Trends in Biotechnology

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Review

Biocontrol manufacturing and agricultural applications of *Bacillus velezensis*

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Many microorganisms have been reported as bioagents for producing ecofriendly, cost-effective, and safe products. Some *Bacillus* species of bacteria can be used in agricultural applications. *Bacillus velezensis* in particular has shown promising results for controlling destructive phytopathogens and in biofungicide manufacturing. Some *B. velezensis* strains can promote plant growth and display antibiotic activities against plant pathogen agents. In this review, we focus on the often-overlooked potential properties of *B. velezensis* as a bioagent for applications that will extend beyond the traditional agricultural uses. We delve into its versatility and future prospects, the challenges such uses may encounter, and some drawbacks associated with *B. velezensis*-based products.

Highlights

Around 80% of the food consumed worldwide is provided by agriculture, and almost 30% of these products are affected by phytopathogenic agents, which cause numerous plant diseases.

Microorganisms have been used as biocontrol agents to eliminate or decrease damage caused by phytopathogens.

For the application of biocontrol agents, such as strains of *Bacillus velezensis*, several parameters need to be considered, including their habitat and molecular and biochemical conditions, such as plant growth promotion and antifungal activity.

B. velezensis strains also have potential as control agents against nematodes in greenhouse cultivation, as probiotic supplements for feed, to pretreat lignocellulosic materials, or in wastewater treatments through the enzymes they produce.

Brief introduction to phytopathogenic agents

The UN Food and Agricultural Organization (FAO) reports that 80% of the food consumed worldwide is provided by agriculture [1]. Almost 30% of these agricultural products are affected by **phytopathogenic agents** (see Glossary), such as fungi, bacteria, viruses, nematodes, and insect pests [2]. *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*Forc*), associated with cucumber root and stem rot diseases; *Pythium aphanidermatum*, a fast-growing fungus and causative agent of root rot disease; and *Phytophthora* are examples of phytopathogenic fungi associated with serious plant diseases with destructive effects on crops both in greenhouses and fields [3–5]. Although chemicals have been developed to control soil-borne phytopathogenic agents, many of these substances are considered hazardous to nature and humans. Soil degradation and destruction of beneficial microorganisms and soil microbial diversity are significant concerns related to the use of chemicals for controlling soil-borne diseases and a powerful motivation for researchers to explore bio-based solutions to replace chemical pesticides [6]. The UN Sustainable Development Goals (SDGs) support the development of environmentally safe agricultural protocols and processes [7,8]. Four main categories are being considered for biocontrol technologies and bio-based solutions to protect agriculture worldwide: (i) microbials, which are products derived from microorganisms, including bacteria, fungi, and protozoans, along with viruses; (ii) **semiochemicals**, which are substances released by plants, animals, and other organisms to facilitate intra- and interspecies communication; (iii) natural substances comprising one or more natural components, including plants, algae or microalgae, animals, minerals, among others, which can be obtained directly from nature or created synthetically; and, finally, (iv) microbial or invertebrate **biocontrol agents (BCAs), which** are natural enemies used for controlling pest populations via predation or parasitism [9].

According to the **International Biocontrol Manufacturers Association (IBMA)**[i], **bioprotection** (which encompasses this set of four biocontrol technologies) is a way toward sustainable and safe agriculture. This makes it a vital approach in the EU's work to support farmers and effectively implement the Sustainable Use of Pesticides Directive (SUD, Directive 128/2009/EC).

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IBMA represents over 250 companies inspired by nature, seeking innovative and effective biocontrol solutions. These companies are joined by allies from around the world, all working on enhancing the sustainability of the global food production system. This effort is aimed at benefiting farmers, consumers, and the environment [10].

Bacillus bacteria are promising BCAs. In 1872, Cohn was the first to report *Bacillus* as heat-resistant, endospore-forming bacteria [11]. The genus *Bacillus* is classified as follows: kingdom Bacteria; phylum Firmicutes; class Bacilli; order Bacillales; family Bacillacae [12]. PCR and denaturing gradient gel electrophoresis (PCR-DGGE) studies revealed that ~95% of Gram-positive bacteria isolated from soil samples were putative *Bacillus* species [13]. The biocontrol potential of the *Bacillus subtilis* group is attributed to the production of ribosomal and nonribosomal secondary metabolites with antagonistic effects against fungi, bacteria, and parasitic nematodes [14,15]. Among other *Bacillus* species, certain strains of *Bacillus velezensis* are known to harbor ribosomal and nonribosomal secondary metabolites, including polyketides and cyclic lipopeptides. These metabolites are of particular interest due to their antagonistic activities against various phytopathogens [16–28]. The metabolic pathways for the biosynthesis of the secondary metabolites surfactin, fengycin, bacillomycin-D, and bacillibactin are shown in Figure 1.

The rhizosphere is the first line of defense for plants by enhancing plant growth and providing protection against infectious microorganisms. Thus, strategies have been developed to harness the potential of rhizosphere microorganisms to prevent disease. *Bacillus* spp. are considered model microorganisms for studying biofilm formation in the rhizosphere. *B. velezensis*, a member of the *B. subtilis* group, can also form endospores, enabling them to survive for long periods in diverse environmental conditions [29–31]. Indeed, the use of BCAs is an integral part of **integrated pest and disease management (IPDM)**, a sustainable alternative to mitigate the detrimental effects of diseases on crop productivity and quality [11]. IPDM combines cultural, chemical, and biological methods in a compatible manner to develop sustainable agriculture and ensure global food security. Given their abundance and diversity in agrosystems, ranging from soil to water, animals, and plants, *Bacillus* spp. have been extensively used for biocontrol purposes. *B. velezensis* offers dual benefits of synthesizing secondary metabolites with antagonistic activities against phytopathogenic agents and engaging in niche competition to suppress the microbial diversity of plant pathogens [6,32].

Previous literature has focused on the molecular mechanisms elucidating the plant growth-promoting and antifungal activities [33] or the molecular pathways leading to the beneficial effects of *B. velezensis.* Here, we summarize and discuss the main gene clusters associated with the biocontrol properties of *B. velezensis* [12,34–37]. In addition, we shed light on the often overlooked and captivating potential properties of *B. velezensis* as a BCA with applications extending beyond its traditionally discussed roles. We delve into its versatility, prospects, the challenges its development may encounter, and drawbacks associated with *B. velezensis*-based products.

Habitats and genotypic molecular identification of *B. velezensis*

Bacillus spp. can be obtained from diverse sources, such as soil, rocks, and water, providing almost unlimited possibilities for their isolation. Members of the *B. subtilis* group have been isolated from different habitats, and among the *B. subtilis* group, *B. velezensis* strains have been reported for their biocontrol and antifungal activity against destructive phytopathogens in field and greenhouse crop cultivation (Table 1).

