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ORIGINAL PAPER

Genome analysis reveals insights of the endophytic *Bacillus toyonensis* BAC3151 as a potentially novel agent for biocontrol of plant pathogens

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Abstract Diseases caused by phytopathogenic microorganisms account for enormous losses for agribusiness. Although *Bacillus* species are recognized as being antimicrobial producers and some may provide benefits to plants, the association between *Bacillus toyonensis* and plants has not been studied. In this study, the whole-genome sequenced endophytic *B. toyonensis* BAC3151, which has demonstrated antimicrobial activity and quorum sensing inhibition of phytopathogenic bacteria, was investigated for its potential for the production of compounds for biocontrol of plant pathogens. Four whole-genome sequenced *B. toyonensis* strains shared 3811 protein-coding DNA sequences (CDSs), while strain-specific CDSs, such as biosynthetic gene clusters of antimicrobials, were associated with specific chromosomal regions and mobile genetic elements of the strains. *B. toyonensis* strains had a higher frequency of putative bacteriocins gene clusters than that of *Bacillus* species traditionally used for the production of antimicrobials. In addition, gene clusters potentially involved in the production of novel bacteriocins were found in BAC3151, as well as biosynthetic genes of several other compounds, including non-ribosomal peptides, *N*-acyl homoserine lactonase and chitinases, revealing a genetic repertoire for antimicrobial synthesis greater than that of other *Bacillus* strains that have demonstrated effective activity against phytopathogens. This study showed for the first time that *B. toyonensis* has potential to produce various antimicrobials, and the analyses performed indicated that the endophytic strain BAC3151 can be useful for the development of new strategies to control microbial diseases in plants that are responsible for large damages in agricultural crops.

Keywords Endophytic · *Bacillus toyonensis* · Genome-mining · Antimicrobials · Secondary metabolites · Enzymes

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Introduction

Microbial diseases are one of the main causes of reduced productivity of many agricultural crops (Vieira et al. 2006) and the control of these diseases is usually carried out by combining crop management and use of pesticides and resistant cultivars (Jayaswal et al. 1990; Vieira et al. 2006). The application of pesticides is the primary control method, but their use is controversial because of harmful effects on the environment and the selection of resistant pathogens (Kim et al. 1999; Yu et al. 2014). There are also microbial diseases for which there are still no products with proven effectiveness (Vieira et al. 2006).

The use of microorganisms that are able to protect plants against phytopathogens has emerged as a more rational and safe alternative to traditional methods (Lucy et al. 2004; Hong et al. 2016). Endophytic bacteria colonize the interior of the host plant without causing apparent damage (Hallmann et al. 1997; Lopes et al. 2017). These bacteria can provide many benefits to plants, including growth promotion (Barka et al. 2002; Tanuja et al. 2013), induction of systemic resistance (Bakker et al. 2007), nitrogen supply by biological fixation (Jha and Kumar 2007), increased tolerance to environmental stresses (Andreolli et al. 2013), and protection against phytopathogenic microorganisms (Coombs et al. 2004; Hong et al. 2015), insects, nematodes (D'Alessandro et al. 2014) and herbivory (Sullivan et al. 2007). In this context, endophytic bacteria are ideal for the biological control of plant diseases because they often occupy the same habitat that phytopathogenic microorganisms and inhibits them without causing damage to the host plant and the environment (Melnick et al. 2008).

Bacillus species has been shown to be a potential source of several antimicrobials, such as ribosomally synthesized antimicrobial peptides (bacteriocins) (Abriouel et al. 2011), non-ribosomally synthesized peptides (NRPs), polyketides (PKs), as well as uncommon antimicrobials and other secondary metabolites (Fickers 2012; Lopes 2017). *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus cereus* and *Bacillus thuringiensis* are the best studied species for antimicrobials production within the genus (Mondol et al. 2013). Although studies have shown that endophytic *B. thuringiensis* and *B. amyloliquefaciens* can increase protection against phytopathogens and promote plant growth (Chen et al. 2007; Jeong et al. 2016), and that *B. thuringiensis* can still increase seed germination (Tanuja et al. 2013) and root nodulation (Mishra et al. 2009; Tanuja et al. 2013), the association between *Bacillus toyonensis* and plants was not investigated yet.

Another way to control microbial diseases in plants is by inhibiting the quorum sensing of phytopathogenic bacteria. Quorum sensing is population density-dependent mechanism for bacterial communication and gene regulation (Fuqua et al. 2001), by which pathogenic bacteria, especially Gram-negative, can modulate the production of virulence factors (Von Bodman et al. 2003). *Bacillus* has demonstrated the ability to produce quorum quenching enzymes which hydrolyze, such as *N*-acyl homoserine lactonase (AHLase), or modify the chemical structure of quorum sensing signaling molecules in Gram-negative bacteria and have variable substrate spectra (Chen et al. 2013). Studies indicate that the use of quorum sensing inhibitors is promising, thus expanding the current methods to control bacterial diseases in plants.

In this study, the whole-genome sequenced endophytic *B. toyonensis* BAC3151, which has demonstrated antimicrobial activity and quorum sensing inhibition of phytopathogenic bacteria (Lopes et al. 2015), was analyzed in silico to determine the genes involved in the synthesis of potential antimicrobial compounds. To our knowledge, this is the first comprehensive analysis focusing on the potential of *B. toyonensis* for the production of antimicrobials and provides valuable insights into how the strain BAC3151 can be used for the development of new strategies to control microbial diseases in plants.

Materials and methods

Bacterial strain and growth conditions

A strain identified initially as *Bacillus thuringiensis* BAC3151 by 16S rRNA gene sequencing was previously isolated from the leaves of the common bean (*Phaseolus vulgaris*) (Costa et al. 2012) and reclassified as *Bacillus toyonensis* BAC3151 by means of pairwise genome calculations of the average nucleotide identity (ANI) (Federhen et al. 2016). This strain is part of the bacterial strain collection of the Laboratory of Microorganism Molecular Genetics/BIOAGRO of the Federal University of Viçosa (Universidade Federal de Viçosa—UFV). *B. toyonensis* BAC3151 was cultured in 10% tryptone soy agar (TSA) (1.5 g/l tryptone, 0.5 g/l soy peptone, 1.5 g/l NaCl, pH 7.3) at 28 °C for the DNA isolation.

DNA isolation and genome sequencing

For genome sequencing, total DNA was isolated from culture grown for 16 h using the Wizard® genomic DNA purification kit (Promega) according to the manufacturer's instructions. The total DNA was sequenced by Macrogen Inc. (Seoul, South Korea) using an Illumina HiSeq 2000 platform (100 bp paired-end). A total of 21,512,378 high-quality reads and 2,172,750,178 high-quality bases were generated, producing ~355× coverage of the genome.

Data processing and assembly

The raw data obtained from sequencing were processed using the Phred-Phrap-Consed package (Rasko et al. 2008). The quality value used for the base-calling program was $Q \geq 20$. In the next step, 112 contigs were initially assembled using SOAPdenovo version 2.4 (Luo et al. 2012) and those < 500 bp were removed, resulting in a total of 78 contigs. The chromosome and plasmids contigs were then separated by Blastn. The chromosome contigs were assembled in the correct order and orientation using the *B. toyonensis* BCT-7112 genome as reference on the program Mauve version 2.3.1 (Darling et al. 2010) with default parameters.

Genome annotation

The annotation process involved the use of various algorithms in a multistep procedure. The structural annotation was performed using the following software: FgenesB: gene predictor (Solovyev and Salamov 2011), RNAmmer: rRNA predictor (Lagesen et al. 2007), and tRNAscan-SE: tRNA predictor (Lowe and Eddy 1997). The functional annotation was performed by blast analyses (Altschul et al. 1990) with the Non-Redundant (NR) database, Conserved Domains Database (CDD), Gene Ontology (GO), Swiss-Prot and Clusters of Orthologous Groups (COG), and InterProScan analysis (Jones et al. 2014) (e-value $< 10^{-5}$). Annotation was also performed using NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (Angiuoli et al. 2008).

Phylogenomic analysis

The phylogenomic analysis of *B. toyonensis* BAC3151 was performed using 699 core genes of 54 *Bacillus* strains deposited at NCBI (http://www.ncbi.nlm.nih.gov/genome/browse/). In the process, the core genes were aligned using Muscle version 3.8.31 (Edgar 2004), and non-matching regions in the alignment were masked and then removed using Gblocks (Castresana 2000). The remaining regions from all of the alignments were compiled to form an alignment of large sequences, which was then used as input for the program Phylip v. 3.2 (Felsenstein 1989). A tree was constructed using the neighbor-joining method with 1000 replicates.

