Delving into the *Bacillus cereus* group biosynthetic gene clusters cosmos: a comparative-genomics-based classification framework

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Abstract

Background: In this study, the *Bacillus* sp. strain BH32 (a plant-beneficial bacterial endophyte) and its closest non-type *Bacillus cereus* group strains were used to study the organization, conservation, and diversity of biosynthetic gene clusters (BGCs) among this group to propose a classification framework of gene cluster families (GCFs) among this intricate group. A dataset consisting of 17 genomes was used in this study. Genomes were annotated using PROKKA ver.1.14.5. The web tool antiSMASH ver. 5.1.2 was used to predict the BGCs profiles of each strain, with a total number of 198 BGCs. The comparison was made quantitatively based on a BGCs counts matrix comprising all the compared genomes and visualized using the Morpheus tool. The constitution, distribution, and evolutionary relationships of the detected BGCs were further analyzed using a manual approach based on a BLASTp analysis (using BRIG ver. 0.95); a phylogenetic analysis of the concatenated BGCs sequences to highlight the evolutionary relationships; and the conservation, distribution and the genomic co-linearity of the studied BGCs using Mauve aligner ver. 2.4.0. Finally, the BIG-SCAPE/CORASON automated pipeline was used as a complementary strategy to investigate the gene cluster families (GCFs) among the *B. cereus* group.

Results: Based on the manual approach, we identified BGCs conserved across the studied strains with very low variation and interesting singletons BGCs. Moreover, we highlighted the presence of two major BGCs synteny blocks (named "*synteny block* A" and "*synteny block* B"), each composed of conserved homologous BGCs among the *B. cereus* group. For the automatic approach, we identified 23 families among the different BGCs classes of the *B. cereus* group, named using a rational basis. The proposed manual and automatic approaches proved to be in harmony and complete each other, for the study of BGCs among the selected genomes.

Conclusion: Ultimately, we propose a framework for an expanding classification of the *B. cereus* group BGCs, based on a set of reference BGCs reported in this work.

Keywords: Comparative genomics, *Bacillus cereus* group, endophyte, Biosynthetic gene clusters (BGCs), Synteny.

Background

The *Bacillus cereus* group of bacteria represents a homogeneous subdivision of the genus *Bacillus* with closely related phylogeny within the Firmicutes phylum [1, 2]. The bacteria constituting this group are Gram-positive, spore-forming, aerobic/facultative anaerobic, and rod-shaped with low-GC content [2]. Numerous bacteria related to *B. cereus* group were shown to produce several interesting compounds and enzymes, metabolize different kinds of pollutants, and promote the growth of both plants and animals when used as biostimulators [3–5]. The first described and most documented members of the group are *B. cereus*, *B. thuringiensis,* and *B. anthracis* [2]. Many bacteria classified as *B. cereus* are ubiquitous in the environment, with apparent soil origin, and present as commensal to intestines of insects or foodborne opportunistic pathogens often related to human poisoning [6, 7]. Other members of the group related to *B. thuringiensis* are insect pathogens widely used in agriculture for the biocontrol of insect pests [7, 8]; while *B. anthracis* is the causative agent of anthrax [2].

Traditionally, pathogenic potential and virulence characteristics permit organism differentiation within the group [1, 2, 7]: *B. cereus* by carrying biosynthetic gene cluster (BGC) for cereulide cytotoxin, plasmids carrying the crystal insecticidal genes for *B. thuringiensis* and presence of anthrax toxin and capsule genes for *B. anthracis* [2]. However, genetic experiments have exhibited a high level of synteny and protein similarity, with limited differences in gene content [1]. Conventional taxonomic markers, such as 16S and 23S rRNA genes, as well as whole-genome DNA hybridization seemed essentially identical, questioning the speciation of the group members [7]. Thus, extensive genomic similarities have contributed to the suggestion that *B. anthracis, B. cereus,* and *B. thuringiensis* are members of a single species, *B. cereus* sensu lato [1]. Moreover, the horizontal genetic transfer of plasmid and chromosome DNA among the strains of the *B. cereus* group has likely related to the diversity of this bacterial group, thus complicating the speciation [2].

In the present context of increased environmental screening with the generalization of whole genome sequencing that revealed the presence of newly identified recombinant forms [2], it is important to explore different approaches for understanding the evolution of the *B. cereus* group that may contribute to a more accurate characterization of these organisms. With the availability of a critical mass of compiled genomic data and the development of advanced computational tools for genome mining, one such approach consists of the bioinformatics exploration of an extensive range of potential biosynthetic gene clusters (BGCs) for the presence among the *B. cereus* group.

BGCs are a physical grouping of all the genes required to produce secondary metabolites, including pathway-specific regulatory genes [9]. BGCs mining is the process of identifying and characterizing these clusters to understand the biosynthesis of natural products and to discover new natural products and biosynthetic pathways [10]. BGCs are the source of many important natural products, such as antibiotics, anti-cancer compounds, and enzymes [11]. BGCs mining can lead to the discovery of new natural products with unique properties, and the development of new methods for natural product production [12]. BGCs can be used as

a source of new enzymes and biosynthetic pathways that can be exploited for biotechnology applications such as the production of biofuels, fine chemicals, and enzymes for bioremediation [13]. Many natural products produced by BGCs have medicinal properties and can be used as leads for drug development. BGCs mining can lead to the discovery of new natural products with potential therapeutic applications [14]. BGCs are often horizontally transferred between bacteria [15]. To gain a genetic advantage, it is postulated that elements in BGCs are horizontally acquired across species for quick adaptation to a new environment [16]**.** Studying their evolution and distribution can provide insights into the evolution and ecology of bacteria [17].

With recent developments in next-generation sequencing and advancements in genome mining tools, it became possible to computationally identify thousands of BGCs and draw a global map of BGCs within a group of bacteria that allow us to systematically explore those of interest [9]. To overcome this challenge, researchers are increasingly using bioinformatics tools such as ClusterFinder [18], antiSMASH [19], and Big-scape [20], which can help automate the process of BGCs identification and classification. Additionally, efforts are being made to establish a standardized nomenclature for BGCs and to create a comprehensive database of BGCs, which lead to the establishment of the MIBiG database [21, 22] which can help facilitate data sharing and comparison among researchers.

Big-scape is a bioinformatics tool that can be used to study BGCs in bacteria using an automated streamlined pipeline [20]. It enables researchers to identify, classify, and compare BGCs across different bacterial strains and can be used to infer the biosynthetic pathways and natural products associated with each BGC, providing valuable insights into the evolution and adaptation of bacteria to different environments, as well as the discovery of new natural products and biosynthetic pathways. Automated bioinformatics pipelines and manual bioinformatics are both useful methods for analyzing biological data, but they have different advantages and disadvantages. Automated pipelines are more efficient and can be run by researchers with minimal bioinformatics experience [23], while manual bioinformatics

is more flexible and customizable, but requires more expertise [24]. The choice of which method to use will depend on the specific research question, the amount and type of data, and the available resources.

Based on the known biosynthesis pathways potentially involved in the production of specialized metabolites by *Bacillus* and closely related species, BGC predictions rely on the development of bioinformatics tools and algorithms design to search for conserved motifs of specific pathways; including peptide synthetases (NRPSs), polyketide synthases (PKSs), and ribosomally synthesized and post-translationally modified peptides (RiPPs) pathways [25]

Despite BGCs being important among the *Bacillus cereus* group, there is currently limited data on their conservation across the different strains of this group. Further research is required to better understand the diversity and distribution of BGCs among this group. Nevertheless, highlighting the synteny of BGCs in bacteria is one challenging yet beneficial task.

Synteny refers to the preservation of gene order and chromosomal location among different organisms [26]. One benefit of studying this phenomenon is to guide the discovery and characterization of new natural products produced by these bacteria. By identifying the synteny of BGCs among different strains, researchers can infer the presence of similar BGCs in other strains, and can then use this information to guide their search for new natural products. Another benefit is that it can provide insight into the evolutionary relationships among different taxa. The conservation of BGCs in terms of chromosomal location and gene organization among different strains can be used to infer evolutionary relationships among these strains, and can also help to understand how these bacteria have adapted to different environments over time.

Advances in sequencing technologies, high-throughput screening techniques, and improved computational methods have led to a rapidly increasing number of BGCs being identified. This has created a growing need for effective and standardized methods for BGC classification [12]**.** There have been several efforts to establish a unified system for BGC

133 classification, such as the antiSMASH platform, but the field is still in its early stages and
134 much work remains to be done to develop a comprehensive and widely-accepted
135 classification system [19].

136 Moreover, during a screening for potentially beneficial endophytic bacteria, the strain *Bacillus*
137 sp. BH32, which belongs to the *B. cereus* group, was isolated from *Atriplex halimus L*., a
138 halophyte sampled from a continental hypersaline region (Sebkha) in Djelfa province, Algeria.
139 The strain, which was proven to help tomato and wheat seedlings tolerate salt stress at
140 various levels, was consecutively genome analyzed to determine putative mechanisms
141 involved in salt tolerance and plant promotion [27], and thus, is part of the genome dataset of
142 this study, along with its closely-related strains.

143 In the present study, we investigated the BGCs of the *Bacillus* sp. strain BH32 at a genomic
144 level along with its closest non-type strains to explore the conservation and putative evolution
145 patterns of BGCs among the *Bacillus cereus* group, and to highlight singletons. Based on a
146 combined strategy (manual and automatic), we aimed to establish the basis of a rational
147 classification of BGCs among the *B. cereus* group.

148 **Methods**

149 **1. Presenting the dataset**

150 The genomic dataset is composed of *Bacillus* sp. BH32 genomes and its closest non-type
151 strains genomes; all part of the *Bacillus cereus* group. *Bacillus* sp. BH32 is a beneficial
152 endophyte, isolated during a previous study from *Atriplex halimus* L., a halophyte sampled
153 from an Algerian continental Sebkha from the province of Djelfa. This strain was proven to
154 help tomato and wheat seedlings tolerate salt stress at various levels [27]. The selection of
155 the closest non-type strains of *Bacillus* sp. BH32 was performed with BLASTn 2.10.0+ [28]**,**
156 using the whole genome of *Bacillus* sp. BH32 as a query against the NCBI's "Complete
157 prokaryotic genomes" database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 02-04-
158 2020), targeting the first most significant 16 hits (high-quality complete genomes only) **(table**
159 **1)**.

2. BGCs counts and distribution

BGCs from the dataset were predicted using antiSMASH ver. 5.1.2 [19]**.** BGCs counts were compared quantitatively, after preparing a BGCs-types/counts matrix comprising all the compared genomes, and visualized using Morpheus (https://software.broadinstitute.org/morpheus/). The generated heatmap showed the counts of BGCs (cyan-white-fuchsia, from 0 to 5) by type, and their distribution among the strains (dendrograms were generated by hierarchical clustering using one minus Spearman rank correlation) **(Fig. 1).**

3. BGCs BLASTp comparisons

A BLASTp (ver. 2.9.0+) comparison of *Bacillus* sp. BH32 BGCs against BGCs of the closest non-type strains was done, using the BGCs amino-acid sequences (for each genome, a multifasta file was used, containing the amino acid sequences of all the genes constituting the whole predicted BGCs, prepared from the GenBank records generated by antiSMASH), using BRIG ver. 0.95 [29]. The reference ring constitutes the detected BGCs in *Bacillus* sp. BH32, while each ring represents BGCs of each genome of the closest non-type strains (the color shades represent sequence identity, the greyer it gets; the lower the percentage identity). The 3 outermost rings (from inside to outside) represent: genes composing BGCs in *Bacillus* sp. BH32 according to their respective location in the genome; unique genes (sequence identity < 30% with 100% of the strains) and rare genes (sequence identity < 30% with 80% the strains) with their locus tag IDs; and the corresponding regions/BGCs types (last ring) as detected by antiSMASH **(Fig. 2)**. The annotation from antiSMASH and PROKKA of each gene was retrieved using their corresponding locus tag IDs.

4. BGCs gains, losses, and rearrangements

Manual approach

First, fasta files were prepared; each containing concatenated amino acid sequences of all BGCs for each strain (with conserved order of occurrence in the respective genome).

Sequences were aligned using MAFFT ver. 7.221.3 [30]. An ML tree was inferred from the aligned sequences by using the Maximum Likelihood method based on the JTT matrix-based model [31]. The tree harboring the highest log likelihood (-248721.7967) was selected. The percentage of trees in which the associated sequences clustered together is displayed next to the branches (branch-support values). Initial tree(s) for the heuristic search were automatically obtained based on Neighbor-Join and BioNJ algorithms applied to a matrix of pairwise distances (estimated by a JTT model), and then selecting the topology with the highest log likelihood value. The tree was drawn to scale, with branch lengths reflecting the number of substitutions per site. The analysis involved 17 amino acid sequences (full record of BGCs from each strain). All positions containing gaps and missing data were eliminated. There were a total of 28898 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [32]**.** The final tree was drawn using Adobe Illustrator CC 18.1.1 **(Fig. 3 a).**

BGCs synteny among the strains was investigated with MAUVE ver. 2.4.0 [33–37], using the alignment of concatenated Genbank sequences of the BGCs of each strain, according to their order of appearance in the respective genome, where each row shows the conservation and orientation of BGCs in the corresponding strain in the ML tree after alignment. *Bacillus thuringiensis* c25 was set as a reference, according to the phylogenetic analysis (most distant). Homologous segments indicating orthologous clusters (from MAUVE alignment diagram) with a locally collinear block (LCB) weight ≥ 773 were confirmed by annotation. BGCs relative orientation and order conservation were highlighted **(Fig. 3 b).**

BIGSCAPE automatic approach and GCFs nomenclature proposal

The selected antiSMASH profiles of the *B. cereus* genomes were used to identify the gene cluster families GCFs using BiG-SCAPE v.1.1.0. with default parameters [20]. The output of the BIG-SCAPE was exploited to propose a classification of GCFs among the *B. cereus* group, based on the respective BGCs classes/types, as organized by the unsupervised Machine Learning clustering approach employed by BIG-SCAPE. Since the BIG-SCAPE

output consists of BGCs grouped into GCFs with arbitrary codes, we propose a systematic and grounded nomenclature of the highlighted families. In our proposal, families names are composed of: a **"**BC" prefix that stands for *Bacillus cereus* (group) ("BCS" in case of singletons), followed by the type (e.g. bacteriocin), then an increasing number that should follow the order of detection/characterization during this study (or future studies). The product's name should appear instead of merely the type, in case the cluster product is known (from the MIBIG database). The proposed reference BGCs are based on '*exemplars'*, according to the affinity propagation clustering approach used by BIG-SCAPE. Harmony between the manual and automatic approaches was assessed manually based on the correspondence between the BGCs region codes as given by antiSMASH and those displayed among the BIG-SCAPE output. The similarity networks were arranged to fit their corresponding figures, while the original BIG-SCAPE output, the used dataset (antiSMASH profiles), and the proposed reference BGCs files (included in the antiSMASH profiles) are available as a supplementary material. Final figures of the GCFs classes distribution (Fig. 5-8) were composed under Adobe Illustrator CC 18.1.1.

