



Scientific Article

# Phylogenomic diversity and genome mining of type *Bacillus* species: Searching for genes associated with biological control of phytopathogens

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## ABSTRACT

**Objective/Background.** *Bacillus* is a cosmopolitan bacterial genus with a great genome diversity. Thus, by exploring its genome background, it is possible to understand more about the physiological and biochemical traits involved in its biological control against phytopathogens. The objective of this work was to correlate the phylogenomic relationships of the type species of the genus *Bacillus* with the presence of gene clusters associated with biological control of plant pathogens, through genome mining.

**Materials and Methods.** Based on the literature, 336 species belonging to the genus *Bacillus* have been reported; however, after re-classification, a total of 123 type species have been recognized, and curated genomes were found in the EzBioCloud platform (<http://www.ezbiocloud.net/>). The overall genome relatedness indices (OGRI) were used for this work, which indicate how similar two sequences of a genome are. Then, the Realphy platform was used to create the phylogenomic tree 1.13 (Action-based phylogeny constructor reference). Finally, the prediction of biosynthetic gene clusters (BGC) associated with the biological control of phytopathogens was carried out using antiSMASH v6.0 ([https://antismash.  
secondarymetabolites.org/](https://antismash.secondarymetabolites.org/)).

**Results.** The present strategy allowed us to correlate and predict the biological control capacity of the *Bacillus* species under study based on their taxonomic affiliation since at a shorter evolutionary distance from *Bacillus subtilis* a high

potential capacity to produce biological control compounds was observed. However, the possibility that they acquire the ability to produce new biocontrol compounds during their evolutionary separation is not ruled out.

**Conclusion.** This work validates the correlation between the taxonomic affiliation of the studied *Bacillus* species and their biological control capacity, which is useful in the bioprospecting stage to design promising biopesticides.

**Keywords:** Biocontrol, Genome mining, *Bacillus*, AntiSMASH, Taxonomy.

## INTRODUCTION

The genus *Bacillus* is a large and diverse group of aerobic, gram-positive bacilli (Tejera-Hernández *et al.*, 2011; Zeigler and Perkins, 2021), belonging to the phylum Firmicutes, class Bacilli, order Bacillales, and family Bacillaceae (Villarreal-Delgado *et al.*, 2018). This bacterial genus was first reported by Ferdinand Cohn in 1872 (Errington and Van, 2020). It comprises ubiquitous bacteria isolated from diverse environments, such as water (salt and fresh), food, and soil (being highly present in the rhizosphere of various crops). These *Bacillus* species are also endospore formers; these are structured by thick walls inside bacterial cells, which are composed of proteins and protect the bacterial genome (Basta and Annamaraju, 2024). They are resistant to extreme temperatures, low water content, ultraviolet (UV), and chemical and physical processing (Cho and Chung, 2020), and allow bacteria to survive when faced with unfavorable conditions (Zeigler and Perkins, 2021).

Thus, the genus *Bacillus* is widely distributed in agroecosystems (colonizing soils, water, and plants), and is the most studied and best-described bacteria genus, based on various analyses and research focused on its genetic and metabolic diversity. Thus, due to its outstanding traits, *Bacillus* is a genus that harbors species that fulfill important biological functions and can produce a variety of enzymes (amylase, glucoamylase, etc.), antibiotics, and metabolites (Celandroni *et al.*, 2019). *Bacillus* has been reported to have a great impact in different areas of life, mainly in agriculture, where it has been widely used due to its metabolic traits associated with the improvement of nutrient availability by plants, induction of plant defense, production of ACC deaminase and synthesis of phytohormones, improvement of plant growth and nutrient assimilation under stress conditions and control of phytopathogenic diseases (Valenzuela-Ruiz *et al.*, 2019; Ibarra-Villarreal *et al.*, 2021).

One of the main functional traits of the genus *Bacillus* in agriculture is the biological control of phytopathogens. Biological control agents (BCA) are beneficial organisms with a genetic capacity for metabolite production, reducing the negative impacts of plant pathogens on crops (Villarreal-Delgado *et al.*, 2018; de los Santos Villalobos *et al.*, 2019). Some of the main ways in which these species prevent the establishment and development of plant pathogenic organisms are through a wide versatility of biological control mechanisms, including the production of antibiotics, siderophores, lytic enzymes, toxins, and induction of plant systemic resistance (Layton *et al.*, 2011; Tejera-Hernández *et al.*, 2011).

Also, *Bacillus* can produce secondary metabolites with biocontrol activity, such as lipopeptides, producing at least 98 different types, the most common are surfactins, iturins, and fengycin (Lam *et al.*, 2021). These molecules have an amphiphilic or amphipathic nature (a hydrophilic end, soluble in water, and hydrophobic). These molecules interfere in the cytoplasmic membrane of bacterial or fungal cells, acting as antimicrobials and inducing systemic resistance in host plants, stimulating defense mechanisms (Villarreal-Delgado *et al.*, 2018; Penha *et al.*, 2020; Valenzuela-Ruiz *et al.*, 2020).

Lipopeptides are integrated into three different families, which are identified as i) fengycins, one of the main types of cyclic lipopeptides produced by *Bacillus* that exhibited a high antibacterial and antifungal activity (Medeot *et al.*, 2020); ii) surfactins, represented with seven amino acid peptides, and includes variants such as pumilacidin, lichenisiin, and halobactin; these lipopeptides are very efficient biosurfactants against the biological membrane structure of pathogens (Fira *et al.*, 2018); and iii) iturins, a potent antifungal lipopeptide composed of a  $\beta$ -amino fatty acid chain with 14-17 carbons, and a cyclic heptapeptide (Dang *et al.*, 2019). Through the release of these cyclic lipopeptide families, the germination of various pathogens is controlled mainly by disrupting their cell membranes, affecting cell development (Tunsagool *et al.*, 2021). Thus, due to the wide secondary metabolites produced by *Bacillus*, this work aimed to predict the presence of these biological capacities based on the taxonomic affiliation of its type species.