Comparative analysis among *Bacillus* strains

Efficient Database framework for comparative Genome Analyses using BLAST score Ratios (EDGAR) (Blom et al. 2009) was used to evaluate the gene sharing among genomes of *Bacillus* strains available at NCBI (http://www.ncbi.nlm.nih.gov/genome/browse/). To analyze putative secondary metabolite gene clusters and bacteriocins, were used antiSMASH (Blin et al. 2017) and BAGEL3 (van Heel et al. 2013) software, respectively, with their default parameters. Compounds with antimicrobial activity were also mined from the published literature, their sequences were obtained from GenBank (https://www.ncbi.nlm.nih.gov/protein/) and homologous sequences were extracted from the analyzed genomes by similarity using Blastp (e-value $< 10^{-5}$; identity $\geq 85\%$; length $\geq 90\%$). The ClustalW algorithm (Larkin et al. 2007) was used to align the sequences.

Nucleotide sequence accession numbers

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession LDKD00000000. The version described in this paper is version LDKD02000000.

Results

General genomic features of *Bacillus toyonensis* BAC3151

The strain BAC3151 was first classified into B. thuringiensis based on a 16S rRNA gene sequence analysis (Costa et al. 2012). However, the whole-genome sequenced strain was 99.493% identical by ANI to the type genome of *B. toyonensis* (strain BCT-7112), and thus it was reclassified as B. toyonensis. The draft genome sequence of *B. toyonensis* BAC3151 has a total length of 5,740,808 bp (1 chromosome scaffold and 37 plasmid contigs), an average G + C content of 34.9%, and an N50 length of 299,415. A total of 5,706 protein-coding DNA sequences (CDSs) was predicted, comprising 4,685,804 bp, which resulted in a protein coding percentage of approximately 81.6%. The G + C content of the CDSs was slightly higher than the total G + C content, at 35.8%. The gene density was 0.993 CDS/kb, with a mean size of 821 bp per CDS. The draft genome also had 46 RNAs genes (40 tRNAs, 1 rRNA and 5 non-coding RNAs) and 303 pseudogenes. General features of the *B. toyonensis* genomes are summarized in Table 1. The strains were found to have similar mean G + C content, coding percentage, length and density of CDSs. However, there were significant differences in gene number and strain-specific genes.

Phylogenomic analysis

The phylogenomic tree inferred from 699 core gene of 54 *Bacillus* strains (including 18 *B. thuringiensis* strains, 12 *B. cereus* strains, 8 *Bacillus anthracis* strains, 4 *B. toyonensis* strains, 4 *B. amyloliquefaciens* strains, 4 *B. subtilis* strains, 1 *Bacillus velezensis* strain, 1 *B. licheniformis* strain, 1 *Bacillus clausii* strain, and 1 *Bacillus* sp. strain) is presented in Fig. 1. The cluster *B. cereus sensu lato* containing the *B. toyonensis, B. thuringiensis, B. anthracis* and *B. cereus* strains apart from other *Bacillus* members under study can be recognized. The endophytic *B. toyonensis* BAC3151 had a closer relationship with the insect strain *B. toyonensis* VU-DES13. *B. thuringiensis* KB1 grouped together with *B. cereus* strains, while *B. velezensis* LS69 and *Bacillus* sp. Pc3 were closely related with *B. amyloliquefaciens* strains.

Gene sharing among *Bacillus* strains

B. toyonensis shared more orthologous genes with *B. thuringiensis* than with other *Bacillus* species (Fig. 2a). Considering the four genomes of *B. toyonensis* strains (BacAer,

Table 1 Genomic features of *Bacillus toyonensis* strains

Genome feature	BacAer	BAC3151	VU-DES13	BCT-7112
Size (bp)	5,875,192	5,740,808	5,452,021	5,025,419
GC content (%)	34.9	34.9	35.1	35.5
Gene number	6260	6055	5808	5377
Protein-coding sequences (CDSs)	5822	5706	5361	4999
CDS mean length (bp)	833	821	817	824
CDS coding percentage	82	81.6	80.4	81.9
CDS density (CDS/kb)	0.98	0.99	0.98	0.99
CDS GC content (%)	35.8	35.8	35.9	36.2
RNA gene number	109	46	71	138
Pseudogene number	329	303	376	240
Isolation source	Human	Plant	Insect	Soil
Importance of the strain	Opportunistic pathogen	Biocontrol of plant pathogens	Insect pathogen	Probiotic in animal nutrition

BAC3151, VU-DES13 and BCT-71-12) available until August 2017 at NCBI, were identified 3811 CDSs shared between them (Fig. 2b), which correspond to 65.5 to 93.0% of the CDSs of the strains. BAC3151 had more CDSs in common with VU-DES13 (4973 CDSs), and fewer with BacAer (4243 CDSs). CDSs involved in the general physiological and survival functions of the species were well conserved in all four strains.

The total number of CDSs of all *B. toyonensis* strains was 6,980. Furthermore, 245 strain-specific CDSs in BCT-7112, 250 in VU-DES13, 416 in BAC3151 and 704 in BacAer were found. Most of the strain-specific CDSs were associated with hypothetical proteins, while several others were involved in response to environmental stresses, antimicrobials biosynthesis, processes of pathogenicity and virulence for animal (in VU-DES13 and, mainly, BacAer), and drug resistance. Among *B. toyonensis* strains, BAC3151 had the highest number of genes involved in microbial antagonism and was the only presenting, e.g., gene clusters of thuricin-like, uncharacterized linear azole-containing peptide (LAP), and lactococcin 972 family bacteriocin in the same strain (Fig. 3).

Putative genes of microbial antagonism found in *B. toyonensis* BAC3151

Considering the four available genomes of *B. toyonensis* strains, the species had a higher frequency of bacteriocin gene clusters than the most reported *Bacillus* species for antimicrobials production (Table 2). In addition, putative biosynthetic genes of several other antimicrobial compounds, including NRPs, AHLase, chitinases and novel bacteriocins, were identified in *B. toyonensis* BAC3151. The strain also showed a gene repertoire involved in antagonist greater than that of other *Bacillus* strains with efficient antimicrobial activity, such as *B. thuringiensis* KB1 and *B. subtilis* fmb60 (Jeong et al. 2016; Yang et al. 2016) (Fig. 3, Table 3).

Bacteriocin gene clusters found in BAC3151 included class I and class II bacteriocins. Class I bacteriocins are small peptides (< 10 kDa) ribosomally synthesized, undergoing posttranslational modifications. According to the modification differences, this class can be subdivided into different subclasses (Arnison et al. 2013). Three class I bacteriocins gene clusters were identified in BAC3151, encoding: one lanthipeptide similar to thuricin, one paeninodin-like (lasso peptide) and one LAP. Other gene clusters encoded two class II bacteriocins (unmodified bacteriocins): BhlA-like and lactococcin 972 family bacteriocin. The gene clusters of lactococcin 972 family bacteriocin and thuricin-like (Fig. 4a, b) are in plasmids, while all other bacteriocin gene clusters are on the chromosome (Table 3). Class III bactericin gene clusters (encoding large bacteriocins, > 10 kDa) (Zhao and Kuipers 2016; Turano et al. 2017) were not identified in BAC3151.

The biosynthetic genes of NRPs (petrobacin and bacillibactin siderophores, Fig. 4c, d, respectively), chitinases and AHLase in BAC3151 can be important for the inhibition of phytopathogens by competition for iron, degradation of fungal chitin and inhibition of bacterial quorum sensing, respectively. For AHLase, it was also observed that the motif ^{106}HXDH-H^{169}-D^{191}-Y^{194} necessary for the enzyme activity (Dong et al. 2000, 2002; Lu et al. 2006) was conserved in BAC3151 and other *Bacillus* species (Fig. 5).

Discussion

The *Bacillus cereus* group (or *Bacillus cereus sensu lato*) comprises eight closely relatated species, including *Bacillus*