Results

1. BGCs distribution and counts in the *B. cereus* group

The BGCs were first compared in terms of distribution and counts among the compared genomes **(Fig. 1)**. All the strains have similar counts of NARPS-like, LAP-bacteriocin, siderophore, and betalacton BGCs (one for each type). *B. cereus* JHU has the highest number of bacteriocin BGCs (4), followed by *B. thuringiensis* L-76-01 and B. thuringiensis c25 (3 each), while the other strains have 2 (except *Bacillus* sp. BH32, only one bacteriocin BGC was detected). *B. cereus* ZB201708 is the only strain where antiSMASH failed to detect a terpene BGC (all the other strains have one). *B. cereus* ZB201708, *B. thuringiensis* YGd 22-03, and *B. thrungiensis* c25 are the only strains to have a lanthipeptid BGC (one for each). NRPS BGC-type has the highest counts among the selected strains, ranging from 3 to 5.

Bacillus cereus A1, *Bacillus bombysepticus* Wang, *Bacillus thuringiensis* c25 and *Bacillus cereus* FORC087 have 3 NRPS BGCs ; *Bacillus* sp. BH32, *Bacillus wiedmannii* PL1, *Bacillus thuringiensis* YGd22-03 and *Bacillus thuringiensis* serovar galleriae 4G5 have 4 ; and the remaining strains have 5.

The strains *Bacillus thuringiensis* serovar morrisoni BGS, *Bacillus cereus* G9842, *Bacillus thuringiensis* BT59, *Bacillus thuringiensis* HD1002, *Bacillus thuringiensis* JW-1, and Bacillus *thuringiensis* HD 789 have the same BGC counts (NRPS = 5, NRPS-like = 1, LAP/bacteriocin = 1, bacteriocin = 2, terpene = 1, betalactone = 1, siderophore = 1, lanthipeptide = 0). Same thing for *Bacillus wiedmannii* PL1 and *Bacillus thuringiensis* serovar galleriae 4G5 (NRPS = 4, NRPS-like = 1, LAP/bacteriocin = 1, bacteriocin = 2, terpene = 1, betalactone = 1, siderophore = 1, lanthipeptide = 0). *Bacillus cereus* A1, *Bacillus bombysepticus* Wang and *Bacillus cereus* FORC087 have the same profile as well (NRPS = 3, NRPS-like = 1, LAP/bacteriocin = 1, bacteriocin = 2, terpene = 1, betalactone = 1, siderophore = 1, lanthipeptide = 0). The remaining strains have different BGC count profiles **(Fig. 1)**.

[Insert Figure 1 here]

2. Amino acid sequence conservation of the *B. cereus* group BGCs

The BLASTp comparison of BGCs from *Bacillus* sp. BH32 vs. its closest non-type strains **(Fig. 2)** shows heterogeneous content in terms of predicted protein sequences from detected BGCs. The most conserved BGC seems to be the terpene BGC (region 2.1), while the less conserved one seems to be the NRPS BGC (region 2.2).

Although the antiSMASH failed to detect the terpene biosynthetic genes, they seem to be present in the BLASTp output of *Bacillus cereus* ZB201708 (14^{th} ring, from the inner ring to the outer ring), which could be due to assembly concerns, or else, the constituting genes of this terpene cluster might be distributed in other BGCs from *Bacillus cereus* ZB201708.

NRPS BGC (region 2.2) from *Bacillus* sp. BH32 has very low sequence identity when compared to its counterparts in *B. bombysepticus* Wang, *B. cereus* A1 and *B. thuringiensis* c25 (<30%). Considering that the other NRPS BGCs from *Bacillus* sp. BH32 (regions 6.1, 8.1, 12.1, and 15.1) have higher sequence identity % with the 3 corresponding BGCs of the aforementioned strains, which confirms the absence of a counterpart of the NRPS BGC from region 2.2 in these strains as suggested by the counts (5 NRPS BGCs in *Bacillus*. sp. BH32 against only 4 in *B. bombysepticus* Wang, *B. cereus* A1, and *B. thuringiensis* c25). This cluster has two rare genes (identity bellow 30% for 80% of the strains): ctg2_300 = unknown/hypothetical protein; ctg2_321 = unknown / IS200/IS605 family transposase ISAsp8.

The siderophore BGC (region 3.1) encoding petrobactin (100%) and the LAP/bacteriocin BGC (region 5.1, unknown encoded product, closest BGCs with overall gene sequence identity >70% from strains *Bacillus cereus* ZB201708, *Bacillus cereus* JHU and *Bacillus thuringiensis* L-7601) seem to be well conserved, with a slight variation on the composing genes. The NRPS BGC (region 6.1) is another example of a well-conserved cluster across the compared strains (closest known cluster = bacillibactine, 46%).

The NRPS-like BGC (region 8.1) is present among all the strains. Nevertheless, *Bacillus* sp. BH32 has one unique gene (sequence identity bellow 30% for 100% of the strains) in this region (ctg8_81 = unknown/hypothetical protein), and a rare one (ctg8_90 = unknown/hypothetical protein).

The NRPS BGC (region 12.1) shows variation in its first 19 genes (~ 1/3 of the total BGC), including one unique gene (ctg12_31 = unknown / Spore germination protein B1), and one rare gene (ctg12_35 = biosynthetic-additional; (smcogs) SMCOG1012:4'-phosphopantetheinyl transferase / 4'-phosphopantetheinyl transferase Sfp).

The NRPS BGC (region 15.1) from *Bacillus* sp. BH32 has one unique gene (ctg15_85 = biosynthetic-additional; (smcogs) SMCOG1028: crotonyl-CoA reductase - alcohol dehydrogenase / Zinc-type alcohol dehydrogenase-like protein). This NRPS BGC doesn't

have a counterpart in *Bacillus cereus* FORC087, confirming the BGCs count (5 NRPS BGCs in *Bacillus* sp. BH32 for only 4 in *Bacillus cereus* FORC087).

The bacteriocin BGC (region 24.1) from *Bacillus* sp. BH32 is another example of a well-conserved cluster across its closely related non-type strains.

Finally, the betalacton BGC (region 27.1) from *Bacillus* sp. BH32 (fengycin, 40%) arbores two unique genes (ctg27_13 = unknown/hypothetical protein; ctg27_20 = unknown/hypothetical protein). Globally, all BGCs from *Bacillus* sp. BH32 are present in at least 13 of its closest strains (>81%), either very well-conserved (regions) or less conserved, with some rare/unique genes **(Fig. 2)**.

[Insert Figure 2 here]

3. Manual approach for *B. cereus* group BGCs synteny

The ML tree based on BGCs amino acids sequences **(Fig. 3 a)** shows 4 clades:

Clade I: *B. thuringeinsis c25, B. cereus* JHU, *B. thuringeinsis* HD1002 and *B. thuringeinsis* BT-59; Clade II: *Bacillus* sp. BH32; Clade III: *B. wiedmannii* PL1, *B. cereus* FORC087, *B. thuringiensis* serovar galleriae 4G5, *B. bombysepticus* Wang, *B. cereus* A1 and *B. thuringiensis* YGd22-03; Clade IV: B. thuringiensis HD 789, B. thuringiensis JW1, *B. thuringiensis* L-7601, *B. cereus* G9842, *B. thuringiensis* serovar morrisoni BGS and *B. cereus* ZB201708.

From the ML tree **(Fig. 3 a)**, *B. thuringiensis* c25 has been considered as a reference for BGCs conservation (the most distant genome among the compared strains). *Bacillus* sp. BH32 which usually clusters with *B. cereus* ZB201708 and *B. thuringiensis* serovar morrisoni BGSC 4AA1 (GBDP and ANI analysis), doesn't seem to be close to these strains when it comes to its BGCs, showing thus more complexity among this intricate group, in terms of BGCs.

The analysis of the BGCs conservation is shown in **(Fig. 3 b)**. Here, BGCs are mentioned according to their attributed BGC tag number **(Fig. 3 b** legends**)**. Considering *B. thuringiensis*

c25 as a reference, we observe that the orthologous BGCs group that seems to be best conserved in order and appearance is the one composed of BGC3 (bacteriocin: unknown), BGC4 (bacteriocin: unknown), BGC5 (betalacton, fengycin), BGC6 (NRPS: gramicidin in *B. cereus* JHU; nostopeptolide A2 in *B. thuringiensis* serovar galleriae 4G5; unknown in the remaining strains), BGC7 (NRPS: bacillibactin), BGC8 (siderophore: petrobactin) and BGC9 (linear azol(in)e-containing peptides "LAP" bacteriocin). This group was named "*synteny block A"*, which appears in this order in all strains except *B. wiedmannii*, where BGC3 appears to be in the beginning, unusually separated from the other BGCs.

The second conserved group is composed of BGC1 (terpene: molybdenum co-factor) and BGC2 (NRPS: polyoxypeptin, except for *B. thringiensis* c25, unknown), named "*synteny block B"*. BGC10 (bacteriocin: unknown) is only present in *B. thuringiensis* C25 (thus, will not be mentioned here again).

In *B. cereus* JHU, BGC1 and BGC2 (*synteny block B*) are still in the same order, as well as for the BGC3, BGC4, BGC5, BGC6, BGC7, BGC8 and BGC9 (*synteny block A*). Meanwhile, 4 new BGCs appeared: BGC13 (bacteriocin: unknown) and BGC14 (bacteriocin: unknown) both in the beginning; BGC15 (NRPS-like 2: unknown) between the two synteny blocks A and B; and BGC16 (NRPS-Polyketide: chejuenolide A / chejuenolide B) at the end. The BGC11 (NRPS-like 1: unknown) moved to the beginning, right before the *synteny block B*. BGC12 (lanthipeptid: cerecidin / cerecidin A1 / cerecidin A2 / cerecidin A3 / cerecidin A4 / cerecidin A5 / cerecidin A6 / cerecidin A7) is lost.

BGC13 and BGC14 (both unknown bacteriocins) are only found in *B. cereus* JHU.

As for B. cereus JHU, *B*. *thuringiensis* HD-1002 and *B. thuringiensis* BT-59 have the two synteny blocks with the BGC15 in between. BGC16 is at the right end of the *synteny block B*. BGC12, BGC13, and BGC14 are lost and the BGC11 jumped to the end.

Since *Bacillus* sp. BH32 genome doesn't constitute a complete one, the observations for the BGCs conservation for this strain concern only their presence, not their order (the use of a reference genome for the reordering of the contigs doesn't take into count the possible

genomic rearrangements, and thus biases the synteny analysis, so we kept the contigs as they resulted from the assembly, from the largest to the smallest one).

In *Bacillus* sp. BH32, the BGC4 (bacteriocin with no closest known cluster) seems to be missing, while conserved across all the analyzed genomes of its closely related non-type strains, suggesting this absence due to the incompleteness of the genome, and should be found once the genomic gaps filled. The remaining BGCs from *synteny blocks A* and *B* are all present in *Bacillus* sp. BH32.

In the BGCs clades III and IV, all BGCs are inversely oriented in comparison with the strains of the first clade (including *B. thuringiensis* C25). In *B. wiedmannii,* BGC11 jumped between *synteny blocks A* and *B*, while BGC3 and BGC15 are right before the *synteny block B*. This strain doesn't have the BGC12.

B. cereus FORC087 has the same homologous BGCs from 1 to 9 (inversely oriented), with BGC11 in the beginning. BGC15 and BGC16 are absent. *B. thuringiensis* serovar galleriae 4G5 has *synteny blocks A* and *B*, and the BGC15 reappears next to BGC2 from *synteny block B*. As for *B. cereus* FORC087, BGC 11 is still in the beginning.

For *B. bombysepticus* Wang, BGC2 is missing. Despite the BGC15 (NRPS-like: unknown) bears homologous segments linking it with the BGC2 (NRPS: polyoxypeptin), after manually checking the annotation, it confirmed the assignment to its closest orthologous BGC, the BGC15. The BGC1 is there, at the end. BGC11 is still in the beginning, before the *synteny block A*.

B. cereus A1 has an arrangement similar to *B. bombysepticus* Wang, except for BGC11 jumping to the end. For *B. thuringiensis* YGd22-03, BGC2 reappears, in the *synteny block B*, while the BGC12 lost since *B. thuringiensis* c25 reappears here.

For clade IV, the *synteny blocks A*, *B,* and BGC15 hold the same position in all its strains. In *B. thuringiensis* HD-789, BGC11 is in the end, next to the *synteny block B*. BGC16 reappears, to stand in the beginning.

B. thuringiensis HD-789, *B. thuringiensis JW-1, B. thuringiensis* serovar morrisoni BGSC 4AA1 *B. cereus* G9842, *B. thuringiensis L76-01* and *B. cereus* ZB201708 have the BGC11 jumping again to the front position, followed by the BGC16, except *B. cereus* ZB201708 where the appearing BGC18 (lanthipeptid: surfactin) appeared between BGC11 and BGC16; and the *synteny blocks A* and *B* with BGC15 in between. *B. thuringiensis L76-01* and *B. cereus* ZB201708 are characterized with a new BGC for each one: BGC17 (bacteriocin: unknown) and BGC19 (bacteriocin: unknown) respectively, at the end.

BGCs of the *synteny blocks A* and *B* being consistently present in all the strains, will naturally tend to occur in chromosomic locations among the analyzed genomes. BGC11 (NRPS-like 1: unknown) is present across all strains, at various positions, suggesting its possible jumping between chromosomic and plasmidic locations.

BGC15 (NRPS-like 2: unknown) is largely present (except in *B. thuringiensis* c25 and *B. cereus* FORC087), and is located most of the time between synteny blocks A and B, suggesting a more likely chromosomic location as well. BGC12 (lanthipeptid: cerecidin / cerecidin A1 / cerecidin A2 / cerecidin A3 / cerecidin A4 / cerecidin A5 / cerecidin A6 / cerecidin A7), when present, is next to BGC11.