## MATERIALS AND METHODS

**Genomic sequences of type species of the genus *Bacillus*.** Based on the literature, 336 species belonging to the genus *Bacillus* have been reported (Mendoza and Pazmiña, 2021); however, after re-classification, a total of 123 type species have been reported and curated genomes were found in the EzBioCloud platform (<http://www.ezbiocloud.net/>). The type strains of *Bacillus* species studied are indicated in Table 1.

**Table 1.** Type strain species belonging to the genus *Bacillus* downloaded from the EzBioCloud platform, which were used in this study.

#	Species	Strain	#	Species	Strain
1	<i>B. abyssalis</i>	DSM 25875(type)	47	<i>B. glycinifermentans</i>	GO-13(Type)
2	<i>B. aquimaris</i>	TF12(Type)	48	<i>B. gobiensis</i>	FJAT-4402(Type)
3	<i>B. aryabhatai</i>	B8W22(Type)	49	<i>B. halotolerans</i>	FJAT-2398(Type)
4	<i>B. camelliae</i>	7578-1(Type)	50	<i>B. haynesii</i>	NRRL B-41327(Type)
5	<i>B. circulans</i>	NBRC 13626(Type)	51	<i>B. humi</i>	DSM 16318(Type)
6	<i>B. endophyticus</i>	DSM 13796(Type)	52	<i>B. inaquosorum</i>	KCTC 13429(Type)
7	<i>B. filamentosus</i>	SGD-14(Type)	53	<i>B. koreensis</i>	DSM 16467(Type)
8	<i>B. halmapalus</i>	DSM 8723(Type)	54	<i>B. korlensis</i>	NBRC 107688(Type)
9	<i>B. horikoshii</i>	DSM 8719(Type)	55	<i>B. kwashiorkori</i>	SIT6(Type)
10	<i>B. idriensis</i>	DSM 19097(Type)	56	<i>B. kyonggiensis</i>	NB22(Type)
11	<i>B. marisflavi</i>	JCM 11544(Type)	57	<i>B. lacus</i>	KCTC 33946(Type)
12	<i>B. megaterium</i>	ATCC 14581(Type)	58	<i>B. lentus</i>	NCTC 4824(Type)
13	<i>B. nealsonii</i>	FO-92(Type)	59	<i>B. licheniformis</i>	ATCC 14580(Type)
14	<i>B. solitudinis</i>	Type (FJAT-45122)	60	<i>B. luciferensis</i>	DSM 18845(Type)
15	<i>B. sporothermodurans</i>	Type (DSM 10599)	61	<i>B. luti</i>	TD41(Type)
16	<i>B. acidicerler</i>	DSM 18954(Type)	62	<i>B. mangrovi</i>	KCTC 33872(Type)
17	<i>B. acidicola</i>	FJAT-2406(Type)	63	<i>B. manliponensis</i>	JCM 15802(Type)
18	<i>B. acidifaciens</i>	3-2-2(Type)	64	<i>B. marasmi</i>	Marseille-P3556(Type)
19	<i>B. acidiproducens</i>	DSM 23148(Type)	65	<i>B. marinisedimentorum</i>	NC2-31(Type)
20	<i>B. aciditolerans</i>	YN-1(Type)	66	<i>B. massiliogabonensis</i>	Marseille-P2639(Type)
21	<i>B. albus</i>	N35-10-2(Type)	67	<i>B. massiliosenegalensis</i>	JC6(Type)
22	<i>B. alkalitelluris</i>	DSM 16976(Type)	68	<i>B. methanolicus</i>	NCIMB 13113(Type)
23	<i>B. altitudinis</i>	41KF2b(Type)	69	<i>B. mobilis</i>	0711P9-1(Type)
24	<i>B. amyloliquefaciens</i>	DSM 7(Type)	70	<i>B. mojavenis</i>	KCTC 3706(Type)
25	<i>B. andreaoultii</i>	SIT1(Type)	71	<i>B. mycoides</i>	ATCC 6462(Type)
26	<i>B. anthracis</i>	Vollum(Type)	72	<i>B. nakamurai</i>	NRRL B-41091(Type)
27	<i>B. atrophaeus</i>	NRRL NRS 213(Type)	73	<i>B. natronophilus</i>	M30(Type)
28	<i>B. aurantiacus</i>	DSM 18675(Type)	74	<i>B. niameyensis</i>	SIT3(Type)
29	<i>B. australimaris</i>	NH71_1(Type)	75	<i>B. nitratireducens</i>	4049(Type)
30	<i>B. badius</i>	MTCC 1458(Type)	76	<i>B. oleivorans</i>	JC228(Type)
31	<i>B. bingmayongensis</i>	FJAT-13831(Type)	77	<i>B. oleronius</i>	DSM 9356(Type)
32	<i>B. cabrialesii</i>	TE3(Type)	78	<i>B. onubensis</i>	0911MAR22V3(Type)
33	<i>B. cereus</i>	ATCC 14579(Type)	79	<i>B. oryzae</i>	ZYK(Type)
34	<i>B. coagulans</i>	ATCC 7050(Type)	80	<i>B. pacificus</i>	EB422(Type)
35	<i>B. coahuilensis</i>	m4-4(Type)	81	<i>B. panaciterrae</i>	DSM 19096(Type)
36	<i>B. cohnii</i>	DSM 6307(Type)	82	<i>B. paralichenformis</i>	KJ-16(Type)
37	<i>B. cytotoxicus</i>	NVH 391-98(Type)	83	<i>B. paramycoides</i>	NH24A2(Type)
38	<i>B. dakarensis</i>	Marseille-P3515(Type)	84	<i>B. paranthracis</i>	Mn5(Type)
39	<i>B. dielmoensis</i>	FF4(Type)	85	<i>B. pasinlerensis</i>	P1(2020)(Type)
40	<i>B. enclensis</i>	SGD-1123(Type)	86	<i>B. piezotolerans</i>	Type (YLB-04)
41	<i>B. flexus</i>	NBRC 15715(Type)	87	<i>B. populi</i>	Type (FJAT-45347)
42	<i>B. fordii</i>	DSM 16014(Type)	88	<i>B. proteolyticus</i>	Type (TD42)
43	<i>B. fortis</i>	DSM 16012(Type)	89	<i>B. pseudomycoides</i>	Type (DSM 12442)
44	<i>B. fungorum</i>	17-SMS-01(Type)	90	<i>B. pumilus</i>	Type (NCTC 10337)
45	<i>B. gaemokensis</i>	KCTC 13318(Type)	91	<i>B. rubiinfantis</i>	Type (MT2)
46	<i>B. glennii</i>	V44-8(Type)	92	<i>B. safensis</i>	Type (FO-36b)

