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Review Article

Biotechnology Section

Bioactive Compounds Produced by *Bacillus subtilis*: A Brief Review

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ABSTRACT

Bacteria generate a wide array of bioactive compounds, each serving vital functions in medicine, agriculture, and industrial applications. *Bacillus subtilis* is one such bacterium that produces extensive and effective bioactive substances, which include lipopeptide molecules like surfactins, iturins, and fengycins, bacteriocins like bacillomycin, bacilysocin and subtilosin. Bacterial bioactive compounds can make an immense contribution to therapeutic applications, playing a key role in drug discovery and the innovation of new antimicrobial treatments. These bioactive compounds are antimicrobial and has potential anticancer properties. This is due to their specific targeting abilities. These molecules usually show diverse mechanisms in destroying cells, usually by cell wall disruption, known for their immunosuppressive and anti-inflammatory activities. Industrial-scale optimisation strategies may improve production efficiency and enhance commercial feasibility.

Keywords: Antimicrobial, Bacteriocins, Fengycins, Iturins, Surfactins

INTRODUCTION

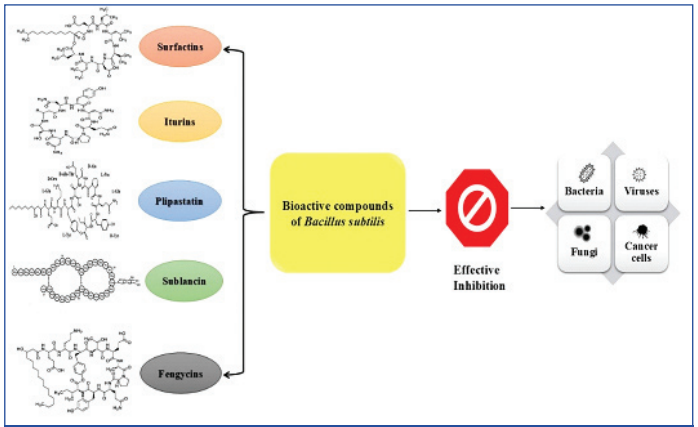
Bioactive substances are chemical compounds that influence living cells in specific ways. These molecules are synthesised by microbes either because of their unique metabolic processes or as a defensive strategy to compete with other microorganisms in densely populated environments. These bioactive substances are produced as secondary metabolites by microbes. Bioactive substances have a wide range of applications in agriculture (biofertilizer and biocontrol agents) and pharmaceutical industries (development of antibacterial, antiviral, antifungal, anticancer, and immunomodulatory peptides/molecules, enzymes, and probiotics). The production of these substances is typically optimised through fermentation [1]. First, specific microbial strains are identified and screened for their ability to produce bioactive compounds. These strains are then cultivated in appropriate growth media, where critical growth parameters are carefully maintained within bioreactors. This controlled environment supports microbial growth and facilitates the production of bioactive compounds as secondary metabolites during the fermentation process [2]. Purification and downstream processing are employed to isolate and recover the purified compound effectively. Only a limited number of bacteria and fungi exhibit these unique characteristics. *Bacillus* species are particularly recognised for their efficient production of bioactive molecules, which include cyclic lipopeptides, polyketides, non ribosomal peptides, and enzymes [3-5]. *B.subtilis* is one of the *Bacillus* species that has a great antiquity of producing efficient metabolites. *B.subtilis* is a non pathogenic, gram-positive, rod-shaped bacterium, which is often used as a model organism [6]. Its industrial applications have a wide range in the production of enzymes, alcohols, and acids, apart from bioactive compounds. *B.subtilis* has a remarkable role in the production of bioactive compounds [Table/Fig-1] like lipopeptides (surfactins, iturins, and fengycins) and bacteriocins (bacillomycin and bacilysocin) [Table/Fig-2]. These include fibrinolytic activity (Nattokinase), anti-arterial calcification (Menaquinone-7), anti-blood coagulation (Dipicolinic acid), antioxidant properties (Poly-gamma-glutamic acid), anti-obesity effects (L-ornithine), and prebiotic activity (2'-fucosyllactose, levan). Due to its diverse bioactive potential, *B. subtilis* aligns with the "One Health" principle, emphasising its significance in human health [7-9]. Many recombinant studies have mostly studied *Bacillus subtilis* due to its high resistance to extreme environmental conditions like temperature, drought, salinity, pH, radiation, and nutrient stress [10]. Since *Bacillus subtilis* grows more easily than many other organisms, managing the strain during upstream processing is relatively easy. Various technologies for producing bioactive molecules have been optimised through fermentation, and ongoing research is focused on scaling up these commercial products. Apart from traditional submerged fermentation, solid-state fermentation is also showing extensive results in bioactive substances' production. The present review aimed to summarise the different bioactive substances produced by *Bacillus subtilis* and their recent scale-up strategies and yields.

METHODOLOGY

This narrative review was conducted using literature retrieved from PubMed, Scopus, Web of Science, Google Scholar, and included books, case reports, and through discussion with industrialists who are experts in troubleshooting and process development.

