

Exploring *Bacillus subtilis*

Akinsemolu, Adenike A.; Onyeaka, Helen; Odion, Samuel; Adebajo, Idris

DOI:

[10.1002/jobm.202300614](https://doi.org/10.1002/jobm.202300614)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Akinsemolu, AA, Onyeaka, H, Odion, S & Adebajo, I 2024, 'Exploring *Bacillus subtilis*: Ecology, biotechnological applications, and future prospects', *Journal of Basic Microbiology*.
<https://doi.org/10.1002/jobm.202300614>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

REVIEW

Exploring *Bacillus subtilis*: Ecology, biotechnological applications, and future prospects

Adenike A. Akinsemolu^{1,2}  | Helen Onyeaka^{1,2}  | Samuel Odion^{2,3} | Idris Adebajo²

¹School of Chemical Engineering, University of Birmingham, Birmingham, UK

²The Green Microbiology Lab, University of Birmingham, Birmingham, UK

³The Green Institute, Ondo, Ondo State, Nigeria

Correspondence

Adenike A. Akinsemolu and Helen Onyeaka, School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, UK.

Email: a.akinsemolu@bham.ac.uk and h.onyeaka@bham.ac.uk

Abstract

From its early identification by Christian Gottfried Ehrenberg to its current prominence in scientific research, *Bacillus subtilis* (*B. subtilis*) has emerged as a foundational model organism in microbiology. This comprehensive review delves deep into its genetic, physiological, and biochemical intricacies, revealing a sophisticated cellular blueprint. With the incorporation of advanced techniques such as clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 and integrative computational methodologies, the potential applications of *B. subtilis* span diverse sectors. These encompass its significant contributions to biotechnology, agriculture, and medical fields and its potential for aiding environmental cleanup efforts. Yet, as we move forward, we must grapple with concerns related to safety, ethics, and the practical implementation of our lab findings in everyday scenarios. As our understanding of *B. subtilis* deepens, it is evident that its contributions will be central to pioneering sustainable solutions for global challenges in the years to come.

KEYWORDS

Bacillus subtilis, biotechnology, CRISPR-Cas9, environmental restoration, microbial therapies

1 | INTRODUCTION

Microorganisms play a crucial role in shaping the environment and are increasingly recognized as valuable tools for studying biological processes and promoting sustainable practices. Among the prominent model organisms in green microbiology, *Bacillus subtilis* is

highly regarded for its contribution to studying ecological processes and advancing sustainability practices [1]. This review article aims to shed light on the reasons behind *B. subtilis*' exceptional track record in green microbiology studies. It focuses on exploring the organism's key features, applications, and implications related to sustainability issues.

Abbreviations: BTEX, benzene, toluene, ethylbenzene, and xylene; CRISPR-Cas9, Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9; GAs, gibberellins; GMOs, genetically modified organisms; HPP, high-pressure processing; IAA, indole-3-acetic acid; ICMS, Integrated Crop Management System; ISR, induced systemic resistance; NRPSs, nonribosomal peptide synthetases; ORFs, open reading frames; PGPR, plant growth-promoting rhizobacteria; PHA, polyhydroxyalkanoates; PHB, polyhydroxybutyrate; PR, pathogenesis-related; SmF, submerged fermentation; SSF, solid-state fermentation.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Journal of Basic Microbiology* published by Wiley-VCH GmbH.

B. subtilis, a Gram-positive bacterium first identified by Ferdinand Cohn in 1872 and widely present across diverse environments such as soil, plants, and animal digestive tracts, has long been studied by scientists for over 100 years [1–3]. As its wide presence contributes to ecological studies of various kinds, its longstanding study makes for fascinating scientific research. However, its significance has recently skyrocketed with the rise of green microbiology, an endeavor designed to harness microorganisms for sustainable practices. Given its genetic accessibility, metabolic versatility, and stress tolerance characteristics—which allow researchers to probe specific genes, pathways, and regulatory networks in *B. subtilis* to uncover its complex molecular mechanisms while uncovering ecological contributions—its place as an experimental organism has only increased exponentially in importance over the years [4–6].

B. subtilis' genetic accessibility is one of the primary reasons behind its selection as a model organism. Following the completion of genome sequencing in 1997 [7], an abundance of genetic information became accessible, permitting in-depth investigations of molecular makeup and regulatory mechanisms within *B. subtilis* itself as well as manipulation tools available today that support its manipulation, allowing greater insight into ecological processes as well as sustainable applications of this organism [4].

B. subtilis' metabolic versatility is another critical characteristic. This bacterium stands out in terms of adapting and flourishing in diverse environments by using various carbon sources for energy and playing an integral part in essential ecological processes like nutrient cycling, bioremediation, waste management, and waste disposal [8, 9]. Furthermore, *B. subtilis* can even be engineered to produce enzymes, biofuels, or other valuable compounds without using nonrenewable resources for production purposes reducing dependence on nonrenewable materials for production and unsustainable sources of energy [10].

B. subtilis exhibits remarkable stress tolerance and resilience, making it a valuable model organism to study how microbes respond to environmental challenges [11, 12]. *B. subtilis* has evolved robust stress response mechanisms, enabling it to cope with adverse environmental conditions like limited nutrients, fluctuating temperatures, toxic exposure, and toxic compounds. By unraveling its stress response pathways, researchers gain key insight into the resilience strategies of microorganisms, thereby providing sustainable practices against changing environmental conditions.

This review seeks not only to explore the genetic accessibility, metabolic versatility, and stress tolerance of *B. subtilis* but also explore its applications in green microbiology. This review will specifically highlight its role in sustainable bioproduction, environmental

bioremediation, and plant–microbe interactions. *B. subtilis*' capacity to produce enzymes, biofuels, and other valuable substances offers viable alternatives to more conventional approaches for manufacturing them. *B. subtilis* plays an invaluable role in environmental bioremediation, contributing to the restoration of polluted ecosystems and improving sustainable agricultural practices.

2 | TAXONOMY AND PHYLOGENY OF *B. SUBTILIS*

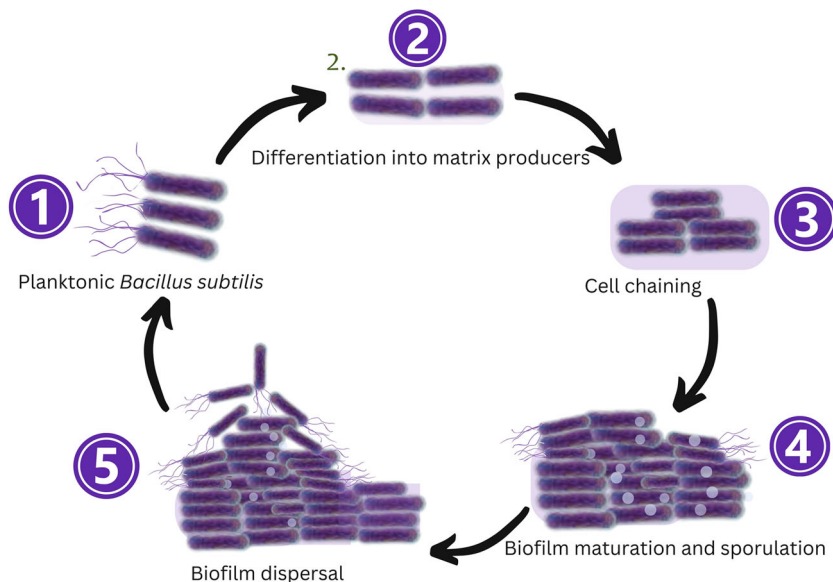
2.1 | Classification and related species

Taxonomy is an integral aspect of biological science that deals with classifying organisms according to shared characteristics and evolutionary relationships. *B. subtilis*, a Gram-positive, rod-shaped catalase-positive bacteria species found throughout soil, water, and air environments worldwide, has undergone periodic revision due to advances in molecular techniques and increased knowledge about microbial diversity [13, 14].

B. subtilis belongs to the firmicutes bacteria genus *Bacillus* and belongs to its diverse group characterized by Gram-positive cell wall structure and endospore formation—two characteristics that enable these species to adapt well in harsh environmental conditions such as extreme temperatures, limited nutrition, and desiccation [11, 15, 16]. Although traditionally classified as its own species in *Bacillus* genus classification schemes; as new information becomes available, this could change. To stay up-to-date and get accurate classification information, it would be prudent to refer to regularly updated taxonomic references or databases to gain accurate classification information [11].

B. subtilis shares both genetic and phenotypic similarities with its close relatives such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus pumilus*, and *Bacillus megaterium* in terms of both genes and physical characteristics, making comparison studies useful to gain deeper insights into *Bacillus* diversity [11, 17]. These traits make them invaluable when studying physiological and biochemical mechanisms relating to family tree activity. *B. amyloliquefaciens* is one of the closest relatives to *B. subtilis* in terms of genetic similarity and metabolic capabilities, sharing many features such as antimicrobial production [18]. Additionally, this strain has been extensively investigated due to potential uses such as agriculture biocontrol or biotechnology applications [19, 20]. *B. licheniformis* and *B. subtilis* also share close genetic and ecological relationships; both species exhibit strong biofilm formation capabilities (Figure 1) as

FIGURE 1 The lifecycle of *Bacillus subtilis* biofilm (authors compilation).



well as producing various enzymes for production purposes [21]. *B. licheniformis* has also been researched for industrial applications related to enzyme production and bioremediation processes [22]. Studying these three bacteria species—with similar physiological and genetic features to *B. subtilis* provides invaluable insights into diversity and adaptation among their species.

Taxonomy is an ever-evolving science; as more genomic and phylogenetic data becomes available, classification and relationships among *Bacillus* species shift with time. New technologies for DNA sequencing offer invaluable insight into their evolution over time.