The similarity plots in the homologous segments are confirming the BLASTp results from the BRIG ring generator **(Fig. 2)**. The present synteny analysis can give direction to the prediction of the presence of certain BGCs, as we showed that they are usually conserved in presence and order. It can also be used to detect the evolution and distribution of homologous BGCs across the complex *B. cereus* group. The observed BGCs positions should be further investigated, in their genomic context, by studying synteny not only for BGCs, but extending the analysis to the whole genomic content.

[Insert Figure 3 here]

4. BIG-SCAPE results

The BIG-SCAPE pipeline was used as well, in line with the manual approach, as a complementary strategy to investigate the gene cluster families (GCFs) among the *B. cereus* group.

There was a total of 198 BGCs, organized into 4 main classes (Fig. 4, table 2):

- NRPS: consisting of a total of 89 BGCs, it is the biggest class representing 44.94% of the total BGCs;
- RiPPs: 58 BGCs fall into this class, representing 29.29% of the sum of the analyzed BGCs;
- Terpenes: represent 5.58% of the total BGCs (with 17 BGCs identified as coding for a terpene);
- Other (remaining classes): composed of 34 BGCs, representing 17.17% of the studied BGCs.

We suggest a framework for an expanding classification of the *B. cereus* group BGCs, based on a set of reference BGCs described in this work (tables 3-4; supplementary figures S1, S2, S3, and S4), as anchoring points to affiliate unknown query BGCs to the proposed families/clans accordingly. Such reference BGCs should be included in future attempts to assign a set of BGCs (from genomes belonging to the *B. cereus* group) using the BIG-SCAPE pipeline into one of the proposed families in the current proposal. Moreover, the same strategy can be reproduced for other bacterial groups.

[Insert Figure 4 here]

Hence, we propose the following families:

The RiPPs gene cluster families of the *Bacillus cereus* group

The BIG-SCAPE output yielded 13 RiPPs GCFs (Fig. 5), with an average of 4 BGCs by family. It is the 2nd largest group of BGCs with 58 clusters (including 7 singletons, table 4), without any known reference BGC in the MIBIG database (Fig. 5a). Following the proposed

nomenclature, the identified families were named as follows (GCF name/ reference BGC strain and genomic region) (Fig. 5b; table 3; supp. Fig. S1):

- BC-Bacteriocin 1: *B. cereus* ZB201708, NZ_CP030982.1.region009
- BC-Bacteriocin 2: *B. thuringiensis* HD1002, NZ_CP009351.1.region005
- BC-Bacteriocin 3: *B. thuringiensis* serovar *galleriae* 4G5, NZ_CP010089.1.region007
- BC-Lasso peptide 1: *B. cereus* A1, NZ_CP015727.1.region001
- BC-Bacteriocin 4: *B. thuringiensis* serovar *morrisoni* BGSC 4AA1, NZ_CP010577.1.region009
- BC-Bacteriocin 5: *B. cereus* ZB201708, NZ_CP030982.1.region010
- BC-Cerecidin: *B. thuringiensis* YGd22-03, NZ_CP019230.1.region011

Families BC-Bacteriocin 1, BC-Bacteriocin 2 and the singleton BC-Bacteriocin 3 are part of the RiPPs Clan I, while the families BC-Bacteriocin 4 and BC-Bacteriocin 5 are part of the RiPPs Clan II (Fig. 5c).

The BC-Cerecidin family was named based on the cerecidin compounds (lanthipeptides), with the closest known BGC (MIBIG database) being that of the cerecidin / cerecidin A1 / cerecidin A2 / cerecidin A3 / cerecidin A4 / cerecidin A5 / cerecidin A6 / cerecidin A7 (lanthipeptides putative class II), with a similarity of 94%.

The BC-Lasso peptide 1 was named based on its similarity with a LAP–bacteriocin BGC (sactipeptide/lassopeptide).

Meanwhile the singletons are (Fig. 5c, table 4; supp. Fig. S1):

- BCS-Bacteriocin 1: *B. cereus* JHU, CP046511.1.region001
- BCS-Surfactin-like 1: *B. cereus* ZB201708, NZ_CP030982.1.region002
- BCS-Bacteriocin 2: *B. cereus* JHU, CP046511.1.region002
- BCS-Bacteriocin 3: *B. thuringiensis* c25, NZ_CP022345.1.region010
- BCS-Bacteriocin 4: *B. cereus* ZB201708, NZ_CP030982.1.region014
- BCS-Bacteriocin 5: B. thuringiensis L-7601, NZ_CP020002.1.region013

The largest family was named BC-Lassopeptide 1 (with 17 analogous BGCs present in all of the studied genomes), followed by BC-Bacteriocin 4 (with 14 analogous BGCs). The remaining families have somewhere between 7 and 1 BGC; while the smallest family is composed of only 2 analogous BGCs (the BC-Cerecidin family) (Fig. 5b,c).

Furthermore, the RiPPs class is characterized by 7 singleton BGCs (Fig. 5c), displaying the highest number of singletons among the analyzed BGCs classes, which makes the RiPPs class the best niche for peculiar and likely to be unique secondary metabolites in the *B. cereus* group, in addition to pinpointing to possible horizontal gene transfer (HGT) at a BGC-level.

The singleton BCS-Surfactin-like 1 was named this way, due to its similarity (8%) with a known lanthipeptide BGC coding for the surfactin lipopeptide.

[Insert Figure 5 here]

The NRPS gene cluster families of the *Bacillus cereus* group

For the NRPS class (Fig. 6), the BIG-SCAPE approach highlighted 6 families, without any reference BGC being known, which pinpoints further the future possible discovery of numerous novel metabolites in this intricate group. The NRPS class has the most represented families among the studied genomes, composed of 89 BGCs with an average number of 15 BGCs by family, and a staggering number of links (634 links) (Fig. 6a). No clans were proposed for this class.

We proposed the following names for the described NRPS families (Fig. 6b, table 3; supp. Fig. S2):

- BC-NRPS 1: *Bacillus.* sp. BH32, SJAS00000000.2.2.region002
- BC-NRPS 2: *Bacillus* sp. BH32, SJAS00000000.2.15.region001
- BC-Bacillibactin-like: *B. cereus* A1, NZ_CP015727.1.region003
- BC-NRPS-like 1: *B. cereus* G9842, NC_011772.1.region001

- BC-NRPS 3: *B. cereus* G9842, NC_011772.1.region006
- BC-NRPS 4: *B. cereus* ZB201708, NZ_CP030982.1.region003

The BC-NRPS 3 (showing low similarity with known BGCs coding for nostocyclopeptide A2: 28%, gramicidine: 16% from the MIBIG database; and the kurstakin C12 with a score of 0.639 according to the norine database) and the BC-Bacillibactin-like (showing a genes similarity of 46% with the known Bacillibactin BGC from the MIBIG database) families are represented in all of the studied genomes (17 analogous BGCs for both of them) (Fig. 6b,c).

It is noteworthy to mention that the BC-NRPS 4 family has a reference BGC showing little similarity with the known BGCs coding for polyketides chejuenolide A / chejuenolide B (7%) from the MIBIG database (table 3; supp. Fig. S2).

[Insert Figure 6 here]

The terpene gene cluster family of the *Bacillus cereus* group

For the terpene BGCs (Fig. 7), they all belong to the same family, named BC-Terpene 1, which bears similarity with the molybdenum cofactor (reference BGC: NZ_CP020002.1.region012 from *B. thuringiensis* L-7601, with a similarity of 17% with the molybdenum cofactor from the MIBIG database) (table 3; supp. Fig. S3).

It is the most conserved GCF (137 link in the corresponding similarity network) (Fig. 7 b) among the studied *B. cereus* genomes. The phylogenetic analysis (Fig. 7 c) confirms this conservation, showing two almost identical clades, with the exception of two different genes bearing 2 different pfam domains: the PF08445 FR47-like protein domain (Fig. 7 c, clade I) and the PF00583 Acetyl transferase GNAT family domain (Fig. 7 c, clade II).

[Insert Figure 7 here]

The remaining siderophores and betalactone gene cluster families of the *Bacillus cereus* group

Regarding the remaining BGC classes (the 3 GCFs gathered under the section “others” in the BIG-SCAPE output)(Fig. 8), namely the BC-Petrobactin 1, BC-Petrobactin 2 families

(both part of the BC-Petrobactin clan, coding for petrobactin siderophores, with a genes similarity of 100% with the known petrobactin BGC) and the BC-Fengycin-like 1 family (coding for betalactone products, with a genes similarity of 40% with known fengycin BGC from the MIBIG database), are another example of well-conserved families across the *B. cereus* group genomes, with an average number of BGCs by family of 11 (Fig. 8a).

The proposed families and their respective reference BGCs are (Fig. 8, table 3; supp. Fig. S4):

- BC-Petrobactin 2: *B. cereus* A1, NZ_CP015727.1.region002
- BC-Petrobactin 1: *Bacillus* sp. BH32, SJAS00000000.2.3.region001
- BC-Fengycin-like 1: *B. cereus* G9842, NC_011772.1.region007

Indeed, for the BC-Petrobactin clan (Fig. 8c), it has analogous BGCs across all of the studied genomes, being part of either the BC-Petrobactin 1 or BC-Petrobactin 2 families; and the BC-Fengycin-like 1 is represented in all of the 17 genomes (Fig. 8b), which is in line with the broad prevalence of Fengycin-like compounds reported in numerous strains belonging to the *B. cereus* group and their closely related taxa [38].

[Insert Figure 8 here]

5. Harmony between the manual and the automatic approach

Based on the manual approach **(Fig. 3)**, we highlighted 2 BGCs synteny blocks "*synteny block A"* and "*synteny block B*".

After performing a BIG-SCAPE analysis of the antiSMASH profiles of the studied genomes (Fig. 5, 6, 7, and 8), we could link the BGCs of the highlighted synteny blocks with their corresponding proposed families as follow:

For the **synteny block A:**

- BGC3 (bacteriocin: unknown) belongs to the **BC-Bacteriocin 4** family;

- BGC4 (bacteriocin: unknown) belongs to the **BC-Bacteriocin 1-2-3** families (BC-Bacteriocin clan I);
- BGC5 (betalacton, fengycin) belongs to the **BC-Fengycin-like 1** family;
- BGC6 (NRPS: gramicidin in *B. cereus* JHU; nostopeptolide A2 in *B. thuringiensis* serovar galleriae 4G5 ; unknown in the remaining strains) belongs to the **BC-NRPS 3** family;
- BGC7 (NRPS: bacillibactin) belongs to the **BC-Bacillibactin-like** family;
- BGC8 (siderophore: petrobactin) belongs to the **BC-Petrobactin** clan; and
- BGC9 (linear azol(in)e-containing peptides "LAP" bacteriocin) belongs to the **BC-Lasso peptide 1** family.

For the second conserved group named ***"synteny block B"***:

- BGC1 (terpene: molybdenum co-factor) belongs to the **BC-Terpene 1** family; and
- BGC2 (NRPS: polyoxypeptin, except for *B. thringiensis* c25, unknown) belongs to the **BC-NRPS 1** family.

That is to say, the synteny block A is a series of BGCs belonging (in order) to the families /clans (with the corresponding number of analogous BGCs found in each family):

BC-Bacteriocin 4 (14 BGCs), BC-Bacteriocin clan I (7+8+1 BGCs of the BC-Bacteriocin 1-2-3 families, respectively), BC-Fengycin-like 1 (17 BGCs), BC-NRPS 3 (17 BGCs), BC-Bacillibactin-like (17 BGCs), BC-Petrobactin clan (families 1 and 2, 17 BGCs), BC-Lasso peptide 1 (17 BGCs);

And the synteny block B is composed of BGCs belonging (in order) to the families:

BC-Terpene 1 (17 BGCs), and BC-NRPS 1 (16 BGCs).

The remaining families are either under-represented across the *B. cereus* group or considered as singletons, which confirms the harmony and complementarities between both manual and automated approaches with BIG-SCAPE.

Discussion

1. The importance of BGCs investigations

Deciphering beneficial features in plant growth-promoting bacteria requires research into the encoded parvome (the secondary metabolome inferred from the genome) [39]**.** Genes responsible for the production of secondary metabolites (SMs) are typically grouped into often quite large and complex BGCs [40]. BGCs are self-contained sets of co-located genes that accomplish the coordinated and regulated biosynthesis of a single set of SM congeners, with some exceptions, including BGCs that lack genes for required modifying enzymes that are located in different parts of the genome; distributed BGCs where two or more sub-clusters located in different parts of the genome collaborate during convergent biosynthesis of a single set of SM congeners; and superclusters that contain intertwined genes for the biosynthesis of more than one set of SM scaffolds [39, 41, 42].

BGCs are organized around genes that encode biosynthetic enzymes that yield the SM carbon skeleton ("backbone" enzymes), such as NRPSs, PKSs, PKS–NRPS hybrids, etc. The BGCs also feature genes encoding various enzymes that further modify the SM carbon skeleton (such as cytochrome P450 monooxygenases, various other oxidoreductases, etc) together with genes for transporters, regulators, and self-resistance determinants. In addition, many BGCs also harbor genes for enzymes that synthesize specialized monomers for the corresponding pathway [43].

2. BGCs screening and mining approaches

A biological approach in BGCs screening is considered the best prospect for resolving the potential of microbial parvomes. However, **Baltz** [44] estimated that 10^7 strains would need to be examined to discover a novel class of antibiotics [44]**.** Thus platforms combining different approaches are the appropriate strategy [45]. Moreover, the apparent failure to uncover the full potential of natural product-producing microorganisms is likely due to the

lack of understanding that is required to activate the expression of their BGCs in the laboratory [46]. Hence, new strategies for microbial SMs discovery are being employed, comprising genomics, metabolomics, and analytical tools that permit the study of complex systems [47].