Bioactive Compounds Produced By *B.Subtilis*

Surfactin: Surfactin is a cyclic lipopeptide characterised by a heptapeptide core linked to a β-hydroxy fatty acid, forming a unique amphiphilic structure. A lactone bridge links the β-hydroxyl group of the acid to the carboxy-terminal functionality of the peptide, giving the molecule its distinct cyclic structure. Surfactin interferes with the membranes of bacteria by creating membrane channels, chelating cations, inserting into lipid bilayers, and causing cell disruption.



[Table/Fig-1]: Bioactive compounds of *B.subtilis* exhibit effective inhibition effect on cells.

Compound class	Produced by *B. subtilis*	Biological role	Applications
Lipopeptides	Surfactin, Fengycin, Iturin	Antimicrobial, biofilm inhibition	Biocontrol in agriculture, antimicrobial agents, bioremediation
Polyketides	Bacillaene, Difficidin	Broad-spectrum antibacterial activity	Potential antibiotics, therapeutic leads
Ribosomally Synthesised Peptides (RiPPs)	Subtilosin A, Lantibiotics (e.g., Subtilin)	Antibacterial activity, quorum sensing regulation	Food preservation, novel antibiotics
Exopolysaccharides	EPS from biofilms	Structural role, stress protection	Biothickeners, biofilm studies, potential medical biomaterials
Volatile Organic Compounds (VOCs)	Acetoin, 2,3-butanediol	Plant growth promotion, signalling	Agriculture (plant growth promoters), biotechnological feedstocks

[Table/Fig-2]: Critical comparison of bioactive compound classes produced by *B.subtilis*.

Surfactins function as biosurfactants due to their amphiphilic surface-active properties. Biosurfactants have attracted significant interest across various industrial sectors due to their exceptional structural diversity and advantageous characteristics. Their ability to be produced under controlled conditions ensures consistent quality and functionality, while their wide range of physicochemical properties enhances their versatility in different applications. Additionally, biosurfactants provide several advantages, such as antimicrobial properties, low potential toxicity, and high biodegradability, making them a sustainable and eco-friendly substitute for synthetic surfactants. Surfactin biosynthesis in *B. subtilis* is mediated by a non ribosomal peptide synthetases, while its export is facilitated by the SwrC transported protein. A study indicates that the SwrC protein requires MeeY, a member of the TerC family of membrane proteins that support the function of secreted and membrane metalloenzymes. MeeY, a member of the TerC membrane protein family, is proposed to bind with SwrC, enabling metal binding to the surfactin-lipopeptide during its export from the cell [9]. There are also findings that quorum sensing effector ComA, regulating biofilm formation and biosurfactin production in *B. subtilis* ASAG 0 [10], when tested by removing the ComA gene, showed significant reduction in surfactin production [9]. Due to the widespread industrial applications of biosurfactants, numerous experiments have been designed to enhance their production yields using *B. subtilis* [11]. The *B. subtilis* 3KP isolate shows strong potential for surfactin-type biosurfactant production. The biosurfactant produced by *B. subtilis* 3KP reduced surface tension from 72 mN/m to 27 mN/m and exhibited a critical micelle concentration of 20.01 mg/L [12,13]. It was observed that magnetic field treatment increased the volumetric surfactin productivity to 136% and specific surfactin formation rate to 217% with the *B. subtilis* ATCC 21332 strain, and a synergistic effect was noticed in the fermentation process between the magnetic field and Mg2+ [14]. In increasing the yield of surfactin, optimising the process parameters plays a major role in bacterial fermentation. It was observed that the oxygen mass transfer coefficient (kLa) influences the surfactin productivity, where optimal surfactin production was achieved at kLa = 21.88 h-1, utilising 0.3 vvm aeration with 250 rpm stirring, yielding peak productivity of 14.1 mg/L·h and a maximum surfactin concentration of 443.1 mg/L. This shows *B. subtilis* can result in high yields of surfactin with low aeration and high agitation in fermentation [15]. As glycerol is used as a carbon source and yeast extract as a nitrogen source in a 3:1 ratio in the fermentation media, growth parameters are maintained with a pH of 6 and a temperature of 30°C followed by agitation at 130 rpm, *B. subtilis* SMP-2 yielded 8.13±0.9 g/L of lipopeptide biosurfactant [16]. Apart from industrial applications, biosurfactants also have potent applications in integrated pest and disease control, including chemical fertilizers. It was observed that biosurfactants produced from *B. subtilis* could be used to increase the shelf life of fruits and vegetables [17]. Few bioinformatics studies have evaluated the drug potential of surfactin produced by *B.subtilis* KLP2016. Surfactin-capped silver nanoparticles (AgNPs) demonstrated significant antimicrobial and anticancer properties, as confirmed by UV–VIS spectroscopy, FE-SEM, and X-ray diffraction analysis [18]. Surfactin derived from *B. subtilis* SF1 effectively suppressed Fusarium foetens mycelial growth at concentrations exceeding 20 µg/µL. Surfactin-like lipopeptide produced by *B. subtilis* strain A52 showed promising antibacterial and antifungal activity against more than 29 strains. The study also suggested that synergistic application of surfactins with synthetic antibiotics gives scope for the development of effective therapeutic drugs [19].