2.2 | Evolutionary history and phylogenetic relationships

These genetic components could play an essential part in helping it be resilient to environmental challenges as well. *B. subtilis* belongs to the Genus *Bacillus* and species *B. subtilis* [11, 23]; its taxonomical relatives include *B. amyloliquefaciens* and *B. licheniformis* [18]. Molecular analysis based on its evolutionary background has placed *B. subtilis* into the Firmicutes phylum [15]; its genetic diversity, adaptive characteristics, ecological significance have all been well studied with comparative genomic research providing further understanding into genetic traits as well as taxonomies/phylogenies of bacteria species such as *B. licheniformis* [2, 24]. *B. subtilis* contains approximately 4100 genes; of these, only 192 were absolutely essential, with another 79 predicted as likely being essential [25]. Essential genes were generally concentrated into several metabolic domains, with half being involved with information processing, one-fifth

being related to cell envelope production/control of shape/division control, and one-tenth being involved with energy utilization for metabolism purposes [25]. *B. subtilis* substrain QB928 contains 4,146,839 DNA base pairs and 4292 genes in its genome [26]. Due to numerous markers [*aroI(aroK)906 purE1 dal(alrA)1 trpC2*], QB928 strain has become a popular choice in genetic research studies [27]. Bsr RNAs were identified among noncoding RNAs identified within the *B. subtilis* genome in 2009 [28]. Microarray analyses conducted between *B. subtilis* strains have demonstrated their remarkable level of genetic variation. FsrA, found within *B. subtilis*, functions as part of its iron-sparing response in times of limited bioavailability to act against iron-containing proteins by downregulating them [29]. *B. subtilis* strain WS1A, an established fish probiotic, successfully inhibited motile *Aeromonas septicemia* infection in *Labeo rohita* fish via de novo assembly to produce 4288 open reading frames and an estimated chromosome size of 4,148,460 base pairs; its de novo assembly also revealed this result [30]. Additionally, genes responsible for biosynthesising riboflavin, vitamin B6, and amino acids as well as carbon utilization can be identified within its genome, including those responsible for biosynthesising proteins. Understanding *B. subtilis*' genetic variety, adaptive features and ecological significance requires understanding its evolutionary background and phylogeny. Molecular techniques like DNA sequencing and comparative genomics make this possible for us today. Firmicutes is an expansive group of Gram-positive bacteria distinguished by comparatively lower genomic DNA G + C concentration. Within it can be found several genera, among them *B. subtilis*; its classification within Firmicutes' class Bacilli, order

Bacillales and family Bacillaceae is determined. Studies on *B. subtilis*' evolution using molecular markers like 16S ribosomal RNA (rRNA) gene sequencing have demonstrated its distinct evolution relative to other species and related taxa of its subgenus; we believe this evidence proves its unique cluster within this subgenus; our investigations revealed greater genetic affinity with *B. amyloliquefaciens* and *B. licheniformis* than with any of its peers within its species' cluster. Studies suggest that horizontal gene transfer events shaped *B. subtilis*' evolutionary history. Horizontal gene transfer involves the acquisition of genetic material from other organisms while vertical inheritance involves passing genetic information from generation to generation through reproduction processes [31]. Horizontal gene transfer events likely contributed significantly to increasing genetic diversity as well as creating adaptive features in *B. subtilis*' evolution. Comparative genomics analyses have become easier thanks to the availability of whole genome sequences from several strains of *B. subtilis*, enabling scientists to examine genetic variants and evolutionary dynamics within this species. Such studies have yielded new information regarding mechanisms behind genetic diversification and adaptation in populations populated with *B. subtilis* bacteria [9, 32, 33]. Studies of *B. subtilis* using comparative phylogenomic analyses have unveiled unique gene clusters and genetic components that contribute to its distinctive traits, such as being capable of building biofilms, producing antibiotics, or resisting environmental challenges.

2.3 | Ecological significance of *B. subtilis*

Lately, research is directed to environmentally friendly alternatives for controlling plant pathogens and improving crop production, which are recommended within an Integrated Crop Management System (ICMS) [34]. As an important component of an ICMS, biological control is defined as the use of beneficial organisms to reduce the negative effects of plant pathogens and promote positive responses by the plant. The most common approach to biological control is the selection of antagonistic microorganisms, evaluation of their mechanisms of action, and development of a biocontrol preparation [35].

Bacillus species are among the most investigated biocontrol agents, particularly in their role as biopesticides, which contribute to the suppression of plant pathogens by antagonism and/or competition [36]. *Bacillus* spp. can inhibit pathogen growth through various mechanisms, including competition for nutrients and space, production of antibiotics, hydrolytic enzymes, siderophores, and/or inducing systemic resistance [37].

They may also act as biofertilizers/biostimulators by helping plants take in certain nutrients from their environment through nitrogen fixation and phosphate solubilization or providing them with compounds through processes such as the biosynthesis of plant hormones [38]. Essentially, *Bacillus* spp. represent a viable natural substitute for synthetic fertilizers and pesticides in stimulating plant growth. Various agricultural crops, including wheat, maize, soybeans, sunflower seeds, common beans, tomatoes, peppers, potatoes, cucumbers, and more, have exhibited positive effects on both their growth and yield when *Bacillus* spp. are utilized [39]. However, the application of *Bacillus* spp. to enhance the productivity of field and vegetable crops faces challenges due to variations in results observed in laboratory, greenhouse, and field settings [40]. In reality, when reintroduced through plant/soil inoculation, only a small percentage, approximately 1%–2%, of rhizobacteria have a beneficial impact on plant growth [37]. Given the numerous factors influencing the effectiveness of *Bacillus* spp., it is essential to gain insight into how different strains execute biocontrol and growth-promotion activities in plants. Additionally, it is crucial to clearly define the traits and criteria for selecting the most effective strains [40].

2.4 | Antibiotics

The antagonistic actions of *Bacillus* spp. are often associated with the synthesis of secondary compounds possessing antibiotic properties. These molecules typically consist of low-molecular-weight peptides produced either ribosomally (in the case of bacteriocins) or nonribosomally (lipopeptides [LPs], peptides, and polyketides).

Bacillus spp. are highly researched antibiotic compounds due to their production of cyclic LPs, which have an impressive track record against various plant diseases [24]. These LPs are synthesized via large nonribosomal peptide synthetases (NRPSs; 25]. The primary mechanisms of LPs' actions typically involve an interaction with the cell membrane of the target pathogens, causing changes in its structure and permeability through disruption, solubilization, or formation of ion-conducting pores [41].

2.5 | Nutrient availability

Bacillus spp. produces a wide range of metabolites that enhance the availability of nutrients to plants, leading to a direct promotion of plant growth and yield. Many of the

essential nutrients required by plants are typically supplied through mineral fertilizers, a practice associated with significant economic losses and environmental challenges. Biofertilizers containing *Bacillus* spp. capable of nitrogen fixation (N_2 -fixing) and phosphorus solubilization (P-solubilizing) present a sensible approach to mitigate these issues without compromising food safety [34]. These specific *Bacillus* spp. strains play a crucial role in nutrient uptake and subsequent growth promotion in various plant species.

2.6 | Phytohormone production

Bacillus spp. have the potential to directly boost plant yield by engaging in mechanisms that involve the production of phytohormones or plant growth regulators (PGRs), including auxins, cytokinins, gibberellins (GAs), ethylene, and abscisic acid. Among these, the production of indole-3-acetic acid (IAA) is a common trait among soil bacteria, with approximately 80% of rhizobacteria known to produce IAA [42]. When IAA-producing *Bacillus* strains were applied in vitro to plant roots, it resulted in increased root length and the development of more lateral roots [43]. Additionally, studies have shown that *B. subtilis* can enhance shoot and root growth, seedling vigor, and leaf area in tomato plants. This enhancement is associated with higher levels of GAs and IAA in the treated plants [44].

GAs, which are plant hormones produced by *B. methylotrophicus* and responsible for its beneficial effects, play a significant role in various developmental processes in higher plants. These processes include seed germination, stem elongation, flowering, and fruiting. The secretion of GAs by these bacteria has been shown to enhance the germination rates of seeds in plants like lettuce, muskmelon, soybean, and vegetable mustard [45]. Furthermore, the same authors demonstrated that the bacterial strain capable of producing GAs increased various aspects of lettuce growth, including shoot length, shoot fresh weight, leaf width, as well as the levels of proteins, amino acids, macro and micro minerals, carotenoids, and chlorophyll in lettuce plants.

2.7 | Plant–*Bacillus* interactions

The effectiveness of applying *Bacillus* spp. in the field is also influenced by the interactions between the bacteria and the plants, which can be hindered when there is insufficient colonization of the rhizosphere [46]. *Bacillus* spp. typically take 24 h to establish a biofilm, a crucial factor in facilitating root colonization and extending the

positive effects of *Bacillus* spp. in the soil [47]. Transcriptomic analysis of the *B. amyloliquefaciens* genome has revealed the presence of numerous genes related to rhizosphere adaptation and traits beneficial to plants. These include the utilization of plant polysaccharides, cell motility, chemotaxis, the synthesis of secondary antibiotics, and clusters relevant to promoting plant growth [48]. Gao et al. [46] demonstrated that both chemotaxis and swarming motility play important roles in the colonization of tomato roots by *B. subtilis*, with swarming being more significant than chemotaxis in this process.

However, root colonization by *Bacillus* strains is more effective when using indigenous strains compared with laboratory or commercial strains. Emerging strategies such as microbiome engineering and the development of crops optimized for microbe interactions can directly or indirectly identify, modify, and enhance the traits and mechanisms that improve the performance of *Bacillus* strains [30]. The genes responsible for root colonization and interactions between plants and *Bacillus* are activated in the presence of root and seed exudates [47, 49, 50]. Recent research on plant–bacteria interactions reveals the plant's ability to shape its rhizosphere (the soil zone influenced by root secretions) and endorhiza (inside the root) microbiome [51].

Certain microorganisms serve as biofertilizers and/or biopesticides, offering benefits to plants and fulfilling the same functions as chemical fertilizers and pesticides. Plant growth-promoting rhizobacteria (PGPR) are capable of significantly enhancing plant growth and represent a mutually beneficial interaction between plants and microbes. *Bacillus* species are a prominent group of rhizobacteria known for their ability to form spores, enabling them to survive in the soil for extended periods, even in harsh environmental conditions.

PGPR contribute to enhanced plant growth through several mechanisms, including the induction of systemic resistance, antibiosis (production of antibiotics), and competitive exclusion of harmful organisms. Consequently, microbial applications can be used to stimulate systemic resistance in plants against biotic agents, such as pathogens, and improve the plants' tolerance to environmental stresses. *B. subtilis*, in particular, employs both direct and indirect biocontrol mechanisms to suppress diseases caused by pathogens, making it a valuable asset in agriculture. The direct mechanism includes the synthesis of many secondary metabolites, hormones, cell-wall-degrading enzymes, and antioxidants that assist the plant in its defense against pathogen attack. The indirect mechanism includes the stimulation of plant growth and the induction of acquired systemic resistance. *B. subtilis* can also solubilize soil phosphorus,

improve nitrogen fixation, and produce siderophores to promote its own growth and inhibit that of pathogens. By activating stress response genes and phytohormones as well as stress-related metabolites to enhance stress tolerance in its plant host hosts, *B. subtilis* improves stress tolerance significantly. This review explores *B. subtilis*' rhizosphere activity as a root colonizer; potential biocontrol techniques; mechanisms of biocontrol implementation and its ability to boost crop output when under either biotic or abiotic stress conditions.

3 | BIOTECHNOLOGICAL APPLICATIONS OF *B. SUBTILIS*

For many years, *B. subtilis*, a Gram-positive bacterium frequently discovered in various settings, including soil and the digestive systems of cattle and humans, has garnered substantial attention in fundamental research endeavors. However, its value extends beyond academic study to practical industrial applications. This versatile microorganism's adaptability and diverse attributes have positioned it as a valuable asset in various industrial and agricultural domains. Its contributions to enzyme production, biocontrol, biopolymer synthesis, probiotic use, and bioremediation showcase its potential in shaping the landscape of modern biotechnology.