Various techniques have been applied to isolate a *Bacillus* species suitable for biocontrol manufacturing, with *B. velezensis* strains being obtained from diverse sources, namely water,

Glossary

Allelopathy: chemical inhibition that occurs when the substances released by one microorganism (or plant) hinder the growth or activity of another, as a result of their coexistence in the environment.
Azoreductase: variety of flavoenzymes, identified in both prokaryotes and eukaryotes, with a primarily role in the detoxification and biotransformation of azo-dyes and azoic drugs.
Biocontrol agents (BCAs): biological agents used to develop technologies to effectively control pests and diseases in an environmentally friendly way in agriculture, forestry, public spaces, and gardens.
Bioflocculant: environmentally friendly biodegradable alternatives to flocculants, comprising polysaccharides, proteins, and glycoproteins.
Bioprotection: global term used for the set of biocontrol technologies; that is, technologies used to protect crops using biosolutions.
Decolorization: bleaching or removing the color from something.
Detoxification: identifying and removing toxins from a medium or body.
Endoglucanase: active enzyme that cleaves internal glycoside bonds in a polymeric chain of glucose.
Endoglycosidase: active enzyme that cleaves glycan groups from glycoproteins.
Flocculant: substrate that facilitates the agglomeration of fine particles in a solution, forming a floc that can be easily sedimented or floated to simplify the removal of the target substance from the liquid.
Integrated pest and disease management (IPDM): entails assessing the pest situation and subsequently devising a systematic approach to address these challenges within the context of crop production. The complexity lies in the need to consider the impact on the entire crop block, as well as the potential side effects on BCAs working 'behind the scenes' to prevent other pests from becoming problematic.
International Biocontrol Manufacturers Association (IBMA): 'IBMA is the experienced voice of Biocontrol for a sustainable and resilient global agriculture for healthier food systems to benefit farmers, environment, and consumers'[i].

soil, and other habitats. Molecular and biochemical analyses, including plant growth promotion and antifungal activity, are commonly used to isolate *B. velezensis* strains [38,39]. Although we do not discuss these topics in detail, we briefly discuss the general process for the molecular genetic identification of isolated bacteria.

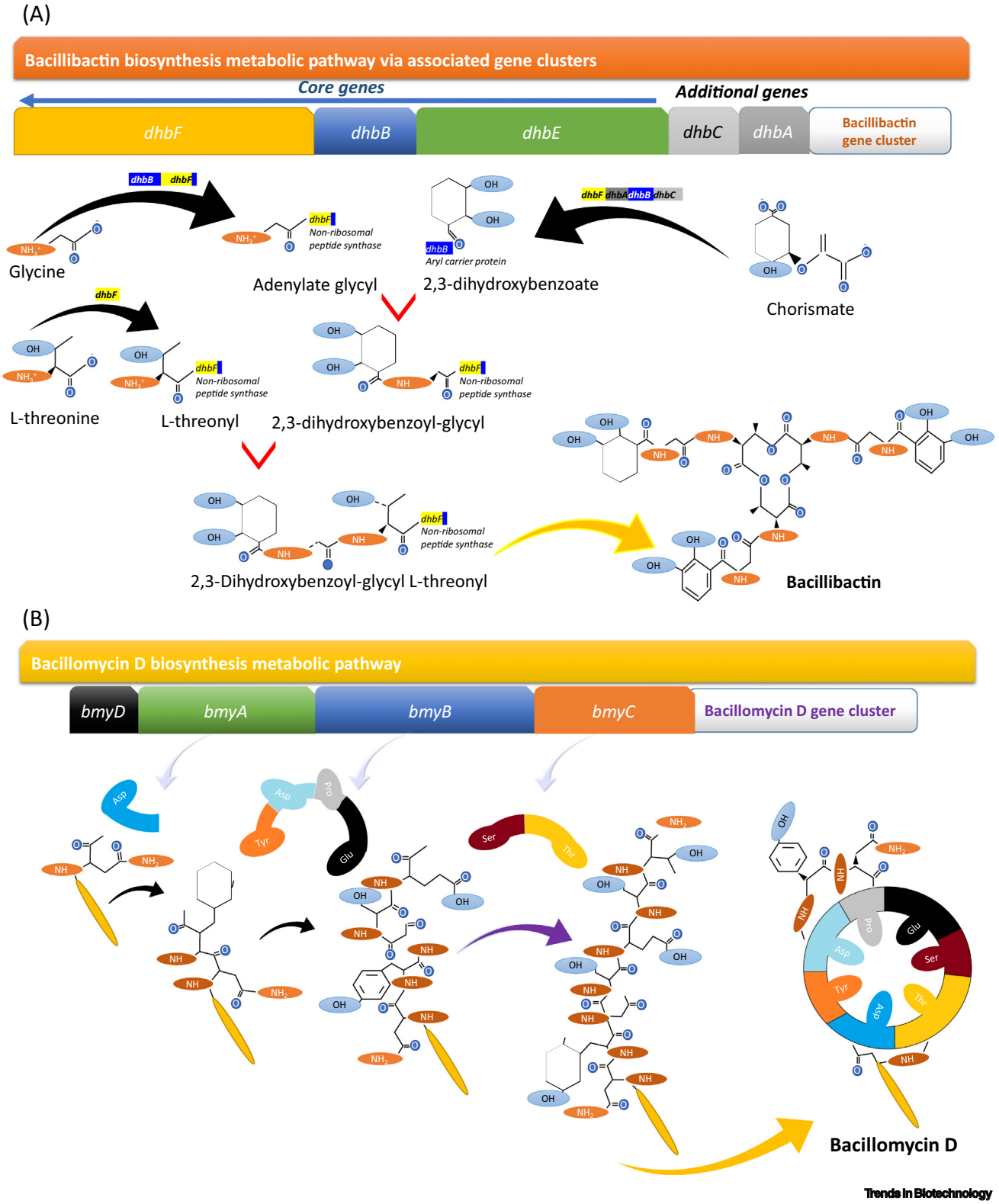
The initial step in molecular genetic identification involves 16s rDNA PCR. Successful PCR requires knowledge and experience, considering essential parameters such as the quality and purity of extracted genomic DNA (using genomic DNA extraction kits or other methods), the ratio and total volume of PCR ingredients (master mix, forward-reverse primers, and distilled water), and sensitive optimization of PCR parameters, including denaturation, annealing, extension time, and temperature, based on the DNA fragment [40,41]. Although universal primers, commonly used by many researchers, are valuable, some authors advocate alternative genomic DNA identifications, such as housekeeping genes, particularly *gyrA* or *gyrB*, instead of 16s rDNA. They argue that these genes exhibit higher conservation and offer better resolution for molecular identification of *Bacillus* spp., enhancing the reliability of the results for further analysis [42,43]. A comparison of PCR conditions for 16s rDNA, *gyrA*, *gyrB*, and *amyA1* is provided in Table 2, with additional information available elsewhere [13,44,45]. Thus, through proper primer design and optimized PCR, it is not only possible, but also recommended to identify the presence of gene clusters associated with the synthesis of secondary metabolites responsible for the antagonistic and antifungal activity of *B. velezensis*.