Polyketides: Polyketides are one of the secondary metabolites produced by the bacteria; these polyketides, produced by sequential condensation of malonyl-CoA units, result in the progressive elongation of a poly- β-ketoacyl chain. Biosynthesis of polyketides happens through Type-1 and Type-2 polyketide synthases. *B. subtilis* synthesises both aliphatic and aromatic polyketides. Aliphatic peptides, such as triketide pyrone, are generated by BpsA N(4)-bis(aminopropyl)spermidine synthase, a type-3 polyketide synthase and non-ribosomal peptide synthase enzyme. Meanwhile, alkylpyrone methyl ethers are produced through the art gene cluster and the bpsA-bpsB operon system [20-22]. *B. subtilis* also synthesises bacillaene, an uncharacterised antibiotic, a polyketide/nonribosomal peptide synthase encoded within the pksX gene cluster [23]. Bacillaene is a linear molecule characterised by two amide bonds: one connecting an α-hydroxy carboxylic acid to a ω-amino carboxylic acid with a conjugated hexaene and the other linking the hexaene-containing carboxylic acid to an (ω-1) amino carboxylic acid featuring a conjugated triene. A new variant of polyketide is observed in the seaweed A. longifolius-associated bacterium *B. subtilis*, i.e., aryl-crowned polyketide 7-O-6'-(2"-acetyl phenyl)-5'-hydroxyhexanoate-macrolactin [24]. Apart from Bacillaene, polyketides include difficidin and macrolactin, where difficidin is a notable unsaturated macrocyclic polyene antibiotic synthesised by specific *Bacillus* species. As a member of the polyketide class, its production is enabled by modular type I Polyketide Synthases (PKS). Oxidative derivatives of difficidins are oxydifficidins; both show strong antimicrobial activities. These difficidins inhibit cell wall synthesis. The pksX gene cluster in *B. subtilis* plays a crucial role in the biosynthesis of bacillaene. The pksX gene cluster encodes PKS and Non Ribosomal Peptide Synthetase (NRPS). It was observed that *B. subtilis* PS-216 showed antagonistic behaviour against the growth of Salmonella typhimurium by producing pks-associated bacillaence [25].

Fengycin and Plipastatin: Plipastatin is a lipopeptide molecule that comes under the fengycin family, classified as a cyclodepsipeptide. Although fengycin's structure is nearly identical to that of plipastatin, the stereochemistry differs at positions 3 and 9. In plipastatin, Tyr3 is in the L-form and Tyr9 is in the D-form, whereas in fengycin, these configurations are reversed. Although fengycin and plipastatin are known to have potent bioactive properties, they exhibit effective antifungal, anticancer, antiviral, and antibacterial activities [26]. In *Bacillus subtilis*, the ppsABCDE operon with 38 gene clusters is responsible for the non ribosomal peptide synthesis of plipastatin. This operon comprises five genes—ppsA, ppsB, ppsC, ppsD, and ppsE—that work together to synthesise the plipastatin molecule. As it has been observed that during the exponential growth phase in *B. subtilis* 168, the transition state regulator AbrB inhibits the expression of the ppsABCDE operon, whereas the regulatory protein DegQ has a positive influence on plipastatin biosynthesis [27]. Apart from degQ

positive stimuli, plipastatin production is also influenced by the surfactin operon srfAA-AD. The substantial decline in plipastatin productivity was observed when the srfAA-AD operon was deleted; a constitutive plipastatin producer strain indicates that optimal plipastatin synthesis depends on surfactin synthetase. In *Bacillus subtilis* 168, initial fengycin production reached 1.81 mg/L. By knocking out pathways related to surfactin and bacillaene biosynthesis and replacing the native PppsA promoter with the stronger Pveg promoter, synthesis was enhanced to 174.63 mg/L. Further upregulation of key genes involved in the fatty acid pathway increased production to 258.52 mg/L. Optimising the process by inhibiting spore and biofilm formation further boosted yields to 302.51 mg/L. Additionally, incorporating the amino acid threonine into the culture medium significantly enhanced fengycin production [28]. The three-strain artificial consortium approach was also used to increase the yields of fengycin. Corynebacterium glutamicum was engineered to increase precursor levels (Thr, Pro, Val, and Ile), which promotes fengycin production, and Yarrowia lipolytica, which is providing amino acid and fatty acid precursors, were co-cultured along with *B. subtilis*, resulting in 2100 mg/L of fengycin production [29]. Since substrate selection plays a crucial role in metabolite production, various supplements can lead to distinct statistical variations in fengycin yield. Fengycin titer was increased from 0 to 130.10 mg/L when grown in culture media containing 20 g/L xylose, 21.9 g/L soybean meal, 3.1 g/L $NaNO^3$, and 0.15 g/L $MnSO^4{\cdot}4H^2O$ [30]. While in *B. subtilis*, 168 yields fengycin with an 87% increase when cultured with xylose as a carbon source [31]. Many gene-editing technologies were also tried to increase the production of fengycin; *B. subtilis* 168 native promoter PppsA was replaced with Pveg promoter through CRISPR/Cas9 editing, resulting in an increase of 5-fold yield, where 40 g/L xylose was supplemented in the media, which showed 430.86 mg/L fengycin titer [32]. Using arabinose as the carbon source and water-soluble soybean cake powder as the nitrogen source, *B. subtilis* 168 achieved a fengycin production of 121.20 mg/L. Modifying the fengycin biosynthetic gene cluster by replacing its promoter with Pylb increased the yield to 137.05 mg/L. Further genetic modifications, including the removal of fadB and the overexpression of yhfL, yngH, and tesA, enhanced fengycin production to 258.41 mg/L [33]. In *B. subtilis* ATCC 21332 upregulated the expression of the fen operon, the engineered strain BSA034 showed an increase in fengycin production of 442.51 mg/L; later supplementing the medium with glutamate further increased the yield to 657.55 mg/L [34]. Besides glucose, sucrose, and xylose, alternative carbon sources such as arabinose, glycerol, fructose, and starch can enhance fengycin production. For nitrogen sources, yeast extract, soybean meal powder, ammonium bicarbonate, and ammonium nitrate, combined with salts like magnesium sulfate, disodium hydrogen phosphate, and sodium dihydrogen phosphate, can further boost yields. In scale-up processes, optimal growth parameters for fengycin production include a temperature of 30°C and pH 7.0. Since *B. subtilis* is aerobic, proper aeration and oxygen supply are essential for its growth.