Two early instances of the applications of *B. subtilis* in biotechnology involve the production of tempeh and natto, traditional fermented soybean products renowned in Indonesia and Japan, respectively. These items are produced via soybean fermentation, facilitated by *B. subtilis* bacteria, while natto is produced using *Bacillus natto*. During fermentation, these bacteria produce enzymes that help break down soybeans, resulting in the unique tastes, consistencies, and nutritional properties found in tempeh and natto [52, 53].

One of the key factors contributing to the industrial utility of *B. subtilis* is its unique simple capacity for genetic manipulation. This bacterium's genome can be readily engineered and modified, allowing researchers to tailor its genetic makeup to produce desired compounds or proteins. Such genetic engineering capabilities have propelled *B. subtilis* into the realm of biotechnology, where it serves as a powerful "cell factory," churning out a wide range of industrially valuable products' remarkable ability to secrete proteins [54].

B. subtilis exhibits favorable characteristics for large-scale fermentation, a crucial aspect of successful industrial production. Its robust growth and adaptability to various environmental conditions enable efficient mass cultivation, making it a cost-effective option for large-scale bioprocesses [54]. Additionally, its ability to utilize

a wide range of carbon sources allows for flexibility in industrial applications, further cementing its status as a microbial workhorse for large-scale production [55].

Apart from its notable characteristics in its active state, the metabolically inactive spores of *B. subtilis* have attracted considerable interest because of their exceptional resistance to various environmental challenges. These spores present an exceptional foundation with extensive potential uses [48]. Their ability to survive harsh conditions, such as high temperatures, desiccation, and radiation, make them appealing for various purposes. The extraordinary resistance of *B. subtilis* spores opens up a realm of opportunities for innovative and sustainable applications.

This section explores the diverse biotechnological applications of *B. subtilis*, including enzyme production, biocontrol and plant growth promotion, probiotic and health benefits, and bioremediation and waste management use. The versatile bacterium showcases its potential in driving innovative solutions across various industries (Figure 2).

3.1 | Industrial enzyme production and biocatalysis

Industrial enzymes play a pivotal role in the biocatalysis and biotransformation sectors. They exhibit remarkable catalytic efficiency, specificity, and environmentally-friendly characteristics during production. *B. subtilis*, known for its wide distribution, safety, ease of cultivation, and amenability to genetic modifications, serves as a cost-effective source of enzymes [56].

Overall, industrial enzymes from *B. subtilis* have a significant impact on the field of biotechnology, driving advancements in various products and enhancing the performance and processes of existing ones. *Bacillus* spp. enzymes, in particular, hold prominence in this regard, accounting for approximately 50% of the overall enzyme market [57].

In biocatalysis, *B. subtilis* enzymes have been applied across numerous industrial sectors. Proteases (which represent approximately 30% of the total sales of enzymes worldwide) from *B. subtilis* aid in the breakdown of protein-based stains and are utilized in food, textile, pharmaceutical, and detergent production [58]. Lipases derived from *B. subtilis* catalyze the conversion of fats and oils into biodiesel fuel, finding applications in biodiesel production [59]. Therefore, they have received considerable attention because of their potential use in industrial processes, especially as biocatalysts [60].

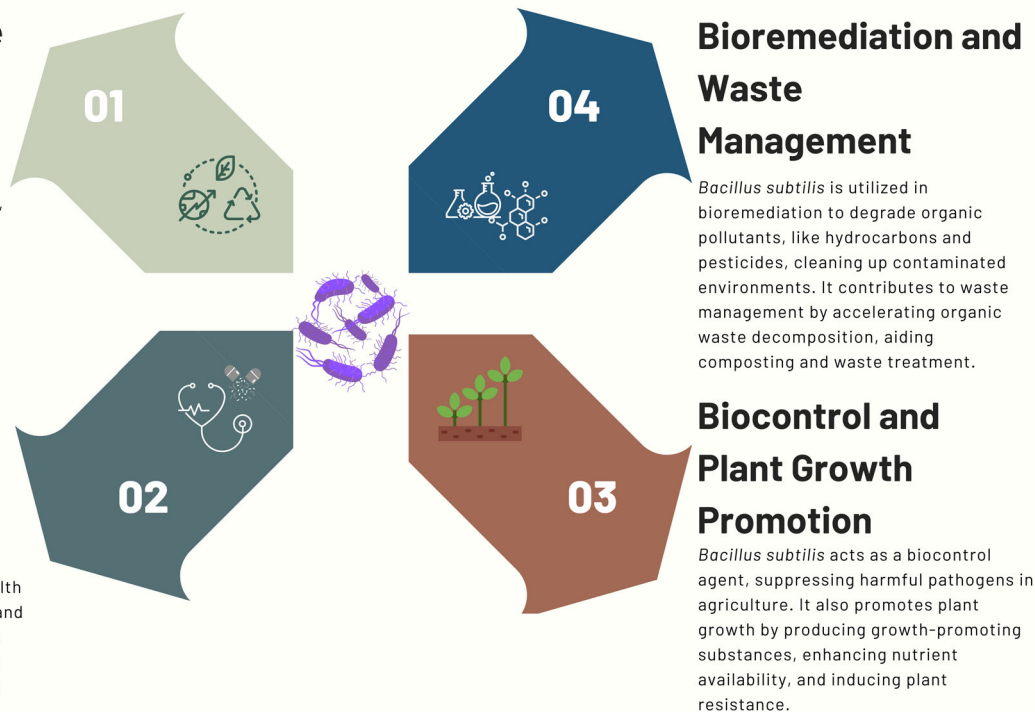
B. subtilis amylases, on the other hand, enhance the production of glucose and other valuable products

Industrial Enzyme Production and Biocatalysis

Bacillus subtilis is employed to produce enzymes such as amylases, proteases, and lipases. These enzymes have applications in various industries, including food processing and detergent production

Probiotics and Pharmaceutical Uses

Bacillus subtilis strains are used as probiotics in animal feed and food supplements. They improve gut health and nutrient absorption in animals and humans. *Bacillus subtilis* strains are used to produce antibiotics such as bacitracin and polymyxin, which are used in medicine.



Bioremediation and Waste Management

Bacillus subtilis is utilized in bioremediation to degrade organic pollutants, like hydrocarbons and pesticides, cleaning up contaminated environments. It contributes to waste management by accelerating organic waste decomposition, aiding composting and waste treatment.

Biocontrol and Plant Growth Promotion

Bacillus subtilis acts as a biocontrol agent, suppressing harmful pathogens in agriculture. It also promotes plant growth by producing growth-promoting substances, enhancing nutrient availability, and inducing plant resistance.

FIGURE 2 Biotechnological applications of *B. subtilis* (authors compilation).

by facilitating starch hydrolysis in the food and starch industry [61]. Amylases, specifically alpha- and beta-amylases, are hydrolases that hold significant industrial importance. Hydrolases, as a broad category of enzymes, are widely utilized in various industrial applications. However, among hydrolases, amylases have received special attention due to their diverse and extensive practical applications [62]. These enzymes, which are generated by a broad variety of microorganisms, catalyze the hydrolysis of starch. However, for industrial purposes, they are often sourced from *Bacillus*. *Bacillus*-produced amylases exhibit notable heat resistance, making them highly valuable in commercial applications where elevated temperatures are necessary [58, 63].

Microbial proteolytic enzymes, characterized by their extensive diversity, have garnered the interest of the scientific community. Despite their extensive research history, these enzymes continue to exhibit significant promise for practical utilization in both the biomedical and agricultural sectors [64].

There are two methods of fermentation by which enzymes are produced from *B. subtilis*. These are solid-state fermentation (SSF) and submerged-state fermentation. SSF is a novel approach, while submerged fermentation (SmF) is a conventional method used for the production of enzymes from microorganisms, which has been employed for a longer duration. In SmF,

enzyme production utilizes liquid substrates such as molasses and broths, allowing the end products of fermentation to be released into the liquid medium. SmF demonstrates rapid substrate utilization, necessitating a continuous supply of substrate throughout the fermentation process [65]. Separating enzymes from substrates can be challenging, and reaction conditions often require strict control, which can lead to enzyme inactivation and hinder reuse. However, a solution lies in displaying enzymes on the surface of spores. The exceptional stress resistance of spores enhances enzyme stability in complex environments and facilitates their reusability, allowing for more efficient and sustainable processes (Table 1) [56].

Biocatalysis using enzymes from *B. subtilis* offers numerous advantages. These enzymes possess high specificity, enabling selective and efficient reactions under mild reaction conditions. They reduce the need for harsh chemicals, lower energy consumption, and minimize environmental impact. *B. subtilis* enzymes also exhibit stability and robustness, allowing for their reuse in multiple cycles. The potential of *B. subtilis* in industrial enzyme production and biocatalysis continues to be explored through genetic engineering and optimization of fermentation processes. Researchers strive to enhance enzyme productivity, tailor enzyme properties, and discover novel enzymes with unique functionalities. These advancements pave the way for the development

TABLE 1 Main compounds produced by industrial fermentation of *B. subtilis* (adapted from Shahid and colleagues [63, 66]).

Products	Industrial application
Enzymes	
Alpha-acetolactate decarboxylase	Beverage
Alpha-amylase	Food, Paper, Starch, Textile, Brewing
Beta-glucanase	Beverage
Beta-glucosidase	Brewing
Cellulase	Detergents
Cyclodextrin glucanotransferase	Food, Pharma, Cosmetics
Galactomannase	Feed, Beverage
Glutaminase	Food, Flavor
Lipases	Detergent
Neutral (metallo-) protease	Detergent, Food
Alkaline (serine-) protease	Detergent, Textile
Penicillin acylase	Pharma
Pullulanase	Starch, Food, Beverage
Poly-gamma-glutamic acid	Food, Pharma, Cosmetics
Urease	Analysis, Beverage
Xylanases	Baking, Feed, Beverage, Brewing, Food
Fine chemicals	
D-ribose	Food, feed, cosmetics, pharma
Poly-gamma-glutamic acid	Food, feed, pharma
Purine nucleosides (inosine, guanosine)	Food
Riboflavin	Food, pharma
Streptavidin	Microarrays
Thaumatococcus	Food, pharma
Surfactants	Pharma, bioremediation
Others	
Natto (fermented soy)	Food
Animal probiotics	Feed

of more sustainable and cost-effective biotechnological processes.

3.2 | Biocontrol and plant growth promotion

Plants are exposed to diverse biotic factors such as viruses, bacteria, nematodes, weeds, and arachnids. These agents

create biotic stress, disrupting the plants' regular metabolic functions. Consequently, this stress hinders plant growth and development, and in severe cases, can even result in plant mortality. Certain biotic agents establish symbiotic or synergistic relationships with their host plants. In some cases, specific microbes exhibit beneficial characteristics and function similarly to chemical fertilizers and pesticides, acting as biofertilizers and/or biopesticides [67]. PGPR exemplify a mutualistic plant-microbe interaction that significantly enhances plant growth. These PGPRs assist in promoting plant development through various mechanisms, such as nutrient solubilization, production of growth-stimulating compounds, biological nitrogen fixation, and disease suppression. The symbiotic association between these beneficial microbes and plants results in mutual benefits, fostering healthier plants and sustainable agricultural practices [68].