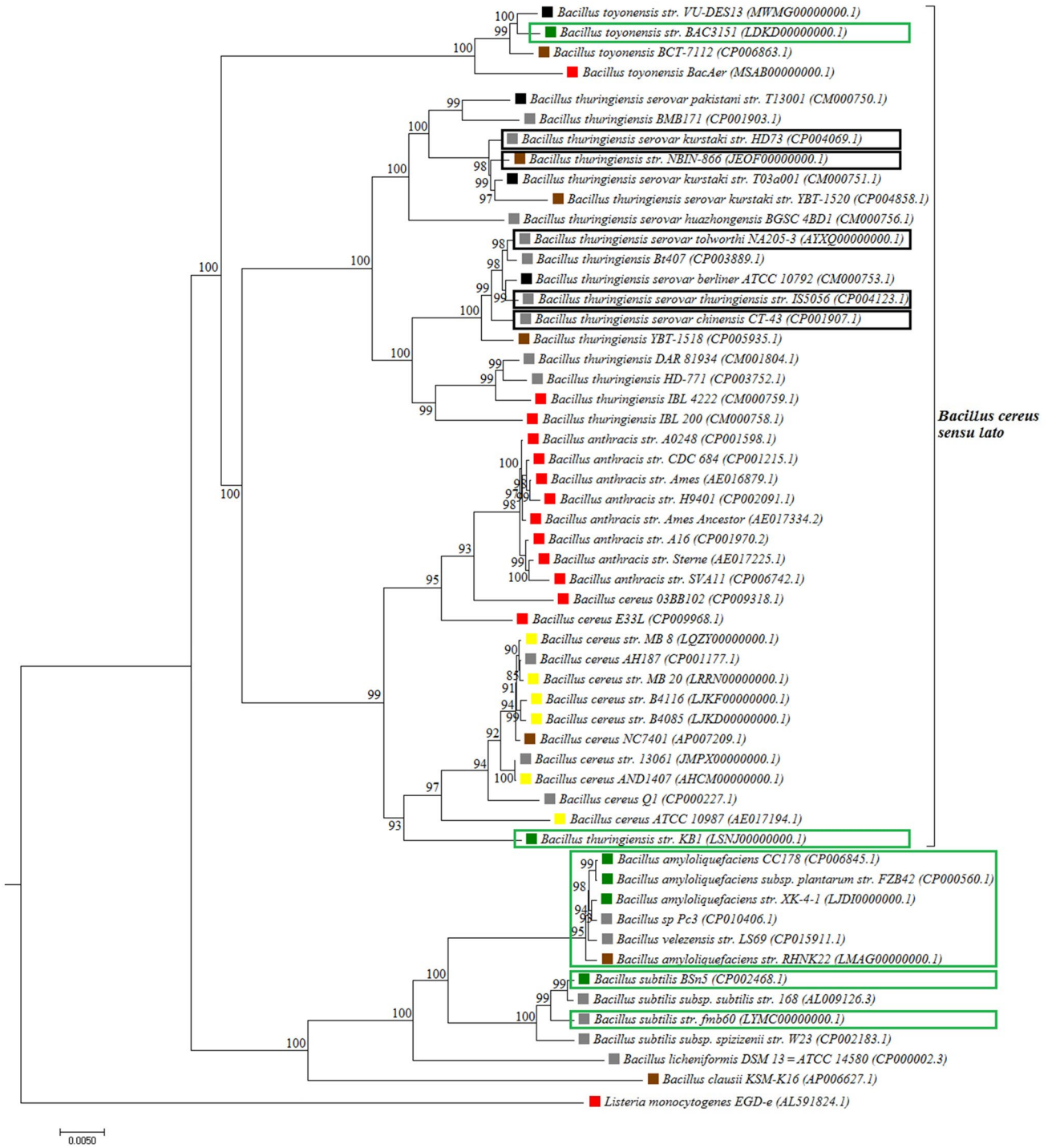

Fig. 1 Phylogenomic tree of 54 *Bacillus* strains based on 699 core genes. The tree was constructed with Phylip (Felsenstein 1989) using the neighbor-joining method with 1000 replicates. *Listeria monocytogenes* EGD-e was used as outgroup. The bootstrap values are shown at the nodes. The scale bar shows five nucleotide substitutions per 1000 nucleotides. Green boxes highlight strains with reported antimicrobial activity; black boxes indicate strains with high insecticidal or nematicidal activity. Green squares show strains isolated from plants; black, insect; brown, soil; yellow, food; red, human or animal; gray, other sources or sources not specified. The accession numbers of the sequences are given in parentheses

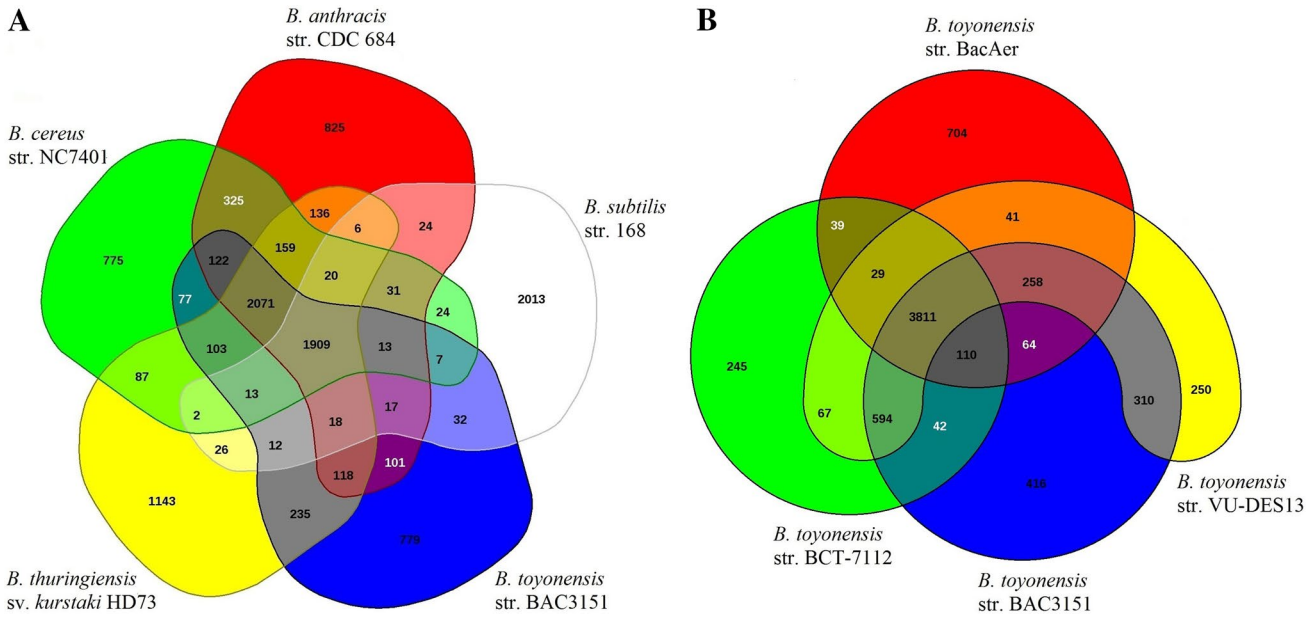

Fig. 2 Venn diagrams showing the sharing CDSs between *Bacillus* species (**a**) and *B. toyonensis* strains (**b**). Overlapping regions represent CDSs that are common between the genomes. Values outside of the overlapping regions indicate the number of CDSs in each genome without orthologs in the other genomes

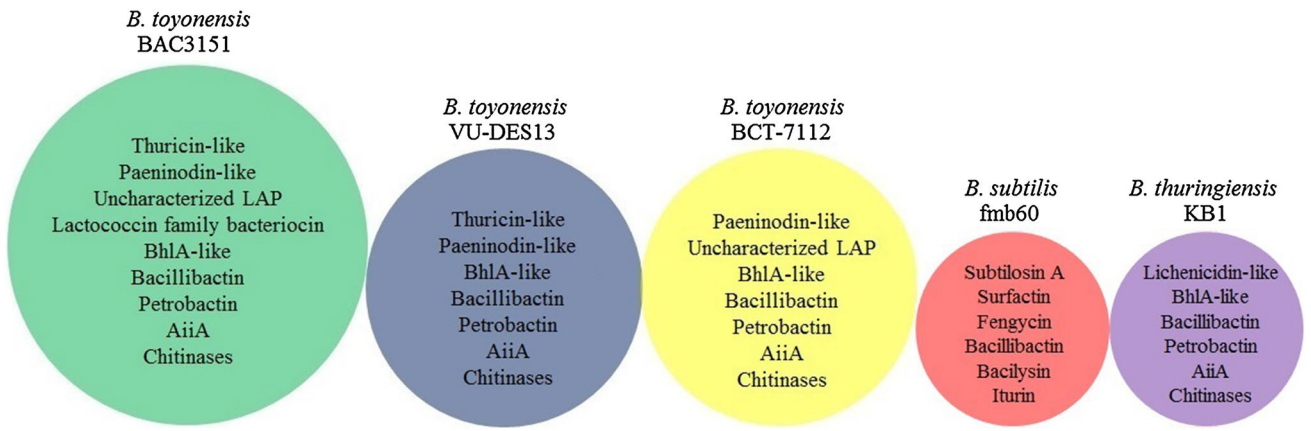

Fig. 3 Sets of antagonism genes present in *Bacillus* strains that show antimicrobial activity and/or strains of agricultural importance

cereus, Bacillus thuringiensis, Bacillus anthracis, Bacillus toyonensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus weihenstephanensis and *Bacillus cytotoxicus* (Helgason et al. 2000; Zwick et al. 2012; Guinebretière et al. 2013; Jiménez et al. 2013). Traditionally, the extensive similarity between them has been revealed by different techniques, such as 16S rRNA gene sequencing (Bavykin et al. 2004), multilocus sequence typing (MLST) (Priest et al. 2004), genomic mapping (Carlson et al. 1996), pulsed-field gel electrophoresis (PFGE) (Carlson et al. 1994), multilocus enzyme electrophoresis (MEE) (Helgason et al. 2000), variable number tandem repeat (VNTR) mapping, BOX-PCR fingerprinting (Kim et al. 2002), amplified fragment length polymorphisms (AFLP) (Ticknor et al. 2001), and, currently, genome sequencing (Zwick et al. 2012; Böhm et al. 2015).