Advances in genomics have unveiled a vast reservoir of BGCs in microbial genomes [46]. There are two main approaches to predicting BGCs. The first is based on pre-computed pHMMs derived from a set of genes known to participate in SM metabolism to identify sequences of interest [48, 49]. The second uses some function-agnostic criteria, such as synteny conservation or shared evolutionary history, to implicate genes as part of a gene cluster [50] which highlights the importance of synteny analysis among BGCs. Moreover, due to common metabolic functions across distant taxa, approaches employed by SMURF and antiSMASH are enormously successful [51]**.** Given the size of genomic data, studying BGCs on a case-by-case basis is no longer interesting. Hence, sequence similarity networking approaches can automatically relate predicted BGCs to gene clusters of known function and group them into gene cluster families (GCFs) [12, 46, 52, 53]. “Old school” methods gave way to new workflows that entail: (1) sequencing of the whole genome of strains that produce interesting SMs; (2) bioinformatic prediction of all BGCs; (3) comparison of the predicted BGCs with the retro-biosynthetic assessment of the structure of the target SM; (4) comparative genome analysis with organisms that produce similar SMs and with taxa that are phylogenetically close but not known to yield the target SM; and (5) comparative analysis of transcriptomes under SM producing vs. nonproducing conditions [39]**.** This led to the differentiation between BGCs that are likely to produce known compounds and BGCs that may encode novel chemistry. However, the number of GCFs to which no known functions can be linked is so great that it is difficult to know which of the BGCs encode the most interesting molecules [46].

3. Original BGC exploration methods

598 Out-of-the-box approaches are encouraged in targeting underexplored environmental niches
599 and bacterial phyla [54]. Rare environments have rendered an interesting source of SMs.
600 The chemical diversity of natural products correlates with the diversity of source
601 microorganisms. This is probably due to the evolution of organism-specific biosynthetic
602 machinery selected based on the adaptation of the microbe to the habitat, where beneficial
603 SMs play an important part [47]**.** Applying an isolate-based genome-mining approach on
604 bacteria obtained from unusual environments -such as deserts and arid regions like the
605 sampling sites of the present study- can be used for screening unique BGCs that may govern
606 the biosynthesis of novel natural products [54].

607 **4. Automated bioinformatics pipelines vs. manual bioinformatics**

608 Automated bioinformatics pipelines and manual bioinformatics are both methods used to
609 analyze biological data, but they have some key differences. Automated bioinformatics
610 pipelines are computer programs that are designed to perform specific bioinformatics tasks,
611 such as sequence alignment, gene annotation, and variant calling, in a pre-defined, step-by-
612 step manner. They are typically used to analyze large amounts of data and can be run on
613 high-performance computing clusters, allowing for efficient and rapid data processing [55].

614 Automated bioinformatics pipelines are also designed to be easy to use and can be run by
615 researchers with minimal bioinformatics experience [56]. Manual bioinformatics, on the other
616 hand, is the process of analyzing biological data using manual methods, such as web-based
617 tools, command-line programs, or custom scripts [57]. This method is typically used for
618 smaller data sets, or when an automated pipeline is not available or not suitable for the task
619 at hand. Manual bioinformatics requires a higher level of bioinformatics expertise and can be
620 more time-consuming, but it can also be more flexible and customizable [58]. Both
621 automated bioinformatics pipelines and manual bioinformatics have their advantages and
622 disadvantages. Automated pipelines can be faster and more efficient at processing large
623 amounts of data, but they may not be as flexible or customizable as manual methods.

Manual bioinformatics requires more expertise and can be more time-consuming, but it can also be more tailored to specific research questions.

During our study, Big-scape has proven to be useful in the exploration of BGCs in the *B. cereus* group thanks to its ability to identify and analyze large numbers of BGCs comprehensively and efficiently.

As Big-scape allowed the comparison of BGCs across different strains of *Bacillus cereus*, it helped us identify conserved and divergent BGCs among the *B. cereus* group, which will ultimately provide insights into the evolution and adaptation of *Bacillus cereus* to different environments.

5. The lack of BGCs classification

Despite the importance of BGCs, they are often difficult to classify and identify due to their complex genetic organization and a large number of different types of BGCs that can be found in different bacterial species [9]. One of the main challenges in BGCs classification is the lack of consensus on the criteria and methods used to classify BGCs [59]. Different researchers may use different criteria, such as gene content, gene organization, or evolutionary relationships, to classify BGCs, which can lead to inconsistent and conflicting results. Another challenge is that many BGCs are not well-annotated, making it difficult to identify the genes and functions associated with each BGC [60]. This can be particularly difficult in the case of novel BGCs, which may not have been previously described in the literature. Additionally, the lack of a standardized nomenclature for BGCs can further complicate the classification process [52].

6. Highlighting the hidden BGC potential and synteny in the *B. cereus* group

Many BGCs are not accounted for in the corresponding parvomes, referred to as “orphan” BGCs. These are either ‘cryptic’ (cannot yet be linked to a product, activity, or phenotype) or ‘silent’ (the compound is known in another organism, but experimental validation is required) [46]. The development of facile strategies to induce the expression of these silent BGCs, and

to assign SM structures to orphan clusters will allow us to elucidate the microbial parvomes [39], including those of the *Bacillus cereus* group.

7. Lack of data about BGCs conservation in *Bacillus cereus* sensu lato

There is currently limited data available about the conservation of biosynthetic gene clusters (BGCs) in the *Bacillus cereus* group [61]**.** One reason for this is that the *Bacillus cereus* group is a large and diverse group of bacteria, comprising several closely related species and subspecies. This diversity can make it challenging to identify and study BGCs across the different strains of *Bacillus cereus*. Additionally, the identification and characterization of BGCs often require sophisticated techniques such as genome sequencing, transcriptomics, and metabolomics, which can be time-consuming and expensive [62]**.**

Therefore, not all strains of *Bacillus cereus* have been fully characterized in terms of their BGCs. Furthermore, the majority of studies focus on certain strains of *Bacillus cereus*, such as *Bacillus anthracis*, *Bacillus thuringiensis*, and *Bacillus cereus* sensu stricto, that are known to produce important natural products [63–69], leaving out other strains.

The availability of genomes allowed the functional validation of identified BGCs based on the structures of known SMs. However, comparative genomics can also be used to derive hypotheses for the structures of the products of orphan BGCs. Thus, *de novo* sequenced BGCs may be assigned to known SM structural families if the core genes and the constituent tailoring genes are orthologous (or even syntenic) to functionally characterized BGCs, as those revealed in the present work. The synteny and the tight phylogenetic distances observed in our study support the conclusion that the BGCs in the *B. cereus* group arose dependently with the acquisition of conserved core component genes.

In the case of the *B. cereus* group, we observed that many of the biosynthetic gene clusters responsible for the production of natural products are syntenic, meaning that they are located in the same chromosomal position and have a similar gene organization among different strains of *B. cereus*. This suggests that these biosynthetic gene clusters have been inherited through common ancestry, and have been conserved over time through selective pressures.

The synteny of these gene clusters can be used to help understand the evolutionary relationships among different strains of *B. cereus*, and can also aid in the identification of new natural products. The growing number of genomes in databases revealed lineage-specific conservation of certain orthologous BGCs [39], as for the synteny bock A and B of the *B. cereus* group highlighted by the present study, or by pointing out the absence of certain widely present BGCs in some species, thereby allowing the generation of evolutionary hypotheses correlating the production of a given SM with the lifestyle and the evolutionary history of the producer [39].

Additionally, the synteny of BGCs can also aid in the development of new antibiotics and other bioactive compounds [10]. By identifying conserved regions of BGCs among different strains of *B. cereus*, researchers can infer the presence of similar biosynthetic pathways and enzymes among these strains, and can then use this information to guide the development of new antibiotics and other bioactive compounds. Overall, highlighting the synteny of BGCs can provide a valuable tool for understanding the biology, evolution, and biotechnology of the *B. cereus* group [70].

For instance, a syntenic BGC with orthologous genes to those of the *B. bassiana* oosporein BGC has recently been identified in the genome of *Cordyceps cycadae*, and the production of oosporein was confirmed by HPLC [71], and comparative genomics of *C. militaris* and *A. nidulans* revealed a syntenic BGC with four orthologous genes each in these fungi [72]**.**

8. The RiPPs in the *B. cereus* group

RiPPs are an important class of natural products produced by the *Bacillus cereus* group. We highlighted a consequent diversity among this BGC class, as it appeared to be the most diversified compared to the remaining classes, with 13 RiPPs GCFs, and 7 singletons. RiPPs have a wide range of biological activities, and they have potential applications in medicine, agriculture, and industry [73]. RiPPs have a wide range of structural diversity and chemical complexity, making them an attractive target for natural product discovery. Additionally, the lack of resistance to RiPPs amongst pathogenic bacteria makes them an attractive target for

the development of new antibiotics. The study of RiPPs biosynthesis and the enzymes responsible for the ribosomal synthesis and post-translational modification of these peptides is an active area of research, which has the potential to lead to the discovery of new RiPPs with unique properties and the development of new methods for RiPPs production [74]**.**

We suggested that some BGCs could be part of mobile elements (BGC11, from the manual approach). One common method of BGCs acquisition is through integrative and conjugative elements (ICEs). The prevalence of these ICEs seems to be partially dictated by their ecological background: bacteria originating from soil, plants, or aquatic environments contain a greater number of ICEs than species from other environments [75].

9. BGCs in endophytes

Endophytes developed a variety of ways to successfully colonize plants; subdue their immunity and their physiology as a nutrient source, and defend the plant host from pathogens and opportunists. BGCs were also reported to mediate crucial functions in plant colonization by beneficial endophytes. These functions are often facilitated by the vast array of SMs produced by root-associated bacteria, which play a key role in inter- and intra-species interactions [76, 77]. To date, a handful of studies have explored the diversity and composition of bacterial SM-encoding BGC in soil [78].

Bioactive metabolites mediate important ecological functions, which are as diverse as their chemical structures. Siderophores enhance iron uptake in environments where the bioavailability of iron is limited [79], pigments protect against ultraviolet radiation and have antioxidant activity [80] and compatible solutes protect against osmotic stress [81]. Besides, it is possible to find in nature several examples of mutualistic relationships that have coevolved whereby the microorganisms are actively cultured in exchange for producing bioactive small molecules [47].

For instance, NRPS and PKS BGCs are responsible for the synthesis of a wide array of siderophores, toxins, pigments, and antimicrobial compounds [82] that are believed to play a pivotal role in bacterial adaptation to soil and rhizosphere ecosystems, and in plant health

and development [83]. However, little is known regarding the distribution of these gene families in the root microbiome, and their functional role in the complex community interactions in root ecosystems remains an enigma [84].

Studying the encoded secondary metabolome of endophytes will amplify our understanding of the multiple roles that SMs play in the biotic and abiotic interactions in plants, leading to the unveiling of natural products that can be used for various applications**.**

Conclusions

The classification of Biosynthetic Gene Clusters (BGCs) is an evolving field that has gained significant attention in recent years. While some initial efforts have been made to classify BGCs, the field is still relatively new and the classification methodologies are constantly being refined and improved [22, 85]. Our work is an objective proposal for a consistent and standardized approach to BGCs classification among the *Bacillus cereus* group, based on a reproducible strategy that can be extended to other taxa, allowing comparison and integration of data from different studies to expand the initial classification scheme that we proposed. The current investigation is a substantive contribution to the discovery and characterization of new natural products and biosynthetic pathways, based on BGCs analogy and synteny.

Supplementary Information

The online version contains available supplementary material.

Additional file 1. Supplementary figures.

Additional file 2. antiSMASH profiles.

Additional file 3. BIG-SCAPE output files.

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Authors' contributions

HAB conceived, designed the study; and analyzed the data. HAB and AY drafted the manuscript; HAB, AY, AZ, and AM reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files). The genomic dataset can be accessed through the corresponding accession numbers. The antiSMASH profiles and the BIG-SCAPE output are available as supplementary material, from which the reference BGCs can be fetched (see table 3).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

References

1. Rasko DA, Altherr MR, Han CS, Ravel J. Genomics of the Bacillus cereus group of organisms. FEMS Microbiology Reviews. 2005.

2. Ehling-Schulz M, Lereclus D, Koehler TM. The Bacillus cereus Group: Bacillus Species with Pathogenic Potential . Microbiol Spectr. 2019.

3. Hong HA, Le HD, Cutting SM. The use of bacterial spore formers as probiotics. FEMS

781 Microbiology Reviews. 2005.

782 4. Guinebretière MH, Auger S, Galleron N, Contzen M, de Sarrau B, de Buyser ML, et al.
783 Bacillus cytotoxicus sp. nov. is a novel thermotolerant species of the Bacillus cereus group
784 occasionally associated with food poisoning. Int J Syst Evol Microbiol. 2013.

785 5. Liu Y, Lai Q, Göker M, Meier-Kolthoff JP, Wang M, Sun Y, et al. Genomic insights into the
786 taxonomic status of the Bacillus cereus group. Sci Rep. 2015.

787 6. Margulis L, Jorgensen JZ, Dolan S, Kolchinsky R, Rainey FA, Lo SC. The Arthromitus
788 stage of Bacillus cereus: Intestinal symbionts of animals. Proc Natl Acad Sci U S A. 1998.

789 7. Priest FG, Barker M, Baillie LWJ, Holmes EC, Maiden MCJ. Population structure and
790 evolution of the Bacillus cereus group. J Bacteriol. 2004.

791 8. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, et al. Bacillus
792 thuringiensis and Its Pesticidal Crystal Proteins . Microbiol Mol Biol Rev. 1998.

793 9. Chen R, Wong H, Burns B. New Approaches to Detect Biosynthetic Gene Clusters in the
794 Environment. Medicines. 2019.

795 10. Ziemert N, Alanjary M, Weber T. The evolution of genome mining in microbes-a review.
796 Natural Product Reports. 2016.

797 11. Albarano L, Esposito R, Ruocco N, Costantini M. Genome mining as new challenge in
798 natural products discovery. Marine Drugs. 2020.

799 12. Cimermancic P, Medema MH, Claesen J, Kurita K, Wieland Brown LC, Mavrommatis K,
800 et al. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic
801 gene clusters. Cell. 2014.

802 13. Ju KS, Gao J, Doroghazi JR, Wang KKA, Thibodeaux CJ, Li S, et al. Discovery of
803 phosphonic acid natural products by mining the genomes of 10,000 actinomycetes. Proc Natl
804 Acad Sci U S A. 2015.

805 14. Cane DE, Walsh CT. The parallel and convergent universes of polyketide synthases and
806 nonribosomal peptide synthetases. Chem Biol. 1999.

807 15. Romero D, Traxler MF, López D, Kolter R. Antibiotics as signal molecules. Chemical
808 Reviews. 2011.

809 16. Tran PN, Yen MR, Chiang CY, Lin HC, Chen PY. Detecting and prioritizing biosynthetic
810 gene clusters for bioactive compounds in bacteria and fungi. Applied Microbiology and
811 Biotechnology. 2019;103:3277–87.