Sublancin: Sublancin is a glycopeptide molecule containing 37 amino acids. It consists of two alpha helices linked with a loop with two disulfide bonds amid four cysteine molecules. It has an S-linked glucose molecule attached to a cysteine residue in the structure, which makes the structure look like an S-linked glycopeptide. Sublancin is known for its antibacterial properties against many gram-positive bacteria. This glycopeptide disrupts the cell wall of the bacteria and negatively affects DNA replication, transcription, and translation [35]. Sublancins are considered glycopeptide bacteriocins, which are glycocins. Sublancins are produced by most of the *B. subtilis* 168 members, which exhibit high antimicrobial activity even against methicillin-resistant Staphylococcus aureus and vancomycin-resistant *Enterococcus* sp [36]. These sublanicins are lantibiotic molecules containing cross-linked lanthionine and dehydroalanine residues in the amino acid chain. These peptides are generated by ribosomes and undergo significant post-translational modifications to attain their final functional state. The sublancin gene cluster is located within the SPβ prophage, a DNA element embedded in the *B. subtilis* genome. Genes include SunI, SunA, SunT, bdbA, sunS, and bdbB, where SunI provides immunity to the producer, SunA initiates the process by encoding the precursor peptide, and SunT manages its export and cleavage. Disulfide bond formation is assisted by bdbA and bdbB, while SunS acts as a glycosyltransferase. The *B.subtilis* 168 Δspβ strain has no sunI gene in the operon, making it highly sensitive to sublancins [37]. As recent developments are a part of synthetic drugs, improving natural bioactive agents produced from microbes have had great significance in facing many highly resistant pathogens. Initially, low levels of sublancin yields were observed in the fermentation process, but by implementing different scale-up strategies, experimental methodologies can help to increase the productivity. In *Bacillus subtilis* 168, high yields were achieved with substrate containing 28.49 g/L of corn powder and 22.99 g/L of soybean meal, with 30.8°C as incubation temperature as a critical growth parameter, boosting production from under 60 mg/L to 129.72 mg/L in the shake flask model. The same experiment in batch mode fermentor level showed much higher yields, i.e., 135.4 mg/L [38]. A new approach by inserting two strong promoters, P43 and P luxS, upstream of sunI and sunT–bdbA–sunS–bdbB, in *B.subtilis* 1A747, which encodes five functional proteins essential for the biosynthesis of mature sublancin. 642 mg of sublancin was recovered from one liter of culture supernatant. Antibacterial activity of sublancin against the most resistant pathogens, like gentamicin–resistant *E. faecalis*, showed an MIC of 6.8±0.29 mg/L, methicillin-resistant *S. aureus* with an MIC of 0.4±0.21 mg/L, erythromycin-resistant *S. agalactiae* with an MIC of 1.4±0.24 mg/L, and erythromycin-resistant *S. pyogenes* with an MIC of 1.0±0.36 mg/L. Some other pathogens also showed effective results, like *E. faecalis* ATCC 29212 with an MIC of 7.3±0.35 mg/L, *S. aureus* ATCC 25923 with an MIC of 0.6±0.14 mg/L, *S. agalactiae* ATCC 27956 with an MIC of 2.1±0.35 mg/L, *S. pyogenes* ATCC 19615 with an MIC of 0.8±0.22 mg/L, and B. cereus ATCC 10987 with an MIC of 3.4±0.21 mg/L [14]. Sublancin produced by *B.subtilis* strain A52 showed effective antibacterial activity in Staphylococcus aureus (MTCC 1430) with MIC 10 µg/mL, *Bacillus subtilis* (MTCC 121) with MIC 5 µg/mL, *Bacillus* cereus (MTCC 430) with MIC 5 µg/mL, *B.coagulans* (MTCC 492) with MIC 10 µg/mL, Micrococcus luteus (MTCC 106) with MIC 2 µg/mL, Streptococcus pyogenes (MTCC 1928) with MIC 2 µg/mL, S. anginosus (MTCC 1929) with MIC 6 µg/mL, S. oralis (MTCC 2696) with MIC 8 µg/mL, and S. mutans (MTCC 497) with MIC 10 µg/mL [39].

