, water, and the gastrointestinal systems of humans and animals. It is also capable of withstanding extreme conditions such as high temperatures, desiccation, and nutrient deprivation. This adaptability is primarily due to its endospore-forming ability, metabolic flexibility, and capacity to interact with various ecosystems. Additionally, *B. subtilis* is recognized for its safety and low pathogenicity, making it an excellent candidate for a wide range of applications in human and animal health [36]. Its long history of use in food, probiotics, and agriculture, along with its regulatory approvals, highlights its status as a beneficial microorganism [57]. Known for its effectiveness as a probiotic, *B. subtilis* survives harsh gastrointestinal environments, influences gut microbiota, and boosts immune responses [58]. Extensive research has demonstrated its benefits in promoting digestive health, inhibiting pathogens, and regulating immune functions in both humans and animals [59].

3.1 Antimicrobial properties

The Bacillus genus is well-known for its production of a diverse array of antimicrobial peptides (AMPs), positioning it as a promising source for the discovery of novel inhibitory compounds [60]. It is estimated that approximately 4–5% of the genetic material in *B. subtilis* strains is dedicated to the synthesis of antimicrobial compounds (AMCs), primarily in the form of AMPs [4]. The production of these bioactive metabolites is heavily dependent on the strain characteristics and the growth of medium conditions. Factors such as the composition of the medium, pH, temperature, and cultivation duration are pivotal in shaping the antibiotic properties of microorganisms [61]. *B. subtilis* produces a variety of antimicrobial compounds that possess potent antibacterial and antifungal activities. These compounds are essential in inhibiting the growth of pathogenic microorganisms in various environments. A crucial survival mechanism of *B. subtilis* is its ability to form highly resilient endospores. In response to environmental stress such as heat, radiation, desiccation, or nutrient scarcity, the bacterium undergoes sporulation, encapsulating its genetic material within a protective spore coat. These endospores can remain dormant for extended periods and reactivate once conditions become more favorable [62]. Furthermore, *B. subtilis* produces antimicrobial substances, including surfactin and subtilin, which effectively inhibit the growth of harmful pathogens like *Clostridium difficile*, Salmonella, and *Escherichia coli*. This antimicrobial activity prevents infections and maintains a balanced gut microbiome [63]. Lipopeptides produced by *B. subtilis* exhibit strong antimicrobial properties, especially against multidrug-resistant bacteria. Owing to their amphiphilic structure, they reduce the surface tension during cultivation and show significant inhibitory effects against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* [64].

3.2 Probiotic potential

The robust endospore of *B. subtilis* enables it to survive in the acidic environment of the stomach, allowing it to colonize the gut. Once established, *B. subtilis* plays a crucial role in modulating the gut microbiome by promoting the growth of beneficial bacteria such as Lactobacillus and Bifidobacterium, which support digestive health, alleviate gastrointestinal disorders, and enhance immune responses. *B. subtilis* supplementation can reduce symptoms of irritable bowel syndrome and lower the incidence of diarrhea [57]. *B. subtilis* is naturally present in the gut microbiota of both humans and animals. Surviving stomach acidity in its spore form, it germinates in the intestines and helps maintain gut homeostasis. Studies indicate that *B. subtilis* regulates immune functions, supports beneficial gut flora growth, and inhibits pathogenic microorganisms [65]. Unlike other Bacillus species that may produce harmful toxins, *B. subtilis* is non-pathogenic and does not produce enterotoxins or hemolysins, ensuring its safety [36]. Clinical trials on humans have shown that even at high doses (~10^9 spores or CFU/day), *B. subtilis* strains did not cause infections in healthy individuals, making it a safe choice for probiotic use [66, 67]. Unlike many probiotics that are vulnerable to stomach acidity, the spores of *B. subtilis* are highly resistant to harsh conditions, enabling them to pass through the stomach and germinate in the intestines. *B. subtilis* modulates immune responses by stimulating immunoglobulin A (IgA) production and enhancing the activity of macrophages and dendritic cells, bolstering overall health and improving defenses against infections [68]. For instance, *B. subtilis* KATMIRA1933 demonstrates strong probiotic potential, with notable bile salt tolerance, acid resistance, and antimicrobial activity against oral pathogens. Their non-mutagenic properties and ability to co-aggregate with pathogens further support their safety for use in healthcare and food applications [69]. Its antimicrobial properties, immune modulation, and ability to normalize gut flora make it a strong candidate for clinical applications, particularly in preventing and treating diarrhea [70]. Furthermore, *B. subtilis* natto is gaining recognition as a probiotic in animal nutrition, offering antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory benefits that contribute to animal health and food safety, supporting sustainable livestock production [6].