812 17. Flórez L V., Biedermann PHW, Engl T, Kaltenpoth M. Defensive symbioses of animals
813 with prokaryotic and eukaryotic microorganisms. Nat Prod Rep. 2015.

814 18. Carey J, Nguyen T, Korchak J, Beecher C, De Jong F, Lane AL. An isotopic ratio outlier
815 analysis approach for global metabolomics of biosynthetically talented actinomycetes.
816 Metabolites. 2019.

817 19. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, et al. AntiSMASH 5.0: Updates
818 to the secondary metabolite genome mining pipeline. Nucleic Acids Res. 2019;47:W81–7.

819 20. Navarro-Muñoz JC, Selem-Mojica N, Mullowney MW, Kautsar SA, Tryon JH, Parkinson
820 EI, et al. A computational framework to explore large-scale biosynthetic diversity. Nat Chem
821 Biol. 2020.

822 21. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, Van Der Hooft JJJ, et al.
823 MIBiG 2.0: A repository for biosynthetic gene clusters of known function. Nucleic Acids Res.
824 2020.

825 22. Terlouw BR, Blin K, Navarro-Muñoz JC, Avalon NE, Chevrette MG, Egbert S, et al.
826 MIBiG 3.0: a community-driven effort to annotate experimentally validated biosynthetic gene
827 clusters. Nucleic Acids Res. 2023;51:D603–10.

828 23. Liu Z, Ma H, Goryanin I. A semi-automated genome annotation comparison and
829 integration scheme. BMC Bioinformatics. 2013.

830 24. Pfeiffer F, Oesterhelt D. A manual curation strategy to improve genome annotation:
831 Application to a set of haloarchael genomes. Life. 2015.

832 25. Sánchez-Hidalgo M, Martín J, Genilloud O. Identification and heterologous expression of

833 the biosynthetic gene cluster encoding the lasso peptide humidimycin, a caspofungin activity
834 potentiator. Antibiotics. 2020;9.

835 26. Makałowski W, Boguski MS. Evolutionary parameters of the transcribed mammalian
836 genome: An analysis of 2,820 orthologous rodent and human sequences. Proc Natl Acad Sci
837 U S A. 1998.

838 27. Belaouni HA, Compant S, Antonielli L, Nikolic B, Zitouni A, Sessitsch A. In-depth genome
839 analysis of Bacillus sp. BH32, a salt stress-tolerant endophyte obtained from a halophyte in a
840 semiarid region. Appl Microbiol Biotechnol. 2022;106:3113–37. doi:10.1007/s00253-022-
841 11907-0.

842 28. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA
843 sequences. Journal of Computational Biology. 2000;7:203–14.

844 29. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator
845 (BRIG): Simple prokaryote genome comparisons. BMC Genomics. 2011;12.

846 30. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7:
847 Improvements in performance and usability. Mol Biol Evol. 2013.

848 31. Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from
849 protein sequences. Bioinformatics. 1992;8:275–82.

850 32. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis
851 Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016;33:1870–4.

852 33. Darling AE, Mau B, Perna NT. Progressivemauve: Multiple genome alignment with gene
853 gain, loss and rearrangement. PLoS One. 2010.

854 34. Deshpande NP, Kaakoush NO, Wilkins MR, Mitchell HM. Comparative genomics of
855 Campylobacter concisus isolates reveals genetic diversity and provides insights into disease
856 association. BMC Genomics. 2013.

857 35. Johnson MC, Tatum KB, Lynn JS, Brewer TE, Lu S, Washburn BK, et al. Sinorhizobium
858 meliloti Phage ΦM9 Defines a New Group of T4 Superfamily Phages with Unusual Genomic

Features but a Common T=16 Capsid. J Virol. 2015.

36. Martínez-Carranza E, Ponce-Soto GY, Servín-González L, Alcaraz LD, Soberón-Chávez G. Evolution of bacteria seen through their essential genes: The case of Pseudomonas aeruginosa and Azotobacter vinelandii. Microbiol (United Kingdom). 2019.

37. de la Haba RR, López-Hermoso C, Sánchez-Porro C, Konstantinidis KT, Ventosa A. Comparative Genomics and Phylogenomic Analysis of the Genus Salinivibrio. Front Microbiol. 2019.

38. Daas MS, Acedo JZ, Rosana ARR, Orata FD, Reiz B, Zheng J, et al. Bacillus amyloliquefaciens ssp. plantarum F11 isolated from Algerian salty lake as a source of biosurfactants and bioactive lipopeptides. FEMS Microbiol Lett. 2018.

39. Zhang L, Yue Q, Wang C, Xu Y, Molnár I. Secondary metabolites from hypocrealean entomopathogenic fungi: Genomics as a tool to elucidate the encoded parvome. Nat Prod Rep. 2020;37:1164–80.

40. Scherlach K, Hertweck C. Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem. 2009.

41. Wiemann P, Guo CJ, Palmer JM, Sekonyela R, Wang CCC, Keller NP. Prototype of an intertwined secondarymetabolite supercluster. Proc Natl Acad Sci U S A. 2013.

42. Rokas A, Wisecaver JH, Lind AL. The birth, evolution and death of metabolic gene clusters in fungi. Nature Reviews Microbiology. 2018.

43. Wang B, Kang Q, Lu Y, Bai L, Wang C. Unveiling the biosynthetic puzzle of destruxins in Metarhizium species. Proc Natl Acad Sci U S A. 2012.

44. Baltz RH. Function of MbtH homologs in nonribosomal peptide biosynthesis and applications in secondary metabolite discovery. Journal of Industrial Microbiology and Biotechnology. 2011.

45. Lewis K. Platforms for antibiotic discovery. Nature Reviews Drug Discovery. 2013;12:371–87.

885 46. van Bergeijk DA, Terlouw BR, Medema MH, van Wezel GP. Ecology and genomics of
886 Actinobacteria: new concepts for natural product discovery. Nature Reviews Microbiology.
887 2020.

888 47. Granada Garc'\ia SD. Metabolitos secundarios microbianos como alternativa de control
889 frente a fitopatógenos del aguacate (Persea americana Mill.). Esc Biociencias. 2019.

890 48. Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, et al. SMURF:
891 Genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol. 2010;47:736–
892 41.

893 49. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, et al. AntiSMASH 4.0 -
894 improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids
895 Res. 2017;45:W36–41.

896 50. Gluck-Thaler E, Slot JC. Specialized plant biochemistry drives gene clustering in fungi.
897 ISME J. 2018.

898 51. Gluck-Thaler E, Haridas S, Binder M, Grigoriev I V., Crous PW, Spatafora JW, et al. The
899 architecture of metabolism maximizes biosynthetic diversity in the largest class of Fungi. Mol
900 Biol Evol. 2020;37:2838–56.

901 52. Ziemert N, Lechner A, Wietz M, Millań-Aguiñaga N, Chavarria KL, Jensen PR. Diversity
902 and evolution of secondary metabolism in the marine actinomycete genus Salinispora. Proc
903 Natl Acad Sci U S A. 2014.

904 53. Doroghazi JR, Albright JC, Goering AW, Ju K-S, Haines RR, Tchalukov KA, et al. A
905 Roadmap for Natural Product Discovery Based on Large-Scale Genomics and Metabolomics
906 HHS Public Access Author manuscript. Nat Chem Biol. 2014.

907 54. Sekurova ON, Schneider O, Zotchev SB. Novel bioactive natural products from bacteria
908 via bioprospecting, genome mining and metabolic engineering. Microbial Biotechnology.
909 2019;12:828–44.

910 55. Roy S, LaFramboise WA, Nikiforov YE, Nikiforova MN, Routbort MJ, Pfeifer J, et al. Next-

911 generation sequencing informatics: Challenges and strategies for implementation in a clinical
912 environment. Archives of Pathology and Laboratory Medicine. 2016.

913 56. Schmid K, Dohmen H, Ritschel N, Selignow C, Zohner J, Sehring J, et al. SangeR: the
914 high-throughput Sanger sequencing analysis pipeline. Bioinforma Adv. 2022;2.

915 57. Neves M, Ševa J. An extensive review of tools for manual annotation of documents.
916 Briefings in Bioinformatics. 2021;22:146–63.

917 58. Cherkasov A. Bioinformatics: A practical guide to the analysis of genes and proteins. Am
918 J Hum Biol. 2005.

919 59. Hannigan GD, Prihoda D, Palicka A, Soukup J, Klempir O, Rampula L, et al. A deep
920 learning genome-mining strategy for biosynthetic gene cluster prediction. Nucleic Acids Res.
921 2019.

922 60. Nguyen DD, Wu CH, Moree WJ, Lamsa A, Medema MH, Zhao X, et al. MS/MS
923 networking guided analysis of molecule and gene cluster families. Proc Natl Acad Sci U S A.
924 2013.

925 61. Xia L, Miao Y, Cao A, Liu Y, Liu Z, Sun X, et al. Biosynthetic gene cluster profiling
926 predicts the positive association between antagonism and phylogeny in Bacillus. Nat
927 Commun. 2022.

928 62. Medema MH, Trefzer A, Kovalchuk A, Van Den Berg M, Müller U, Heijne W, et al. The
929 sequence of a 1.8-Mb bacterial linear plasmid reveals a rich evolutionary reservoir of
930 secondary metabolic pathways. Genome Biol Evol. 2010.

931 63. Stohl EA, Milner JL, Handelsman J. Zwittermicin A biosynthetic cluster. Gene. 1999.

932 64. Hoton FM, Fornelos N, N'Guessan E, Hu X, Swiecicka I, Dierick K, et al. Family portrait
933 of Bacillus cereus and Bacillus weihenstephanensis cereulide-producing strains. Environ
934 Microbiol Rep. 2009.

935 65. Arias AA, Ongena M, Devreese B, Terrak M, Joris B, Fickers P. Characterization of
936 amylolysin, a novel lantibiotic from Bacillus amyloliquefaciens GA1. PLoS One. 2013.

937 66. Mei X, Xu K, Yang L, Yuan Z, Mahillon J, Hu X. The genetic diversity of cereulide
938 biosynthesis gene cluster indicates a composite transposon Tnces in emetic Bacillus
939 weihenstephanensis. BMC Microbiol. 2014.

940 67. Zhang L, Teng K, Wang J, Zhang Z, Zhang J, Sun S, et al. CerR, a single-domain
941 regulatory protein of the LuxR family, promotes cerecidin production and immunity in Bacillus
942 cereus. Appl Environ Microbiol. 2018.

943 68. Farlow J, Bolkvadze D, Leshkasheli L, Kusradze I, Kotorashvili A, Kotaria N, et al.
944 Genomic characterization of three novel Basilisk-like phages infecting Bacillus anthracis 06
945 Biological Sciences 0604 Genetics. BMC Genomics. 2018.

946 69. Prasertanan T, Palmer DRJ. The kanosamine biosynthetic pathway in Bacillus cereus
947 UW85: Functional and kinetic characterization of KabA, KabB, and KabC. Arch Biochem
948 Biophys. 2019.

949 70. Steinke K, Mohite OS, Weber T, Kovács ÁT. Phylogenetic Distribution of Secondary
950 Metabolites in the Bacillus subtilis Species Complex. mSystems. 2021.

951 71. Lu Y, Luo F, Cen K, Xiao G, Yin Y, Li C, et al. Omics data reveal the unusual asexual-
952 fruiting nature and secondary metabolic potentials of the medicinal fungus Cordyceps
953 cicadae. BMC Genomics. 2017.

954 72. Asai T, Chung YM, Sakurai H, Ozeki T, Chang FR, Wu YC, et al. Highly oxidized
955 ergosterols and isariotin analogs from an entomopathogenic fungus, Gibellula formosana,
956 cultivated in the presence of epigenetic modifying agents. Tetrahedron. 2012.

957 73. Inoue M. Total synthesis and functional analysis of non-ribosomal peptides. Chem Rec.
958 2011.

959 74. Ortega MA, Van Der Donk WA. New Insights into the Biosynthetic Logic of Ribosomally
960 Synthesized and Post-translationally Modified Peptide Natural Products. Cell Chemical
961 Biology. 2016.

962 75. Ghinet MG, Bordeleau E, Beaudin J, Brzezinski R, Roy S, Burrus V. Uncovering the

963 prevalence and diversity of integrating conjugative elements in actinobacteria. PLoS One.
964 2011.

965 76. Tyc O, Song C, Dickschat JS, Vos M, Garbeva P. The Ecological Role of Volatile and
966 Soluble Secondary Metabolites Produced by Soil Bacteria. Trends in Microbiology. 2017.

967 77. Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. Going back to the roots:
968 The microbial ecology of the rhizosphere. Nature Reviews Microbiology. 2013.

969 78. Dror B, Wang Z, Brady SF, Jurkevitch E, Cytryn E. Elucidating the Diversity and Potential
970 Function of Nonribosomal Peptide and Polyketide Biosynthetic Gene Clusters in the Root
971 Microbiome. mSystems. 2020.

972 79. Guerinot ML. Microbial iron transport. Annual Review of Microbiology. 1994.

973 80. Li C, Ji C, Tang B. Purification, characterisation and biological activity of melanin from
974 Streptomyces sp. FEMS Microbiol Lett. 2018.

975 81. Sadeghi A, Soltani BM, Nekouei MK, Jouzani GS, Mirzaei HH, Sadeghizadeh M.
976 Diversity of the ectoines biosynthesis genes in the salt tolerant Streptomyces and evidence
977 for inductive effect of ectoines on their accumulation. Microbiol Res. 2014.

978 82. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014.
979 Journal of Natural Products. 2016.

980 83. Chowdhury SP, Hartmann A, Gao XW, Borriss R. Biocontrol mechanism by root-
981 associated Bacillus amyloliquefaciens FZB42 - A review. Frontiers in Microbiology. 2015.

982 84. Charlop-Powers Z, Pregitzer CC, Lemetre C, Ternei MA, Maniko J, Hover BM, et al.
983 Urban park soil microbiomes are a rich reservoir of natural product biosynthetic diversity.
984 Proc Natl Acad Sci U S A. 2016.

985 85. Medema MH, Kottmann R, Yilmaz P, Cummings M, Biggins JB, Blin K, et al. Minimum
986 Information about a Biosynthetic Gene cluster. Nature Chemical Biology. 2015.