Iturin: *B.subtilis* are gram-positive bacteria which produce biosurfactant compounds like surfactin, iturin, and fenthromycin, which show effective antimicrobial efficacy. Iturins are cyclic lipopeptides recognised for their strong antibacterial and antifungal effects. Beyond these benefits, they also exhibit anticancer properties, making them valuable across various industries, including food, agriculture, and pharmaceuticals [40]. Iturins are bioactive substances produced by *Bacillus* species, mostly in *B.subtilis* and *B.amyloliquefaciens*. Structurally, iturins are cyclic peptide molecules that consist of seven α-amino acids and one β-amino acid. There are different members of iturin, like A, C, D, and E, as well as various members. Iturin A is a specific member of this class that consists of an amino acid heptapeptide sequence Asn-Tyr-Asn-Gln-Pro-Asn- Ser with a β-aminofatty acid. Iturin A is produced through the biosynthetic pathway of Non Ribosomal Peptide Synthetases (NRPSs), utilising the ituD, ituA, ituB, and ituC genes. The ituD gene encodes an essential enzyme in fatty acid synthesis, i.e., malonyl coenzyme A transacylase. The ituA gene encodes a protein moiety that is involved in three functions, i.e., fatty acid synthetase, amino acid transferase, and peptide synthetase activities. ItuB encodes a 609 kDa protein with four amino acid components, while ItuD encodes a 297 kDa protein with two amino acid components. Iturin interacts with the cytoplasmic membrane (phospholipid membrane) of the cell, creates ion-permeable channels, and causes cell death. There are eight naturally occurring isomeric forms of iturin A, ranging from

A1 to A8; these structures differ with different molecular weights [41]. The production of iturin via a fermentation approach shows effective results in both solid-state fermentation and submerged models. In fed-batch fermentation using *B.subtilis* ZK-H2, the addition of amino acid supplements at 12 hours showed a 32.81% increase in productivity; the addition of 0.0752 g/L asparagine, 0.1992 g/L l-glutamine, and 0.1464 g/L l-proline enhanced the yield of Iturin A to 0.85 g/L [42]. The co-culturing strategy has seriously contributed to the high production of iturin exhibited by the *Bacillus* vallismortis strain TU-Orga21 and the *B.subtilis* strain TU-Orga1 with a 350% rise. The co-culture of *B.vallismortis* strain TU-Orga21 and *B. subtilis* strain TU-Orga1 exhibited a significant inhibitory effect on Pythium aphanidermatum strain, reducing mycelium growth by up to 39.26% [43]. Iturin produced by *B.subtilis* Z-14 showed effective antifungal activity against the plant pathogen. Gaeumannomyces graminis var. Tritici: microscopic observation reveals iturin showed cell wall disappearance, intracellular material shrinkage, and hyphal fragmentation [44].

Bacteriocins: Bacteriocins are ribosomally synthesised peptide molecules produced by bacteria to inhibit the growth of other microbes. *B.subtilis* is well known for producing bacteriocins like bacilysocin, a 1-(12-methyltetradecanoyl)-3-phosphoglyceroglycerol molecule that interacts with the plasma membrane of the cell and causes cell death. It is a phospholipid antibiotic that is highly effective in inhibiting fungal growth [45]. Subtilosin is another bacteriocin, which is a cyclic peptide featuring three residue bridges, where two cysteine residues are connected to two phenylalanine groups, while the third cysteine is bonded to a threonine residue. The sboA gene resides within the larger sbo-alb cluster, alongside the albA, albB, albC, albD, albE, and albF genes, which play crucial roles in the processing, modification, and export of subtilosin A [46]. Subtilosin A from *B. subtilis* has potential against gram-positive bacteria. It was also identified that subtilosin A is produced by *B. subtilis* strain 168, containing intramolecular thioether bonds and a head-to-tail macrocyclic peptide bond [47]. Bacillomycin D is another kind of bacteriocin, which is a lipopeptide molecule that has high antifungal properties. The gene operon of bacillomycin D is the bmy operon, comprising four genes: bmyD, bmyA, bmyB, and bmyC. The bacteriocin produced by *B. subtilis* exhibited strong antimicrobial activity, particularly against *S. pyogenes*, followed by *S. Typhi* and *P. aeruginosa*, while showing the lowest effectiveness against A. baumannii. In comparison to standard antibiotics, the *B. subtilis*-derived bacteriocin displayed a notable inhibitory effect against the tested pathogens [48]. As a scale-up criterion, bacteriocin synthesis increased by 29%, 45%, and 34% with sucrose as a carbon source, along with modifications to NaCl concentration and maintaining the pH at 7. Enhancing bacteriocin activity depended on optimising sucrose concentration, NaCl levels, and incubation duration [49]. Bacteriocin exhibited stability within a temperature range of 24–50°C and a pH of 5–8. Its purified molecular weight was determined to be between 13 and 35 kDa. The purified protein from *B. subtilis* HD15 demonstrated potent antimicrobial activity against Listeria monocytogenes and *Bacillus* cereus [50].