PGPR exert their influence on plant growth through both direct and indirect mechanisms, as described by Gonzalez-Covarrubias et al. [69]. Direct mechanisms encompass bacterial traits that directly stimulate plant growth, such as the production of auxin, ACC deaminase, cytokinin, GA, nitrogen fixation, phosphorous solubilization, and the sequestration of iron through bacterial siderophores. These mechanisms directly contribute to the promotion of plant growth.

On the other hand, indirect mechanisms refer to bacterial traits that inhibit the activity of plant pathogens, including fungi and bacteria. These mechanisms act by hindering the functioning of one or more pathogenic organisms. Indirect mechanisms include ACC deaminase, antibiotics, cell wall degrading enzymes, competition, hydrogen cyanide production, induced systemic resistance (ISR), quorum quenching, and the production of siderophores [69].

Bacillus strains demonstrate their biocontrol capabilities primarily through two key mechanisms. To begin, they exhibit the capacity to impede the growth and propagation of plant pathogens. This hindrance is attributable to the production of substances with antimicrobial properties, encompassing antibiotics, antifungal peptides, and enzymes. These agents function directly to target and stifle pathogen growth, as documented by Gaballa et al. [41].

These antimicrobial agents disrupt the development, progression, and viability of pathogenic entities, effectively thwarting their establishment and dissemination. *B. subtilis*, when colonizing plant surfaces, assumes the role of a protective barrier akin to a biofilm. This biofilm structure serves to obstruct entry points exploited by pathogens, a phenomenon elucidated by Muras et al. [70]. By limiting the growth of plant pathogens, *Bacillus* strains help prevent disease development and protect the

health of plants. Second, *Bacillus* strains induce systemic resistance in plants. This process, referred to as ISR, encompasses the activation of the plant's inherent defense mechanisms. When *Bacillus* strains establish colonization within the plant's rhizosphere or other plant tissues, they initiate signaling pathways that prompt the synthesis of defense-associated compounds like phytohormones, pathogenesis-related (PR) proteins, and secondary metabolites. These defense molecules enhance the plant's ability to resist and combat pathogenic attacks. ISR offers extended safeguarding to plants and exhibits efficacy against a diverse spectrum of pathogens [41].

The inherent abilities of *B. subtilis* to counteract plant pathogens have rendered it a valuable asset as a biocontrol agent in agriculture. It has displayed considerable potential in safeguarding crops from diverse diseases, thereby lessening dependence on synthetic pesticides and fostering the adoption of sustainable agricultural methods. In addition to its biocontrol functions, *B. subtilis* displays remarkable capabilities in fostering plant growth and development. It synthesizes a wide array of substances that promote plant growth, among them phytohormones like auxins, cytokinins, and GAs [71].

These hormones trigger diverse physiological processes within plants, encompassing cell division, elongation, and differentiation, ultimately culminating in heightened growth and development. Moreover, *B. subtilis* facilitates the acquisition and utilization of nutrients by dissolving phosphate compounds, liberating bound nutrients in the soil, and fixing atmospheric nitrogen, thus augmenting nutrient availability for plants [69]. Furthermore, it produces enzymes that enhance nutrient uptake and metabolic processes in plants, contributing to improved nutrient efficiency. The bacterium also exhibits inherent stress tolerance mechanisms and can produce stress-responsive proteins and enzymes, enabling plants to better withstand adverse environmental conditions such as drought, salinity, and temperature extremes. This stress tolerance conferred by *B. subtilis* enhances plant resilience and survival, promoting overall plant health and productivity [72].

The utilization of *B. subtilis* for biocontrol and enhancing plant growth presents a sustainable and comprehensive method for agriculture. By tapping into the beneficial attributes of this bacterium, farmers can reduce their dependence on synthetic pesticides, alleviate ecological risks, and enhance soil and ecosystem health. *B. subtilis* demonstrates remarkable efficacy in combating pathogens, enhancing nutrient availability, and enhancing stress tolerance in plants, underscoring its significant potential in shaping the trajectory of sustainable agriculture.

3.3 | Probiotics and health benefits

Probiotics are microorganisms that provide beneficial effects on human health by supporting the body in maintaining a harmonious community of microorganisms, including gut microbes' balance when used in specific quantities [73]. *B. subtilis*, an intriguing strain with promising implications for improving wellness and overall quality of life, has garnered significant interest. The therapeutic benefits and health-enhancing characteristics associated with *B. subtilis*, as a probiotic strain, are widely recognized [6].

An important benefit of *B. subtilis* as a probiotic lies in its skill to uphold a harmonious gut microbiota. By inhabiting the intestines, *B. subtilis* engages in competition with detrimental bacteria for nourishment and adhesion points, thereby thwarting the excessive proliferation of pathogens and fostering a wide-ranging and advantageous microbial assemblage. Consequently, this supports the development of a more robust gut milieu and enhances overall gut well-being [74].

B. subtilis has been noted for its contribution to optimizing digestive capabilities. It is known to generate a diverse spectrum of digestive enzymes, encompassing proteases, lipases, and amylases, which facilitate the decomposition of intricate dietary elements [75]. This enzymatic engagement contributes to the effective breakdown and assimilation of nutrients, ultimately resulting in enhanced nutrient utilization and promoting general digestive wellness.

Nattokinase, an enzyme sourced from *B. subtilis*, exhibits remarkable anticlotting properties. This enzymatic activity enables the dissolution of blood clots, presenting a valuable therapeutic option for addressing cardiovascular conditions. Nattokinase operates through the degradation of fibrin and plasmin, resulting in the transformation of prourokinase into urokinase. This sequence also encompasses the degradation of plasminogen activator inhibitor-1 (PAI-1) and augmentation in tissue plasminogen activator (t-PA), thus fostering fibrinolysis. It is noteworthy to emphasize that one distinguishing attribute of nattokinase is its minimal or inconsequential undesirable impacts relative to alternative fibrinolytic enzymes. This emphasizes its potential as a safe and effective option for clinical use [13]. The utilization of *B. subtilis* natto DG101, in combination with suitable medication, has emerged as a promising and innovative intervention in combating the global diabetes epidemic [76].

B. subtilis, recognized as a probiotic, presents an added benefit with its capacity to positively influence the immune system. Probiotics can trigger immune factor production, including cytokines and immunoglobulins, fostering an equilibrium in immune reactions and bolstering the body's resistance against infections [77].

This immune-enhancing effect may contribute to improved resistance to common illnesses and support overall immune system functionality.

3.4 | Bioremediation and waste management

The increase in urbanization, combined with the industrial revolution and the mounting challenges of population growth, have placed substantial strain on the global environment. As society advances, numerous unsustainable practices have arisen, leading to the creation of extensive waste volumes that play a role in the contamination of our surroundings [30]. Microbial bioremediation stands out as an economically viable, flexible, rapid, and environmentally conscious approach to eliminating pollutants from the surroundings. The exceptional attributes of *B. subtilis* have positioned it as a potential asset in the realm of bioremediation and effective waste control. This versatile bacterium exhibits unique characteristics and metabolic pathways that make it highly valuable for addressing environmental pollution and managing various types of waste, contributing to sustainable solutions and ecosystem restoration.

B. subtilis exhibits remarkable proficiency in bioremediation, particularly in degrading and metabolizing a range of organic pollutants. This involves various substances such as hydrocarbons, petroleum compounds, polycyclic aromatic hydrocarbons (PAHs), pesticides, and industrial chemicals. *B. subtilis* demonstrates a diverse range of metabolic capabilities, which empower it to degrade and transform these pollutants into less detrimental compounds. As a result, it plays a vital role in rehabilitating and revitalizing polluted environments [2].

The biodegradative potential of *B. subtilis* is due to its ability to synthesize diverse enzymes, bio-surfactants, and bio-degradative compounds. This intricate enzymatic and metabolic interplay enables *B. subtilis* to efficiently dismantle complex organic pollutants, transforming them into simpler and less detrimental forms [78]. By doing so, *B. subtilis* effectively reduces the toxicity and environmental impact of these pollutants. This biodegradation process not only facilitates the remediation of contaminated sites but also plays a crucial role in the restoration and preservation of ecosystems [79].

Through its transformative capabilities, *B. subtilis* contributes to the reduction of pollutants, promoting the health and sustainability of the environment. *B. subtilis* has emerged as a promising solution for addressing the environmental repercussions of oil spills [27]. This adaptable microorganism possesses a distinctive talent

for metabolizing hydrocarbons, encompassing both crude oil and petroleum derivatives, for sustenance. This metabolic flexibility equips *B. subtilis* to play an active role in remediating environments tainted by oil, facilitating the disintegration and elimination of pollutants while aiding the recovery of affected ecosystems [27]. Through leveraging its enzymatic proficiencies, *B. subtilis* adeptly breaks down hydrocarbons within oil spills, converting them into less complex and less hazardous byproducts [27]. The utilization of *B. subtilis* in oil spill clean-up offers several advantages over conventional methods. Instead of relying solely on mechanical means, such as containment and physical removal, the introduction of *B. subtilis* harnesses the power of nature to actively degrade the pollutants. This approach is more sustainable and environmentally friendly, as it reduces the need for harsh chemicals and minimizes disturbance to sensitive ecosystems [80].

The application of *B. subtilis* capitalizes on its inherent capabilities, amplifying bioremediation effectiveness and contributing to the sustained recovery of ecosystems. This approach aligns with sustainability principles, utilizing natural processes to address oil spill environmental challenges. *B. subtilis* demonstrates promise as an eco-friendly solution, effectively utilizing hydrocarbons, degrading pollutants, and actively participating in the bioremediation process.

B. subtilis offers valuable contributions to waste treatment and management. It serves as a potent agent for decomposing and breaking down diverse organic waste materials, including agricultural and food waste, as well as sewage sludge. The bacterium's enzymatic capabilities effectively dismantle complex organic compounds, transforming them into more environmentally benign forms. This dual action not only reduces waste volume but also addresses potential odor concerns linked to waste buildup. The utilization of *B. subtilis* supports the advancement of eco-friendly waste disposal and recycling methods, enhancing the overall sustainability and efficiency of waste management practices [81].

B. subtilis also demonstrates the potential for heavy metal remediation in contaminated environments [66]. It exhibits the capacity to endure and eliminate heavy metals like lead, cadmium, and copper from contaminated areas. *B. subtilis* can effectively capture and isolate heavy metal ions using diverse approaches, encompassing biosorption and precipitation mechanisms. This process reduces the bioavailability of heavy metals, minimizing their potential harm to living organisms and ecosystems. The application of *B. subtilis* in heavy metal remediation offers a promising approach to restoring and rehabilitating sites affected by heavy metal contamination [66, 82].