**Table 1.** Continue.

#	Species	Strain	#	Species	Strain
93	<i>B. salaceticis</i>	Type (SKP7-4)	109	<i>B. thermotolerans</i>	SGZ-8(Type)
94	<i>B. salsus</i>	Type (IBRC-M10078)	110	<i>B. thuringiensis</i>	ATCC 10792(Type)
95	<i>B. shackletonii</i>	Type (LMG 18435)		( <i>gv. thuringiensis</i> )	
96	<i>B. siamensis</i>	Type (KCTC 13613)	111	<i>B. timonensis</i>	10403023(Type)
97	<i>B. sinesaloumensis</i>	Type (Marseille-P3516)	112	<i>B. toyonensis</i>	BCT-7112(Type)
98	<i>B. siralis</i>	Type (171544)	113	<i>B. tropicus</i>	N24(Type)
99	<i>B. smithii</i>	Type (DSM 4216)	114	<i>B. vallismortis</i>	DSM 11031(Type)
100	<i>B. solimangrovi</i>	Type (GH2-4)	115	<i>B. velezensis</i>	NRRL B-41580(Type)
101	<i>B. solisilvae</i>	Type (NEAU-cbsb5)	116	<i>B. vietnamensis</i>	NBRC 101237(Type)
102	<i>B. sonorensis</i>	Type (NBRC 101234)	117	<i>B. weihaiensis</i>	Alg07(Type)
103	<i>B. spizizenii</i>	Type (TU-B-10)	118	<i>B. wiedmannii</i>	FSL W8-0169(Type)
104	<i>B. subtilis</i>	NCIB 3610(Type)	119	<i>B. wudalianchiensis</i>	FJAT-27215(Type)
105	<i>B. swezeyi</i>	NRRL B-41294(Type)	120	<i>B. xiamenensis</i>	HYC-10(Type)
106	<i>B. taxi</i>	M5HDSG1-1(Type)	121	<i>B. xiapuensis</i>	FJAT-46582(Type)
107	<i>B. tequilensis</i>	KCTC 13622(Type)	122	<i>B. yapensis</i>	XXST-01(Type)
108	<i>B. terrae</i>	LMG 29 oki 736(Type)	123	<i>B. zhangzhouensis</i>	DW5-4(Type)

**Phylogenomic analysis, and overall genome relatedness index (OGRIs).** The overall genome relatedness indices (OGRIs) indicate how similar two sequences of a genome are, and thus identify whether we are dealing with a previously reported species, which was first determined by Chun and collaborators in 2018. This work was based on obtaining the type genomes of each related studied type strain using a similarity value  $\geq 98.7\%$  in the 16S rRNA gene to the strain of interest (Chun *et al.*, 2018; Morales *et al.*, 2021). These indices include the obtention of the average nucleotide identity (ANI), which was obtained by using the ANI Calculator in the Ezbiocloud platform. ANI and OrthoANI are comparable algorithms because they share the same species demarcation limit of 95 - 96% (EzBioCloud, 2017). Also, an ANI analysis was performed to obtain a reliable measure of the evolutionary distance of the species. The software used to calculate these ANI values was JspeciesWS, which allows us to analyze and compare species boundaries between genomes, draft genomes, or partial sequences of random genomes, and allows the measurement of the probability that two or more genomes belong to the same species by comparing ANI values (Richter and Rosselló-Móra, 2009; Arahal, 2014).

Besides, the studied strains were subjected to a comprehensive analysis using version 2.1 of the web server genome-to-genome distance calculator (<https://ggdc.dsmz.de/>), reported for the first time by Meier-Kolthoff and collaborators in 2013. This software applies an *in silico* method for the delimitation of subspecies or species (scilicet, a query, and a reference) that limits the DNA-DNA hybridization values (DDH) for a universal and specific restriction of prokaryotic species or