Challenges in Industrial Translation, Alongwith Regulatory And Safety Highlights

Industrial translation encounters challenges in sustaining high yields, stability, and cost-effectiveness at scale, despite *B. subtilis*'s status as one of the top microbial hosts for enzyme production owing to its non pathogenicity and efficient secretion mechanism [Table/Fig-3]. *B. subtilis* is Generally Recognised As Safe (GRAS) and industrially considered safe; however, any recombinant development with the strain requires proper biosafety regulations, and industries should comply with regulatory guidelines. Although *B. subtilis* is regarded as a safe and efficient host for enzyme production, regulatory approval is contingent upon the demonstration of genetic stability, the absence of deleterious metabolites, and consumer safety through rigorous toxicological and allergenicity testing. Enzymes must undergo toxicological evaluation, including oral toxicity, genotoxicity, and allergenicity. Industries should also qualify biosafety and occupational safety standards [51].

Challenge area	Description	Industrial impact
Fermentation scale-up	Optimisation at the lab level does not always transfer to the industrial level. Nutrient gradients, pH regulation, and oxygen transfer become crucial.	Decrease in the efficiency of production
Genomic integrity	Loss of plasmids in recombinant strains due to repeated subculturing	Affects the production of the desired product
Metabolic stress	An increase in enzyme production can sometimes affect the growth of the strain	Limits scalability
Protease effect	Protease can degrade the desired enzymes during fermentation	Loss in specific enzyme recovery
Downstream purification	Development of proper purification steps	Cost burden and time-consuming

[Table/Fig-3]: Summary table of main challenges in the production of bioactive substances and industrial impacts.

Limitation(s)

The study provides a detailed description of individual bioactive compounds produced by *B.subtilis*. Chemical structures, their physical nature, and genes responsible for producing these bioactive compounds were discussed. The review does not comprehensively discuss industrial commercialisation case studies, as the study does not focus on large-scale development this can be considered as a limitation. Additionally, the review primarily focuses on fermentation modes, substrate selection, feed strategies, and critical process parameters, along with their respective yields.

CONCLUSION(S)

B. subtilis is a pervasive bacterial strain, that has high potential in the production of different bioactive metabolites. Understanding the metabolisms of emerging *B. subtilis* strains from diverse sources aids in identifying various bioactive substances. As these strains exhibit antagonistic behaviour, they produce distinct bioactive compounds as a survival mechanism, ultimately leading to the formation of unique substances Recent studies have highlighted the growing use of gene knockout mechanisms and CRISPR/Cas9 technology in developing recombinant strains. These advancements enhance strain efficiency, leading to higher yields of bioactive compounds. Scaling up through techniques such as co-culturing, media optimisation, and advanced fermentation strategies can significantly enhance productivity.

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REFERENCES

[1] Put H, Gerstmans H, Vande Capelle H, Fauvart M, Michiels J, Masschelein J. Bacillus subtilis as a host for natural product discovery and engineering of biosynthetic gene clusters. Natural Product Reports. 2024;41(7):1113-51.
[2] Arsov A, Armenova N, Gergov E, Petrov K, Petrova P. Cloning systems in Bacillus: Bioengineering of metabolic pathways for valuable Recombinant products. Fermentation. 2024;10(1):50.
[3] Ortiz A, Sansinenea E. The Industrially important enzymes from bacillus species. InBacilli in Agrobiotechnology: Plant Stress Tolerance, Bioremediation, and Bioprospecting. Cham: Springer International Publishing; 2022. pp. 89-99.
[4] Raphel S, Halami PM. Bioactive compounds from food-grade Bacillus. Journal of the Science of Food and Agriculture. 2025;105(8):4085-95.
[5] Xiao S, Chen N, Chai Z, Zhou M, Xiao C, Zhao S, Yang X. Secondary metabolites from marine-derived Bacillus: A comprehensive review of origins, structures, and bioactivities. Marine drugs. 2022;20(9):567.
[6] Bremer E, Calteau A, Danchin A, Harwood C, Helmann JD, Médigue C, Palsson BO, Sekowska A, Vallenet D, Zuniga A, Zuniga C. A model industrial workhorse: Bacillus subtilis strain 168 and its genome after a quarter of a century. Microbial biotechnology. 2023;16(6):1203-31.
[7] Razafindralambo H, Seerengaraj V, Rabetafika H. Bacillus subtilis: A Promising. Bacillus subtilis-Functionalities and One Health Applications: Functionalities and One Health Applications. 2025:3.