B. subtilis in bioremediation and waste management represents a significant step toward sustainable environmental practices. This versatile bacterium's ability to degrade organic pollutants, aid in oil spill clean-up, facilitate waste decomposition, and assist in heavy metal remediation showcases its immense potential in addressing environmental challenges. However, site-specific conditions, optimization of process parameters, and a comprehensive understanding of *B. subtilis*' interactions with target pollutants are crucial factors in achieving successful bioremediation and waste management outcomes.

4 | RECENT ADVANCES IN BACILLUS RESEARCH

In recent times, the exploration of *B. subtilis* has undergone remarkable strides, giving rise to a wealth of novel insights and captivating breakthroughs across various domains. These contemporary advancements have illuminated the intricate facets of *B. subtilis* and its potential utilities across diverse spheres. Substantial headway has been achieved in the realm of novel genetic manipulation tools tailored for *B. subtilis*. These innovative instruments have streamlined the process of engineering *B. subtilis* for the generation of sought-after outcomes, including recombinant proteins, antibiotics, and biofuels.

Notably, scientists have made significant progress in comprehending the metabolic intricacies of *B. subtilis* in recent years. This heightened understanding has spurred the formulation of novel strategies aimed at elevating the production of desired outputs by *B. subtilis* [83].

In 2023, researchers used clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR-Cas9) technology to engineer *B. subtilis* to produce a recombinant protein that could be used to prevent a type of cancer. This was a significant advance, as it showed that CRISPR-Cas9 could be used to modify the genome of *B. subtilis* in a precise and efficient manner [56]. This technology has the potential to be used to develop new treatments for a variety of diseases including various forms of cancers such as prostate and cervical cancer [84, 85].

With the advent of high-throughput sequencing tools for genomic studies, researchers have been able to give a thorough insight into the genetic makeup of *B. subtilis* [57]. Scientists have developed a better grasp of its regulatory networks, metabolic processes, and functional capabilities because of comprehensive genome sequencing studies. The availability of specific *B. subtilis* strain genome sequences has paved the way for *B. subtilis*

targeted genetic engineering and manipulation, allowing researchers to modify its genetic material and metabolic pathways for specific purposes [86].

B. subtilis has been gaining attention for its environmental applications. Its ability to degrade a variety of pollutants, including hydrocarbons and heavy metals has positioned it as a potential candidate for bioremediation and waste management [81]. Also, the escalating environmental issues associated with conventional petroleum-based plastics have urged scientists to explore alternative strategies for sustainable plastic production.

Bioplastics, derived from renewable resources, have emerged as a promising avenue to mitigate plastic pollution and reduce dependence on fossil fuels. Among the numerous candidates investigated, *B. subtilis*, a gram-positive, spore-forming bacterium, has recently gained considerable attention for its potential in bioplastic synthesis [67, 87].

Researchers have focused on leveraging its ability to accumulate polyhydroxyalkanoates (PHA), a class of biodegradable polyesters, and more specifically, polyhydroxybutyrate (PHB). PHB exhibits properties akin to traditional petroleum-based plastics but possesses the distinct advantage of being biodegradable and sourced from renewable feedstocks [14, 88].

Significant progress has been made in understanding the genetic, physiological, and environmental factors influencing bioplastic production in *B. subtilis*. Genetic engineering approaches have been employed to optimize PHB production and tailor the material's properties for specific applications. Moreover, advances in fermentation technologies and downstream processing have enabled scalable and cost-effective bioplastic synthesis from *B. subtilis*.

B. subtilis' biotechnological application is expanding rapidly. Its robust growth, genetic tractability, and ability to secrete valuable proteins have positioned it as a desirable host for recombinant protein production. The enzymes and metabolites it produced have found uses in the food and beverage, textile, detergent, and pharmaceutical industries. Current research in this field aims to further improve the biotechnological potential of *B. subtilis* for industrial-scale production.

4.1 | Synthetic biology and genetic engineering tools

The field of synthetic biology encompasses the integration of engineering concepts into the formation and advancement of new biological systems. Through the utilization of genetic engineering instruments, the manipulation of genetic frameworks in living organisms

is facilitated, ushering in the possibility of forging new capabilities or refining those already in existence. With its ability to adapt for growth and modification inside controlled laboratory conditions as well as its thoroughly researched genetic makeup, *B. subtilis* holds a desirable position within the field of synthetic biology.

Recent progress in *B. subtilis* research has been significantly influenced by the fields of synthetic biology and metabolic engineering. Leveraging these domains, scientists have successfully crafted *B. subtilis* strains endowed with heightened traits and customized capabilities. This breakthrough has ushered in promising prospects for generating valuable substances, including biofuels, pharmaceuticals, and biopolymers, as highlighted by Liu et al. in 2019 [56]. The capacity to reconfigure *B. subtilis*' genetic apparatus has instigated a transformative wave in biotechnology, introducing eco-friendly and sustainable alternatives to established industrial procedures.

There are several genetic engineering tools that can be used to modify the genome of *B. subtilis*. These tools include Gene transfer methods and there are a number of methods that can be used to transfer DNA into *B. subtilis*. These methods include transformation, transduction, and conjugation [75].

Restriction enzymes: Restriction enzymes are proteins that cut DNA at specific sequences. They can be used to cleave the genome of *B. subtilis* at specific locations, which can then be repaired or replaced with new DNA [89].

DNA ligases: DNA ligases are enzymes that join DNA fragments together. They can be used to repair DNA that has been cleaved by restriction enzymes or to insert new DNA into the genome [90].

Researchers have also harnessed *B. subtilis* in tandem with innovations like CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9). These cutting-edge genome editing tools, including CRISPR/Cas9, have empowered researchers to make precise and focused changes to the bacterial genetic structure. This has streamlined the process of editing particular genes. This breakthrough has ushered in fresh avenues for delving into the intricacies of *B. subtilis*' biology and crafting innovative biotechnological uses. These tools have streamlined strain engineering and expedited the development of specific *B. subtilis* variants [7].

The evolution of these genetic manipulation techniques has opened up avenues to create a diverse array of genetically altered strains of *B. subtilis*. These strains have found utility in the production of an array of bio-based outputs, encompassing enzymes, antibiotics, and vaccines. Moreover, they have proven instrumental in

dissecting bacterial genetics and physiology, while also birthing innovative approaches for bioremediation and biotransformation.

Advancements made in the field of synthetic biology along with the development of genetic engineering tools have propelled *B. subtilis* to a position as a robust platform for nurturing new bio-derived goods and technologies. This microbe is positioned to play a progressively significant role in the landscape of synthetic biology in the coming times.

However, despite this substantial progress, certain challenges still impede the complete realization of *B. subtilis*' potential in the field of synthetic biology. These challenges include improving transformation efficiency, reducing genome instability, and developing standardized genetic parts for predictable biological performance [91]. Addressing these challenges will undoubtedly pave the way for *B. subtilis* to become an even more robust tool in synthetic biology and genetic engineering.

4.2 | Omics approaches and systems biology studies

Omics approaches refer to a collection of techniques used for studying the expression and function of genes, proteins, and other biological molecules. Systems biology is a field that utilizes omics approaches to investigate the interactions between biological molecules and processes [92]. The progress made in "omics" technologies like genomics, transcriptomics, proteomics, and metabolomics has greatly advanced our comprehension of *B. subtilis* at a level [93].

The combination of omics approaches with systems biology has provided researchers with an understanding of the biology of *B. subtilis*. By integrating genomics, transcriptomics, proteomics, and metabolomics approaches researchers have gained insights into the networks and metabolic pathways that control the physiology of this model bacterium [92].

This knowledge has paved the way for developing strategies to manipulate the bacterium's genome and enhance its production of bioproducts [75].

4.2.1 | Genomics and transcriptomics

The introduction of sequencing technologies has greatly advanced the study of genomics and transcriptomics in *B. subtilis*. Now through genome sequencing and RNA sequencing, we have such an amount of data that illuminate what is genetic about this bacterium, and how it regulates gene expression. These discoveries

triggered elements, noncoding RNAs as well as gene clusters involved with specialized metabolite production [50]. As a result, we have gained an understanding of how this bacterium responds to environmental cues and stressful conditions, including processes, like sporulation, stress response, and nutrient metabolism. Comprehensive transcriptomics analysis of *B. subtilis* salt stress adaptation by Hannes et al. showed an unparalleled and abundant foundation is now available, offering a remarkable opportunity for profound exploration into the physiological and genetic reactions of *B. subtilis* toward hyperosmotic stress [52]. Additional research has made significant strides in the field of *B. subtilis* transcriptomics. By comparing the transcriptome profiles of *B. subtilis* from two distinct missions to the International Space Station, scientists sought to gain insights into how these bacteria respond to the unique environmental conditions of spaceflight [12].

4.2.2 | Proteomics

High-throughput proteomics approaches have facilitated the comprehensive analysis of *B. subtilis* proteomes. Quantitative proteomics has allowed researchers to monitor protein abundance changes under various conditions, providing insights into cellular adaptation and response mechanisms. Protein-protein interaction studies have unveiled key players in complex regulatory networks, unraveling the functional organization of the bacterium [32]. Hannes's et al. comprehensive proteomics analysis of *B. subtilis* salt stress adaptation has yielded an unprecedented wealth of data. This rich foundation now provides a remarkable opportunity to delve deeply into the physiological and genetic responses of *B. subtilis* to hyperosmotic stress [52]. By conducting a thorough analysis of the *B. subtilis* proteome, researchers have not only identified novel open reading frames (ORFs) but have also traced the evolutionary history of modified proteins [28]. Further investigations in the field of high-pressure processing (HPP) technology for the food industry have been carried out, including studies on the Proteomic Response of *B. subtilis* Spores under High-Pressure Combined with Moderate Temperature and Random Peptide Mixture LK Treatment. These research efforts aim to enhance and refine the HPP techniques used in the food sector [94].

4.2.3 | Metabolomics

The study of metabolomics, in *B. subtilis* has provided information about how the bacterium responds to

environmental factors and genetic changes. Metabolomics involves examining all the molecules, called metabolites that exist within *B. subtilis* cellular system. This research field aims to understand the metabolic pathways, interactions, and regulatory mechanisms that govern *B. subtilis*' cellular metabolism. By analyzing and characterizing the organism's metabolites scientists can gain insights into its processes. How it reacts to various environmental conditions or genetic modifications. Metabolomics is essential for unraveling *B. subtilis* complex metabolic network and exploring its applications, in biotechnology bioengineering and biomedicine [69].

By profiling the cellular metabolite composition, metabolomics has identified critical metabolic pathways and key metabolites involved in various physiological processes. Integration with transcriptomics and proteomics data has led to more comprehensive models of *B. subtilis* metabolism.

Several research has been conducted with a focus on the metabolomics of *B. subtilis*. A study was conducted using metabolomic approaches to understand the SSF of okara using *B. subtilis* WX-17 for enhanced nutritional profile [36]. Similarly, the profiling of metabolites of *Bacillus* spp. has been undertaken to evaluate potential for their application in sustainable plant growth promotion and biocontrol [95].