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Tables

Table 1. Dataset for the comparative analysis

Preferred name	Deposit	Base pairs	% G+C	Proteins	Bioproject accession	Biosample accession	Assembly accession
Bacillus wiedmannii	PL1	5,309,441	35.26	5272	PRJDB9286	SAMD00204526	GCA_011405335.1
Bacillus sp.	BH32	5,661,597	34.86	5682	PRJNA523918	SAMN10992522	GCF_004367825.2
Bacillus cereus	JHU	5,323,903	35.26	5489	PRJNA591929	SAMN13391868	GCA_009738575.1
Bacillus cereus	G9842	5,387,334	35.26	5379	PRJNA224116	SAMN02604060	GCF_000021305.1
Bacillus thuringiensis	HD-789	5,495,278	35.26	5550	PRJNA171844	SAMN02603564	GCA_000292705.1
Bacillus bombysepticus	Wang	5,295,783	35.25	5265	PRJNA242213	SAMN02691825	GCF_000831065.1
Bacillus thuringiensis	HD1002	5,491,311	35.25	5548	PRJNA236049	SAMN03010437	GCF_000835025.1
Bacillus thuringiensis serovar galleriae	4G5	5,701,188	35.29	5770	PRJNA224116	SAMN03074947	GCF_000803665.1
Bacillus thuringiensis serovar morrisoni	BGSC 4AA1	5,652,292	35.33	5693	PRJNA224116	SAMN03274640	GCF_000940785.1
Bacillus cereus	A1	5,352,307	35.28	5273	PRJNA242371	SAMN02693464	GCA_000635895.2
Bacillus thuringiensis	YGd22-03	5,420,545	35.23	5455	PRJNA224116	SAMN06197294	GCF_002184245.1
Bacillus thuringiensis	L7601	5,790,408	35.18	5846	PRJNA224116	SAMN06473083	GCF_002025105.1
Bacillus thuringiensis	c25	5,334,660	35.32	5300	PRJNA388287	SAMN07317425	GCA_002222555.1

Bacillus cereus	FORC087	5,271,204	35.27	5272	PRJNA470818	SAMN09111977	GCA_006384875.1
Bacillus cereus	ZB201708	5,466,652	35.22	5423	PRJNA480899	SAMN09652006	GCA_004006495.1
Bacillus thuringiensis	BT-59	5,500,615	35.26	5549	PRJNA534189	SAMN11479299	GCA_005155285.1
Bacillus thuringiensis	JW-1	5,500,376	35.26	5552	PRJNA574201	SAMN12860338	GCA_009025915.1

Table 2. BGC classes/families overview

	RiPPS	NRPS	Terpene	others	total
# of families:	13	6	1	3	23
Average # of BGCs per family:	4	15	17	11	8
Max # of BGCs in a family:	17	17	17	17	-
Families with MIBiG Reference BGCs:	0	0	0	0	0

1037 **Table 3. Reference BGCs per class/family**

BGC families	strain	region	genomic location (nt)	most similar known cluster	similarity
RiPPs					
BC-Bacteriocin 1	*B. cereus* ZB201708	NZ_CP030982.1.region009	2,468,894–2,477,159	-	-
BC-Bacteriocin 2	*B. thuringiensis* HD1002	NZ_CP009351.1.region005	1,379,714–1,389,169	-	-
BC-Bacteriocin 3	*B. thuringiensis* 4G5	NZ_CP010089.1.region007	2,741,425–2,750,241	-	-
BC-Lasso peptide 1	*B. cereus* A1	NZ_CP015727.1.region001	568,125–591,631	-	-
BC-Bacteriocin 4	*B. thuringiensis* BGSC 4AA1	NZ_CP010577.1.region009	2,725,350–2,735,610	-	-
BC-Bacteriocin 5	*B. cereus* ZB201708	NZ_CP030982.1.region010	2,580,757–2,589,106	-	-
BC-Cerecidin	*B. thuringiensis* YGd22-03	NZ_CP019230.1.region011	4,472,628–4,495,786	cerecidin / cerecidin A1 / cerecidin A2 / cerecidin A3 / cerecidin A4 / cerecidin A5 / cerecidin A6 / cerecidin A7	94%
NRPS families					
BC- NRPS 1	*Bacillus* . sp. BH32	SJAS00000000.2.2.region002	286,950–346,871	polyoxypeptin	5%
BC- NRPS 2	*Bacillus* sp. BH32	SJAS00000000.2.15.region001	61,643–91,585	-	-
BC- Bacillibactin-like	*B. cereus* A1	NZ_CP015727.1.region003	1,501,835–1,550,463	bacillibactin	46%
BC- NRPS-like 1	*B. cereus* G9842	NC_011772.1.region001	386,831–430,076	-	-
BC-NRPS 3	*B. cereus* G9842	NC_011772.1.region006	2,287,034–2,352,013	-	-
BC-NRPS 4	*B. cereus* ZB201708	NZ_CP030982.1.region003	791,630–836,873	chejuenolide A/ chejuenolide B	7%
Terpene family					
BC- terpene 1	*B. thuringiensis* L-7601	NZ_CP020002.1.region012	3,779,510–3,801,363	molybdenum cofactor	17%
Siderophore families					
BC- Petrobactin 1	*B. cereus* G9842	NC_011772.1.region004	1,852,404–1,866,111	petrobactin	100%
BC- Petrobactin 2	*B. cereus* A1	NZ_CP015727.1.region002	1,182,329–1,196,036	petrobactin	100%
Betalactone family					
BC- Fengycin-like 1	*B. cereus* G9842	NC_011772.1.region007	2,387,411–2,412,649	fengycin	40%

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Table 4. Reference BGCs for RiPPs singletons

RiPPs singletons	strain	region	genomic location (nt)	most similar known cluster	similarity
BCS-Bacteriocin 1	*B. cereus* JHU	CP046511.1.region001	24,944 – 34,346	-	-
BCS-Surfactin-like 1	*B. cereus* ZB201708	NZ_CP030982.1.region002	748,698 – 771,271	-	-
BCS-Bacteriocin 2	*B. cereus* JHU	CP046511.1.region002	128,885 – 139,342	-	-
BCS-Bacteriocin 3	*B. thuringiensis* c25	NZ_CP022345.1.region010	4,376,042 – 4,388,249	-	-
BCS-Bacteriocin 4	*B. cereus* ZB201708	NZ_CP030982.1.region014	5,325,429 – 5,338,733	-	-
BCS-Bacteriocin 5	*B. thuringiensis* L-7601	NZ_CP020002.1.region013	5,353,484 – 5,365,667	-	-

Figure captions

Fig. 1 Distribution of BGCs classes and counts. The heatmap shows the number of BGCs by type (from 0 to 5), and their distribution among the strains (dendrograms were generated by hierarchical clustering using *one minus Spearman* rank correlation)

Fig. 2 BLASTp comparisons of *Bacillus* sp. BH32 BGCs against BGCs of the closest non-type strains. The reference ring constitutes the detected BGCs in *Bacillus* sp. BH32 (translated to amino acid sequence), while individual rings represent BGCs of the closest non-type strains (the color represents sequence identity on a sliding scale, the greyer it gets; the lower the percentage identity). The 3 outermost rings depict (from inside to outside): genes composing BGCs in *Bacillus* sp. BH32 (in blue); *unique genes* (sequence identity bellow 30% for 100% of the strains, in green) and *rare genes* (sequence identity bellow 30% for 80% of the strains, in light velvet) with their locus tag IDs; and the *regions/BGCs types* (as detected by antiSMASH). Unique and rare genes have the following annotation (from antiSMASH / prokka, with the same location, or overlapping locations): ctg8_81 = unknown/hypothetical protein; ctg12_31 = unknown / Spore germination protein B1; ctg15_85 = biosynthetic-additional (smcogs) SMCOG1028:crotonyl-CoA reductase - alcohol dehydrogenase / Zinc-type alcohol dehydrogenase-like protein; ctg27_13 = unknown/hypothetical protein; ctg27_20 = unknown/hypothetical protein; ctg2_300 = unknown/hypothetical protein; ctg2_321 = unknown / IS200/IS605 family transposase ISAsp8 ; ctg8_90 = unknown / hypothetical protein; ctg12_35 = biosynthetic-additional (smcogs) SMCOG1012:4'-phosphopantetheinyl transferase / 4'-phosphopantetheinyl transferase Sfp

Fig. 3 BGCs phylogeny and conservation. **a)** Maximum Likelihood (ML) phylogenetic tree generated from concatenated BGCs amino acid sequences. Numbers on branches represent bootstrap values (average value: 92.21%). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. **b)** BGCs conservation among the strains (MAUVE alignment of concatenated genebank sequences of the BGCs of each strain). Each

row represents the conservation and orientation of BGCs of the corresponding strain in the ML tree (left) after alignment, in comparison to *Bacillus thuringiensis* c25 (bottom row, set as reference). Red bars represent BGC sequence limits. Colored blocks refer to homologous segments with similarity plots (from MAUVE alignment diagram), with a *locally collinear block* (LCB) weight ≥ 773. Each arrow symbolizes the BGC relative orientation with its tag number. BGCs with conserved order are framed in purple. BGC tag number, type, and most similar known clusters are shown on legends. The distance scale is shown under the alignment diagram (in amino acids)

Fig. 4 BGCs fractions (counts and percentages by class, according to BIG-SCAPE output).

Fig. 5 RiPPs BGC families/clans. **a)** RiPPs BGCs families statistics, **b)** proposed RiPPs families distribution matrix (presence: 1, absence: 0) among the *B. cereus* group genomes, **c)** RiPPs BGCs families/clans cluster networks (generated by the highest cutoff selected) and singletons (separate dots).

Fig. 6 NRPS BGCs families. **a)** NRPS BGCs families statistics, **b)** proposed NRPS families distribution matrix (presence: 1, absence: 0) among the *B. cereus* group genomes, **c)** NRPS BGCs families cluster networks (generated by the highest cutoff selected).

Fig. 7 BC-Terpene 1 family features. **a)** BC-Terpene 1 BGCs family statistics, **b)** BC-Terpene 1 BGCs cluster network generated by the highest cutoff selected, **c)** CORASON-like tree generated for the BC-Terpene 1 GCF. This tree was created using the sequences of the Core Domains in the BC-Terpene 1 gene cluster family. These are defined as the domain type(s) that (1) appeared with the highest frequency in the BC-Terpene 1 gene cluster family and (2) were detected in the exemplar cluster (defined by the affinity propagation cluster), which is, in this case, that of *B. thuringiensis* L-7601 (in red). All copies of the Core Domains in the exemplar were automatically concatenated, as well as those from the best-matching domains of the rest of the BGCs in the BC-Terpene 1 gene cluster family (aligned domain sequences were used). The tree was inferred using FastTree43 with default parameters. Visual alignment was attempted based on the position of the ‘longest common information’

from the distance calculation step (between the exemplar BGCs vs. each of the remaining clusters). The Pfam domains of the 2 main clades are reported at the bottom part of this figure. The Pfam domains' color significance can be retraced from a list, available here: https://git.wageningenur.nl/medema-group/BiG-SCAPE/blob/master/domains_color_file.tsv

Fig. 8 BC-Petrobactin 1, BC-Petrobactin 2, and BC-Fengycin BGCs families/clan. **a)** the remaining BGCs families statistics, **b)** proposed BC-Petrobactin 1, BC-Petrobactin 2, and BC-Fengycin families distribution matrix (presence: 1, absence: 0) among the *B. cereus* group genomes, **c)** BC-Petrobactin 1, BC-Petrobactin 2 and BC-Fengycin BGCs families/clan cluster networks (generated by the highest cutoff selected).

Fig. S1 RiPPs reference BGCs for each proposed family. For each reference BGC, from top to bottom: reference BGC info (from left to right are mentioned: the name of the proposed RiPPs family; the strain bearing the reference BGC; the genomic region; and the most similar known cluster with the similarity %); a depiction of the BGCs regions/distribution among the genome; and the BGC organization (from antiSMASH output).

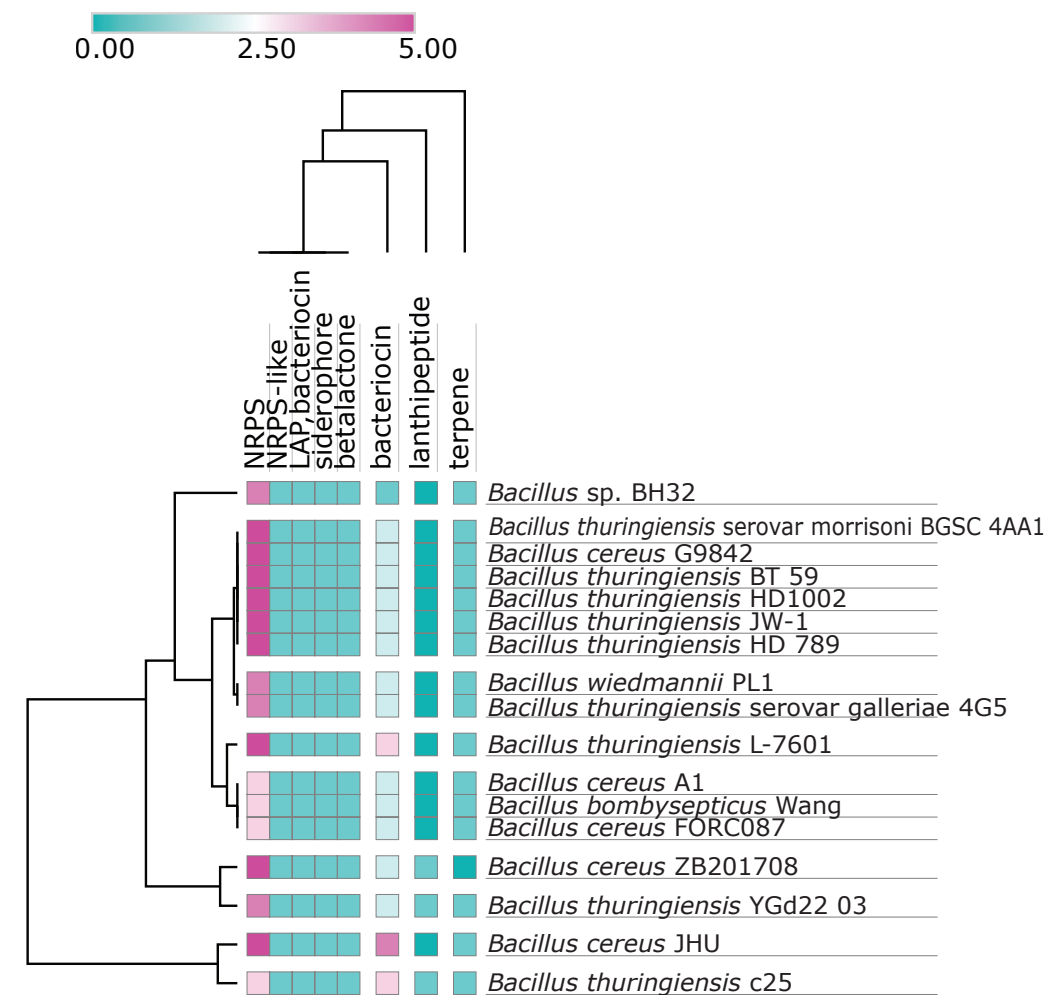
Fig. S2 NRPS reference BGCs for each proposed family. For each reference BGC, from top to bottom: reference BGC info (from left to right are mentioned: the name of the proposed NRPS family; the strain bearing the reference BGC; the genomic region; and the most similar known cluster with the similarity %); a depiction of the BGCs regions/distribution among the genome; and the BGC organization (from antiSMASH output).