Antagonistic activities of *B. velezensis*

In 2007, the Gram-positive BCA *Bacillus amyloliquefaciens* strain FZB42 was the first to have its complete genome sequenced. This strain demonstrated the production of promising bioactive secondary molecules that hold significant potential for agricultural applications [46]; later work revealed its promising biocontrol activity and plant growth-promoting properties [47]. Notably, many *B. velezensis* strains are reported to have gene clusters associated with the secretion of secondary metabolites with antifungal activities, which have been used to produce biocontrol products exhibiting a variety of antagonistic properties against phytopathogenic agents (Figure 2). Among these gene clusters, *srf*, *bmy*, *fen*, *nrs*, *and dhb* (137 kb) are responsible for synthesizing cyclic lipopeptides, namely surfactin, bacillomycin-D, fengycin, iron-siderophore bacillibactin, and certain unidentified peptides, respectively. Additionally, three other gene clusters, *mln*, *bae*, and *dfn* (199 kb), are responsible for the synthesis of antibacterial polyketides, such as macrolactin, bacillaene, and difficidin. Moreover, the *bac* cluster (6.9 kb) directly contributes to the production of bacilysin, an antibacterial dipeptide [48–53]. Surfactin, a cyclic lipopeptide known for its antimicrobial properties, is a potent surfactant widely used as an antibiotic. Iturin, another lipopeptide, exhibits robust antifungal activity, while fengycin and kurstakin are additional examples of secondary metabolites with antagonistic effects against plant pathogenic agents [14]. Genomic analysis of *B. velezensis* strain LM2303 revealed 13 gene clusters associated with its antifungal activity against *Fusarium graminearum*. This finding demonstrates the remarkable capacity of this strain to synthesize secondary metabolites with antagonistic properties against plant pathogenic agents (Figure 3).

The pivotal factor in considering *B. velezensis* as a potent bioagent for manufacturing biocontrol products lies in its capacity to produce specific gene clusters associated with secondary metabolites actively involved in suppressing the phytopathogenic activities of various phytopathogenic agents (Box 1). The antibacterial properties of certain macrolactin and bacillaene compounds produced by *B. velezensis*, associated with specific gene clusters (Box 1), are not fully understood, and warrant further investigation. Plantazolicin, a novel antimicrobial and nematocidal agent, is synthesized by a cluster of 12 genes spanning nearly 10 kb. Amylocyclicin, another

Levanase: enzyme responsible for metabolism of levan (a naturally occurring homopolymer of fructose).
Ochratoxin: foodborne mycotoxin produced by some fungi and responsible for contaminating a variety of agricultural commodities around the globe.
Phosphogalactosidase: hydrolytic enzyme specifically able to hydrolyze *O*- and *S*-glycosyl compounds to cleave disaccharides (lactose) to produce galactose and glucose.
Phosphoglucosidase: hydrolytic enzyme specifically able to hydrolyze *O*- and *S*-glycosyl compounds and to catalyze the degradation of phosphorylated glucosides or fiber-related disaccharides.
Phytopathogenic agents: fungal phytopathogens that can be classified as biotrophs that survive by utilizing nutrients in the plant; hemibiotrophs live as biotrophs during the first half of the plant life cycle, whereas necrotrophs live as biotrophs during the second half and survive by killing the plant tissue to access nutrients.
Semiochemicals: chemical substances produced by animals or plants that evoke the same biological or behavioral response in individuals from the same or another species.
Sucrose-phosphate hydrolase: enzyme required for the metabolism of phosphorylated sucrose.


(A)
Bacillibactin biosynthesis metabolic pathway via associated gene clusters
Core genes
Additional genes
dhbF
dhbB
dhbE
dhbC
dhbA
Bacillibactin gene cluster
Glycine
Adenylate glycyl
Aryl carrier protein
2,3-dihydroxybenzoate
Chorismate
Non-ribosomal peptide synthase
L-threonine
L-threonyl
2,3-dihydroxybenzoyl-glycyl
2,3-Dihydroxybenzoyl-glycyl L-threonyl
Bacillibactin
(B)
Bacillomycin D biosynthesis metabolic pathway
bmyD
bmyA
bmyB
bmyC
Bacillomycin D gene cluster
Asp
Tyr
Pro
Glu
Ser
Thr
Bacillomycin D
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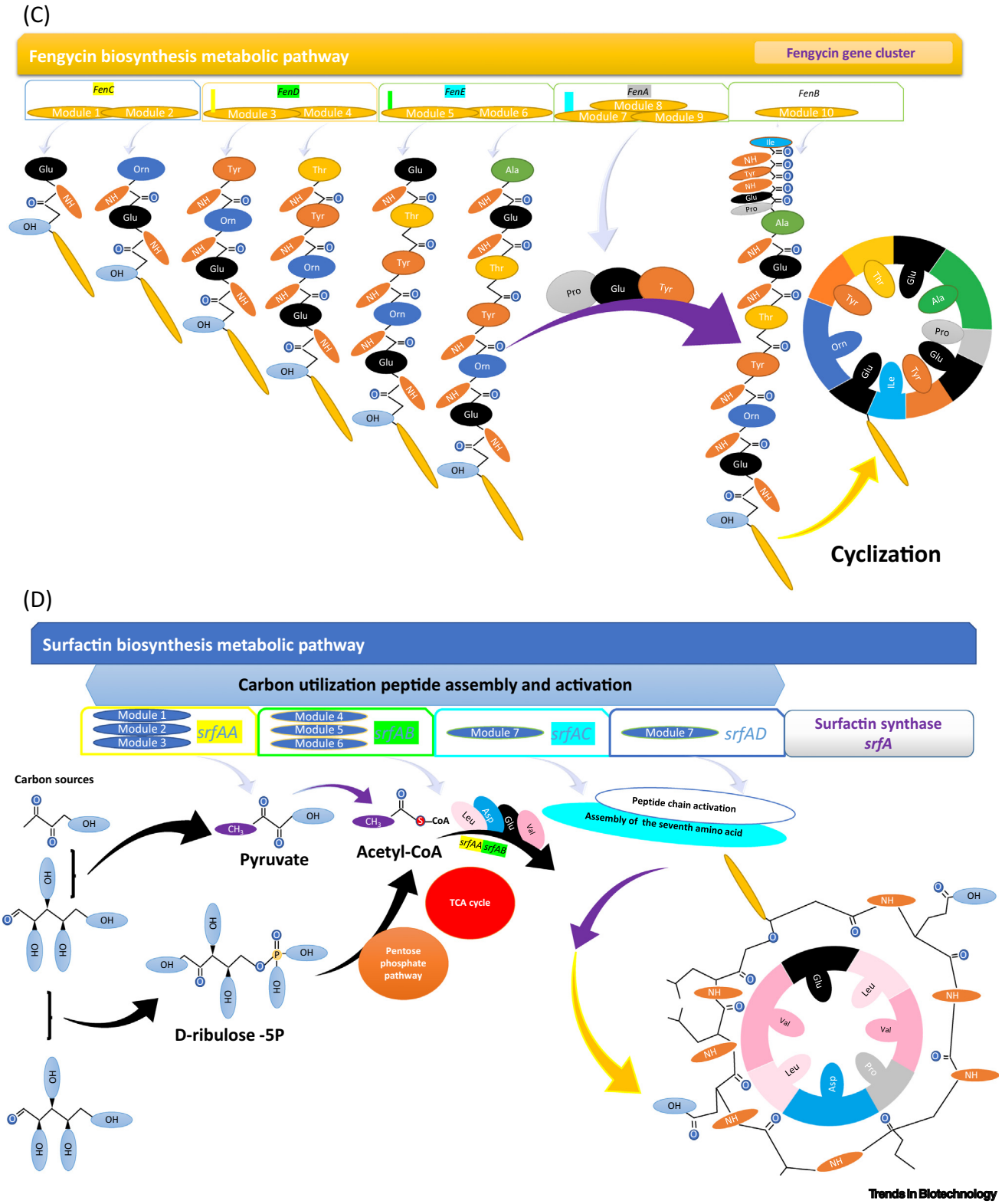