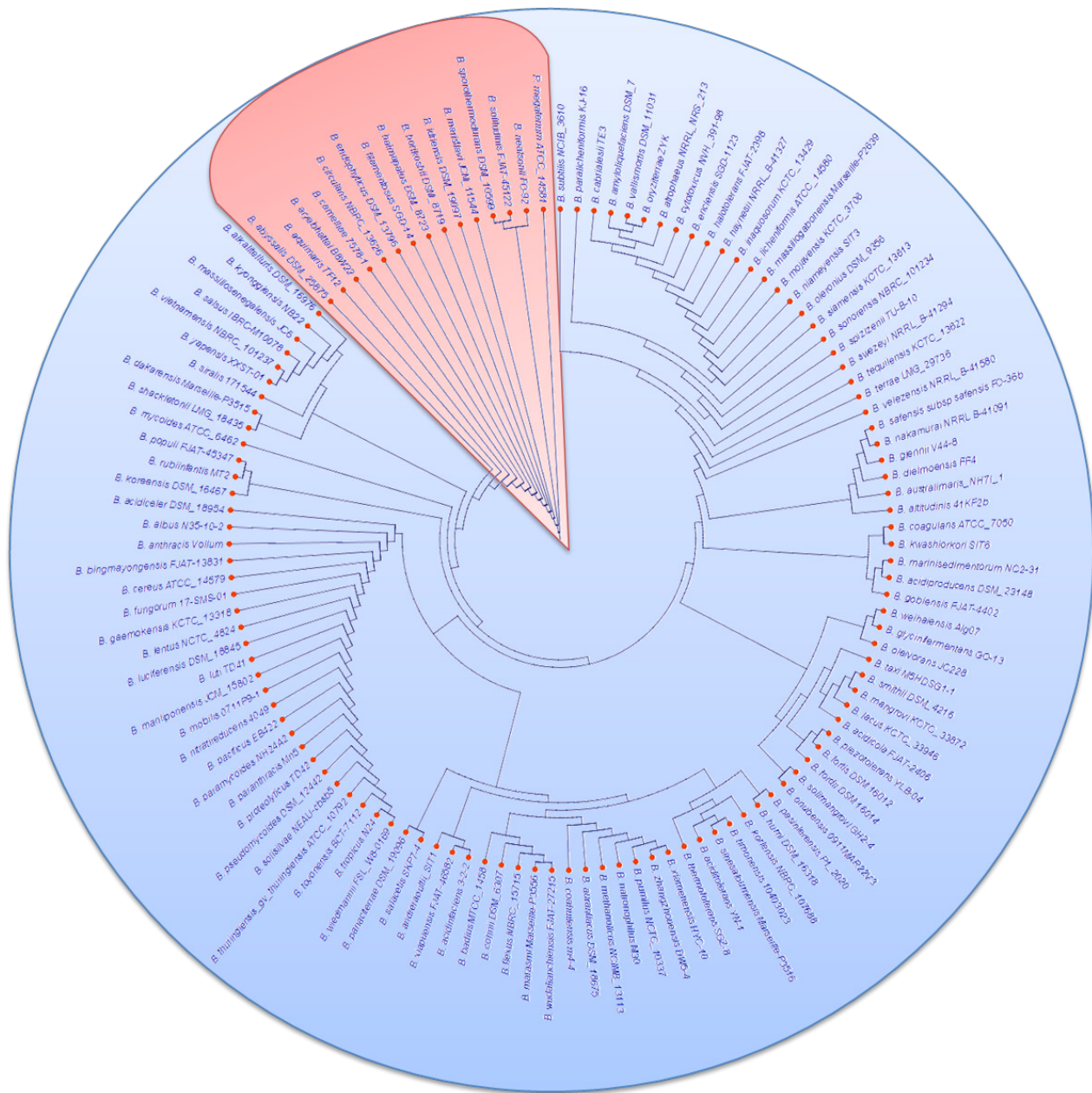
subspecies, as well as calculates the intergenomic distance based on three different formulas (Meier-Kolthoff *et al.*, 2013; Zhang *et al.*, 2018). Here, two genomes were placed, one of reference and the other of interest, as a result, the relationship between the genomes was analyzed by using the formula 2, which is the sum of all the identities found in the HSPs and multiplies it by 2, divided by the sum of the total length of the HSPs of both genomes (Morales *et al.*, 2021). In this work, formula 2 was used because it is independent of genome length and is the only one that can be used with incompletely sequenced genomes, thus avoiding errors (Auch *et al.*, 2010).

The Realphy platform was used to create the phylogenomic tree 1.13 (Action-based phylogeny constructor reference). This software allows the creation of phylogenomic trees from genomic sequence data, to carry out this process it was necessary to introduce several reference genomes in FASTA or Genbank format (Bertels *et al.*, 2014). The phylogenomic tree was first performed in the CLC Sequence Viewer v8.0 software (<https://clc-sequence-viewer.software.informer.com/8.0/>), which provides access to the genomic analysis in the FASTQ format, which provides access to basic bioinformatics analysis (QIAGEN, 2017).

**Genome mining.** The prediction of biosynthetic gene clusters (BGC) associated with the biological control of phytopathogens was carried out using antiSMASH v6.0 (<https://antismash.secondarymetabolites.org/>) (Blin *et al.*, 2021). This strategy allows the identification, detection, and characterization of rapid annotations and analysis of biosynthetic gene clusters responsible for biosynthesis, regulation, resistance, and the transport of metabolites. Files in FASTA format containing the genome of the studied type *Bacillus* species were used (Roger and Castillo, 2017; Menezes *et al.*, 2021).

## RESULTS AND DISCUSSION

**Genome-based phylogeny.** These analyses helped to visualize the correlation of genes between different *Bacillus* species that can biocontrol plant pathogens, as mentioned by Syazwan and collaborators (2021). The phylogenomic tree was divided into two distinct groups (Figure 1). The first group belongs to *Bacillus megaterium*, which had a genus reclassification to *Priestia megaterium* (Gupta *et al.*, 2020). It is reported in the literature that *P. megaterium* does not have biocontrol capacity, and for this reason, it was placed as the root in the phylogenomic tree. It can be seen in Figure 1 that there is a distance between *P. megaterium* and *B. subtilis*. Unlike *P. megaterium*, *B. subtilis* is reported as a biological control agent, placing *P. megaterium* as a root allowed us to visualize which species are related to



**Figure 1.** Phylogenomic tree with the 123 studied type *Bacillus* species, where two main groups were identified: i) *B. megaterium* (pink color) and ii) *B. subtilis* (blue color).

it, which according to the literature are not reported with biocontrol, being that the rest of the species usually have the capacity for biological control.