4. *B. subtilis* applications in One Health

4.1 Human health applications

4.1.1 Probiotic formulations for gut health

B. subtilis has been extensively studied for its probiotic properties, demonstrating resilience due to its ability to form spores that withstand gastric acidity, thereby facilitating effective colonization of the gut [59]. Its probiotic benefits include modulation of gut microbiota, enhancement of nutrient absorption, and mitigation of gastrointestinal disorders such as diarrhea and inflammatory bowel diseases [29]. Furthermore, *B. subtilis* improves intestinal barrier integrity by upregulating tight junction proteins and reducing inflammation [71]. A randomized, double-blind, placebo-controlled trial investigated the gastrointestinal (GI) viability and tolerance of *B. subtilis* R0179 in healthy adults across a dose range of 0.1 to 10×10^9 CFU/day. The results indicated no adverse effects on general wellness or GI function, with a dose-dependent increase in fecal viable counts, confirming the strain's ability to survive passage through the GI tract [72]. Another study assessed the safety and tolerability of *B. subtilis* HU58 in patients with hepatic encephalopathy undergoing lactulose treatment. The probiotic group exhibited a trend toward reduced blood ammonia levels, with a significant decrease observed in individuals with baseline ammonia levels above 60 μg/dL, highlighting its potential in managing hepatic encephalopathy [73]. The effects of a probiotic formulation (MegaDuo™) containing *Bacillus coagulans* SC208 and *B. subtilis* HU58 were evaluated on intestinal permeability and immune markers using in vitro models. Under antibiotic-induced dysbiosis, MegaDuo™ improved gut membrane barrier function and reduced levels of pro-inflammatory cytokines TNFα and IL-6. Notably, these benefits were observed after 2 weeks of treatment, whereas no significant effects were detected under normal conditions [74]. *B. subtilis* plays a beneficial role in inflammatory bowel disease management [75]. For instance, a probiotic formulation containing *B. subtilis* alongside *Streptococcus thermophilus*, *Lactobacillus casei*, *Bifidobacterium breve*, and *Bifidobacterium animalis* subsp. lactis in an experimental colitis model showed comparable efficacy to Vivomixx®, reducing colitis symptoms, lowering pro-inflammatory cytokines (IL-6, TNF-α), and increasing anti-inflammatory markers (IL-10) and regulatory T cells (Tregs).

4.1.2 Antibacterial and anti-inflammatory properties

Lipopeptides from *B. subtilis* exhibit potent antimicrobial activity against pathogenic bacteria, including *Escherichia coli*, *Clostridium difficile*, and *Salmonella* spp., by disrupting microbial membranes and inhibiting pathogen colonization [76]. In addition to its direct antimicrobial properties, *B. subtilis* exerts immunomodulatory effects by regulating cytokine production, thereby mitigating inflammatory responses in conditions such as ulcerative colitis and Crohn's disease [77]. A study evaluating the functional properties of various *B. subtilis* strains demonstrated their anti-inflammatory, antioxidant, and antimicrobial activities and effectively inhibited the growth of skin pathogens, underscoring their potential as a functional probiotic material [78]. A comparative study assessed the effects of *Bacillus licheniformis* and *B. subtilis* on IPEC-J2 cells challenged with *E. coli* or *Salmonella Typhimurium*. Both strains demonstrated anti-inflammatory and antioxidant effects, as well as inhibited pathogen adhesion, although they had minimal impact on paracellular permeability. These findings suggest that *Bacillus* species could enhance probiotic efficacy in multispecies formulations [79].

4.1.3 Potential in functional foods and therapeutic agents

B. subtilis has been widely integrated into functional foods and dietary supplements due to its ability to produce beneficial enzymes, vitamins, and bioactive compounds. Fermented foods containing *B. subtilis* have been linked to improved digestion and immune function, making them valuable components of health-promoting diets [80]. Additionally, *B. subtilis* fermentation enhances the bioavailability of nutrients and bioactive compounds, contributing to the overall nutritional value of functional foods. A study on soybean fermentation using protease-producing *B. subtilis* strains (MTCC5480 and MTCC1747) from the Sikkim Himalayan region demonstrated significant improvements in antioxidant activity. The fermentation process increased radical scavenging activity, total antioxidant capacity, and reduced power, attributed to enhanced protein hydrolysis, elevated levels of free phenolics, and the release of amino acids such as proline and tyrosine. Furthermore, the fermented by-products exhibited strain-specific antioxidant properties, indicating their potential as functional food and feed additives with health benefits [81]. Beyond its role in food fermentation, *B. subtilis* has garnered interest in biotechnology due to its potential as a delivery system for therapeutic agents, including vaccines and recombinant proteins. Engineered *B. subtilis* strains are being actively investigated for their ability to express and deliver antigens in oral vaccination strategies, offering a promising approach for mucosal immunization [82]. The robust spore-forming nature of *B. subtilis* enhances its stability and viability, making it an attractive candidate for the development of oral vaccine platforms and biologics.