Fig. S3 BC-Terpene 1 reference BGC. From top to bottom: reference BGC info (from left to right are mentioned: the name of the proposed family; the strain bearing the reference BGC; the genomic region; and the most similar known cluster with the similarity %); a depiction of the BGCs regions/distribution among the genome; and the BGC organization (from antiSMASH output).

Fig. S4 Siderophore/Betalactone reference BGCs for each proposed family. For each reference BGC, from top to bottom: reference BGC info (from left to right are mentioned: the name of the proposed family; the strain bearing the reference BGC; the genomic region; and

1156 the most similar known cluster with the similarity %); a depiction of the BGCs

1157 regions/distribution among the genome; and the BGC organization (from antiSMASH output).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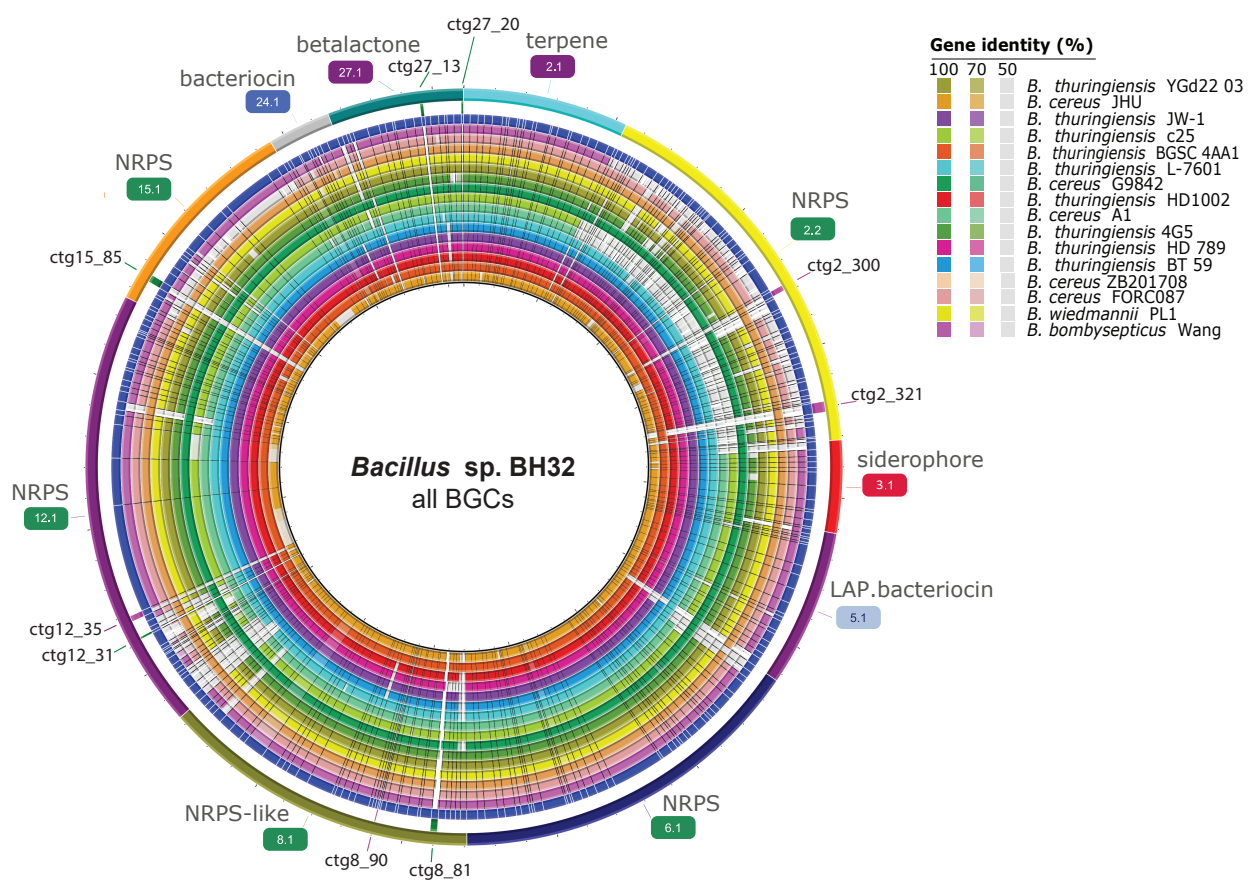
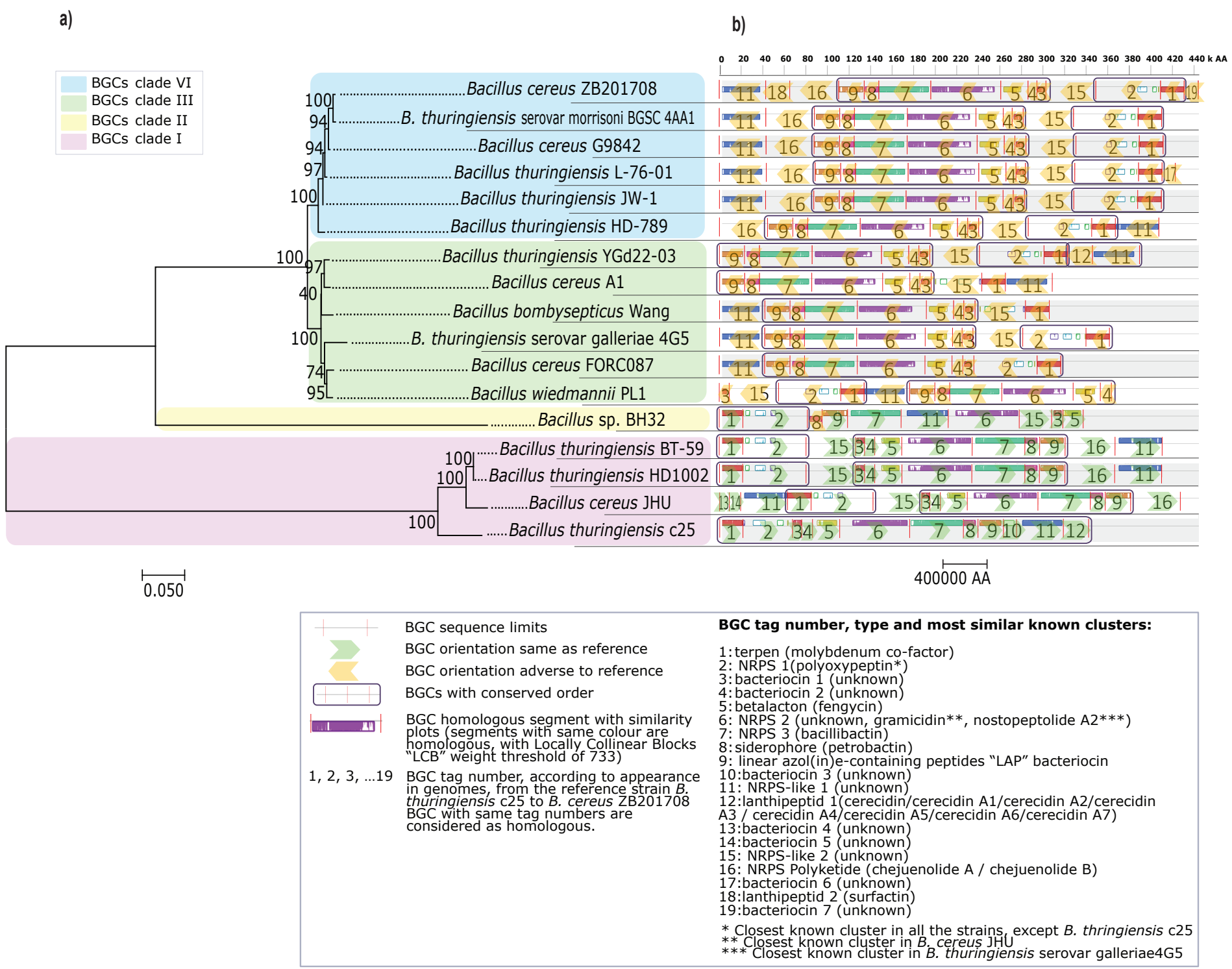
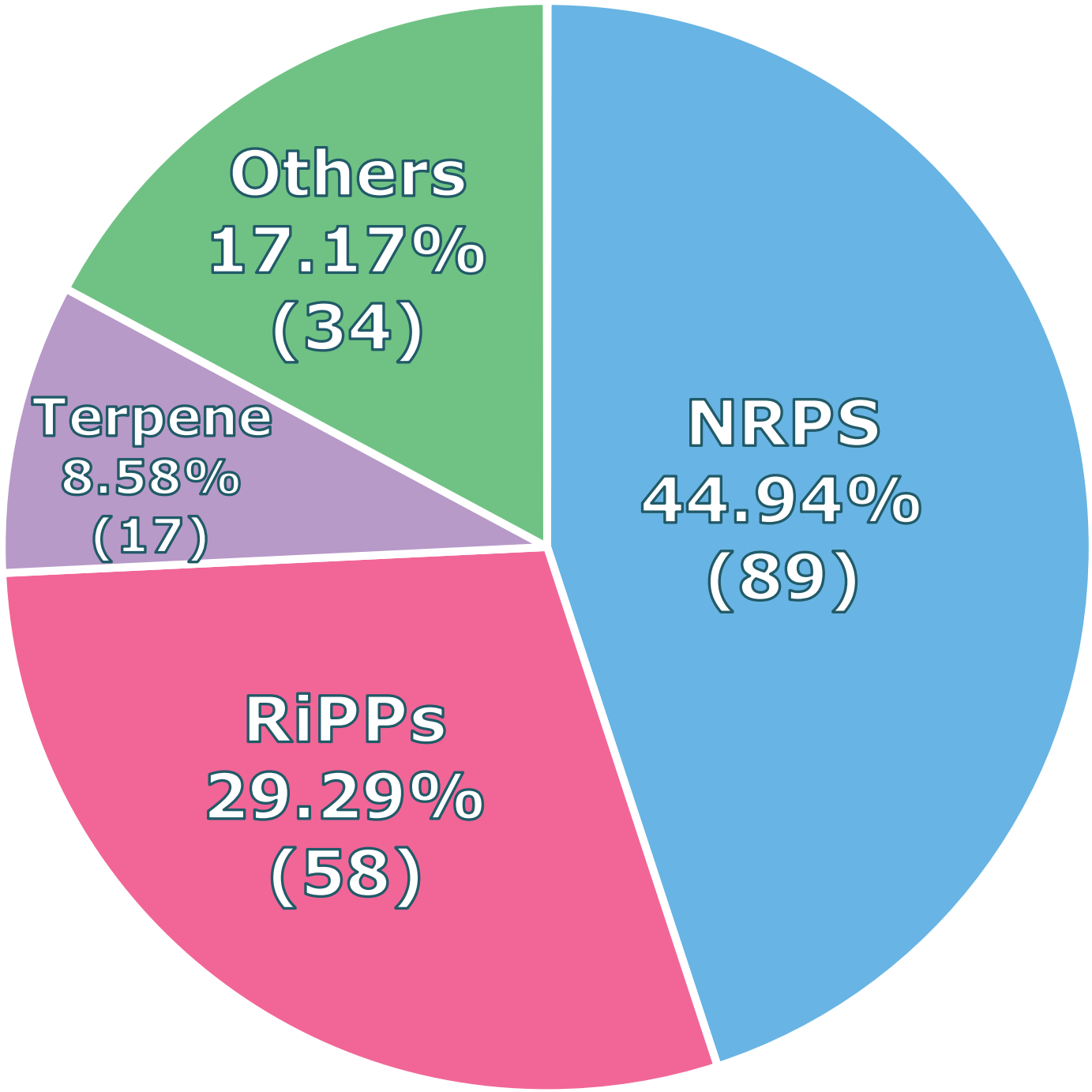
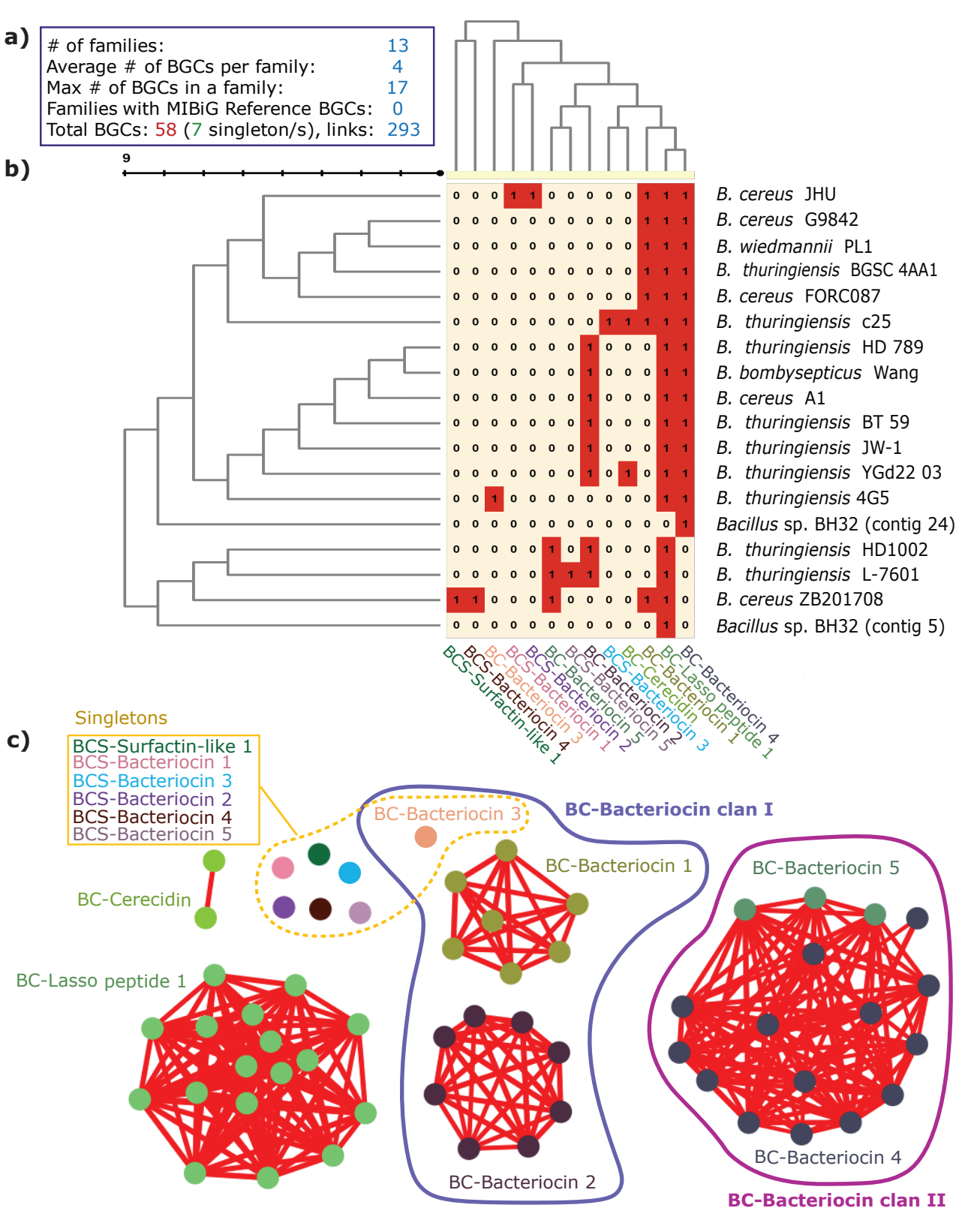
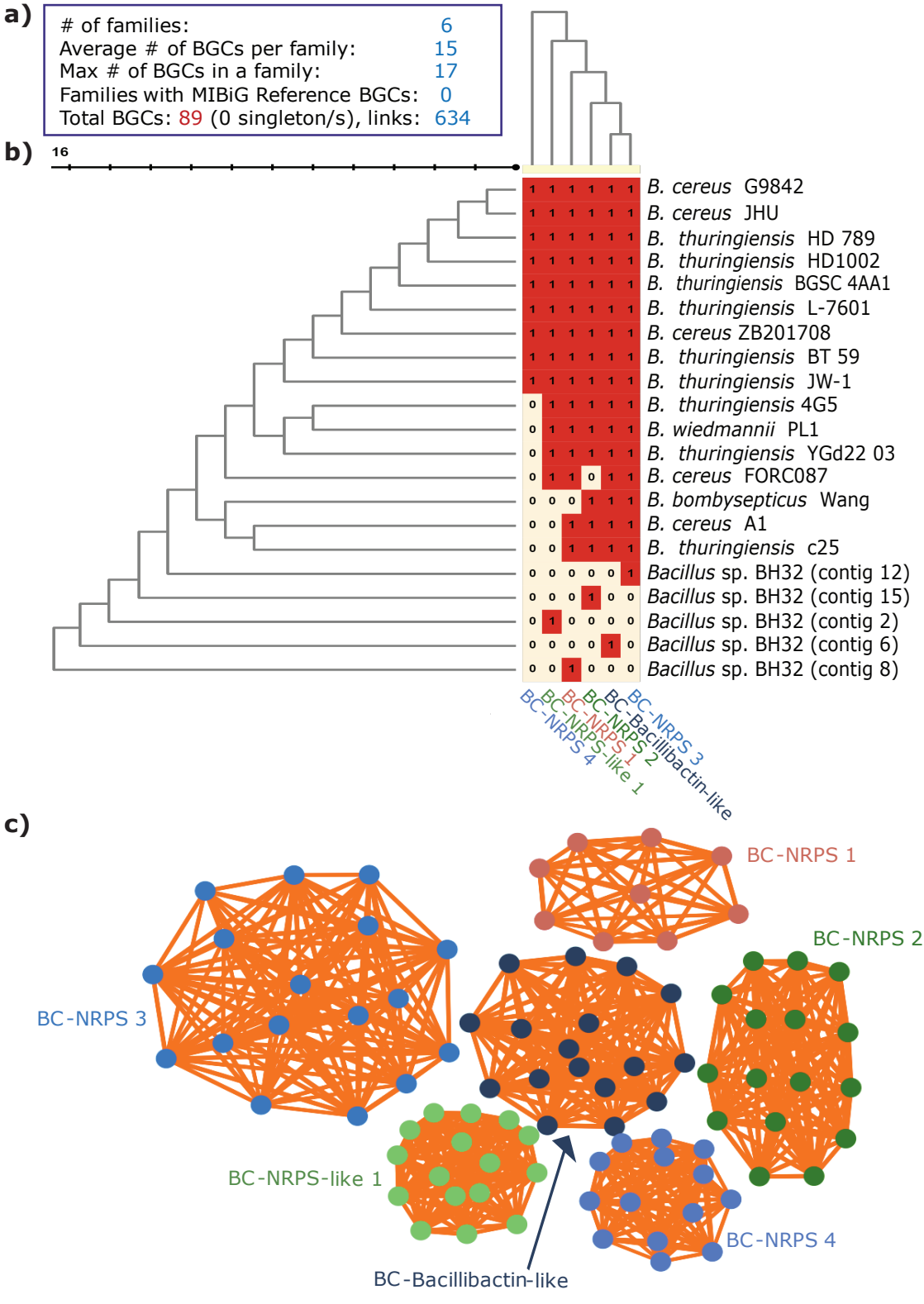
Bacillus thuringiensis serovar morrisoni BGSC 4AA1
Bacillus cereus G9842
Bacillus thuringiensis BT 59
Bacillus thuringiensis HD1002
Bacillus thuringiensis JW-1
Bacillus thuringiensis HD 789
Bacillus wiedmannii PL1
Bacillus thuringiensis serovar galleriae 4G5
Bacillus thuringiensis L-7601
Bacillus cereus A1
Bacillus bombysepticus Wang
Bacillus cereus FORC087
Bacillus cereus ZB201708
Bacillus thuringiensis YGd22 03
Bacillus cereus JHU
Bacillus thuringiensis c25