[8] Miyazawa T, Abe C, Bhaswant M, Ikeda R, Higuchi O, Miyazawa T. Biological functions of compounds from Bacillus subtilis and its subspecies, Bacillus subtilis natto. Food bioengineering. 2022;1(3-4):241-51.
[9] He B, Sachla AJ, Ruesewald SB, Kearns DB, Helmann JD. The TerC family metal chaperone MeeY enables surfactin export in Bacillus subtilis. Journal of Bacteriology. 2025;207(5):e00088-25.
[10] Zhang T, Gong Z, Zhou B, Rao L, Liao X. Recent progress in proteins regulating the germination of Bacillus subtilis spores. Journal of Bacteriology. 2025;207(2):e00285-24.
[11] Pandit NK, Meena SS. Exploring sustainable biosurfactant production through waste valorization: Emerging research trends and industrial applications. Waste and Biomass Valorization. 2026;17(2):585-619.
[12] Yuliani H, Nimatuzahroh N, Darmokoesoemo H, Suwito H, Nugroho EA, Affandi M, et al. Isolation and characterization of a surfactin-like biosurfactant produced by Bacillus subtilis 3KP isolated from oil-contaminated soil in Indonesia. Bioscience Reports. 2025:BSR20241227.
[13] Bai X, Yi L, Zhao S, Xiu J, Li P, Shi R, et al. Identification and comparative genomic analysis of two Bacillus subtilis producing antifungal lipopeptide. Pesticide Biochemistry and Physiology. 2025;213:106470.
[14] Jin Y, Chen S, Zhao Q, Yang N, Ning Y, Xu X. Mechanism investigation into static magnetic field effects on enhancing surfactin productivity by Bacillus subtilis. Food Bioscience. 2025;66:106316.
[15] Martins TB, Debon J, Schmidell W, Soares HM. Effect of oxygen mass transfer coefficient (kLa) on surfactin production by Bacillus subtilis ATCC 21332. Brazilian Journal of Chemical Engineering. 2025:01-02.
[16] Sonbhadra S, Pandey LM. Isolation, identification, and characterization of Bacillus subtilis SMP-2 from panitenga and exploring its potential for biosurfactant production. Food and Bioproducts Processing. 2025;149:144-57.
[17] Caretta TD, Unterholzner M, Zuluaga MY, Spitaler U, Ceccon A, Cesco S, et al. Antifungal activity of surfactin produced by Bacillus subtilis against phytopathogenic fungi in apple and strawberry: An in vitro and in vivo study. International Journal of Food Science and Technology. 2025;60(1):vvaf087.
[18] Chauhan V, Pandey A, Mahajan G, Dhiman V, Kanwar SS. Synergistic exploration of Surfactin-capped silver nanoparticles: Bioinformatics insights, antibacterial potency, and anticancer activity. 3 Biotech. 2025;15(1):13.
[19] Meena KR, Sharma A, Kanwar SS. Antitumoral and antimicrobial activity of surfactin extracted from Bacillus subtilis KLP2015. International Journal of Peptide Research and Therapeutics. 2020;26(1):423-33.
[20] Dai C, Shu Z, Ma C, Yan P, Huang L, He R, et al. Isolation of a surfactin-producing strain of Bacillus subtilis and evaluation of the probiotic potential and antioxidant activity of surfactin from fermented soybean meal. Journal of the Science of Food and Agriculture. 2024;104(14):8469-79.
[21] Liu L, Jin X, Lu X, Guo L, Lu P, Yu H, et al. Mechanisms of surfactin from Bacillus subtilis SF1 against Fusarium foetens: A novel pathogen inducing potato wilt. Journal of Fungi. 2023;9(3):367.
[22] Nakano C, Ozawa H, Akanuma G, Funa N, Horinouchi S. Biosynthesis of aliphatic polyketides by type III polyketide synthase and methyltransferase in Bacillus subtilis. Journal of bacteriology. 2009;191(15):4916-23.
[23] Butcher RA, Schroeder FC, Fischbach MA, Straight PD, Kolter R, Walsh CT, et al. The identification of bacillaene, the product of the PksX megacomplex in Bacillus subtilis. Proceedings of the national academy of sciences. 2007;104(5):1506-9.
[24] Chakraborty K, Thilakan B, Kizhakkekalam VK. Antibacterial aryl-crowned polyketide from Bacillus subtilis associated with seaweed Anthophycus longifolius. Journal of applied microbiology. 2018;124(1):108-25.
[25] Podnar E, Erega A, Danevčič T, Kovačec E, Lories B, Steenackers H, et al. Nutrient availability and biofilm polysaccharide shape the bacillaene-dependent antagonism of Bacillus subtilis against Salmonella Typhimurium. Microbiology spectrum. 2022;10(6):e01836-22.
[26] Gao L, Han J, Liu H, Qu X, Lu Z, Bie X. Plipastatin and surfactin coproduction by Bacillus subtilis pB2-L and their effects on microorganisms. Antonie Van Leeuwenhoek. 2017;110(8):1007-18.
[27] Vahidinasab M, Lilge L, Reinfurt A, Pfannstiel J, Henkel M, Morabbi Heravi K, et al. Construction and description of a constitutive plipastatin mono-producing Bacillus subtilis. Microbial Cell Factories. 2020;19(1):205.
[28] Gao GR, Hou ZJ, Ding MZ, Bai S, Wei SY, Qiao B, et al. Improved production of fengycin in Bacillus subtilis by integrated strain engineering strategy. ACS Synthetic Biology. 2022;11(12):4065-76.