Some of the species related to *P. megaterium* were *B. abyssalis*, *B. camelliae*, *B. solitudinis*, *B. nealsonii*, and *B. halmपालus*, among others, which are not reported

with biocontrol (Saeki *et al.*, 2002; Venkateswaran *et al.*, 2003; Biedendieck *et al.*, 2021). *Bacillus subtilis* corresponds to the second group identified in Figure 1, the species within this group correspond to *B. dakarensis*, *B. siralis*, *B. yapensis*, *B. vietnamensis*, *B. salsus*, and *B. alkalitelluris*, and the species most closely related to the species of *B. subtilis* are *B. thuringiensis*, *B. velezensis*, *B. cabrialesii*, *B. tequilensis*, *B. spizizenii*, *B. mojavensis*, *B. inaquosorum*, *B. halotolerans*, and *B. atrophaeus*, as it has higher biocontrol capacity according to the literature (Gupta *et al.*, 2020).

The obtained results show phylogenetically related *Bacillus* species in group 1 that share morphological traits. This characteristic is relevant because it allows us to compare genes between different species and thus identify those that meet the biocontrol capacity. *Bacillus* species are known to have potential biocontrol capacity (Syazwan *et al.*, 2021); some examples are *B. thuringiensis*, *B. amyloliquefaciens*, *B. nakamurai*, *B. siamensis*, and *B. velezensis*. These strains have in common the production of lytic enzymes and also produce lipopeptides such as surfactin, which means that they can induce a systemic response in the plant, which helps in the colonization of the rhizosphere (Sánchez, 2016). As mentioned above, AntiSMASH results showed that *P. megaterium* does not possess biocontrol activity.

Another *Bacillus* species located in group 1 was the bacterium *Bacillus swezeyi* isolated by Dunlap and collaborators (2017). This *Bacillus* species is closely related to *B. licheniformis*, which also has a wide variety of metabolites with applications in pharmaceutical, food, biomedicine, aquaculture, and agriculture, among others. In addition, *Bacillus licheniformis* can show a high production of antioxidant catalase, which eliminates the presence of hydrogen peroxide, and cancels the absence of microorganisms and therefore can kill beneficial microorganisms. Other examples in this clade are 10 *Bacillus* species isolated from Xinjiang, China, such as *Bacillus tamaricis* sp. nov., *Bacillus populi* sp. nov., *Bacillus capparidis* sp. nov., *Bacillus urumqiensis* sp. nov., *Bacillus gobiensis* sp. nov., *Bacillus solani* sp. nov., *Bacillus salitolerans* sp. nov., *Bacillus shacheensis* sp. nov., *Bacillus deserti* sp. nov. and *Bacillus korlensis* sp. nov. (Liu *et al.*, 2019).

Biological control agents are focused on reducing or eliminating the effect of phytopathogenic agents (Serrano and Galindo, 2007). However, polyphasic characterization is important in terms of the application of safe microorganisms for mankind. To determine the capacity to produce biocontrol compounds by the studied type strains of the genus *Bacillus*, genomic analyses for the identification of non-ribosomal peptide biosynthetic gene clusters (NRPs) were performed through AntiSMASH v 6.0 (Antibiotic and Secondary Metabolites Analysis Shell) (Medema *et al.*, 2011).

The genome analysis of the studied strains provided a total of 85 biological control compounds (Table 2). The most prevalent was the siderophore Bacillibactin, which



**Table 2.** *Bacillus* species with metabolites associated with the biocontrol of phytopathogens, according to AntiSMASH, as well as the mechanisms corresponding to them based on the literature.

Group	Species	Identified clusters (%)	Modes of action	Reported by
1	<i>B. aquimaris</i>	Paeninodin (100%)	Lasso peptide	Zhu <i>et al.</i> , 2016
	<i>B. endophyticus</i>	Paeninodin (80%)	Lasso peptide	Zhu <i>et al.</i> , 2016
	<i>B. filamentosus</i>	Paeninodin (80%)	Lasso peptide	Zhu <i>et al.</i> , 2016
	<i>B. albus</i>	Petrobactin (100%) Thurincin H (70%)	Siderophore Antimicrobial	Ortiz <i>et al.</i> , 2022; Manck <i>et al.</i> , 2022
2	<i>B. alkalielluris</i>	Paeninodin (80%)	Lasso peptide	Zhu <i>et al.</i> , 2016
	<i>B. altitudinis</i>	Bacilicidin (85%)	Antibiotic	Basso <i>et al.</i> , 2018
	<i>B. amyloliquefaciens</i>	Bacillibactin (100%) Fengycin (93%) Surfactin (82%) Bacilicidin (100%)	Siderophore Antifungal Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Butcher <i>et al.</i> , 2007; Pedraza-Herrera <i>et al.</i> , 2020
	<i>B. anthracis</i>	Bacillaene (100%) Petrobactin (100%)	Siderophore	Manck <i>et al.</i> , 2022
	<i>B. atrophaeus</i>	Bacillibactin (100%) Fengycin (86%) Surfactin (98%) Subtilosin A (87%)	Siderophore Antifungal Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022; Butcher <i>et al.</i> , 2007.
	<i>B. australimaris</i>	Bacillisin (85%)	Antibiotic	Basso <i>et al.</i> , 2018
	<i>B. badius</i>	Plantazolicin (75%)	Bacteriocin	Farzand <i>et al.</i> , 2019
	<i>B. cabrialesii</i>	Bacillibactin (100%) Fengycin (100%) Surfactin (90%) Subtilosin A (100%) Bacillisin (100%)	Siderophore Antifungal Antifungal Antifungal Antifungal and Nematicide	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022; Borisova <i>et al.</i> , 2010; Butcher <i>et al.</i> , 2007.
	<i>B. cereus</i>	Petrobactin (100%) Thiocillin I (100%)	Siderophore Antimicrobial	Manck <i>et al.</i> , 2022; Wieland <i>et al.</i> , 2009
	<i>B. dakarensis</i>	Ectoin (75%) Paeninodine (100%)	Organic compound Lasso peptide	Sadeghi <i>et al.</i> , 2019; Zhu <i>et al.</i> , 2016
	<i>B. flexus</i>	Plantazolicin (100%)	Bacteriocin	Farzand <i>et al.</i> , 2019
	<i>B. fungorum</i>	Paeninodine (100%)	Lasso peptide	Zhu <i>et al.</i> , 2016
	<i>B. gaemokensis</i>	Paenilarvins (75%)	Antifungal	Sood <i>et al.</i> , 2014
	<i>B. glycinifermentans</i>	Fengycin (82%) Lichenisin (100%)	Antifungal Lasso peptide	Ley-López, 2022; Bedoya-Cardona, 2019
	<i>B. gobiensis</i>	Teicuronic acid (87%) Bacillibactin (100%)	Anionic polymers	Santos <i>et al.</i> , 2019 Luciana, 2014;
	<i>B. halotolerans</i>	Fengycin (93%) Surfactin (86%) Subtilosin A (100%) Bacillisin (100%) Bacillaeno (100%)	Siderophore Antifungal Antifungal Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Butcher <i>et al.</i> , 2007; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022
	<i>B. haynesii</i>	Fengycin (100%) Bacitracin (100%) Lichenisin (100%)	Antifungal Antibiotic Lasso peptide	Ley-López, 2022; Fira <i>et al.</i> , 2018; Bedoya-Cardona, 2019
	<i>B. inaquosorum</i>	Bacillibactin (100%) Fengycin (100%) Surfactin (82%) Subtilosin A (100%) Bacillisin (100%)	Siderophore Antifungal Antifungal Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Butcher <i>et al.</i> , 2007; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022
	<i>B. lentus</i>	Bacillaeno (100%) Paeninodina (100%)		
	<i>B. licheniformis</i>	Lichenisin (100%)	Lasso peptide	Zhu <i>et al.</i> , 2016 Bedoya-Cardona, 2019