4.2 Animal health applications

4.2.1 Role in livestock health in improving growth and immunity

The use of *B. subtilis*-based probiotics in animal husbandry has gained significant attention as a sustainable alternative to antibiotic growth promoters. Research in poultry and livestock has demonstrated that dietary supplementation with *B. subtilis* enhances feed conversion efficiency, promotes a balanced gut microbiota, and strengthens immune responses, thereby increasing resistance to enteric pathogens [83]. By stimulating the production of short-chain fatty acids (SCFAs) and reducing intestinal inflammation, *B. subtilis* contributes to improved gut health and reduced dependency on antibiotics in farmed animals.

As a Gram-positive, facultative aerobic bacterium recognized as generally safe, *B. subtilis* plays a crucial role in livestock management. Its probiotic functions include modulation of the gut microbiome, enhancement of immunity, inhibition of pathogenic microorganisms, and secretion of bioactive compounds that support gastrointestinal health and disease resistance. Additionally, *B. subtilis* spores have been explored as vaccine carriers for antigen delivery, further expanding their applications in veterinary medicine [84]. A six-week feeding trial in broilers supplemented with *B. subtilis* BYS2 resulted in a 17.19% increase in body weight, improved intestinal morphology, enhanced immunity (elevated IgG and IgM levels), and greater resistance to Escherichia coli and Newcastle virus infections, leading to improved survival rates [85]. In rabbits, dietary supplementation with *B. subtilis* improved growth performance, strengthened immune responses (higher IgG and IgA levels), and enhanced disease resistance against E. coli. The study suggested that *B. subtilis* supplementation might boost innate immunity through β-defensin induction [47].

A 21-day feeding trial in chickens demonstrated that *B. subtilis* supplementation led to improved growth, immune responses (increased IgA and IgM levels), and intestinal health, as evidenced by increased villus height and a well-balanced microbiota. Additionally, *B. subtilis* improved disease resistance following an E. coli challenge, potentially through Toll-like receptor 4 (TLR4) activation [86]. In broilers, supplementation with *B. subtilis* (0.1 g/kg diet) resulted in improved body weight and feeding conversion ratios. Treated birds also exhibited enhanced immune responses, including higher antibody titers against Newcastle disease, improved lymphoid organ development, and better gut morphology [87].

4.2.2 Use in veterinary probiotics and feed additives

The inclusion of *B. subtilis* in animal feed has been widely explored as a probiotic strategy to improve digestion, prevent gastrointestinal infections, and enhance overall animal health. The bacterium contributes to gut homeostasis by modulating microbiota, promoting beneficial bacterial populations, and inhibiting pathogenic microorganisms. Additionally, *B. subtilis* aids in breaking down indigestible feed components, such as fiber and non-starch polysaccharides, thereby improving nutrient bioavailability and supporting intestinal health in livestock [88]. A study evaluating broiler diets containing both *B. subtilis* and an antimicrobial growth promoter (AGP) reported improved feed conversion efficiency and weight gain compared to diets with either *B. subtilis* or AGP alone, though carcass yield remained unaffected [89]. Supplementation with *B. subtilis* C-3102 spores in broilers, for instance, improved growth performance, feed efficiency, and nutrient digestibility, while also increasing Lactobacillus populations and reducing *Escherichia coli* and Salmonella levels in the gut. Additionally, this probiotic reduced ammonia emissions, demonstrating its potential for improving both animal health and environmental sustainability [90]. Another strain, *B. subtilis* AMS6, isolated from fermented soybeans, exhibited antibacterial activity against *Salmonella enterica*. Its cellulolytic activity suggests a potential role in enhancing fiber digestion and gut health when used as an animal feed additive [91]. A study evaluating *B. subtilis* RBT-7/32 and *B. licheniformis* RBT-11/17 as probiotic feed additives found both strains to exhibit strong antagonistic activity against *E. coli* and *Staphylococcus aureus*. Additionally, *B. subtilis* RBT-7/32 demonstrated increased cellulolytic and amylolytic activity. Both strains showed high spore survivability and resistance to veterinary antibiotics, highlighting their potential for use in livestock nutrition [92]. In aquaculture, *B. subtilis* has been utilized to improve water quality, enhance the immune response of fish and shrimp, and reduce the incidence of bacterial diseases [93]. The probiotic modulates gut microbiota, increases nutrient utilization efficiency, and supports intestinal barrier function in aquatic species. Its ability to degrade organic matter and promote biofloc formation further enhances water quality, thereby creating a more sustainable and disease-resistant environment [88].