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Table 1. *Bacillus velezensis* strains with biocontrol activity against specific phytopathogenic agents and acting as bioflocculants

Strain	Country and year of origin; habitat isolated from	Phytopathogenic agent	Associated plant disease	Refs
BR-01	China, 2022	*Magnaporthe oryzae*	Rice blast	[76]
		Ustilaginoidea virens	Rice false smut	
		Fusarium fujikuroi	Rice bakanae disease	
		Rhizoctonia solani	Rice sheath blight	
		Xanthomonas oryzae pv. oryzicol	Rice bacterial leaf streak	
		Xanthomonas oryzae pv. oryzae	Rice bacterial blight	
9D-6	Canada, 2012	*Pseudomonas syringae*	Bacterium Infection	[77]
		Xanthomona ampestresis	Black rot in cruciferous vegetables	
		Xanthomonas euvesicatoria	Bacterial spot of tomato	
		Clavibacter michiganensis	Bacterial canker of tomato	
		Monilinia fructicola	Brown rot in stone fruit	
		Alternaria solani	Early blight of tomato and potato	
		Phaeotrichoconis terrestris	Pink rot of onion	
GUMT319	China, 2019; tobacco rhizosphere	*Colletotrichum scovillei*	Anthracnose of chili in Asia	[78]
		Colletotrichum capsica	Leaf blight on *Chlorophytum borivilianum*, basil, chickpea, pepper	
		Sclerotinia sclerotiorum	White mold, leaf spots, rot, and blight on many plant parts	
E68	Canada, 2020; oil battery	*Fusarium graminearum*	*Fusarium* head blight	[79,80]
RC 218	Argentina, 2004; wheat anthers	*F. graminearum* (*Gibberella zeae*)	Wheat and barley head blight disease	[14,81]
QST713	Commercial compost; in possession of Bayer AG	*Trichoderma aggressivum*	Green mold disease of mushrooms; also as biocontrol for scab, gray mold, and several mildews	[81–83]
40B	Egypt; brackish water, Qarun Lake	–	Flocculation properties, proposed as carbohydrate bioflocculant	[69,81]
LM2303	China; wild yak dung, Qinghai-Tibet plateau	*F. graminearum*	Fusarium head blight	[49,81,84]
CC09	Leaf tissue of *Cinnamomum camphora*	*Glomerella glycines*, *Rhizoctonia solani*, *B. graminis* f. sp. *tritici*, *Alternaria alternate*	Wheat powdery mildew disease, plant growth-promoting bacteria, potential biocontrol vaccine	[81,85,86]

antibiotic, is a highly hydrophobic cyclic peptide synthesized by a cluster of six genes spanning ~4.5 kb [46,47]. Investigation of *B. velezensis* strain ZF2, isolated from a healthy cucumber plant stem, demonstrated activity against the cucumber leaf spot fungus *Corynespora cassiicola*. Genomic and molecular analyses revealed close similarities to other *B. velezensis* strains, with 14 gene clusters associated with the production of secondary metabolites exhibiting antimicrobial activities and a broad spectrum of antagonistic activities against 14 plant bacterial and fungal diseases [54]. According to Palazzini and coworkers [14], the core genomes of *B. velezensis*, *B. amyloliquefaciens*, and *Bacillus siamensis* contain gene clusters responsible for the synthesis

Figure 1. Metabolic pathways for the biosynthesis of the four main nonribosomal secondary metabolites produced by *Bacillus velezensis*: (A) surfactin, (B) bacillibactin, (C) bacillomycin D, and (D) fengycin. These secondary metabolites are lipopeptides with antagonistic activities. All steps and intermediates in the pathways are described, including bioconversion (black arrow), multi-step bioconversion (violet arrows), gene(s) associated with the bioprocess (gray arrows), dimerizations (red arrows), and cyclizations (yellow arrows). Fatty acid chains are represented by the yellow ellipse.

Table 2. PCR parameters for partial genomic DNA amplification of *Bacillus velezensis* strains

B. velezensis strain	Genomic DNA	Length (bp)	Forward-reverse primers	PCR setup					Refs
				Initial temperature (time)	Denaturation temperature (time)	Annealing temperature (time)	Extension temperature (time)	Total no. of cycles	
K68	16s rRNA	1504	F5′-CCTACGGGAG GCAGCAGTAG-3′ R5′-CAACAGAGCTTTACGATCCGAAA-3′	95°C (5 min)	95°C (15 s)	60°C (1 min)	60°C (5 min)	40	[44]
ES2-4	*gyrA*	950	F: 5′- GCAATGAGCGTTATCGTATCCCGG-3′ R: 5′-TCAATCTTTTCGCGCTCCAGATCC-3′	94°C (2 min)	94°C (30 s)	52°C (30 s)	72°C (2 min)	40	[42]
BR-01	16S rDNA	1500	27F5′ -AGAGTTTGATCCTGGCTCAG-3′ 1492R5′-GGTTACCTTGTTACGACTT-3′	95°C (5 min)	95°C (30 s)	60°C (30 s)	72°C (30 s)	30	[76]
				94°C (5 min)	94°C (30 s)	60°C (30 s)	72°C (30 s)	25	[40]
	gyrB	1190	UP1 5′-GAAGTCATCATGACCGTT CTGC AYGCNGGNGGNAARTTYGA-3′ UP2r 5′-AGCAG GATACGGATGTGCGAGCC RTCNACRTCNGCRTCNGTCAT-5′	94°C (5 min)	94°C (1 min)	60°C (1 min)	72°C (2 min)	30	[41,76]
9D-6	16S rDNA	1500	8F 5′- AGAGTTTGATCCTGGCTCAG-3′ 1492R 5′-GGTTACCTTGTTACGACTT-3′	95°C (5 min)	94°C (30 s)	57°C (45 s)	72°C (1 min)	30	[77]
GUMT319	16S rDNA	1400	27F 5′-AGAGTTTGATCCTGGCTCAG-3′ 1492R 5′-GGTTACCTTGTTACGACTT-3′	95°C (10 min	94°C (30 s)	56°C (30 s)	72°C (90 s)	30	[78]
	gyrA	1000	F5′-CAGTCAGGAAATGCGTACGTCCTT-5′ R 5′-CAAGGTAATGCTCCAGGCATTGCT-3′						
GF610	*amyA1*	132	F5′-ATGTCAATTATTGAAACATGG–3′ R5′-CTAATTACAGCTACTTTGAC–3′ F5′-ATGTCAAATCGTGAAAAAGC–3′ R5′-GCAGCTTGATGTACATTTGG–2′	95°C (3 min)	95°C (15 s)	45°C (15 s)	72°C (20 s)	35	[87]
ZSY-1	16S rRNA	1200	F5′-GAAGGCGGNACNCAYGAAG-3′ R5′-CTTCRTGNGTNCCGCCTTC-3′	95°C (5 min)	94°C (45 s)	55°C (45 s)	72°C (90 s)	30	[88]