B. toyonensis BCT-7112, which has been used as active ingredient of the feed additive preparation Toyocerin, was primarily identified as *Bacillus cereus*. However, the strain showed significant genomic differences from the type strains of the *B. cereus* group (ANI < 92%) and it was reclassified as a novel species (Jiménez et al. 2013). *B. toyonensis, B. pseudomycoides* and *B. cytotoxicus* have been distinguishable at species level (ANI > 96%), while other species within the *B. cereus* group, as observed in the phylogenetic analysis

Table 2 Number of putative bacteriocin gene clusters identified in *Bacillus* genomes

Species[a]	Class I								Class II	Class III	Total	Frequency	References
	Lanthipeptides type I	Lanthipeptides type II	Head to tail cyclized	Sactipeptides	Glycocins	Lasso peptides	LAPs	Thiopeptides					
Bacillus toyonensis (4)		2				4	3		7		16	4.0	This study
Bacillus amyloliquefaciens (13)		**16**					**1**		**12**	**1**	**30**	**2.31**	Zhao and Kuipers (2016)
Bacillus thuringiensis (46)	**4**	**8**	**6**	**5**	**3**	**4**	**24**		**31**	**14**	**99**	**2.15**	Zhao and Kuipers (2016)
Bacillus subtilis (39)	**6**			**53**	**15**		**1**	**1**	**8**		**84**	**2.15**	Zhao and Kuipers (2016)
Bacillus cereus (55)	**1**	**16**	**11**	**4**	**1**	**9**	**34**	**3**	**22**	**4**	**105**	**1.91**	Zhao and Kuipers (2016)
Bacillus licheniformis (3)		**1**				**3**					**4**	**1.33**	Zhao and Kuipers (2016)
Bacillus anthracis (39)							25				25	0.64	Zhao and Kuipers (2016)
Bacillus megaterium (5)	1					1					2	0.4	Zhao and Kuipers (2016)

[a]Numbers in parentheses indicate the number of genomes analyzed per species. More reported species with antimicrobial activity are indicated in bold

Table 3 Biosynthetic gene clusters and individual genes involved in antagonist found in *Bacillus* species with antimicrobial activity

Compounds	Genome localization	Locus			
		B. toyonensis BAC3151	*B. thuringiensis* KB1	*B. subtilis* fmb60	*B. velezensis* LS69
Bacteriocins					
Thuricin-like	Plasmid	ABH17_027075–27100			
Lichenicidin-like	Chromosome		AYK81_03545–03575		
Amylolysin	Chromosome				A8142_05960–05995
Paeninodin-like	Chromosome	ABH17_016365–016490			
Uncharacterized LAP	Chromosome	ABH17_022715–022755			
Subtilosin A	Chromosome			BG616_18680–18710	
Amylocyclicin	Chromosome				A8142_14425–14450
Lactococcin 972 family bacteriocin	Plasmid	ABH17_027510–027525			
BhlA-like	Chromosome	ABH17_016500	AYK81_14815		
NRPs					
Surfactin	Chromosome			BG616_01505–01540	A8142_01675–01710
Fengycin	Chromosome			BG616_09385–09405	A8142_09035–09055
Bacillibactin	Chromosome	ABH17_019005–019040	AYK81_07980–08015	BG616_15750–15780	A8142_14330–14465
Petrobactin	Chromosome	ABH17_020625–020660	AYK81_22575–25590		
Bacilysin	Chromosome			BG616_18895–1892	A8142_17315–17345
Iturin	Chromosome				A8142_08905–08940
PKs					
Macrolactin	Chromosome				A8142_07020–07060
Aurantinin	Chromosome			BG616_04340–04440	
Bacillaene	Chromosome				A8142_08290–08355
Difficidin	Chromosome				A8142_10745–10815
Enzymes					
N-Acyl homoserine lactonase (AiiA)	Chromosome	ABH17_015220	AYK81_05535		
Chitinase	Chromosome	ABH17_016765	AYK81_15880		
		ABH17_026160	AYK81_23900		

of this study (Fig. 1), do not match individual phylogenic groups (Böhm et al. 2015). For the indistinguishable species by ANI, differences in phenotype and pathological effects, are mainly resulted from plasmid-mediated characteristics rather than in chromosome in many cases (Helgason et al. 2000; Böhm et al. 2015).

Differences in the number of genes and strain-specific genes, as we observed for *B. toyonensis* strains (Table 1; Fig. 2b), can be primarily explained by mobile genetic elements, such as plasmids, transposons, phages, integrons and genomic islands (Frost et al. 2005; Fang et al. 2011; Böhm et al. 2015). Moreover, the large number of hypothetical proteins identified in *B. toyonensis* strains may also be the result of horizontal gene transfer, including genes from phylogenetically distant organisms for which there is currently no molecular characterization. Indeed, *Bacillus* can easily acquire genetic material from other bacteria (Gonzalez et al. 1982; Haack et al. 1996; Vilas-Bôas et al. 1998; Böhm et al. 2015) and the acquisition of foreign genes can contribute to improved adaptation of populations to specific niches.

In the environment, microorganisms compete for limited resources within a community and competition mechanisms are characterized by antagonistic effects. Direct microbial antagonism occurs by the production of compounds that kill or inhibit the growth of other microorganisms, while indirect antagonism occurs by competition for nutrients and space. Several *Bacillus* species have shown activity against other microorganisms (Jeong et al. 2016; Yang et al. 2016; Liu et al. 2017). We previously reported that *B. toyonensis* BAC3151 had antimicrobial activity against Gram-negative and Gram-positive bacteria, including important phytopathogenic bacteria (Lopes et al. 2015). In this study, the genome

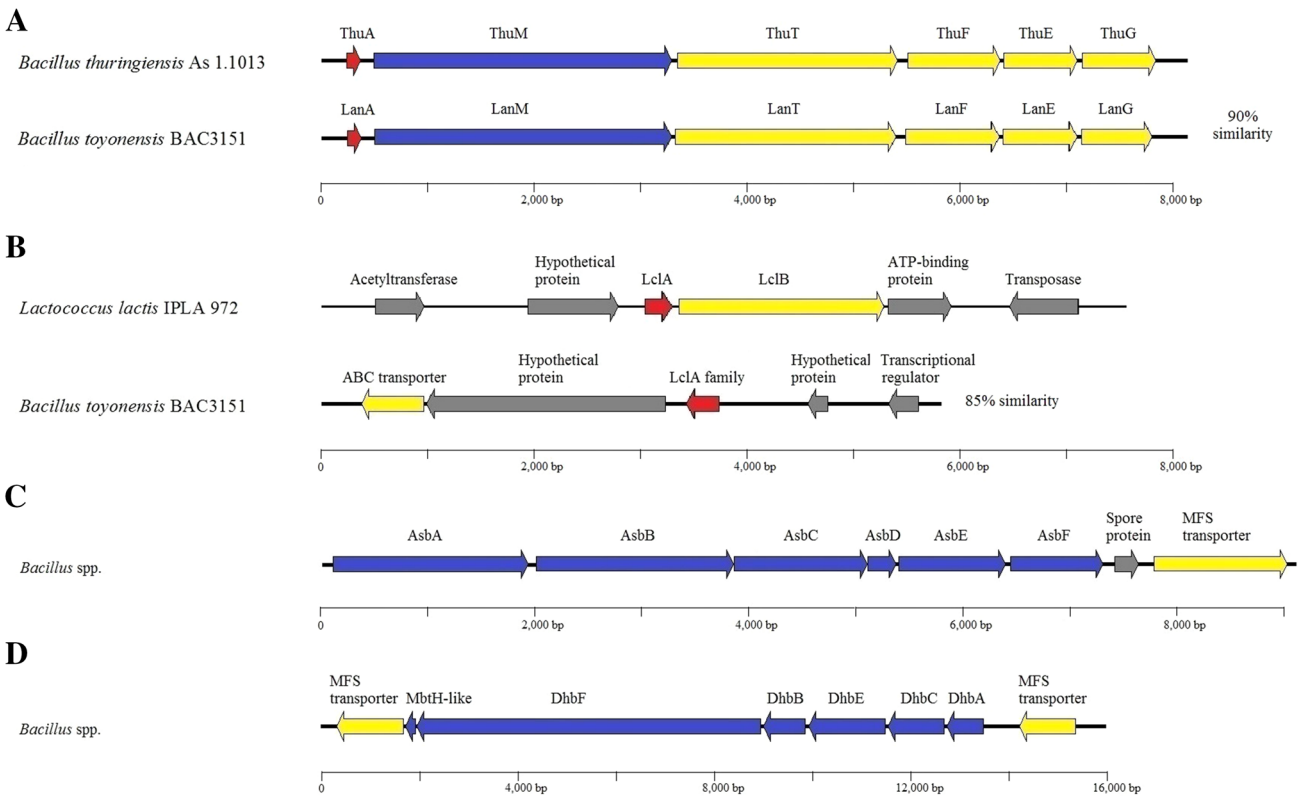

Fig. 4 Biosynthetic gene clusters of putative antimicrobials of *B. toyonensis*. **a** Schematic representation of the previously characterized thuricin gene cluster of *B. thuringiensis* As 1.1013 (GenBank accession: KJ504104.1) and thuricin-like lanthipeptide gene cluster of *B. toyonensis* BAC3151. **b** Lactococcin 972 gene cluster of *Lactococcus lactis* IPLA 972 (GenBank accession: AJ002203.2) and lactococcin 972 family bacteriocin gene cluster of *B. toyonensis* BAC3151. Gene clusters of petrobactin (**c**) and bacillibactin (**d**) found in *Bacillus* species. Red, precursor peptide genes; blue, genes involved in biosynthesis/modification; yellow, transport/immunity; gray, other genes

mining revealed that the strain has potential to produce a variety of antimicrobials, mainly bacteriocins.