[29] Wei SY, Gao GR, Ding MZ, Cao CY, Hou ZJ, Cheng JS, et al. An Engineered Microbial Consortium provides precursors for Fengycin production by Bacillus subtilis. Journal of Natural Products. 2024;87(1):28-37.
[30] Tan W, Yin Y, Wen J. Increasing fengycin production by strengthening the fatty acid synthesis pathway and optimizing fermentation conditions. Biochemical Engineering Journal. 2022;177:108235.
[31] Gao W, Yin Y, Wang P, Tan W, He M, Wen J. Production of fengycin from D-xylose through the expression and metabolic regulation of the Dahms pathway. Applied microbiology and biotechnology. 2022;106(7):2557-67.
[32] Yin Y, Wang P, Wang X, Wen J. Construction of Bacillus subtilis for efficient production of fengycin from xylose through CRISPR-Cas9. Frontiers in Microbiology. 2024;14:1342199.
[33] Jin J, Yin Y, Wang X, Wen J. Metabolic engineering of Bacillus subtilis 168 for the utilization of arabinose to synthesize the antifungal lipopeptide fengycin. Biochemical Engineering Journal. 2022;185:108528.
[34] Li Y, Wen J. Metabolomic analysis of the effect glutamate on fengycin-overproducing Bacillus subtilis ATCC 21332 with an enhanced fatty acid synthesis pathway. Biochemical Engineering Journal. 2023;196:108957.
[35] Wu C, Biswas S, Garcia De Gonzalo CV, Van Der Donk WA. Investigations into the mechanism of action of sublancin. ACS infectious diseases. 2018;5(3):454-59.
[36] Li J, Chen J, Yang G, Tao L. Sublancin protects against methicillin-resistant Staphylococcus aureus infection by the combined modulation of innate immune response and microbiota. Peptides. 2021;141:170533.
[37] Biswas S, Wu C, Van Der Donk WA. The antimicrobial activity of the glycocin sublancin is dependent on an active phosphoenolpyruvate-sugar phosphotransferase system. ACS infectious diseases. 2021;7(8):2402-12.
[38] Ji S, Li W, Xin H, Wang S, Cao B. Improved production of sublancin 168 biosynthesized by Bacillus subtilis 168 using chemometric methodology and statistical experimental designs. BioMed Research International. 2015;2015(1):687915.
[39] Sharma D, Singh SS, Baindara P, Sharma S, Khatri N, Grover V, et al. Surfactin like broad spectrum antimicrobial lipopeptide co-produced with sublancin from Bacillus subtilis strain A52: Dual reservoir of bioactives. Frontiers in microbiology. 2020;11:1167.
[40] Wan C, Fan X, Lou Z, Wang H, Olatunde A, Rengasamy KR. Iturin: Cyclic lipopeptide with multifunction biological potential. Critical Reviews in Food Science and Nutrition. 2022;62(29):7976-88.
[41] Yaraguppi DA, Bagewadi ZK, Patil NR, Mantri N. Iturin: A promising cyclic lipopeptide with diverse applications. Biomolecules. 2023 Oct 12;13(10):1515.
[42] Yue H, Zhong J, Li Z, Zhou J, Yang J, Wei H, et al. Optimization of iturin A production from Bacillus subtilis ZK-H2 in submerge fermentation by response surface methodology. 3 Biotech. 2021;11(2):36.
[43] Thepbandit W, Nawong S, Athinuwat D. Potential of a Microbial Co-Culture Composed of Bacillus vallismortis TU-Orga21 and Bacillus subtilis TU-Orga1 to Improve the Efficacy of Controlling Damping-Off Caused by Pythium aphanidermatum in Kale. Plant Pathology. 2025;74(6):1527-43.
[44] Xiao J, Guo X, Qiao X, Zhang X, Chen X, Zhang D. Activity of fengycin and iturin A isolated from Bacillus subtilis Z-14 on Gaeumannomyces graminis var. tritici and soil microbial diversity. Frontiers in microbiology. 2021;12:682437.
[45] Nannan C, Vu HQ, Gillis A, Caulier S, Nguyen TT, Mahillon J. Bacilysin within the Bacillus subtilis group: Gene prevalence versus antagonistic activity against Gram-negative foodborne pathogens. Journal of Biotechnology. 2021;327:28-35.
[46] Alajlani MM. Characterization of subtilosin gene in wild type Bacillus spp. and possible physiological role. Scientific Reports. 2022;12(1):10521.
[47] Ishida K, Nakamura A, Kojima S. Crystal structure of the AlbEF complex involved in subtilosin A biosynthesis. Structure. 2022;30(12):1637-46.
[48] Ghazaei C. Study of the Effect of Bacteriocin-producing Bacillus subtilis Strains on Beta-lactamase-producing Pathogenic Bacteria. J Clin Res Paramed Sci. 2022;11(2):e130208. Doi: 10.5812/jcrps-130208.
[49] Chandrika K, Sachan A. Enhanced production of bacteriocin by Bacillus subtilis ZY05. 3 Biotech. 2024;14(2):37.
[50] Hong SW, Kim JH, Cha HA, Chung KS, Bae HJ, Park WS, et al. Identification and Characterization of a Bacteriocin from the Newly Isolated Bacillus subtilis HD15 with Inhibitory Effects against Bacillus cereus. Journal of Microbiology and Biotechnology. 2022;32(11):1462.
[51] Liu ZY, Yu XZ. Engineering Bacillus subtilis for high-value bioproduction: Recent advances and applications. Microbial Cell Factories. 2025;24(1):182.

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