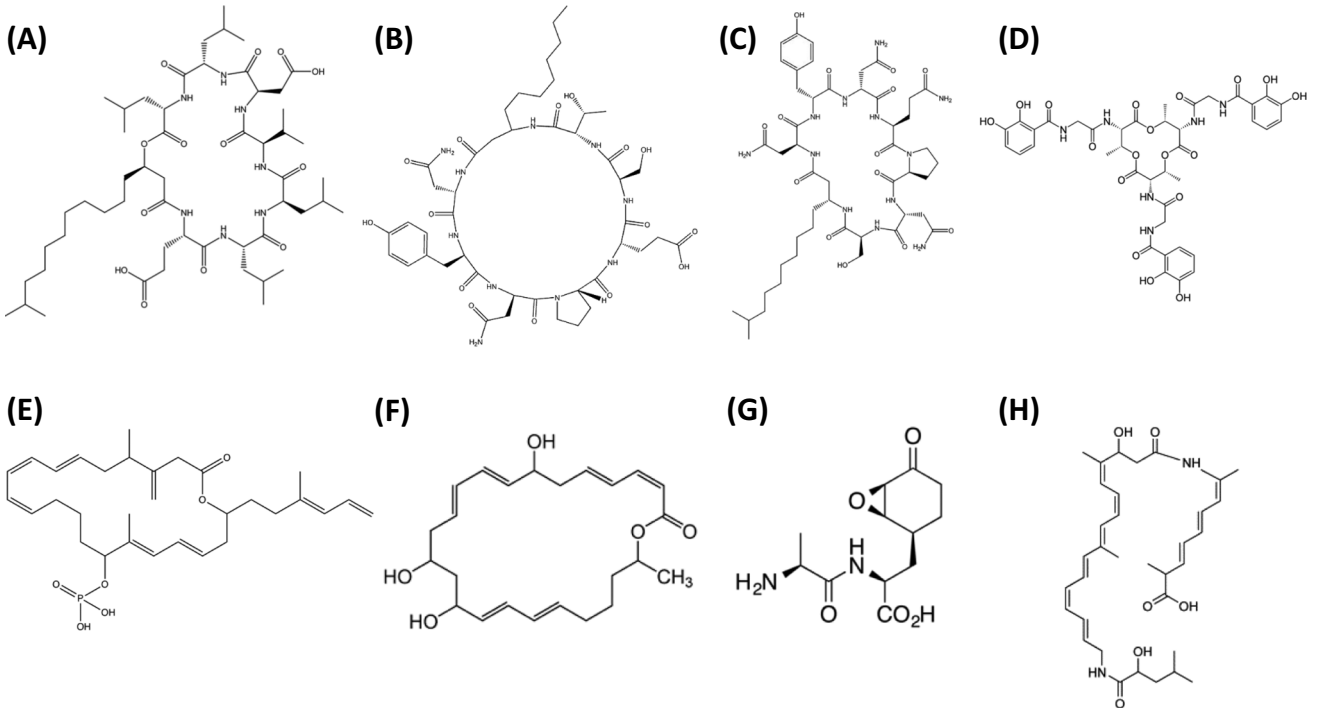

Figure 2. Chemical structures of the secondary metabolites synthesized by *Bacillus velezensis* strains most commonly reported in the literature. These metabolites are recognized as having biocontrol potential due to their antagonistic effects against different phytopathogenic agents. The images highlight the variety of structures involved, from cyclic to non-cyclic. (A) surfactin; (B) bacillomycin D; (C) fengycin; (D) iron-siderophore bacillibactin; (E) difficidin; (F) macrolactin; (G) bacilysin; and (H) bacillaene.

of surfactin, bacillibactin, bacillaene, amylocyclicin, and an iturin-like substrate. Strains of *B. velezensis* also have gene clusters for the synthesis of bacilysin, fengycin, macrolactin, and difficidin [14,15]. Further genomic analysis of *B. velezensis* identified an additional nine gene clusters associated with the biosynthesis of secondary metabolites, including polyketides, such as macrolactin, bacillaene, and difficidin, which exhibit antibiotic properties [55,56]. Among the isolated strains of *B. velezensis*, several secondary metabolites exhibit significant similarities and can be compared based on their compound activity, secretion source, and size of the associated gene cluster (Table 3). The presence of these gene clusters in *B. velezensis*, coupled with reported similar antagonistic activities, indicates that this bacterium holds immense potential as a reliable source of biocontrol products for use in agricultural applications.

Other applications of *B. velezensis*

The agricultural use of *B. velezensis*, with emphasis on its antifungal and plant growth-promoting properties, has attracted consistent attention. However, the current focus on *B. velezensis* goes beyond its antifungal capabilities; it also shows promising potential for nematode control. For example, the *B. velezensis* strain BV-DS1, isolated from tidal soil, demonstrated significant nematocidal activity against the root knot nematode (RKN), *Meloidogyne incognita*, particularly targeting its reproduction and hatching stages. In laboratory pod assays, BV-DS1 resulted in a mortality rate of 71.62% in second-stage RKN juveniles [30]. Similarly, the *B. velezensis* strain Bv-25, isolated from cucumber rhizosphere soil, exhibited inhibitory activity against *M. incognita* egg hatching and achieved a robust mortality rate of 100% in second-stage juveniles within 12 h of exposure to the fermented broth of the isolated strain. Furthermore, the application of this