Table 2. Continue.

Group	Species	Identified clusters (%)	Modes of action	Reported by
	<i>B. luti</i>	Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. mantiponensis</i>	Paeninodine (80%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. mobilis</i>	Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. mojavensis</i>	Bacillibact (100%) Fengycin (86%) Surfactin (86%) Subtilosina A (100%) Bacillisin (100%)	Siderophore Antifungal Antifungal Antibiotic Antifungal	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022
	<i>B. mycooides</i>	Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. nakamurai</i>	Bacillibactina (100%) Bacillisin (100%) Bacillaene (100%) Surfactin (81%) Fengycin (80%)	Catechol-type Siderophore Antifungal Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020; Butcher <i>et al.</i> , 2007.
	<i>B. niameyensis</i>	Paeninodine (80%) Bacitracina (100%)	Lasso peptide Antibiotic	Zhu <i>et al.</i> , 2016
	<i>B. paralicheniformis</i>	Lichenisin(100%) Fengycin (92%)	Lipopeptide Antifungal	Ley-López, 2022; Fira <i>et al.</i> , 2018; Bedoya-Cardona, 2019
	<i>B. nitratireducens</i>	Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. pasinlerensis</i>	Geobacillin (80%)	Bacteriocin	Vaičikauskaitė <i>et al.</i> , 2019
	<i>B. populi</i>	Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Zhu <i>et al.</i> , 2016
	<i>B. proteolyticus</i>	Paeninodine (80%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2016
	<i>B. pseudomycooides</i>	Pseudomycoicidin (100%) Plantazolicina (91%)	Lantibiotic Bacteriocin	Basi-Chipalu <i>et al.</i> , 2015 Basso <i>et al.</i> , 2018; Bedoya-Cardona, 2019; Farzand <i>et al.</i> , 2019
	<i>B. pumilus</i>	Bacillisin (85%) Lichenisin (85%) Plantazolicina (91%)	Antifungal Lipopeptide Bacteriocin	Basso <i>et al.</i> , 2018; Bedoya-Cardona, 2019; Farzand <i>et al.</i> , 2019
	<i>B. safensis</i>	Bacillisin (85%) Lichenisin (85%)	Antifungal Lipopeptide	Basso <i>et al.</i> , 2018; Bedoya-Cardona, 2019; Farzand <i>et al.</i> , 2019
	<i>B. salsus</i>	Paeninodine (80%) Fengycin (100%)	Lasso peptide Antifungal	Zhu <i>et al.</i> , 2016
	<i>B. siamensis</i>	Bacillaene (100%) Surfactin (90%)	Antifungal Antifungal	Luciana, 2014; Ley-López, 2022; Butcher <i>et al.</i> , 2007.
	<i>B. solitudinis</i>	Carotenoid (83%) Bacillibactin (100%) Subtilosin A (100%) Bacillisin (100%) Bacillaene (100%) Mycosubtiline (100%) Surfactin (82%)	Antioxidant Catechol-type Siderophore Antibiotic Antifungal Antifungal Antifungal	Sontsa-Donhoung <i>et al.</i> , 2022 Luciana, 2014; Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022; Butcher <i>et al.</i> , 2007.

Table 2. Continue.