The integration of omics data in systems biology studies has led to a holistic understanding of *B. subtilis* at the systems level. Computational modeling and network analysis have been employed to construct detailed regulatory networks and metabolic models. These systems-level analyses have revealed novel regulatory hubs and feedback loops that govern the bacterium's responses to dynamic environments [82].

Advances in omics approaches and systems biology have hugely influenced the study of *B. subtilis*. New methods to alter the genome of this bacterium, among other enhancements to its production of bioproducts, resulted from advancements made in these two fields. Such developments are likely to grow further through the years ahead, allowing a deeper understanding of such an important bacterium [93].

4.2.4 | Biotechnological and medical applications

The insights gained from omics and systems biology studies in *B. subtilis* have promising applications in biotechnology and medicine. *B. subtilis* has been engineered to produce biofuels, industrial enzymes, and bioactive compounds more efficiently. Furthermore,

systems biology approaches are aiding in the identification of potential drug targets and understanding host–pathogen interactions for medical applications.

5 | EMERGING RESEARCH AREAS AND FUTURE DIRECTIONS

Evidently, as one of the most studied model organisms, *B. subtilis* has provided fundamental insights into key basis in microbiological research. It has, furthermore, become a logical step forward in its discovery by Christian Gottfried Ehrenberg to modern-day applications in biotechnology with agriculture medicine as well as environmental bioremediation, *B. subtilis* prominent features in scientific understanding along with the practical application [24].

Throughout the years, extensive progress has been made in the genetic, physiological, and biochemical characterization of *B. subtilis*, culminating in a profound comprehension of its intricate cellular machinery. The elucidation of its genome, transcriptome, proteome, and metabolome has significantly contributed to a comprehensive, systems-level understanding of the behavior of this highly adaptable bacterium. Moreover, the emergence of integrative approaches, such as systems biology and computational modeling, has bestowed researchers with a holistic perspective on the complex regulatory networks and dynamic interactions that govern the physiology of *B. subtilis*.

Genomics and transcriptomics have been pivotal in identifying novel genes, noncoding RNAs, and regulatory elements that underpin *B. subtilis*' diverse phenotypes and responses to various environmental cues [50]. Furthermore, the application of proteomics and metabolomics has not only expanded our knowledge of the bacterial proteome and metabolite repertoire but also allowed us to decipher the intricate metabolic pathways and responses to stress that contribute to its survival in challenging conditions [93].

Advancements in genome editing technologies, such as CRISPR-Cas9, are opening new horizons for *B. subtilis* research [56]. Researchers are now exploring precise genome engineering to introduce targeted modification, including gene knockouts, knock-in, and regulatory element manipulations. These advancements offer the potential to tailor *B. subtilis* strains for specific biotechnological applications, fine-tuning their capabilities and enhancing their efficiency in enzyme production or bioremediation.

The introduction of synthetic biology and genetic engineering has ushered in exciting possibilities for customizing *B. subtilis* strains, enhancing their characteristics to suit diverse biotechnological purposes [96]. These genetically modified strains have shown promising

potential as efficient cell factories, facilitating the production of enzymes, pharmaceuticals, and biofuels, thereby making valuable contributions to the bioeconomy and fostering sustainable industrial practices.

Beyond biotechnology, *B. subtilis* has garnered significant interest in agriculture, where its use as a biofertilizer and biocontrol agent holds promise for enhancing crop yield and combatting plant pathogens in a sustainable and eco-friendly manner [97].

In the medical field, emerging research points to *B. subtilis*' potential as a probiotic and therapeutic agent, offering avenues for addressing gastrointestinal disorders and infections and fostering gut health [98].

In response to the global coronavirus disease 2019 (COVID-19) pandemic, researchers have been investigating the potential of *B. subtilis* as a therapeutic agent for COVID-19 treatment [42]. This emerging research area shows promise due to several compelling aspects of *B. subtilis* that make it an attractive candidate for further study. *B. subtilis* has demonstrated immunomodulatory properties, meaning it can regulate and enhance the immune response.

This could be beneficial in managing the overactive immune response often seen in severe COVID-19 cases, where cytokine storms contribute to adverse outcomes. By modulating the immune system, *B. subtilis* may help prevent excessive inflammation and improve patient outcomes. Although its specific efficacy against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus responsible for COVID-19 is not fully established, early findings suggest that *B. subtilis* may have inhibitory effects on viral replication, which warrants further investigation. It is crucial to note that while these emerging research areas show promise, the use of *B. subtilis* to cure COVID-19 is still in the experimental phase. Extensive clinical trials and studies are necessary to assess safety, efficacy, and optimal dosages before any widespread therapeutic application can be considered.

The environmental significance of *B. subtilis* cannot be underestimated, as studies have highlighted its ability to degrade various pollutants, making it a potential candidate for environmental bioremediation and contributing to efforts aimed at preserving and restoring ecosystem balance. In 2023, Salmazo et al. [21] demonstrated that *B. subtilis* exhibited the ability to degrade benzyl, toluene, ethylbenzene and xylenes (BTEX) compounds while simultaneously producing biosurfactants. Furthermore, this remarkable bacterium showcased its potential for various industrial applications. Bioremediation proves to be a highly effective, cost-efficient, and adaptable solution for addressing BTEX contamination, encompassing Benzene, toluene, ethylbenzene, and xylene pollutants [21].

Primarily, the paramount importance of research on *B. subtilis* lies in advancing fundamental science but also

TABLE 2 Ecological, biotechnology and future challenges in harnessing *Bacillus subtilis*.

Challenges	Description
<i>Ecological significance challenges</i>	
Competition with other microbes	<i>Bacillus subtilis</i> faces competition with other microorganisms for resources and niches in various ecosystems [25, 69].
Environmental stress	The microbe must endure environmental stressors like temperature fluctuations, pH changes, and nutrient availability [16].
Antibiotic resistance	There is a concern that <i>B. subtilis</i> could develop antibiotic-resistance genes, which could impact ecosystems [99].
Disruption of microbial communities	The introduction of <i>B. subtilis</i> for bioremediation or other applications could disrupt existing microbial communities [20].
<i>Biotechnological application challenges</i>	
Scalability of production	Upscaling the production of <i>B. subtilis</i> for industrial purposes may be challenging while maintaining quality [100].
Consistency in product output	Achieving consistent yields of desired bioproducts can be difficult due to variations in microbial behavior.
Regulatory approval	Regulatory hurdles may arise for novel biotechnological applications involving <i>B. subtilis</i> in various industries [98, 101].
Potential allergenicity	In some applications, products derived from <i>B. subtilis</i> might trigger allergic reactions in sensitive individuals [46].
<i>Future challenges</i>	
Emergence of novel pathogens	The potential for <i>B. subtilis</i> to evolve into new pathogenic strains could pose risks to both human health and ecosystems.
Sustainability and ethics	Balancing the benefits of <i>B. subtilis</i> applications with environmental and ethical considerations requires attention.
Technological advancements	Rapid advancements in biotechnology could render certain <i>B. subtilis</i> -based applications obsolete or less competitive.
Intellectual property and access	Issues related to intellectual property rights and equitable access to <i>B. subtilis</i> -based technologies might arise.

in its potential to address critical challenges in various sectors. Nonetheless, amidst these exciting prospects, there are considerable challenges faced by the scientific community that includes those related to safety, ethical consideration as well as translating fundamental discoveries into real-world applications (Table 2).

6 | CHALLENGES AND PROSPECTS

While *B. subtilis* is a promising model organism, its biology does not universally apply to all bacterial species. While providing valuable insights, its unique mechanisms and genetic pathways may not translate directly to other microbes. Despite advances in genomics, proteomics, and other omics techniques, there is an inherent complexity in the regulatory networks and cellular machinery of *B. subtilis* that still requires comprehensive elucidation.

Potential concerns related to safety and ethical considerations in genetic engineering and synthetic biology. Gaining social acceptance and addressing concerns about genetically modified organisms (GMOs) requires rigorous scientific communication and public engagement. Translating laboratory findings to real-world applications remains challenging. While *B. subtilis* shows great potential in various sectors, its effectiveness and scalability in actual environments, like agricultural fields or clinical settings, need further investigation.

6.1 | Novel technologies and interdisciplinary approaches for sustainable development

Modern research into *B. subtilis* stands at the nexus of biology, computational science, and engineering. The emergence of systems biology and computational

modeling offers interdisciplinary methods to understand the complex interactions within this bacterium. These approaches can be instrumental in designing bacterial strains with desired traits, optimizing pathways for biofuel production, and creating predictive models for bacterial behavior under different environmental conditions.

Moreover, advancements in synthetic biology offer the ability to craft custom genetic circuits within *B. subtilis*, enabling it to perform tailored tasks, from environmental sensing to synthesizing complex molecules. These breakthroughs can potentially revolutionize industries, driving them toward sustainable and eco-friendly practices.

6.2 | Potential applications yet to be explored

- The potential role of *B. subtilis* in addressing new and emerging global challenges, such as newer infectious diseases beyond COVID-19.
- Leveraging its bio-remediatory properties to tackle other forms of environmental pollution, beyond BTEX compounds.
- Exploring its potential in bioplastics production or other sustainable alternatives to current industrial processes.

7 | CONCLUSION

Since its discovery by Christian Gottfried Ehrenberg, *B. subtilis* has become a cornerstone of microbiological research. Genomic, transcriptomic, proteomic, and metabolomic studies, have increased our understanding of its cellular machinery. This bacterium has shown significant versatility in its applications, from enhancing agricultural practices through biocontrol mechanisms such as the synthesis of many secondary metabolites, hormones, cell-wall-degrading enzymes, and antioxidants that assist the plant in its defense against pathogen attacks to its promising roles in medicine through the production of probiotics by leveraging its ability to regulate gastrointestinal flora, and bioremediation by using the bacterium as an anaerobic decomposer to degrade pollutants. The potential applications of *B. subtilis* go beyond agriculture, medicine, and waste remediation. Its genetic plasticity and adaptability present multiple possibilities for applications in these and other industries. However, potential future applications of the bacterium face some challenges, including ethical concerns, safety issues, and the challenge of

translating laboratory success to real-world efficiency. Nevertheless, the continued interdisciplinary collaboration between fields promises a future where *B. subtilis* might play an even more central role in sustainable practices and innovative solutions to global challenges. Ultimately, the study of *B. subtilis* stands as a testament to the immense potential held within even the smallest of nature's creations. As research unfolds, the bacterium's promise to address some of humanity's most pressing issues becomes more palpable, marking a bright path for science and application.