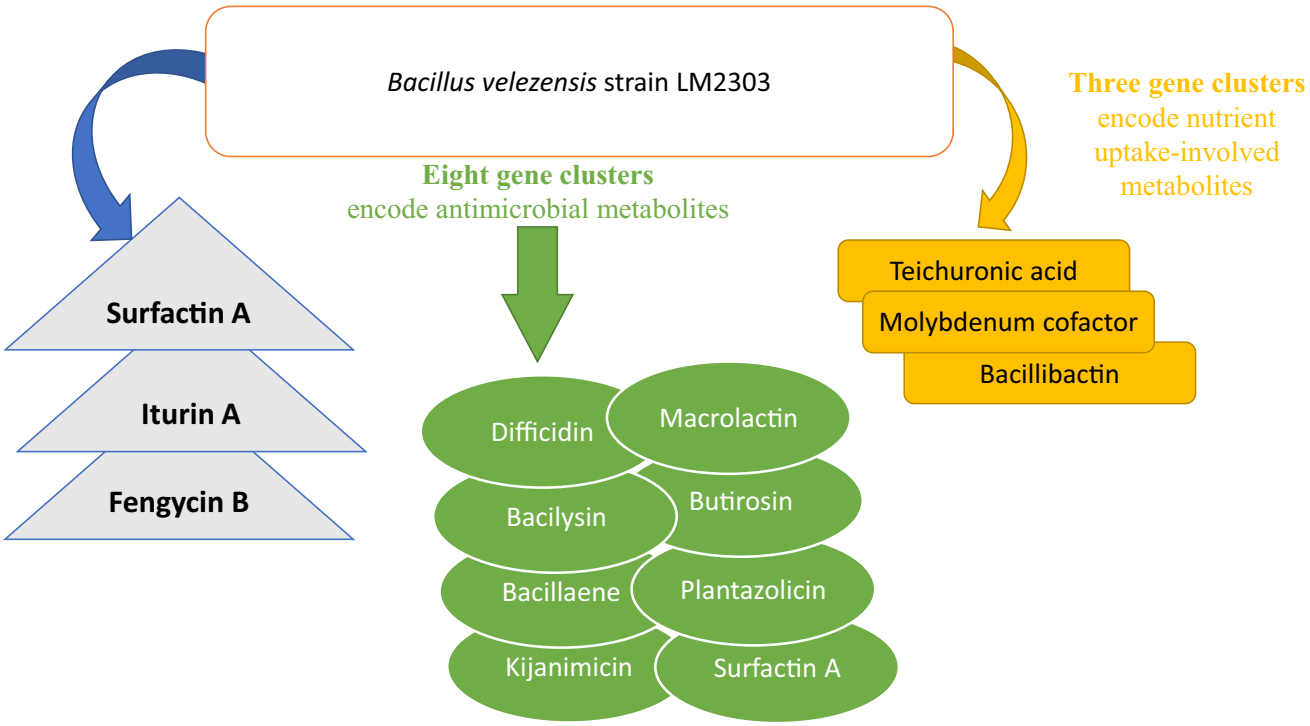

Figure 3. Representation of the 13 gene clusters associated with the biosynthesis of secondary metabolites by the *Bacillus velezensis* strain LM2303. These represent cluster genes involved in antifungal activity against plant pathogenic agents, such as *Fusarium graminearum* [49].

bacterium resulted in the upregulation of cucumber defense response genes, such as *prl*, *pr3*, and *lox1*, leading to induced systemic resistance to *M. incognita* in split-root trials [57]. These investigations have the potential to address a major challenge in greenhouse cultivation, namely contamination by nematodes (which are pervasive plant pathogens). These promising results pave the way for more in-depth research into the molecular mechanisms behind this biocontrol activity. This is especially crucial for addressing various contamination levels and stages, ranging from the severity of contamination to the effectiveness of *B. velezensis* on nematodes throughout their lifecycle, from eggs and hatching stages to fully grown adults. Moreover, these studies consider the diverse contexts of crop cultivation and climates.

Box 1. Gene clusters produced by different strains of *Bacillus velezensis*

Gene clusters of *fenABCDE*; *bmyCBAD*; *dhbABCDEF*; *dfnAYXBCDEFGHIJKLM*; *bacABCDE-ywfG*; *acnBACDEF*; and *pznFKGHIAJCDBEL* associated with the synthesis of fengycin, bacillomycin D, bacillibactin, difficidin, bacilysin, amylocycelicin, and plantazolicin, respectively were identified via molecular genetic analysis of *Bacillus velezensis* strain FZB42 [15,30,40,47,57]. The gene clusters *ituDABC* and *fenABCDE* were identified in *B. velezensis* strain RC218, associated with the synthesis of iturin, and fengycin respectively, and the gene clusters *fenABCDE*; *bmyCBAD*; *dhbABCDEF*; *dfnAYXBCDEFGHIJKLM*; *bacABCDE-ywfG*; *acnBACDEF*; and *pznFKGHIAJCDBEL* were detected in *B. velezensis* strain GUMT319, associated with the production of fengycin, bacillomycin D, bacillibactin, difficidin, bacilysin, amylocycelicin, and plantazolicin, respectively [89,90].

B. velezensis strain ES2-4 was also identified as a BCA due to gene clusters such as *srfAA,AB,AC,AD*, and *sfp*; *ituABCD*; *fenABCDE*; *dhbACEB*; *bacABCDEFG*; *acnBACDE*; *LanBT,C,A,S,I,F,E,G,R,K*; *mlnABCDEFGHI*; *baeBCDE,acpK*, and *baeGHIJLMNRS*; and *dfnAYXBCDEFGHIJKML* associated with the production of surfactin, iturin, fengycin, bacillibactin, bacilysin, amylocyclicin, ericin, macrolactin, bacillaene, and difficidin, respectively [89]. *B. velezensis* strains E68, and 9D-6 are reported to have similar gene clusters, while *B. velezensis* strain TSA32-1 is reported to have gene clusters *ppsA,C,D,E*; and *mycA,B,C* associated with the synthesis of the secondary metabolites plipastatin, and mycosubtilin, respectively [56,81,89].

Table 3. *Bacillus velezensis* strains identified for the synthesis of secondary metabolites[a]