Group	Species	Identified clusters (%)	Modes of action	Reported by
<i>B. subtilis</i>		Bacillibactin (100%)	Catechol-type	Luciana, 2014; Ley-López, 2022; Basso <i>et al.</i> , 2018; Moldenhauer <i>et al.</i> , 2010; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022; Wieland <i>et al.</i> , 2009; Butcher <i>et al.</i> , 2007.
		Fengycin (100%) Subtilosin A (100%) Bacillisin (100%) Bacillaene (100%) Bacillicatin (100%)	Siderophore Antifungal Antibiotic Antifungal Siderophore	
		Esporulation (100%)	Sporulation	
		Surfactin (82%) Thiocillin I (70%)	Antifungal Antimicrobial	
			Antifungal	
			Antibiotic	
<i>B. swiezeyi</i>		Bacillaene (100%)	Antifungal	Moldenhauer <i>et al.</i> , 2010; Fira <i>et al.</i> , 2018; Diabankana <i>et al.</i> , 2022; Butcher <i>et al.</i> , 2007.
		Bacitracin (88%)	Antibiotic	
		Mersacidin (100%)	Antibiotic	
<i>B. tequilensis</i>		Bacillibactin (100%)	Catechol-type	Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020; Wang <i>et al.</i> , 2022; Borisova <i>et al.</i> , 2010; Kugler <i>et al.</i> , 1990
		Fengycin (86%) Surfactin (90%) Subtilosina A (100%) Bacillisin (100%) Rhizocticin A (70%)	Siderophore Antifungal Antifungal Antibiotic	
			Antibiotic	
			Organic compound	
			Siderophore	
<i>B. thermotolerans</i>		Ectoin (75%)	Organic compound	Sadeghi <i>et al.</i> , 2019
<i>B. thuringiensis</i> (gv. <i>thuringiensis</i> )		Petrobactin (100%) Turicin (100%) Zwittermicin A (96%)	Siderophore Bacteriocins Antibiotic	Manck <i>et al.</i> , 2022; Stabb <i>et al.</i> , 1994
			Antibiotic	
<i>B. timonensis</i>		Paeninodine (80%)	Lasso peptide	Zhu <i>et al.</i> , 2016
<i>B. toyonensis</i>		Petrobactin (100%) Paeninodine (80%)	Siderophore Lasso peptide	Manck <i>et al.</i> , 2022
<i>B. tropicus</i>		Petrobactin (100%)	Siderophore	
<i>B. vallismortis</i>		Bacillibactin (100%)	Catechol-type	Basso <i>et al.</i> , 2018; Pedraza-Herrera <i>et al.</i> , 2020
		Fengycin (100%) Bacillisin (100%) Subtilin (100%) Surfactin (82%)	Siderophore Antifungal Antibiotic Antifungal	
			Siderophore	
			Antifungal	
			Antifungal	
<i>B. velezensis</i>		Bacillibactin (100%)	Catechol-type	Ley-López, 2022; Basso <i>et al.</i> , 2018; Moldenhauer <i>et al.</i> , 2010; Pedraza-Herrera <i>et al.</i> , 2020; Diabankana <i>et al.</i> , 2022; Nagao <i>et al.</i> , 2001; Butcher <i>et al.</i> , 2007.
		Fengycin (100%) Bacillisin (100%) Mersacidin (100%) Difficidin (100%) Macrolactin H (100%) Surfactin (94%) Bacillaene (85%)	Siderophore Antifungal Antibiotic Antibiotic Antimicrobial Antimicrobial Antifungal	
			Antifungal	
			Antioxidant	
			Antimicrobial	
			Antifungal	
<i>B. weihaiensis</i>		Carotenoid (83%) Cerecidin A1-A7 (76%)	Antioxidant Antimicrobial	Sontsa-Donhoung <i>et al.</i> , 2022; Wang <i>et al.</i> , 2014
<i>B. wiedmannii</i>		Paeninodine (100%) Petrobactin (100%)	Lasso peptide Siderophore	Manck <i>et al.</i> , 2022
<i>B. xiapuensis</i>		Mersacidin (100%)	Antibiotic	Diabankana <i>et al.</i> , 2022
<i>B. yapensis</i>		Paeninodine (80%)	Lasso peptide	Zhu <i>et al.</i> , 2016
<i>B. zhangzhouensis</i>		Bacillisin (85%)	Antifungal	Basso <i>et al.</i> , 2018

is a catechol siderophore [an organic compound with the formula  $C_6H_4(OH)_2$ ]. It participates in the chelation of ferric iron ( $Fe^{3+}$ ) from the surrounding environment and is subsequently transferred to the bacterial cytoplasm through the use of transporters (Valenzuela-Ruiz *et al.*, 2022). In this analysis, 12 *Bacillus* species showed this capacity, with 100% similarity in the DNA sequence of this cluster, *B. velezensis*, *B. amyloliquefaciens*, *B. siamensis*, *B. nakamurai*, *B. atrophaeus*, *B. mojavensis*, *B. halotolerans*, *B. vallismortis*, *B. tequilensis*, *B. cabrialesii*, *B. spizizenii*, and *B. inaquosorum*.

As is already known, *Bacillus* is a genus rich in metabolic diversity that is mainly associated with its prevalence in different ecosystems. This is one of the most studied characteristics of this bacterial genus, mainly due to its ability to reduce the incidence of diseases in crops mostly for the direct or indirect repression of the growth of the agents that cause the disease. Lipopeptides, such as surfactins, iturins, and fengycins, protect the plant in pre- and post-harvest conditions (Valenzuela-Ruiz *et al.*, 2020). Based on the results of AntiSMASH, the bacteria that presented the highest percentages in terms of the production of these lipopeptides, such as:

- a) **Fengycin.** It is an antifungal that inhibits filamentous fungi but is ineffective against yeasts and bacteria (Ariza and Sánchez, 2012). Among these antimicrobial compounds are cyclic lipopeptides, which include various members of the surfactin, iturin, and fengycin chemical families. However, not all species belonging to the genus *Bacillus* produce these antimicrobial lipopeptides as a defense mechanism (Ley-López *et al.*, 2022).
- b) **Surfactin.** Biosurfactants are substances of microbial origin that have surface activity. A particularly effective group of biosurfactants are lipopeptides, where surfactin stands out, they are produced by bacteria of the *Bacillus* genus through the transformation of renewable substrates (Luciana, 2014). Surfactin exhibits antimicrobial, antitumor, and antiviral functions, and inhibits the formation of biofilms of other bacteria by interfering with the attachment of cells to surfaces (Chen *et al.*, 2022).
- c) **Iturin.** Iturin family are cyclic lipopeptides (CLPs) that are generated from *Bacillus subtilis* and closely related bacterial strains, they are known for their inhibitory properties against phytopathogens (Yaraguppi *et al.*, 2023).