4.3 Agricultural applications

4.3.1 Biocontrol agent against plant pathogens

B. subtilis is a well-established biological control agent known for its ability to suppress plant pathogens through multiple mechanisms, including competition for nutrients and space, the induction of systemic resistance, and the production of antimicrobial lipopeptides [94]. Its application in agriculture has been effective in managing fungal pathogens such as Fusarium, Rhizoctonia, and Botrytis, reducing crop losses while minimizing reliance on chemical pesticides. Additionally, *B. subtilis* colonizes plant roots and forms biofilms, creating a protective barrier against infections. *B. subtilis* has been shown to suppress plant pathogens through plant growth promotion, antibiosis, and induced systemic resistance, providing an eco-friendly alternative to synthetic pesticides [95]. A strain of *B. subtilis* isolated from the rhizosphere of chili plants exhibited, for instance, strong antagonistic activity against *Colletotrichum gloeosporioides* through the production of mycolytic enzymes. It significantly reduced fungal growth and improved chili seed germination, demonstrating the potential for biocontrol applications [96]. *B. subtilis* strains isolated from *Zea mays* exhibited over 40% in vitro inhibition of Botrytis cinerea, primarily through lipopeptide production, siderophore activity, and biofilm formation. These strains also promoted maize growth and reduced *B. cinerea* infection in *Phaseolus vulgaris* [97]. *B. subtilis* strains AI01 and AI03, isolated from the eggplant rhizosphere, exhibited strong antagonistic activity against *Fusarium solani*. They inhibited multiple fungal pathogens, produced antifungal metabolites and plant growth-promoting compounds, and reduced wilt incidence by up to 72% in sterilized soil, highlighting their potential as bioinoculants for sustainable disease management [98]. Six *B. subtilis* strains isolated from natural environments in China exhibited over 50% biocontrol efficacy against *Ralstonia solanacearum* in tomato plants. These strains formed robust biofilms on plant roots, demonstrating strong antagonistic activity. Biofilm formation and matrix production were crucial for plant protection, emphasizing their role in bacterial colonization and disease suppression [99].

4.3.2 Fertilizer production and plant growth promotion

As a plant growth-promoting rhizobacterium (PGPR), *B. subtilis* enhances plant root development by producing phytohormones such as indole-3-acetic acid (IAA) and solubilizing phosphorus, thereby increasing nutrient availability for plants [100]. Additionally, its nitrogen-fixing capabilities contribute to soil fertility, making it an essential component of sustainable agricultural practices. The application of *B. subtilis*-based biofertilizers has been associated with improved crop yield, enhanced stress tolerance, and reduced dependency on chemical fertilizers. *B. subtilis* enhances plant growth and stress tolerance through direct and indirect biocontrol mechanisms. It produces secondary metabolites, enzymes, and hormones that suppress pathogens while inducing systemic resistance. Additionally, it improves soil nutrient availability and promotes root colonization, making it a valuable biofertilizer and biopesticide [101]. For instance, a biofertilizer (BOF) containing *B. subtilis* F2 significantly enhanced strawberry plant growth, nutrient accumulation, and peroxidase activity. BOF also altered the rhizosphere microbial community's β-diversity, suggesting strain F2 played a crucial role in plant health improvement [102]. Another strain (*B. subtilis* L2) significantly improved ginger growth by increasing plant height, leaf length, number of leaves, and chlorophyll content. Inoculation enhanced physiological properties, supporting its potential as a promising biofertilizer for ginger cultivation [103]. Field trials in Tajikistan showed that *B. subtilis* FZB 24® increased cotton yield by up to 30% compared to conventional NPK fertilizer. The treatment enhanced root growth and phosphorus mobilization, demonstrating its potential as an eco-friendly alternative to chemical fertilizers [104]. *B. subtilis*, alone or in combination with nutrients, effectively controlled early blight in tomatoes (67–83%) and enhanced plant growth (20–77%). The treatment boosted phenolic content and defensive enzyme activity, highlighting its synergistic effect with NPK and Zn (2X) in disease management and plant health improvement [105].