Strain	Metabolite	Bioactivity	Enzyme	Size (kb)	Refs
ES2-4	Surfactin	Multiple antagonistic activities	NRPS	26.8	[14]
	Iturin	Antifungal activities	NRPS/PKS	36.9	
	Fengycin	Antifungal activities	NRPS	37.6	
	Bacillibactin	Siderophore	NRPS	11.7	
	Bacilysin	Antibacterial	NRPS	6.7	
	Amylocyclicin	Antibacterial	RSP	4.4	
	Ericin	Antibacterial	RSP	14.2	
	Macrolactin	Antibacterial	PKS	53.1	
	Bacillaene	Antibacterial	PKS/NRPS	71.6	
	Difficidin	Antibacterial	PKS	69.1	
E68	Surfactin	Multiple antagonistic activities	NRPS	26.2	[80]
	Iturin	Antifungal activities	NRPS/PKS	37.2	
	Fengycin	Antifungal activities	NRPS	37.7	
	Bacillibactin	Siderophore	NRPS	12.7	
	Bacilysin	Antibacterial	NRPS	6.7	
	Amylocyclicin	Antibacterial	RSP	4.5	
	Ericin	Antibacterial	RSP	14.2	
	Macrolactin	Antibacterial	PKS	53.2	
	Bacillaene	Antibacterial	PKS/NRPS	72.5	
	Difficidin	Antibacterial	PKS	69.5	
TSA32-1	Surfactin	Multiple antagonistic activities	NRPS	26.1	[89]
	Bacilysin	Antibacterial	NRPS	6.7	
	Plipastatin	Antifungal activities	NRPS	37.6	
	Bacillaene	Antibacterial	PKS-NRPS	43.3	
	Mycosubtilin	Antibacterial	PKS-NRPS	64.6	
9D-6	Surfactin	Multiple antagonistic activities	NRPS	26.2	[77]
	Iturin	Antifungal activities	NRPS/PKS	37.2	
	Fengycin	Antifungal activities	NRPS	37.7	
	Bacillibactin	Siderophore	NRPS	12.7	
	Bacilysin	Antibacterial	NRPS	6.7	
	Amylocyclicin	Antibacterial	RSP	4.5	
	Ericin	Antibacterial	RSP	14.2	
	Macrolactin	Antibacterial	PKS	53.2	
	Bacillaene	Antibacterial	PKS/NRPS	72.5	
	Difficidin	Antibacterial	PKS	69.5	
157	Surfactin	Multiple antagonistic activities	NRPS	65.4	[70]
	Macrolactin	Antibacterial	Transatpks	82.2	
	Bacillaene	Antibacterial	NRPS	102.7	
	Fengycin	Antifungal activities	Transatpks	137.8	
	Difcidin	Antibacterial	Transatpks	100.4	
	Bacillibactin	Siderophore	Bacteriocin-Nrps	66.8	

[a]Abbreviations: NRPS, nonribosomal peptide synthase; PKS, polyketide synthase; RSP, ribosomally synthesized peptide.

Alongside its biofungicidal activities and antagonistic effects against phytopathogens, *B. velezensis* has a longstanding history of being used as a probiotic supplement in fish diets, yielding safe, effective, and promising results. The bactericidal activity of *B. velezensis* against *Vibrio anguillarum* strain 507, a pathogenic bacterium in fish, was also assessed, providing clear evidence of its beneficial immune effects and probiotic properties [58]. Furthermore, the antimicrobial properties of *B. velezensis* strain ANSB01E were evaluated using the Oxford cup method. The results demonstrated its antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. This study suggests the use of *B. velezensis* as a probiotic in animal feed, highlighting its potential for modulating the gut microbiota and gastrointestinal tract, which is a key site for probiotic colonization. The stabilization of the gut microbiota is proposed to contribute to disease resistance and overall adaptability of animals to their environment [59]. In a separate study, the probiotic potential of *B. velezensis* AP193 was explored in channel catfish, leading to the stabilization of intestinal microbiota and improved water quality in the pond. This research suggests the use of *B. velezensis* as a probiotic for fish species [60]. *B. velezensis* JW exhibited antimicrobial activity against fish pathogenic bacteria, such as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Lactococcus garvieae*, and *Streptococcus agalactiae*. Additionally, this strain produced four bacteriocins and three polyketide synthetases (PKSs), and had five nonribosomal peptide synthase (NRPS) gene clusters. These findings demonstrate the probiotic properties of *B. velezensis* JW, which makes it suitable for administration in aquaculture settings [61]. The administration of *B. velezensis* BV-7 to mice resulted in a decrease in the population of pathogenic microbes, leading to an improvement in the overall microbiota in mice [62]. Furthermore, an isolated strain of *B. velezensis* obtained from pigeon feces was orally administrated to pigeons for 60 days. Subsequently, the group was challenged with pigeon circovirus (PiCV), a viral pathogen. In another experiment, pigeons that were orally administrated with this strain of *B. velezensis* showed an enhanced T helper type 1 immune response and a reduction in the viral load of PiCV compared with the control group [63]. Furthermore, the probiotic properties of *B. velezensis* were evaluated in a study involving 468 Hy-Line Brown chickens, aged 49 weeks. The chickens were divided into groups receiving different concentrations of *B. velezensis* (0.1% and 0.2%), a regular diet, or an antibiotic (flavomycin) at a dosage of 50 mg/kg^{-1}. The results indicated the potential application of *B. velezensis* as a viable alternative to the use of antibiotics in the poultry industry [64].

B. velezensis has been also studied as a probiotic bioagent. This could offer a viable alternative, either reducing or replacing synthetic antibiotics, not only in livestock, but also in humans to mitigate associated risks. Additionally, the versatility of *B. velezensis* extends beyond agriculture, holding promise for various industrial processes, especially those involving expensive enzymes. Specific strains of *B. velezensis* could be utilized to modify industrial processes based on specific needs, leveraging its synthesis of diverse hydrolytic enzymes for applications beyond biocontrol manufacturing. For instance, laccases have been reported for their oxidizing activity on a range of substrates and it would be interesting to explore possible industrial applications for hydrolytic enzymes derived from 24 strains of *B. velezensis* (Table 4).

New horizons and drawbacks to overcome to the use of *B. velezensis*

Although not discussed here, there is substantial evidence supporting the beneficial interactions between *B. velezensis* and plants, including plant growth promotion, biofilm formation, and enzyme synthesis through specific metabolic pathways, as well as their significant environmental impacts and, thus, environmental applications. However, further investigation in these areas could be warranted [34,65]. A novel recombinant laccase gene (rLac), originally extracted from *B. velezensis strain TCCC 111904,* was heterologously expressed in *E. coli*. This recombinant gene exhibited significant thermal and pH stability, making it potentially valuable for a variety of industrial applications. Additionally, the recombinant laccase showed high tolerance to most metal

Table 4. Hydrolytic, lignocellulolytic, and pectate lyase enzymes identified from 24 strains of *Bacillus velezensis*[a]

Enzyme type	Potential use	Gene	Enzyme name	No. identified
Lignocellulolytic	Cellulose and hemicellulose degradation	*GH5*	Endo-1,4-β-glucanase	1
		GH30	Glucan endo-1,6-β-glucosidase	1
		GH4	6-Phospho-β-glucosidase, 6-phospho-α-glucosidase	2
		GH1	6-Phospho-β-galactosidase	1
		GH16	β-Glucanase	1
		GH32	Two sucrose-6-phosphate hydrolases and levanase	3
	Hemicellulose degradation	*GH43*	1,4-β-Xylosidase; except strain 9912D	4
		GH11	Endo-1,4-β-xylanase	1
		GH43	Two arabinan endo 1,5-α-L-arabinosidases and one arabinoxylan arabinofuranohydrolase	3
		GH51	α-*N*-arabinofuranosidase	2
		GH30	Glucuronoxylanase	1
		GH26	β-Mannosidase	1
		GH53	Arabinogalactan endo-1,4-β-galactosidase	1
Pectate lyase	Potential for pectin degradation	*PL1*	Pectate lyase	2
		PL9	Pectate lyase	1

[a]Adapted from [70].