The microbial activity of these lipopeptides is produced mainly by the interaction that exists with the cytoplasmic membrane of bacterial or fungal cells, which generates the formation of pores and an osmotic imbalance, triggering the cell death of phytopathogenic microorganisms (Villarreal-Delgado *et al.*, 2018).

Comparing the results of data mining with the phylogenetic results, it can be inferred that the ability to produce numerous biological control compounds is

inhibited by evolutionary separation. The greater the evolutionary separation of the species, the lower the production of biological control compounds is observed, and vice versa. However, the possibility that during the evolutionary separation, the species develop the ability to produce these compounds of interest again is not ruled out.

The analysis of genomic data for the detection of the presence of biosynthetic pathways that allow organisms to produce lipopeptides (fengycin, surfactin, and iturin), is an essential study that complements identification approaches with biotechnological purposes, to help us understand the functioning of the genus *Bacillus*. *Bacilli* have a strategy to survive extreme environmental conditions (temperature, radiation, pressure, pH, and humidity). In addition, their adaptation is possible due to the generation of endospores that maintain survival under unfavorable conditions (Borriss, 2020). The use of AntiSMASH v 6.0 served as a tool for the phylogenomic study and comparison of the DNA obtained from the EzBioCloud platform, helping us to understand the variations of the 123 strains studied with the help of the GGDC analysis version 2.1 where it was proven that the strains can be divergent or evolutionarily distant, thanks to data mining and the comparison of phylogenomic results, in addition to ANI v 3.8.2 comparisons.

**Phylogeny and biocontrol correlation.** The objective of phylogeny is the study of the genome from an evolutionary perspective, which is to understand the mechanisms of change that occur in the genome, as well as the interrelation with its evolution and phenotypic characteristics. The result of the phylogenomic tree is the relationship between taxa in the phylogenomic tree by descent from a recent common ancestor, and less related if they have a less recent common ancestor (Samolski, 2014). The phylogenomic tree shows the 123 species of the genus *Bacillus*, which results in the visualization of the comparison of sequences from different organisms and allows grouping them into families that correspond to groups of genes with some similarity descended from a common ancestral gene (Figure 1 and Table 2). One of the inheritances that occur between species is the biocontrol capacity, which consists of being able to control diseases produced by different microorganisms (Alcaraz *et al.*, 2010; Teem *et al.*, 2020; Bernal, 2021). Based on the results obtained, of the 123 *Bacillus* species downloaded from EzBioCloud only 58 species showed genes associated with biocontrol activity; in addition, of the total of these species in the literature, only 39 *Bacillus* species have been reported with biocontrol, where only 15 *Bacillus* species could produce lipopeptides, such as fengycin and surfactin.

The genus *Bacillus* presents a large number of species with biocontrol capacity, likewise, there is a correlation between the phylogeny and biocontrol of *Bacillus* species, for example, *B. subtilis* was the first strain characterized, and other strains

with biocontrol capacity were identified: *B. thuringiensis*, *B. licheniformis*, *B. tropicus*, among other species. These are also phylogenomically close to *B. subtilis*; thus, it is concluded that there is a correlation between phylogeny and biocontrol. For example, *Bacillus thuringiensis*, a Gram-positive, endospore-forming, facultatively anaerobic bacterium, is widely used for the biocontrol of insect pests, which contributes to agricultural biosecurity when used as a biopesticide-like other species of the genus *Bacillus* (Lacey *et al.*, 2015; Córdova-Albores *et al.*, 2020).

Some of the most studied potential species are *B. subtilis*, *B. pumilus*, *B. licheniformis*, *B. amyloliquefaciens*, *B. cabrialesii*, *B. japonicum*, *B. cereus*, *B. thuringiensis*, *B. anthracis*, *B. mycoides*, and *B. pseudomycoides*, as they have been shown to have antagonistic activity against various phytopathogenic microorganisms of agricultural interest (Villarreal-Delgado *et al.*, 2018; Morales-Barrón *et al.*, 2019; Valenzuela-Ruiz *et al.*, 2019). For example, in the study by Villa-Rodríguez and collaborators in 2019, strain TE3<sup>T</sup> shows promising traits against *Bipolaris Sorokiniana* TPQ<sup>3</sup>, the causal agent of wheat stain. In terms of biosafety, potential strains should be studied extensively before being used as biological control agents in the field. Importantly, the *Bacillus subtilis* group, which includes agriculturally important species such as *B. subtilis*, *B. licheniformis*, and *B. pumilus*, are not traditionally considered pathogenic to humans. *B. subtilis* has even received QPS (Qualified Presumption of Safety) status from the European Food Safety Authority (EFSA, 2015). However, there are some isolated cases of intoxications forming digestive manifestations, so a strict and thorough analysis is necessary (Villarreal-Delgado *et al.*, 2018).

## CONCLUSIONS

The present strategy allowed us to correlate and predict the biological control capacity of the *Bacillus* species under study based on their taxonomic affiliation since at a shorter evolutionary distance from *Bacillus subtilis* a high potential capacity to produce biological control compounds was observed. However, the possibility that they acquire the ability to produce new biocontrol compounds during their evolutionary separation is not ruled out. The genus *Bacillus* is genetically and metabolically diverse, so it is of great importance to perform genomic mining to predict the biological control capacity of the sequenced species and to be able to use them for biotechnological purposes.

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