Bacteriocins found in *Bacillus* are becoming increasingly important due to their spectrum of inhibition, which is sometimes broader than many lactic acid bacteria bacteriocins and may include Gram-negative bacteria, yeast and filamentous fungi, in addition to Gram-positive bacteria (Abriouel et al. 2011). To date, bacteriocins of the *B. cereus* group have been identified and characterized mainly in *B. cereus* and *B. thuringiensis* strains.

Class I bacteriocins belonging to lantipeptide subclass are peptides containing unusual amino acids, such as dehydroalanine/dehydrobutyrine and lanthionine/methyl-lanthionine residues, introduced by posttranslational modifications (Arnison et al. 2013). While class I lanthipeptides are modified by two distinct enzymes (dehydratase LanB and cyclase LanC), class II lanthipeptides are modified by a bifunctional lanthionine-introducing enzyme (LanM) (Knerr ànd van der Donk 2012). Lanthipeptides with antimicrobial activity are named lantibiotics (McAuliffe et at. 2001). Many lantibiotics exhibit broad-spectrum antimicrobial activity against clinical Gram-positive pathogens, including multidrug-resistant strains (Dischinger et al. 2014; Sandiford 2014). So far, reports about various bacteriocins in the *B. cereus* group have been published, but few were about lantibiotics. Some studies suggested that the *B. cereus* group may contain unidentified lanthipeptide gene clusters (Wang et al. 2014; Xin et al. 2015). In BAC3151, one thuricin-like lanthipeptide gene cluster was found in plasmid. Thuricins are typically produced by *B. thuringiensis* and may be encoded on chromosome or large plasmids (Favret and Yousten 1989; Murphy et al. 2011), but thuricin-like clusters have also been described in other species (Ugras et al. 2013). These putative clusters have not yet been experimentally verified and may be a source of novel lantibiotics.

Class I bacteriocins of the lassopeptide subclass have an N-terminal macrolactam ring that is threaded by the C-terminal tail resulting in a unique lasso structure isolated from proteo- and actinobacterial sources (Hegemann et al. 2015). In general, lasso peptide synthesis requires at least three genes, encoding a precursor peptide A, a cysteine protease B, and an ATP-dependent lactam synthetase C (Maksimov et al. 2012). Known lasso peptides display antimicrobial activity by enzyme inhibition, and microcin J25 produced

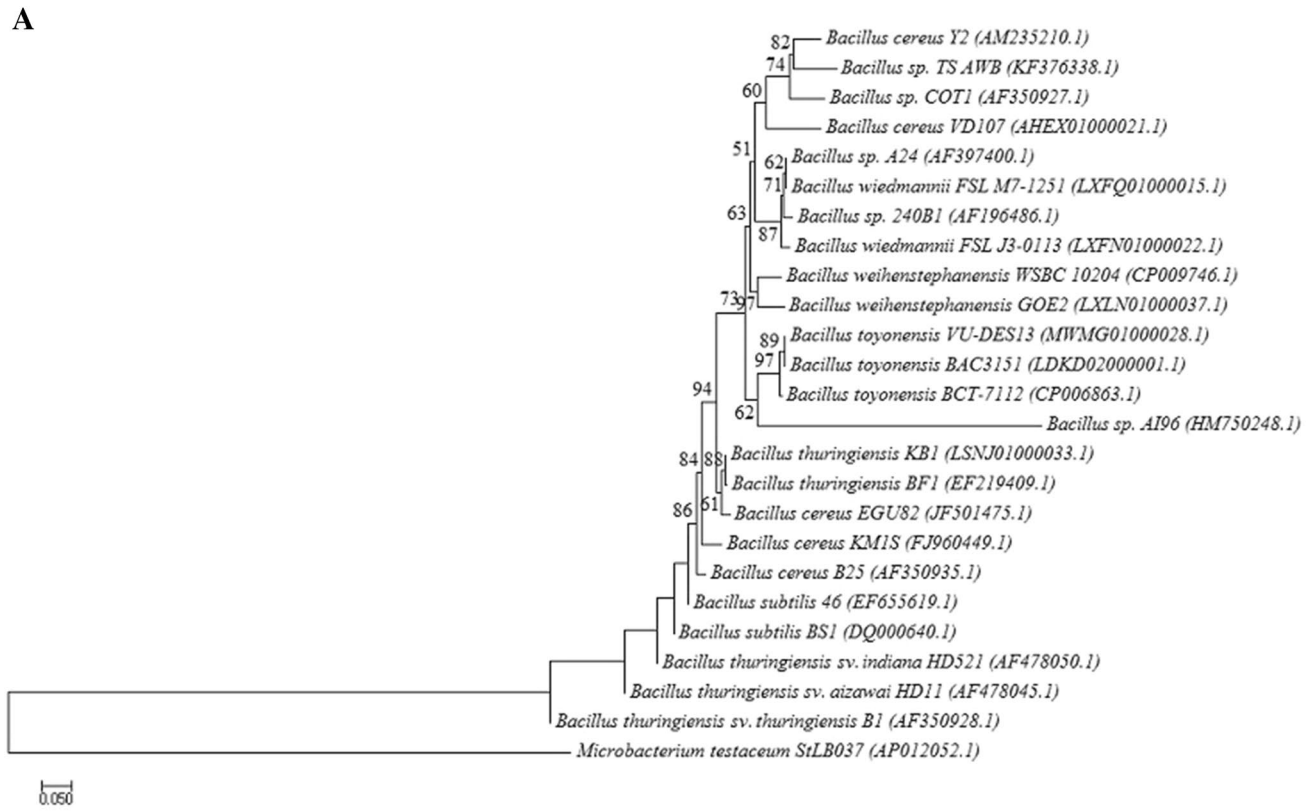

Fig. 5 Analysis of AHLases from *Bacillus* sp. **a** Phylogenetic tree of AHLase genes generated on the basis of the neighbor-joining method with 1000 repetitions using MEGA 7.0. Bootstrap values > 50 are shown. The GenBank accession numbers of the sequences are indicated in parentheses. **b** Multiple alignment of predicted AHLases of various *Bacillus* species. Gray shading indicates sites that were not completely conserved. The amino acid residues necessary for AHL-lactonase activity are boxed. *Bt, Bacillus toyonensis; Bw, Bacillus weihenstephanensis; Bth, Bacillus thuringiensis; Bc, Bacillus cereus; Bwi, Bacillus wiedmannii; Bs, Bacillus subtilis*

by *Escherichia coli* AY25 has served as a model for studies (Delgado et al. 2001; Mukhopadhyay et al. 2004). There is one paeninodin-like lasso peptide gene cluster in BAC3151. The paeninodin is a novel lasso peptide recently described from *Paenibacillus dendritiformis* and has potential antimicrobial activity, although this activity has not yet been confirmed (Zhu et al. 2016).

Class I bacteriocins of the LAP subclass have a distinguishing heterocyclic ring of oxazoles and thiazoles (Melby et al. 2011; Banala et al. 2013). Microcin B17 produced by *E. coli* and streptolysin S produced by lactic acid bacteria are model of representive LAPs (Nizet et al. 2000; Heddle et al. 2001; Cox et al. 2015). LAP subclass has been extended with plantazolicin A and B produced by *B. amyloliquefaciens* FZB42, and they have a unknown mechanism of action against other *Bacillus*, in addition to being associated with nematicidal activity (Banala et al. 2013; Liu et al. 2013). Only these two LAPs have been characterized in *Bacillus* before. However, a putative gene cluster of LAP was identified in BAC3151, meaning that novel LAPs can be found in *B. toyonensis*.