ions tested, without exhibiting significant inhibitory effects, enhancing its potential for industrial use. Notably, it demonstrated remarkable efficiency in **decolorizing** certain dyes found in wastewater from sewers or industrial outfalls, making *B. velezensis* a promising candidate for further detailed investigation in the field of effluent and wastewater management [66]. The primary reason for the isolation of *B. velezensis* strain AB was its direct **detoxification** activity toward dyes; this strain contains **azoreductase**, an enzyme crucial for degrading azo dyes known for their toxicity and carcinogenic properties. These azo dyes are commonly present in textile industry wastewaters, and their removal from industrial effluents is under investigation. A *B. velezensis* strain with azoreductase activity, capable of degrading azo dye (DR28) was identified, highlighting the potential for discovering new strains with significant decolorization and detoxification abilities [67,68]. Additionally, domestic and industrial wastewater management practices often involve the use of hazardous **flocculants**; thus seeking alternatives, such as **bioflocculants**, offers numerous advantages. Notably, a bacterium found in brackish waters, exhibiting a remarkable flocculant activity of >98%, was revealed by 16S rRNA-sequencing analysis as another *B. velezensis* strain [69]. Chen *et al.* investigated various *B. velezensis* strains for their ability to pretreat lignocellulosic materials and efficiently degrade cellulose due to the presence of genes encoding various lignocellulolytic enzymes. These enzymes, including **endoglucanases**, **endoglycosidases**, **sucrose-phosphate hydrolases**, **phosphoglucosidases**, **phosphogalactosidases**, and **levanases**, enabled the degradation of agroindustrial byproducts, including wheat straw, rice husks, maize flour, maize straw, soybean meal, wheat bran, and sugarcane bagasse, making them appropriate for use in biofuel production. During solid-state fermentation, *B. velezensis* strain 157 produced several industrial enzymes capable of synthesizing hydrolytic enzymes, such as cellulase, pectinase, xylanase, and α-amylase, with lignocellulosic activities, which show promise for transforming lignocellulosic biomass into fuels, organic acids, and useful chemicals [70].

However, while the biocontrol properties of *Bacillus* spp., particularly *B. velezensis*, are considered a significant advance in the manufacturing of various biocontrol products, there are certain drawbacks that need to be considered when producing biocontrol solutions for agricultural applications.

BCAs are typically tailored to specific pests and can become cost-effective once the initial setup is in place. However, due to the intricate molecular interactions between the bioagent and its environment, there is always potential for failure or only partial success in certain situations, and complete pest elimination may not always be attainable. In addition, the rehabilitation or controlling effect of the BCA may progress at a slower pace, which can pose a significant concern for farmers. Moreover, the initial setup costs can be expensive due to the infrastructure and equipment required. Some biocontrol products have a limited storage period, and their quality may deteriorate over time, posing challenges in terms of product shelf-life. Furthermore, the use of such products could disrupt soil microorganism diversity and the bacteria niche, which may not be beneficial for plants. Thus, it is important to consider the ecological impacts and potential consequences on the soil ecosystem when utilizing BCAs [71].

As we investigate the utilization of BCAs, it is important to understand the ecological consequences of such utilizing in soil ecosystems. It is crucial to recognize the potential impact of BCAs on the bacterial community structure of the rhizosphere. Furthermore, the use of such products may impact soil microorganism diversity and the bacteria niche, which could be harmful for plants. As the critical interface between plant roots and soil, the rhizosphere is pivotal in supporting plant viability/growth; therefore, the interaction between the BCAs and the existing bacterial community may need to be verified and balanced. Moreover, the potential for **allelopathy** in plant disease management should also be considered. The release of bioactive compounds by BCAs to inhibit plant pathogen growth might affect beneficial organisms or influence soil nutrient cycling. Thus, it is crucial to fully evaluate the ecological impacts and potential consequences on the soil ecosystem. Assessing both short- and long-term effects of introducing BCAs involves not only monitoring changes in bacterial community structure/diversity, but also anticipating how these alterations impact the ecosystem as a whole [71,72].

Concluding remarks

Due to the increasing toxicity of certain chemical fungicides and pesticides, coupled with the urgent need to address climate change and global population growth, the consumption of chemical substances is being reconsidered. Both authorities and manufacturers are now exploring the use of BCAs as a promising alternative for manufacturing such biocontrol products (see Outstanding questions). BCAs have been utilized for decades, and some microorganisms have proven to be highly reliable in this role. *Bacillus* spp. are found in diverse habitats and offer significant potential for different industrial, pharmaceutical, and agricultural applications. *B. velezensis* has been reported widely for exhibiting strong antagonistic activities against specific phytopathogen agents. Genomic analysis revealed that this bacterium has gene clusters associated with the biosynthesis of various secondary metabolites, showing promising antifungal and antibiotic activities against plant pathogens. Therefore, we suggest that the use of *B. velezensis* could meet real-world sustainability demands and contribute to meeting the UN SDGs.

In addition to its application in the agricultural sector, this microorganism could also be explored in other areas, particularly the pharmaceutical sector, because some of its secondary metabolites have yet to be fully explored in this context. Given the potential of currently known *B. velezensis* strains, there is potential to discover new strains with enhanced performance for the industrial manufacturing of bio-based products. We propose exploring extreme habitats, such as arid deserts, deep oceans, and volcanoes, as potential sources of such strains. For example,

Outstanding questions

What environmental factors affect the efficiency of *Bacillus velezensis*-based biocontrol?

How can gene clusters associated with the biocontrol properties of *B. velezensis* help us better understand bioagents for use in agriculture?

Is it possible to enhance the production of secondary metabolites via gene clusters to increase the antagonistic impacts of the applied bioagent?

Can we genetically manipulate the gene clusters or the molecular mechanisms responsible for secreting secondary metabolites and precisely guide the end-product to meet our specific requirements?

What are the adverse effects of using *B. velezensis*-based biocontrol products on soil microbial interactions and populations, as well as on plant root systems?

Are there any adverse effects associated with the long-term application of *B. velezensis*-based products on the soil and plant roots?

B. velezensis strain GUIA was collected from the deep sea and found to have the ability to degrade polyurethane [73], which could be a simple but important approach to plastic degradation. Another example involves the recently discovered *B. velezensis* IS-6 [74]), which was detected in a pool of 350 bacterial isolates. This strain was found to detoxify food contaminated with **ochratoxin** A, a global food safety concern. Additionally, a novel cold-adapted beta-galactosidase was extracted from *B. velezensis* SW5 [75], showing potential as a biocatalyst for efficiently hydrolyzing lactose in milk, which would be particularly beneficial for the dairy industry. These findings offer valuable insights, suggesting the likelihood of discovering other *B. velezensis* strains with unique activities or enzymes capable of addressing diverse needs across various sectors in a biologically, environmentally friendly, and cost-effective manner. Nevertheless, it will be crucial to confront large-scale production issues, including the quality consistency of bacteria-based products after long-term storage, the final product quality, and the efficiency of bacterial performance under different conditions and circumstances. These aspects need further investigation to establish sustainable methods for the mass production of *B. velezensis*-based products, ensuring reliable outcomes in a cost-effective manner.

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Declaration of interests

None declared by authors.

Resources

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