AUTHOR CONTRIBUTIONS

Adenike A Akinsemolu: Conceptualization; writing—original draft; writing—review & editing. **Helen Onyeaka:** Conceptualization; supervision; writing—review & editing. **Samuel Odion:** Writing—original draft. **Idris Adebajo:** Writing—original draft.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Adenike A. Akinsemolu  <https://orcid.org/0000-0001-6360-9356>

Helen Onyeaka  <http://orcid.org/0000-0003-3846-847X>

REFERENCES

- [1] Errington J, Wu LJ. Cell cycle machinery in *Bacillus subtilis*. *Subcell Biochem.* 2017;84:67–101.
- [2] Elenga-Wilson PS, Kayath CA, Mokemiabeka NS, Nzaou SAE, Nguimbi E, Ahombo G. Profiling of indigenous biosurfactant-producing bacillus isolates in the bio-remediation of soil contaminated by petroleum products and olive oil. *Int J Microbiol.* 2021;2021:1–15.
- [3] Hashem A, Tabassum B, Fathi Abd_Allah E. *Bacillus subtilis*: a plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi J Biol Sci.* 2019;26:1291–7.
- [4] Hu G, Wang Y, Blake C, Nordgaard M, Liu X, Wang B, et al. Parallel genetic adaptation of *Bacillus subtilis* to different plant species. *Microbial Genomics.* 2023;9(7):001064. <https://doi.org/10.1101/2023.03.17.533125>
- [5] Khalid A, Arshad M, Zahir ZA. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J Appl Microbiol.* 2004;96:473–80.
- [6] Kuddus M. Enzymes in food biotechnology: production, applications, and future prospects. London: Elsevier Academic Press Inc.; 2018.
- [7] Song Y, He S, Jopkiewicz A, Setroikromo R, van Merkerk R, Quax WJ. Development and application of CRISPR-based

- genetic tools in *Bacillus* species and *Bacillus* phages. *J Appl Microbiol.* 2022;133:2280–98.
- [8] Miljaković D, Marinković J, Balešević-Tubić S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms.* 2020;8:1037.
- [9] Nordgaard M, Blake C, Maróti G, Hu G, Wang Y, Strube ML, et al. Experimental evolution of *Bacillus subtilis* on *Arabidopsis thaliana* roots reveals fast adaptation and improved root colonization. *Iscience.* 2022;25:104406.
- [10] Karlapudi AP, Venkateswarulu TC, Tammineedi J, Kanumuri L, Ravuru BK, Dirisala V, et al. Role of biosurfactants in bioremediation of oil pollution—a review. *Petroleum.* 2018;4:241–9.
- [11] Martins SJ, Medeiros FHV, Lakshmanan V, Bais HP. Impact of seed exudates on growth and biofilm formation of *Bacillus amyloliquefaciens* ALB629 in common bean. *Front Microbiol.* 2018;8:2631.
- [12] Morrison MD, Fajardo-Cavazos P, Nicholson WL. Comparison of *Bacillus subtilis* transcriptome profiles from two separate missions to the international space station. *NPJ Microgravity.* 2019;5:1.
- [13] Weng Y, Yao J, Sparks S, Wang K. Nattokinase: an oral antithrombotic agent for the prevention of cardiovascular disease. *Int J Mol Sci.* 2017;18:523.
- [14] Vijay R, Tarika K. Microbial production of polyhydroxyalkanoates (PHAs) using kitchen waste as an inexpensive carbon source. *Biosci. Biotechnol. Res. Asia.* 2019;16:155–66.
- [15] Belda E, Sekowska A, Le Fèvre F, Morgat A, Mornico D, Ouzounis C, et al. An updated metabolic view of the *Bacillus subtilis* 168 genome. *Microbiology.* 2013;159:757–70.
- [16] Etesami H, Jeong BR, Glick BR. Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops—a review. *Curr Res Biotechnol.* 2023;5:100128.
- [17] Gu Y, Xu X, Wu Y, Niu T, Liu Y, Li J, et al. Advances and prospects of *Bacillus subtilis* cellular factories: from rational design to industrial applications. *Metab Eng.* 2018;50:109–21.
- [18] Toldra F, Kim SK. Marine enzymes biotechnology: production and industrial applications, Part II. In: Toldra F, editor. *Marine organisms producing enzymes.* San Diego: Elsevier Academic Press Inc; 2016.
- [19] Radhakrishnan R, Lee IJ. Gibberellins producing *Bacillus methylotrophicus* KE2 supports plant growth and enhances nutritional metabolites and food values of lettuce. *Plant Physiol Biochem.* 2016;109:181–9.
- [20] Mahapatra S, Yadav R, Ramakrishna W. *Bacillus subtilis* impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. *J Appl Microbiol.* 2022;132:3543–62.
- [21] Salmazo P, de Marco N, Soeiro VS, Castanho NRCM, Leite FG, Chaud MV, et al. Evaluation of *Bacillus subtilis* as a tool for biodegrading diesel oil and gasoline in experimentally contaminated water and soil. *Curr Microbiol.* 2023;80:94.
- [22] Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, et al. Microbial enzymes used in bioremediation. *J. Chem.* 2021;2021:1–17.
- [23] Glick BR. The enhancement of plant growth by free-living bacteria. *Can J Microbiol.* 1995;41:109–17.
- [24] Stülke J, Grüppen A, Bramkamp M, Pelzer S. *Bacillus subtilis*, a Swiss army knife in science and biotechnology. *J Bacteriol.* 2023;205:e00102–23.
- [25] Kobayashi K. Diverse LXG toxin and antitoxin systems specifically mediate intraspecies competition in *Bacillus subtilis* biofilms. *PLoS Genet.* 2021;17:e1009682.
- [26] Chowdappa P, Mohan Kumar SP, Jyothi Lakshmi M, Upreti KK. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. *Biol Control.* 2013;65:109–17.
- [27] Zahed MA, Matinvafa MA, Azari A, Mohajeri L. biosurfactant, a green and effective solution for bioremediation of petroleum hydrocarbons in the aquatic environment. *Discover Water.* 2022;2:5.
- [28] Ravikumar V, Nalpas NC, Anselm V, Krug K, Lenuzzi M, Šestak MS, et al. In-depth analysis of *Bacillus subtilis* proteome identifies new ORFs and traces the evolutionary history of modified proteins. *Sci Rep.* 2018;8:17246.
- [29] Gao S, Wu H, Yu X, Qian L, Gao X. Swarming motility plays the major role in migration during tomato root colonization by *Bacillus subtilis* SWR01. *Biol Control.* 2016;98:11–7.
- [30] Raj A, Jhariya M, Bargali S. Climate smart agriculture and carbon sequestration. In Pandey CB, Mahesh KG, Goyal RK editors. *Climate change and agroforestry: adaptation mitigation and livelihood security.* New Delhi: New India publishing agency (NIPA); 2018. p. 1–19.
- [31] Luo L, Zhao C, Wang E, Raza A, Yin C. *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: an overview for its mechanisms. *Microbiol Res.* 2022;259:127016.
- [32] Huang Y, Swarge BN, Roseboom W, Bleeker JD, Brul S, Setlow P, et al. Integrative metabolomics and proteomics allow the global intracellular characterization of *Bacillus subtilis* cells and spores. *J. Proteome Res.* 2023;23(2):596–608. <https://doi.org/10.1101/2023.06.19.545067>
- [33] Mnif I, Ghribi D. Potential of bacterial derived biopesticides in pest management. *Crop Prot.* 2015;77:52–64.
- [34] Stein T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol Microbiol.* 2005;56:845–57.
- [35] Alabouvette C, Olivain C, Steinberg C. Biological control of plant diseases: the European situation. *Eur J Plant Pathol.* 2006;114:329–41.
- [36] Mok WK, Tan YX, Lee J, Kim J, Chen WN. A metabolomic approach to understand the solid-state fermentation of okara using *Bacillus subtilis* WX-17 for enhanced nutritional profile. *AMB Express.* 2019;9:60.
- [37] Beneduzi A, Ambrosini A, Passaglia LMP. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genet Mol Biol.* 2012;35:1044–51.
- [38] Borriss R. Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in agriculture. In: Maheshwari DK, editor. *Bacteria in agrobiolgy: Plant growth responses.* Berlin, Heidelberg: Springer; 2011. p. 41–76. https://doi.org/10.1007/978-3-642-20332-9_3
- [39] Aloo BN, Makumba BA, Mbega ER. The potential of bacilli rhizobacteria for sustainable crop production and environmental sustainability. *Microbiol Res.* 2019;219:26–39.