4.4 Environmental applications

4.4.1 Bioremediation of environmental pollutants

The application of *B. subtilis* in environmental biotechnology has garnered attention due to its capacity to degrade hydrocarbons and detoxify heavy metals. It plays a crucial role in breaking down oil spills, pesticide residues, and industrial pollutants through enzymatic biodegradation [106]. The production of biosurfactants further enhances its bioremediation potential by aiding in the emulsification and breakdown of hydrophobic contaminants, making them more accessible for microbial degradation. *B. subtilis* isolated from a polymer dump site in Chennai, India, degraded 80% of crude oil and reduced viscosity by 60% within 10 days. Its biosurfactant production, high emulsification efficiency, and stability under extreme conditions highlight its potential for oil spill remediation [107]. *B. subtilis*, widely utilized in bioengineering and biotechnology, forms robust biofilms and spores that enhance its resilience and versatility. These biofilms, composed of exopolysaccharides, proteins, extracellular DNA, and poly-γ-glutamic acid, support applications in bioremediation, biosensing, and biocatalysis. Advances in synthetic biology have enabled the engineering of *B. subtilis* biofilms for self-regenerating and environmentally responsive biomaterials, broadening their utility in sustainable technologies [108]. Biosurfactant concentrations of 19–19.5 mg/kg stimulated hydrocarbon-degrading microbial populations and accelerated aliphatic hydrocarbon degradation. However, its effect on aromatic hydrocarbon degradation was limited [109]. Two *B. subtilis* strains (M16K and M19F) demonstrated simultaneous hydrocarbon degradation and heavy metal (Co^{2+} and Ni^{2+}) removal in contaminated environments. These strains achieved >94% crude oil and >85% phenanthrene degradation while concurrently detoxifying heavy metals within 18–28 days. This study represents the first report of metal-resistant *B. subtilis* strains with dual bioremediation capabilities in sub-Saharan Africa, underscoring their potential for multi-polluted site restoration [110].

4.4.2 Waste management and decomposition

B. subtilis contributes significantly to organic waste degradation by producing cellulases and proteases that accelerate biodegradation processes. Its application in composting enhances nutrient cycling, improves soil fertility, and reduces environmental waste accumulation [111]. Additionally, its ability to break down agricultural residues and convert organic matter into biofertilizers supports sustainable waste management practices. Inoculating compost with *B. subtilis* at a 2% concentration enhanced fermentation but suppressed the synthesis of total organic carbon and humus. In contrast, a 0.5% inoculation significantly improved carbon humification, resulting in a 12.5% increase in total organic carbon and a 20.2% increase in humic substances compared to the 2% treatment [112]. Parameters such as pH and microbial metabolism played key roles in carbon sequestration during composting. In a sequencing batch reactor (SBR), *B. subtilis* improved sludge treatment in recirculating aquaculture systems (RAS). Biofloc crude protein content increased by 23.97% over the control, while removal rates of dissolved inorganic nitrogen (1.17×), total organic nitrogen (1.71×), and dissolved organic carbon (1.95×) were significantly enhanced. Floc volume after 5 minutes reached 22.67% at 19 days [113]. India faces significant post-harvest losses, with approximately 30% of fruit and vegetable production wasted annually. A study exploring *B. subtilis* isolated from pomegranate peel demonstrated its potential as a plant growth-promoting rhizobacterium (PGPR) for agricultural waste management. Treated wheat exhibited increased yield, while microgreens showed higher phenol and antioxidant levels, highlighting *B. subtilis* as a biofertilizer for sustainable agriculture [114]. Solid waste management, particularly concerning petroleum-based plastic and fish solid waste (FSW), remains a global challenge. *B. subtilis* strain KP172548 was shown to convert FSW extract into 1.62 g/L of polyhydroxybutyrate (PHB), a biodegradable biopolymer. The PHB was biocompatible, suggesting its potential as an eco-friendly alternative to conventional plastics [115].