Class II bacteriocins present in BAC3151 were lactococcin 972 family bacteriocin and BhlA-like. Lactococcin 972 is plasmid-encoded and typically produced by *Lactococcus lactis* (Martínez et al. 1999). The lactococcin 972 gene cluster comprises structural and immunity/transport genes (Martínez et al. 1999; Sánchez et al. 2000) (Fig. 4b). This bacteriocin has a potent antimicrobial activity against lactococci and *Lactobacillus* (Martínez et al. 1995; Martínez et al. 1996). Unlike others class II bacteriocins, its primary target is not the cytoplasmic membrane. Instead, lactococcin 972 binds to the cell wall precursor lipid II and inhibits septum biosynthesis (Martínez et al. 2008). To our knowledge, similar bacteriocin has not yet been reported in *Bacillus* species. On the other hand, genes coding for BhlA have been described in *Bacillus pumilus* and *B. licheniformis*, and structural analysis of their sequence revealed features similar to holins (Kyogoku and Sekiguchi 1996; Aunpad and Panbangred 2012), which are phage-encoded proteins involved in the disruption of cytoplasmic membrane (Ziedaite et al. 2005). The peptide BhlA from *Bacillus licheniformis* showed antibacterial activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Micrococcus luteus*, by destroying cell membrane (Anthony et al. 2010).

We also found gene clusters putatively encoding NRPs petrobacin and bacillibactin in BAC3151. Petrobactin and bacillibactin are catecholate siderophores, which are molecules of iron acquisition, encoded by the *asb* and *dhb* operons, respectively (Wilson et al. 2006; Chen et al. 2007). Although siderophores are produced by various strains of the *B. cereus* group, siderophores originating from endophytic bacteria may be especially important for biocontrol in agriculture, given that these bacteria are naturally associated with plants. In this way, iron competition, an essential growth element, could indirectly suppress the growth of phytopathogens, such as fungi, which produce lower affinity siderophores (Kloepper et al. 1980; Compant et al. 2005). In addition, plant-associated bacteria can increase the supply of iron for the plant (Raddadi et al. 2007; Jin et al. 2014).

Another key gene identified was *aiiA*, which encodes the enzyme AHLase. This enzyme is presumably responsible for the ability of BAC3151 to inhibit the quorum sensing in Gram-negative bacteria, as previously reported by us (Lopes et al. 2015). The observed conservation of the motif ^{106}HXDH-H^{169}-D^{191}-Y^{194} (Fig. 5) reinforces evidence of AHLase activity from BAC3151. Gram-negative phytopathogenic bacteria use the quorum sensing as a regulatory mechanism for many biological activities, including the production of virulence factors (Von Bodman et al. 2003). The quorum sensing depends on the production, diffusion, and recognition of small signal molecules, usually *N*-acyl homoserine lactones (AHLs) in Gram-negative bacteria (Fuqua et al. 2001). AHLs share identical homoserine lactone rings, but vary in length and the substitution of the acyl side chain (Whitehead et al. 2001; Zhang 2003). The enzymes AHLases hydrolyse the lactone rings producing molecules that can no longer be used for signalling (Dong et al. 2000; Chen et al. 2013). Thus, the inhibition of the quorum sensing can be an effective strategy to control plant diseases. In addition to using the AHLase producer to suppress the quorum sensing-dependent virulence of phytopathogenic bacteria (Dong et al. 2004), genetically modified plants expressing *aiiA* can also efficiently quench bacterial quorum sensing and attenuate the virulence (Dong et al. 2001; Quiñones et al. 2005; Ouyang and Li 2016). These findings illustrate the promising potential to explore quorum sensing inhibitors for the control and prevention of infectious plant diseases, especially against bacteria that are not susceptible to treatment with available chemical agents.

With regard to chitinases, they may also be valuable for the control of plant diseases. Chitinases hydrolyse the glycosidic bonds of chitin, an important component of fungal cell wall, leading to cell lysis, and have been demonstrated to successfully inhibit the growth of phytopathogenic fungi. Chitinase-producing bacteria have been reported as biocontrol agents for different kinds of fungal diseases of plants (Kobayashi et al. 2002; Tang et al. 2012, 2017). In the *B. cereus* group, some strains have shown promising for this purpose. The chitinase-producing endophytic *B. cereus* strain 65, e.g., was found effective against *Rhizoctonia solani* in cotton (Pleban et al. 1997). Similarly, *B. thuringiensis* strains inhibited several fungi, such as *Sclerotium rolfsii, Pyricularia grisea, Physalospora piricola, Fusarium gramineum* and *Fusarium oxysporum* (Reyes-Ramirez et al. 2004; Tang et al. 2012, 2017). In addition, chitinases have

been used to increase the insecticidal activity of *B. thuringiensis*, since chitin is a major component of the exoskeleton of insects (Liu et al. 2002; Juárez-Hernández et al. 2015; Ni et al. 2015). Chitinases from *B. toyonensis* have not yet been characterized and future experimental investigations will be able to analyze their effectiveness for the control of phytopathogenic fungi as well as insect pests.

Conclusions

This study showed that *B. toyonensis* has a potential not previously reported for the species to produce antimicrobial compounds. Multiple putative genes involved in different types of antagonism are present in the strain BAC3151, including known clusters previously characterized in other bacteria and novel gene clusters. Future studies focusing on the biochemical and structural properties of these antimicrobials may reveal their spectrum of activity and mode of action and to develop new strategies to control pathogenic microorganisms in agriculture or even in other areas.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abriouel H, Franz CM, Ben Omar N, Galvez A (2011) Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol Rev 35:201–232

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Andreolli M, Lampis S, Poli M, Gullner G, Biro B, Vallini G (2013) Endophytic *Burkholderia fungorum* DBT1 can improve phytoremediation efficiency of polycyclic aromatic hydrocarbons. Chemosphere 92:688–694

Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G et al (2008) Toward an online repository of standard operating procedures (sops) for (meta)genomic annotation. OMICS 12:137–141

Anthony T, Chellappa GS, Rajesh T, Gunasekaran P (2010) Functional analysis of a putative holin-like peptide-coding gene in the genome of *Bacillus licheniformis* AnBa9. Arch Microbiol 192:51–56

Arnison PG, Bibb MJ, Bierbaum G, Bowers AA, Bugni TS, Bulaj G et al (2013) Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. Nat Prod Rep 30:108–160

Aunpad R, Panbangred W (2012) Evidence for two putative holin-like peptides encoding genes of *Bacillus pumilus* strain WAPB4. Curr Microbiol 64:343–348

Bakker PA, Pieterse CM, van Loon LC (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97:239–343

Banala S, Ensle P, Sussmuth RD (2013) Total synthesis of the ribosomally synthesized linear azole-containing peptide plantazolicin A from *Bacillus amyloliquefaciens*. Angew Chem Int Ed Engl 52:9518–9523

Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A (2002) Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biol Control 24:135–142

Bavykin SG, Lysov YP, Zakhariev V, Kelly JJ, Jackman J, Stahl DA et al (2004) Use of 16S rRNA, 23S rRNA, and *gyrB* gene sequence analysis to determine phylogenetic relationships of *Bacillus cereus* group microorganisms. J Clin Microbiol 42:3711–3730

Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA et al (2017) Antismash 4.0-improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res. doi:10.1093/nar/gkx319

Blom J, Albaum SP, Doppmeier D, Puhler A, Vorholter FJ, Zakrzewski M et al (2009) EDGAR: a software framework for the comparative analysis of prokaryotic genomes. BMC Bioinform 10:154

Böhm ME, Huptas C, Krey VM, Scherer S (2015) Massive horizontal gene transfer, strictly vertical inheritance and ancient duplications differentially shape the evolution of *Bacillus cereus* enterotoxin operons *hbl, cytk* and *nhe*. BMC Evol Biol 15:246

Carlson CR, Caugant DA, Kolsto AB (1994) Genotypic diversity among *Bacillus cereus* and *Bacillus thuringiensis* strains. Appl Environ Microbiol 60:1719–1725

Carlson CR, Johansen T, Kolsto AB (1996) The chromosome map of *Bacillus thuringiensis* subsp. *canadensis* HD224 is highly similar to that of the *Bacillus cereus* type strain ATCC 14579. FEMS Microbiol Lett 141:163–167

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I et al (2007) Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nat Biotechnol 25:1007–1014

Chen F, Gao Y, Chen X, Yu Z, Li X (2013) Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. Int J Mol Sci 14:17477–17500

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Coombs JT, Michelsen PP, Franco CMM (2004) Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. *tritici* in wheat. Biol Control 29:359–366

Costa LEO, Queiroz MV, Borges AC, Moraes CA, Araújo EF (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). Braz J Microbiol 43:1562–1575

Cox CL, Doroghazi JR, Mitchell DA (2015) The genomic landscape of ribosomal peptides containing thiazole and oxazole heterocycles. BMC Genom 16:778

D'Alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J et al (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant Cell Environ 37:813–826

Darling AE, Mau B, Perna NT (2010) ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS ONE 5:e11147