- [40] Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond risk: bacterial biofilms and their regulating approaches. *Front Microbiol.* 2020;11:928.
- [41] Gaballa A, Antelmann H, Aguilar C, Khakh SK, Song KB, Smaldone GT, et al. The *Bacillus subtilis* iron-sparing response is mediated by a Fur-regulated small RNA and three small, basic proteins. *Proc Natl Acad Sci.* 2008;105:11927–32.
- [42] Khodavirdipour A, Chamanrokh P, Alikhani MY, Alikhani MS. Potential of *Bacillus subtilis* against SARS-CoV-2—a sustainable drug development perspective. *Front Microbiol.* 2022;13:718786.
- [43] Bhutani N, Maheshwari R, Negi M, Suneja P. Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. *Israel J Plant Sci.* 2018;65:83–96.
- [44] Danilova I, Sharipova M. The practical potential of bacilli and their enzymes for industrial production. *Front Microbiol.* 2020;11:1782.
- [45] Rahman MM, Paul SI, Akter T, Tay ACY, Foysal MJ, Islam MT. Whole-genome sequence of *Bacillus subtilis* WS1A, a promising fish probiotic strain isolated from marine sponge of the Bay of Bengal. *Microbiol Resour Announc.* 2020;9. <https://doi.org/10.1128/mra.00641-20>
- [46] Gill DM. Bacterial toxins: a table of lethal amounts. *Microbiol Rev.* 1982;46:86–94.
- [47] Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R. *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proc Natl Acad Sci.* 2013;110:E1621–30.
- [48] Zhang N, Yang D, Wang D, Miao Y, Shao J, Zhou X, et al. Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. *BMC Genomics.* 2015;16:685.
- [49] Zhang X, Al-Dossary A, Hussain M, Setlow P, Li J. Applications of *Bacillus subtilis* spores in biotechnology and advanced materials. *Appl Environ Microbiol.* 2020;86:e01096-20.
- [50] Mazin PV, Fisunov GY, Gorbachev AY, Kapitskaya KY, Altukhov IA, Semashko TA, et al. Transcriptome analysis reveals novel regulatory mechanisms in a genome-reduced bacterium. *Nucleic Acids Res.* 2014;42:13254–68.
- [51] Abedinzadeh M, Etesami H, Alikhani HA. Characterization of rhizosphere and endophytic bacteria from roots of maize (*Zea mays* L.) plant irrigated with wastewater with biotechnological potential in agriculture. *Biotechnol Rep.* 2019;21:e00305.
- [52] Hahne H, Mäder U, Otto A, Bonn F, Steil L, Bremer E, et al. A comprehensive proteomics and transcriptomics analysis of *Bacillus subtilis* salt stress adaptation. *J Bacteriol.* 2010;192:870–82.
- [53] Earl AM, Losick R, Kolter R. Ecology and genomics of *Bacillus subtilis*. *TIM.* 2008;16:269–75.
- [54] Tsonis I, Karamani L, Xaplanteri P, Kolonitsiou F, Zampakis P, Gatzounis G, et al. Spontaneous cerebral abscess due to *Bacillus subtilis* in an immunocompetent male patient: a case report and review of literature. *World J Clin Cases.* 2018;6:1169–74.
- [55] Hachmeister KA, Fung DYC. Tempeh: a mold-modified indigenous fermented food made from soybeans and/or cereal grains. *Crit Rev Microbiol.* 1993;19:137–88.
- [56] Liu D, Huang C, Guo J, Zhang P, Chen T, Wang Z, et al. Development and characterization of a CRISPR/Cas9n-based multiplex genome editing system for *Bacillus subtilis*. *Biotechnol Biofuels.* 2019;12:197.
- [57] Sharma A, Satyanarayana T. Comparative genomics of *Bacillus* species and its relevance in industrial microbiology. *Genomics Insights.* 2013;6:GEI.S12732.
- [58] Barros FF, Simiqueli AP, de Andrade CJ, Pastore GM. Production of enzymes from agroindustrial wastes by biosurfactant-producing strains of *Bacillus subtilis*. *Biotechnol Res Int.* 2013;2013:103960.
- [59] Adetunji AI, Olaniran AO. Production strategies and biotechnological relevance of microbial lipases: a review. *Braz J Microbiol.* 2021;52:1257–69.
- [60] Lin P, Yuan H, Du J, Liu K, Liu H, Wang T. Progress in research and application development of surface display technology using *Bacillus subtilis* spores. *Appl Microbiol Biotechnol.* 2020;104:2319–31.
- [61] Konsoula Z, Liakopoulou-Kyriakides M. Co-production of α -amylase and β -galactosidase by *Bacillus subtilis* in complex organic substrates. *Bioresour Technol.* 2007;98:150–7.
- [62] Kovács ÁT. *Bacillus subtilis*. *TIM.* 2019;27:724–5.
- [63] Shahid I, Han J, Hanoq S, Malik KA, Borchers CH, Mehnaz S. Profiling of metabolites of *Bacillus* spp. and their application in sustainable plant growth promotion and biocontrol. *Front Sustain Food Syst.* 2021;5:605195.
- [64] Dimidi E, Cox S, Rossi M, Whelan K. Fermented foods: definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. *Nutrients.* 2019;11:1806.
- [65] Fira D, Dimkić I, Berić T, Lozo J, Stanković S. Biological control of plant pathogens by *Bacillus* species. *J Biotech.* 2018;285:44–55.
- [66] Zhang B, Dong C, Shang Q, Han Y, Li P. New insights into membrane-active action in plasma membrane of fungal hyphae by the lipopeptide antibiotic bacillomycin L. *Biochim Biophys Acta Biomembr.* 2013;1828:2230–7.
- [67] Law KH, Cheng YC, Leung YC, Lo WH, Chua H, Yu HF. Construction of recombinant *Bacillus subtilis* strains for polyhydroxyalkanoates synthesis. *Biochem Eng J.* 2003;16:203–8.
- [68] Henry CS, Zinner JF, Cohoon MP, Stevens RL. iBsu1101: an improved genome-scale metabolic model of *B. subtilis* based on SEED annotations. *Genome Biol.* 2009;10:R69.
- [69] Gonzalez-Covarrubias V, Martínez-Martínez E, del Bosque-Plata L. The potential of metabolomics in biomedical applications. *Metabolites.* 2022;12:194.
- [70] Muras A, Romero M, Mayer C, Otero A. Biotechnological applications of *Bacillus licheniformis*. *Crit Rev Biotechnol.* 2021;41:609–27.
- [71] Mirouze N, Dubnau D. Chance and necessity in *Bacillus subtilis* development. *Microbiol Spectr.* 2013;1(10):1128.
- [72] Harwood CR. Introduction to the biotechnology of bacillus. In: Harwood, CR, editors. *Bacillus. Biotechnology Handbooks(2)*. Boston, MA: Springer; 1989. p. 1–4. https://doi.org/10.1007/978-1-4899-3502-1_1
- [73] AFRC RF. Probiotics in man and animals. *J Appl Bacteriol.* 1989;66:365–78.

- [74] Ji L, Zhang L, Liu H, Shen J, Zhang Y, Lu L, et al. *Bacillus subtilis* M6 improves intestinal barrier, antioxidant capacity and gut microbial composition in AA broiler. *Front Nutr*. 2022;9:965310.
- [75] Sun D. Pull in and push out: mechanisms of horizontal gene transfer in bacteria. *Front Microbiol*. 2018;9:2154.
- [76] Cardinali N, Bauman C, Rodriguez Ayala F, Grau R. Two cases of type 2 diabetes mellitus successfully treated with probiotics. *Clin Case Rep*. 2020;8:3119–24.
- [77] Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*. 2023;12:184.
- [78] Jalilzadeh YR, Sekhavaţjou M, Maktabi P, Arbab SN, Khadivi S, Pourjafarian V. The biodegradation of crude oil by *Bacillus subtilis* isolated from contaminated soil in hot weather areas. *Int J Environ Res*. 2014;8:509–14.
- [79] Sharma I. Bioremediation techniques for polluted environment: concept, advantages, limitations, and prospects. In: Murillo-Tovar A, Saldarriaga-Noreña H, Saeid A, editors. *Trace metals in the environment-new approaches and recent advances*. London: IntechOpen; 2020;221–30.
- [80] Kalia A, Sharma S, Semor N, Babele PK, Sagar S, Bhatia RK, et al. Recent advancements in hydrocarbon bioremediation and future challenges: a review. *3Biotech*. 2022;12:135.
- [81] Rana Chhetri B, Silwal P, Jyapu P, Maharjan Y, Lamsal T, Basnet A. Biodegradation of organic waste using *Bacillus* species isolated from soil. *Int J Appl Sci Biotechnol*. 2022;10:104–11.
- [82] Imam SSA. Comparative study of heavy metal bioremediation in soil by *Bacillus subtilis* and *Saccharomyces cerevisiae*. *Indian J Sci Technol*. 2016;9:1–7.
- [83] Blázquez B, San León D, Rojas A, Tortajada M, Nogales J. New insights on metabolic features of *Bacillus subtilis* based on multistrain genome-scale metabolic modeling. *Int J Mol Sci*. 2023;24:7091.
- [84] Kapała A, Szlendak M, Motacka E. The anti-cancer activity of lycopene: a systematic review of human and animal studies. *Nutrients*. 2022;14:5152.
- [85] Kamada M, Hase S, Fujii K, Miyake M, Sato K, Kimura K, et al. Whole-genome sequencing and comparative genome analysis of *Bacillus subtilis* strains isolated from non-salted fermented soybean foods. *PLoS One*. 2015;10:e0141369.
- [86] Hamdy AA, Esawy MA, Elattal NA, Amin MA, Ali AE, Awad GEA, et al. Complete genome sequence and comparative analysis of two potential probiotics *Bacillus subtilis* isolated from honey and honeybee microbiomes. *J Genet Eng Biotechnol*. 2020;18:34.
- [87] Acharjee SA, Bharali P, Gogoi B, Sorhie V, Walling B, Alemtoshi. PHA-based bioplastic: A potential alternative to address microplastic pollution. *Water, Air, Soil Pollut*. 2023;234:21.
- [88] Kojuri SA, Issazadeh K, Heshmatipour Z, Mirpour M, Zarrabi S. Production of bioplastic (polyhydroxybutyrate) with local *Bacillus megaterium* isolated from petrochemical wastewater. *Iran J Biotechnol*. 2021;19:e2849.
- [89] Loenen WAM, Dryden DTF, Raleigh EA, Wilson GG, Murray NE. Highlights of the DNA cutters: a short history of the restriction enzymes. *Nucleic Acids Res*. 2014;42:3–19.
- [90] Bhagavan NV, Ha CE. DNA replication, repair and mutagenesis. In: Ha CE, Bhagavan NV, editors. *Essentials of medical biochemistry*. Cambridge, Massachusetts: Academic Press. 2; 2022. p. 401–17. <https://doi.org/10.1016/B978-0-12-095461-2.00022-9>
- [91] Cai D, Rao Y, Zhan Y, Wang Q, Chen S. Engineering *Bacillus* for efficient production of heterologous protein: current progress, challenge and prospect. *J Appl Microbiol*. 2019;126:1632–42.
- [92] Pinu FR, Beale DJ, Paten AM, Kouremenos K, Swarup S, Schirra HJ, et al. Systems biology and multi-omics integration: viewpoints from the metabolomics research community. *Metabolites*. 2019;9:76.
- [93] Bate AR, Bonneau R, Eichenberger P. *Bacillus subtilis* systems biology: applications of-omics techniques to the study of endospore formation. *Microbiol Spectr*. 2016;2:129–44.
- [94] Pang Y, Wu R, Cui T, Zhang Z, Dong L, Chen F, et al. Proteomic response of *Bacillus subtilis* spores under high pressure combined with moderate temperature and random peptide mixture LK treatment. *Foods*. 2022;11:1123.
- [95] Schallmey M, Singh A, Ward OP. Developments in the use of *Bacillus* species for industrial production. *Can J Microbiol*. 2004;50:1–17.
- [96] Liu Y, Liu L, Li J, Du G, Chen J. Synthetic biology toolbox and chassis development in *Bacillus subtilis*. *Trends Biotechnol*. 2019;37:548–62.
- [97] Nowocień K, Sokołowska B. *Bacillus* spp. as a new direction in biocontrol and deodorization of organic fertilizers. *AIMS Environ Sci* 2022;9:95–105.
- [98] Elshaghabe FMF, Rokana N, Gulhane RD, Sharma C, Panwar H. *Bacillus* as potential probiotics: status, concerns, and future perspectives. *Front Microbiol*. 2017;8:1490.
- [99] Adimpong DB, Sørensen KI, Thorsen L, Stuer-Lauridsen B, Abdelgadir WS, Nielsen DS, et al. Antimicrobial susceptibility of *Bacillus* strains isolated from primary starters for African traditional bread production and characterization of the bacitracin operon and bacitracin biosynthesis. *Appl Environ Microbiol*. 2012;78:7903–14.
- [100] Abuhena M, Al-Rashid J, Azim MF, Khan MNM, Kabir MG, Barman NC, et al. Optimization of industrial (3000 L) production of *Bacillus subtilis* CW-S and its novel application for minituber and industrial-grade potato cultivation. *Sci Rep*. 2022;12:11153.
- [101] Arora M, Baldi A. Regulatory categories of probiotics across the globe: a review representing existing and recommended categorization. *Indian J Med Microbiol*. 2015;33:S2–S10.

How to cite this article: Akinsemolu AA, Onyeaka H, Odion S, Adebajo I. Exploring *Bacillus subtilis*: ecology, biotechnological applications, and future prospects. *J Basic Microbiol*. 2024;e202300614. <https://doi.org/10.1002/jobm.202300614>