5. Safety and regulation aspects

B. subtilis has a long history of safe use in food fermentation and as a probiotic. Strains such as *B. subtilis* DE111 have been administered in doses up to 10 billion colony-forming units daily for periods ranging from 2 to 8 weeks without adverse effects [59]. It is approved for use in food in certain European countries [116] and is also naturally present in traditional Asian foods such as natto and kimchi [66, 117]. This safety profile has led to its recognition as a safe organism in various applications, including food production and dietary supplementation [116]. In the U.S., the Food and Drug Administration (FDA) grants Generally Recognized As Safe (GRAS) status to *B. subtilis* strains through assessments that include strain characterization, genome sequencing, screening for undesirable attributes and metabolites, and experimental safety evaluations conducted in appropriately designed studies. At least five GRAS notifications for *B. subtilis* in food applications have been approved without questions from the FDA [118].

European Food Safety Authority (EFSA) has established the Qualified Presumption of Safety (QPS) approach to assess the safety of microorganisms. To meet QPS status requirements, any new *B. subtilis* strain must be identified at both the strain and species levels, be free of transferable antimicrobial resistance, and exhibit no toxigenic activity [67]. *B. subtilis* is included in the list of QPS-recommended microorganisms and considered to have QPS status when used as an animal feed additive, owing to its lack of toxigenic activity [119].

Several studies have assessed the safety of *B. subtilis* strains for human consumption, including both in vitro and clinical evaluations. For example, *B. subtilis* CU1 was shown to be free of antibiotic resistance and caused no physiological or biological issues in adults consuming 2 x 10^9 spores per day for 40 days [67]. *B. subtilis* MB40 was also found to be safe at a daily intake of 2.6 x 10^{11} spores/day for a 70 kg adult [66].

As a feed additive, EFSA's safety evaluation did not reveal any toxigenic activity or antibiotic resistance in *B. subtilis*, and it is therefore considered safe for animals [120, 121]. Moreover, *B. subtilis* strains have been approved by the Environmental Protection Agency (EPA) as biocides and are exempt from tolerance limits in food crops, as they are not considered toxic or pathogenic to humans, animals, or plants [122].

However, the use of *Bacillus* species raises safety concerns due to the potential production of enterotoxins and emetic toxins, which are strain-specific [123]. Therefore, it is crucial to assess and confirm the safety of potential probiotics at a strain level prior to their use. Adhering to regulatory standards and ensuring safety are critical for guaranteeing the safety of probiotics. These regulations are guided by the criteria set by national authorities, including the European Food Safety Authority (EFSA), the U.S. Food and Drug Administration (FDA), and the Canadian Natural and Non-Prescription Health Products Directorate [124].

Emerging concerns about the safety of probiotics, particularly their potential risks for immunocompromised individuals, underline the need for in-depth safety studies [125]. Recommendations for future regulatory frameworks have been proposed, emphasizing the importance of assessing both acute and long-term safety. Probiotics intended for specific patient populations should undergo rigorous testing to ensure they meet quality standards, ideally verified by an independent third party. Additional research is essential to fill existing knowledge gaps, including identifying the most suitable animal models for safety testing, assessing the potential transfer of antibiotic resistance genes, and exploring the effects of probiotics on microbiomes, drug interactions, and colonization [126].

6. Conclusion

B. subtilis is a bacterial species with immense potential for producing a high diversity of bioactive compounds, ranging from the simple organic and inorganic metabolites to more complex systems such as nanoparticles and vesicles. These compounds serve a variety of properties and functions. Recognized as safe, *B. subtilis* is a promising natural candidate for the One Health approach, which seeks to optimize the health of people, animals, plants, and the environment. The resilience and adaptability of *B. subtilis* make it a highly valuable microorganism with diverse applications across biotechnology, agriculture, and human health. Its ability to withstand extreme conditions and colonize a wide range of environments—from soil to the gastrointestinal tracts of humans and animals—highlights its essential role in maintaining ecosystem stability. In human and animal health, *B. subtilis* demonstrates significant probiotic potential, offering health benefits for both the prevention and treatment of various diseases. In agriculture, *B. subtilis* plays a crucial role as a biocontrol agent and biofertilizer, promoting plant growth, enhancing soil fertility, and reducing the need for chemical pesticides and fertilizers. As biotechnological advancements progress, the range of *B. subtilis* applications is expected to expand, providing innovative solutions for global health, food security, and environmental sustainability. Future research should focus on optimizing microbial formulations, utilizing genetic engineering for strain improvement, and integrating *B. subtilis* into precision agriculture, sustainable livestock production, and large-scale environmental restoration projects. By leveraging its natural capabilities, future innovations could enhance public health, promote resilient food systems, and contribute to a more sustainable world.

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Conflict of interest

The authors declare no conflict of interest.

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