Delgado MA, Rintoul MR, Farias RN, Salomon RA (2001) *Escherichia coli* RNA polymerase is the target of the cyclopeptide antibiotic microcin J25. J Bacteriol 183:4543–4550

Dischinger J, Basi Chipalu S, Bierbaum G (2014) Lantibiotics: promising candidates for future applications in health care. Int J Med Microbiol 304:51–62

Dong YH, Xu JL, Li XZ, Zhang LH (2000) Aiia, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci USA 97:3526–3531

Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH (2001) Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. Nature 411:813–817

Dong YH, Gusti AR, Zhang Q, Xu JL, Zhang LH (2002) Identification of quorum-quenching *N*-acyl homoserine lactonases from *Bacillus* species. Appl Environ Microbiol 68:1754–1759

Dong YH, Zhang XF, Xu JL, Zhang LH (2004) Insecticidal *Bacillus thuringiensis* silences *Erwinia carotovora* virulence by a new form of microbial antagonism, signal interference. Appl Environ Microbiol 70:954–960

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Fang Y, Li Z, Liu J, Shu C, Wang X, Zhang X et al (2011) A pangenomic study of *Bacillus thuringiensis*. J Genet Genom 38:567–576

Favret ME, Yousten AA (1989) Thuricin: the bacteriocin produced by *Bacillus thuringiensis*. J Invertebr Pathol 53:206–216

Federhen S, Rossello-Mora R, Klenk H-P, Tindall BJ, Konstantinidis KT, Whitman WB et al (2016) Meeting report: Genbank microbial genomic taxonomy workshop (12–13 may, 2015). Stand Genom Sci 11:15

Felsenstein J (1989) PHYLIP: phylogeny inference package (version3.2). Cladistics 5:164–166

Fickers P (2012) Antibiotic compounds from *Bacillus*: why are they so amazing? Am J Biochem Biotechnol 8:38–43

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Gonzalez JM Jr, Brown BJ, Carlton BC (1982) Transfer of *Bacillus thuringiensis* plasmids coding for delta-endotoxin among strains of *B. thuringiensis* and *B. cereus*. Proc Natl Acad Sci USA 79:6951–6955

Guinebretière MH, Auger S, Galleron N, Contzen M, De Sarrau B, De Buyser ML et al (2013) *Bacillus cytotoxicus* sp. nov. is a novel thermotolerant species of the *Bacillus cereus* group occasionally associated with food poisoning. Int J Syst Evol Microbiol 63:31–40

Haack BJ, Andrews RE, Loynachan TE (1996) Tn916-mediated genetic exchange in soil. Soil Biol Biochem 28:765–771

Hallmann J, Quadt-Hallmann A, Mahafee WF, Kloepper J (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Heddle JG, Blance SJ, Zamble DB, Hollfelder F, Miller DA, Wentzell LM et al (2001) The antibiotic microcin B17 is a DNA gyrase poison: characterisation of the mode of inhibition. J Mol Biol 307:1223–1234

Hegemann JD, Zimmermann M, Xie X, Marahiel MA (2015) Lasso peptides: an intriguing class of bacterial natural products. Acc Chem Res 48:1909–1919

Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M et al (2000) *Bacillus anthracis, Bacillus cereus*, and *Bacillus thuringiensis*: one species on the basis of genetic evidence. Appl Environ Microbiol 66:2627–2630

Hong CE, Jo SH, Moon JY, Lee JS, Kown SY, Partk JM (2015) Isolation of novel leaf-inhabiting endophytic bacteria in *Arabidopsis thaliana* and their antagonistic effects on phytophathogens. Plant Biotechnol Rep 9:451–458

Hong CE, Kwon SY, Park JM (2016) Biocontrol activity of *Paenibacillus polymyxa* AC-1 against *Pseudomonas syringae* and its interaction with *Arabidopsis thaliana*. Microbiol Res 185:13–21

Jayaswal RK, Fernandez MA, Schroeder RG (1990) Isolation and characterization of a *Pseudomonas* strain that restricts growth of various phytopathogenic fungi. Appl Environ Microbiol 56:1053–1058

Jeong H, Jo SH, Hong CE, Park JM (2016) Genome sequence of the endophytic bacterium *Bacillus thuringiensis* strain KB1, a potential biocontrol agent against phytopathogens. Genome Announc 4:e00279–e00316

Jha PN, Kumar A (2007) Endophytic colonization of *Typha australis* by a plant growth-promoting bacterium *Klebsiella oxytoca* strain GR-3. J Appl Microbiol 103:1311–1320

Jiménez G, Urdiain M, Cifuentes A, Lopez-Lopez A, Blanch AR, Tamames J et al (2013) Description of *Bacillus toyonensis* sp. nov., a novel species of the *Bacillus cereus* group, and pairwise genome comparisons of the species of the group by means of ANI calculations. Syst Appl Microbiol 36:383–391

Jin CW, Ye YQ, Zheng SJ (2014) An underground tale: contribution of microbial activity to plant iron acquisition via ecological processes. Ann Bot 113:7–18

Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C et al (2014) Interproscan 5: genome-scale protein function classification. Bioinformatics 3:1236–1240

Juárez-Hernández EO, Casados-Vázquez LE, del Rincón-Castro MC, Salcedo-Hernández R, Bideshi DK, Barboza-Corona JE (2015) *Bacillus thuringiensis* subsp. *israelensis* producing endochitinase ChiA74deltasp inclusions and its improved activity against *Aedes aegypti*. J Appl Microbiol 119:1692–1699

Kim BS, Moon SS, Hwang BK (1999) Isolation, antifungal activity, and structure elucidation of the glutarimide antibiotic, streptimidone, produced by *Micromonospora coerulea*. J Agric Food Chem 47:3372–3380

Kim W, Hong YP, Yoo JH, Lee WB, Choi CS, Chung SI (2002) Genetic relationships of *Bacillus anthracis* and closely related species based on variable-number tandem repeat analysis and BOX-PCR genomic fingerprinting. FEMS Microbiol Lett 207:21–27

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. Curr Microbiol 4:317–320

Knerr PJ, van der Donk WA (2012) Discovery, biosynthesis, and engineering of lantipeptides. Annu Rev Biochem 81:479–505

Kobayashi DY, Reedy RM, Bick J, Oudemans PV (2002) Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. Appl Environ Microbiol 68:1047–1054

Kyogoku K, Sekiguchi J (1996) Cloning and sequencing of a new holin-encoding gene of *Bacillus licheniformis*. Gene 168:61–65

Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Liu M, Cai QX, Liu HZ, Zhang BH, Yan JP, Yuan ZM (2002) Chitinolytic activities in *Bacillus thuringiensis* and their synergistic effects on larvicidal activity. J Appl Microbiol 93:374–379

Liu Z, Budiharjo A, Wang P, Shi H, Fang J, Borriss R et al (2013) The highly modified microcin peptide plantazolicin is associated with nematicidal activity of *Bacillus amyloliquefaciens* FZB42. Appl Microbiol Biotechnol 97:10081–10090

Liu G, Kong Y, Fan Y, Geng C, Peng D, Sun M (2017) Whole-genome sequencing of *Bacillus velezensis* LS69, a strain with a broad inhibitory spectrum against pathogenic bacteria. J Biotechnol 249:20–24

Lopes RBM (2017) Quem é o chefe aqui? Os avanços biotecnológicos e perspectivas genômicas do uso de Bacillus thuringiensis na agricultura. Novas Edições Acadêmicas. Chişinău, pp 45–51

Lopes RBM, Costa LEO, Vanetti MC, de Araujo EF, de Queiroz MV (2015) Endophytic bacteria isolated from common bean (*Phaseolus vulgaris*) exhibiting high variability showed antimicrobial activity and quorum sensing inhibition. Curr Microbiol 71:509–516

Lopes R, Cerdeira LT, Fernandes MR, Perez-Chaparro PJ, McCulloch JA, Lincopan N (2017) Draft genome sequence of a CTX-M-15-producing endophytic Klebsiella pneumoniae ST198 isolate from commercial lettuce. J Glob Antimicrob Resist 10:19–20

Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964

Lu X, Yuan Y, Xue XL, Zhang GP, Zhou SN (2006) Identification of the critical role of Tyr-194 in the catalytic activity of a novel *N*-acyl-homoserine lactonase from marine *Bacillus cereus* strain Y2. Curr Microbiol 53:346–350

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1–25

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J et al (2012) SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 1:18

Maksimov MO, Pan SJ, James Link A (2012) Lasso peptides: structure, function, biosynthesis, and engineering. Nat Prod Rep 29:996–1006

Martínez E, Sudrer JE, Rodríguez A (1995) Antagonistic activities of wild lactococcal strains isolated from homemade cheeses. J Food Prof 58:1118–1123

Martínez B, Suárez JE, Rodríguez A (1996) Lactococcin 972: a homodimeric lactococcal bacteriocin whose primary target is not the plasma membrane. Microbiology 142:2393–2398

Martínez B, Fernández M, Suárez JE, Rodríguez A (1999) Synthesis of lactococcin 972, a bacteriocin produced by *Lactococcus lactis* IPLA 972, depends on the expression of a plasmid-encoded bicistronic operon. Microbiology 145:3155–3161

Martínez B, Bottiger T, Schneider T, Rodríguez A, Sahl HG, Wiedemann I (2008) Specific interaction of the unmodified bacteriocin lactococcin 972 with the cell wall precursor lipid II. Appl Environ Microbiol 74:4666–4670

McAuliffe O, Ross RP, Hill C (2001) Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol Rev 25:285–308

Melby JO, Nard NJ, Mitchell DA (2011) Thiazole/oxazole-modified microcins: complex natural products from ribosomal templates. Curr Opin Chem Biol 15:369–378

Melnick RL, Zidack NK, Bailey BA, Maximova SN, Guiltinan M, Backman PA (2008) Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. Biol Control 46:46–56

Mishra PK, Mishra S, Selvakumar G, Bisht JK, Kundu S, Gupta HS (2009) Coinoculation of *Bacillus thuringiensis*-KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.). World J Microbiol Biotechnol 25:753–761

Mondol MA, Shin HJ, Islam MT (2013) Diversity of secondary metabolites from marine *Bacillus* species: chemistry and biological activity. Mar Drugs 11:2846–2872

Mukhopadhyay J, Sineva E, Knight J, Levy RM, Ebright RH (2004) Antibacterial peptide microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel. Mol Cell 14:739–751

Murphy K, O'Sullivan O, Rea MC, Cotter PD, Ross RP, Hill C (2011) Genome mining for radical SAM protein determinants reveals multiple sactibiotic-like gene clusters. PLoS ONE 6:e20852

Ni H, Zeng S, Qin X, Sun X, Zhang S, Zhao X et al (2015) Molecular docking and site-directed mutagenesis of a *Bacillus thuringiensis* chitinase to improve chitinolytic, synergistic lepidopteran-larvicidal and nematicidal activities. Int J Biol Sci 11:304–315

Nizet V, Beall B, Bast DJ, Datta V, Kilburn L, Low DE et al (2000) Genetic locus for streptolysin S production by group A streptococcus. Infect Immun 68:4245–4254

Ouyang LJ, Li LM (2016) Effects of an inducible *aiiA* gene on disease resistance in *Eucalyptus urophylla* × *Eucalyptus grandis*. Transgenic Res 25:441–452

Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of *Bacillus cereus*. Lett Appl Microbiol 25:284–288

Priest FG, Barker M, Baillie LW, Holmes EC, Maiden MC (2004) Population structure and evolution of the *Bacillus cereus* group. J Bacteriol 186:7959–7970

Quiñones B, Dulla G, Lindow SE (2005) Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. Mol Plant Microbe Interact 18:682–693

Raddadi N, Cherif A, Ouzari H, Marzorati M, Brusetti L, Boudabous A et al (2007) *Bacillus thuringiensis* beyond insect biocontrol: plant growth promotion and biosafety of polyvalent strains. Ann Microbiol 57:481–494

Rasko DA, Rosovitz MJ, Myers GS, Mongodin EF, Fricke WF, Gajer P et al (2008) The pangenome structure of *Escherichia coli*: Comparative genomic analysis of *E. coli* commensal and pathogenic isolates. J Bacteriol 190:6881–6893

Reyes-Ramirez A, Escudero-Abarca BI, Aguilar-Uscanga G, Hayward-Jones PM, Barboza-Corona JE (2004) Antifungal activity of *Bacillus thuringiensis* chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. J Food Sci 69:M131-M134

Sánchez C, Hernández de Rojas A, Martínez B, Arguelles ME, Suárez JE, Rodríguez A et al (2000) Nucleotide sequence and analysis of pBL1, a bacteriocin-producing plasmid from *Lactococcus lactis* IPLA 972. Plasmid 44:239–249

Sandiford SK (2014) Advances in the arsenal of tools available enabling the discovery of novel lantibiotics with therapeutic potential. Expert Opin Drug Discov 9:283–297

Solovyev V, Salamov A (2011) Automatic annotation of microbial genomes and metagenomic sequences. In: Li RW (ed) Metagenomics and its applications in agriculture, biomedicine and environmental studies. Nova Science Publishers, Hauppauge, pp 61–78

Sullivan TJ, Rodstrom J, Vandop J, Librizzi J, Graham C, Schardl CL et al (2007) Symbiont-mediated changes in *Lolium arundinaceum* inducible defenses: evidence from changes in gene expression and leaf composition. New Phytol 176:673–679

Tang Y, Zou J, Zhang L, Li Z, Ma C, Ma N (2012) Anti-fungi activities of *Bacillus thuringiensis* H3 chitinase and immobilized chitinase particles and their effects to rice seedling defensive enzymes. J Nanosci Nanotechnol 12:8081–8086

Tang M, Sun X, Zhang S, Wan J, Li L, Ni H (2017) Improved catalytic and antifungal activities of *Bacillus thuringiensis* cells with surface display of Chi9602deltasp. J Appl Microbiol 122:106–118

Tanuja R, Bisht SC, Mishra PK (2013) Ascending migration of endophytic *Bacillus thuringiensis* and assessment of benefits to different legumes of N.W. Himalayas. Eur J Soil Biol 56:56–64

Ticknor LO, Kolsto AB, Hill KK, Keim P, Laker MT, Tonks M et al (2001) Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. Appl Environ Microbiol 67:4863–4873

Turano H, Gomes F, Barros-Carvalho GA, Lopes R, Cerdeira L, Netto LES et al (2017) Tn6350, a novel transposon carrying pyocin S8 genes encoding a bacteriocin with activity against carbapenemase-producing *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 61:e00100-17

Ugras S, Sezen K, Kati H, Demirbag Z (2013) Purification and characterization of the bacteriocin Thuricin Bn1 produced by *Bacillus thuringiensis* subsp. *kurstaki* Bn1 isolated from a hazelnut pest. J Microbiol Biotechnol 23:167–176

van Heel AJ, de Jong A, Montalban-Lopez M, Kok J, Kuipers OP (2013) Bagel3: automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. Nucleic Acids Res 41:W448-W453

Vieira C, Paula Júnior TJD, Bórem A (2006) Feijão, 2nd edn. UFV, Viçosa, pp 241–263

Vilas-Bôas GFLT, Vilas-Bôas LA, Lereclus D, Arantes OMN (1998) *Bacillus thuringiensis* conjugation under environmental conditions. FEMS Microbiol Ecol 25:369–374

Von Bodman SB, Bauer WD, Coplin DL (2003) Quorum sensing in plant-pathogenic bacteria. Annu Rev Phytopathol 41:455–482

Wang J, Ma H, Ge X, Zhang J, Teng K, Sun Z et al (2014) Bovicin HJ50-like lantibiotics, a novel subgroup of lantibiotics featured by an indispensable disulfide bridge. PLoS ONE 9:e97121

Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25:365–404

Wilson MK, Abergel RJ, Raymond KN, Arceneaux JE, Byers BR (2006) Siderophores of *Bacillus anthracis, Bacillus cereus* and *Bacillus thuringiensis*. Biochem Biophys Res Commun 348:320–325

Xin B, Zheng J, Xu Z, Song X, Ruan L, Peng D et al (2015) The *Bacillus cereus* group is an excellent reservoir of novel lanthipeptides. Appl Environ Microbiol 81:1765–1774

Yang J, Zhu X, Cao M, Wang C, Zhang C, Lu Z et al (2016) Genomics-inspired discovery of three antibacterial active metabolites, aurantinins B, C, and D from compost-associated *Bacillus subtilis* fmb60. J Agric Food Chem 64:8811–8820

Yu J, Hu S, Ma K, Sun L, Hu H, Zou F et al (2014) Ribosomal protein S29 regulates metabolic insecticide resistance through binding and degradation of CYP6N3. PLoS ONE 9:e94611

Zhang LH (2003) Quorum quenching and proactive host defense. Trends Plant Sci 8:238–244

Zhao X, Kuipers OP (2016) Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. BMC Genom 17:882

Zhu S, Hegemann JD, Fage CD, Zimmermann M, Xie X, Linne U et al (2016) Insights into the unique phosphorylation of the lasso peptide paeninodin. J Biol Chem 291:13662–13678

Ziedaite G, Daugelavicius R, Bamford JK, Bamford DH (2005) The holin protein of bacteriophage PRD1 forms a pore for small-molecule and endolysin translocation. J Bacteriol 187:5397–5405

Zwick ME, Joseph SJ, Didelot X, Chen PE, Bishop-Lilly KA, Stewart AC et al (2012) Genomic characterization of the *Bacillus cereus* sensu lato species: backdrop to the evolution of *Bacillus anthracis*. Genome Res 22:1512–1524