Gene identity (%)
100 70 50
B. thuringiensis YGd22 03
B. cereus JHU
B. thuringiensis JW-1
B. thuringiensis c25
B. thuringiensis BGSC 4AA1
B. thuringiensis L-7601
B. cereus G9842
B. thuringiensis HD1002
B. cereus A1
B. thuringiensis 4G5
B. thuringiensis HD 789
B. thuringiensis BT 59
B. cereus ZB201708
B. cereus FORC087
B. wiedmannii PL1
B. bombysepticus Wang
Bacillus sp. BH32
all BGCs
betalactone
27.1
ctg27_20
ctg27_13
terpene
2.1
bacteriocin
24.1
NRPS
15.1
ctg15_85
NRPS
2.2
ctg2_300
ctg2_321
siderophore
3.1
NRPS
12.1
LAP.bacteriocin
5.1
ctg12_35
ctg12_31
NRPS-like
8.1
ctg8_90
ctg8_81
NRPS
6.1


a)
b)
BGCs clade VI
BGCs clade III
BGCs clade II
BGCs clade I
Bacillus cereus ZB201708
B. thuringiensis serovar morrisoni BGSC 4AA1
Bacillus cereus G9842
Bacillus thuringiensis L-76-01
Bacillus thuringiensis JW-1
Bacillus thuringiensis HD-789
Bacillus thuringiensis YGd22-03
Bacillus cereus A1
Bacillus bombysepticus Wang
B. thuringiensis serovar galleriae 4G5
Bacillus cereus FORC087
Bacillus wiedmannii PL1
Bacillus sp. BH32
Bacillus thuringiensis BT-59
Bacillus thuringiensis HD1002
Bacillus cereus JHU
Bacillus thuringiensis c25
100
94
94
97
100
100
97
40
100
74
95
100
100
100
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 k AA
0.050
400000 AA
BGC sequence limits
BGC orientation same as reference
BGC orientation adverse to reference
BGCs with conserved order
BGC homologous segment with similarity plots (segments with same colour are homologous, with Locally Collinear Blocks "LCB" weight threshold of 733)
1, 2, 3, ...19
BGC tag number, according to appearance in genomes, from the reference strain B. thuringiensis c25 to B. cereus ZB201708 BGC with same tag numbers are considered as homologous.
BGC tag number, type and most similar known clusters:
1: terpen (molybdenum co-factor)
2: NRPS 1(polyoxypeptin*)
3: bacteriocin 1 (unknown)
4: bacteriocin 2 (unknown)
5: betalacton (fengycin)
6: NRPS 2 (unknown, gramicidin**, nostopeptolide A2***)
7: NRPS 3 (bacillibactin)
8: siderophore (petrobactin)
9: linear azol(in)e-containing peptides "LAP" bacteriocin
10: bacteriocin 3 (unknown)
11: NRPS-like 1 (unknown)
12: lanthipeptid 1(cerecidin/cerecidin A1/cerecidin A2/cerecidin A3 / cerecidin A4/cerecidin A5/cerecidin A6/cerecidin A7)
13: bacteriocin 4 (unknown)
14: bacteriocin 5 (unknown)
15: NRPS-like 2 (unknown)
16: NRPS Polyketide (chejuenolide A / chejuenolide B)
17: bacteriocin 6 (unknown)
18: lanthipeptid 2 (surfactin)
19: bacteriocin 7 (unknown)
* Closest known cluster in all the strains, except B. thringiensis c25
** Closest known cluster in B. cereus JHU
*** Closest known cluster in B. thuringiensis serovar galleriae4G5


Others
17.17%
(34)
NRPS
44.94%
(89)
Terpene
8.58%
(17)
RiPPs
29.29%
(58)

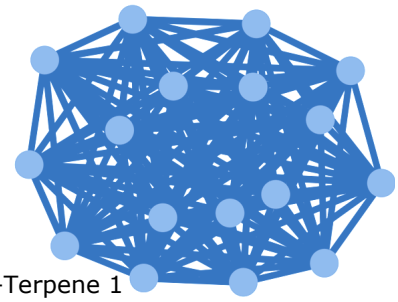

a)
# of families: 13
Average # of BGCs per family: 4
Max # of BGCs in a family: 17
Families with MIBiG Reference BGCs: 0
Total BGCs: 58 (7 singleton/s), links: 293
b)
9
B. cereus JHU
B. cereus G9842
B. wiedmannii PL1
B. thuringiensis BGSC 4AA1
B. cereus FORC087
B. thuringiensis c25
B. thuringiensis HD 789
B. bombysepticus Wang
B. cereus A1
B. thuringiensis BT 59
B. thuringiensis JW-1
B. thuringiensis YGd22 03
B. thuringiensis 4G5
Bacillus sp. BH32 (contig 24)
B. thuringiensis HD1002
B. thuringiensis L-7601
B. cereus ZB201708
Bacillus sp. BH32 (contig 5)
BCS-Surfactin-like 1
BCS-Bacteriocin 4
BC-Bacteriocin 3
BCS-Bacteriocin 1
BCS-Bacteriocin 3
BCS-Bacteriocin 2
BCS-Bacteriocin 5
BC-Cerecidin
BCS-Bacteriocin 3
BC-Lasso peptide 1
BC-Bacteriocin 4
c)
Singletons
BCS-Surfactin-like 1
BCS-Bacteriocin 1
BCS-Bacteriocin 3
BCS-Bacteriocin 2
BCS-Bacteriocin 4
BCS-Bacteriocin 5
BC-Cerecidin
BC-Lasso peptide 1
BC-Bacteriocin 3
BC-Bacteriocin clan I
BC-Bacteriocin 1
BC-Bacteriocin 2
BC-Bacteriocin 5
BC-Bacteriocin 4
BC-Bacteriocin clan II


a)
# of families: 6
Average # of BGCs per family: 15
Max # of BGCs in a family: 17
Families with MIBiG Reference BGCs: 0
Total BGCs: 89 (0 singleton/s), links: 634
b)
16
B. cereus G9842
B. cereus JHU
B. thuringiensis HD 789
B. thuringiensis HD1002
B. thuringiensis BGSC 4AA1
B. thuringiensis L-7601
B. cereus ZB201708
B. thuringiensis BT 59
B. thuringiensis JW-1
B. thuringiensis 4G5
B. wiedmannii PL1
B. thuringiensis YGd22 03
B. cereus FORC087
B. bombysepticus Wang
B. cereus A1
B. thuringiensis c25
Bacillus sp. BH32 (contig 12)
Bacillus sp. BH32 (contig 15)
Bacillus sp. BH32 (contig 2)
Bacillus sp. BH32 (contig 6)
Bacillus sp. BH32 (contig 8)
BC-NRPS 3
BC-NRPS 2
BC-Bacillibactin-like
BC-NRPS 1
BC-NRPS-like 1
BC-NRPS 4
c)
BC-NRPS 1
BC-NRPS 2
BC-NRPS 3
BC-NRPS-like 1
BC-NRPS 4
BC-Bacillibactin-like

a)

# of families:	1
Average # of BGCs per family:	17
Max # of BGCs in a family:	17
Families with MIBiG Reference BGCs:	0
Total BGCs: 17 (0 singleton/s), links:	136

b)



c)

BC-Terpene 1 clades

Clade I

Clade II

B. bombysepticus Wang
B. thuringiensis c25
B. thuringiensis 4G5
B. thuringiensis YGd22 03
B. wiedmannii PL1
B. cereus FORC087
B. cereus A1
B. thuringiensis BT 59
B. thuringiensis JW-1
B. thuringiensis HD1002
B. thuringiensis HD 789
B. cereus G9842
B. cereus JHU
Bacillus sp. BH32 (contig 2)
B. cereus ZB201708
B. thuringiensis L-7601
B. thuringiensis BGSC 4AA1

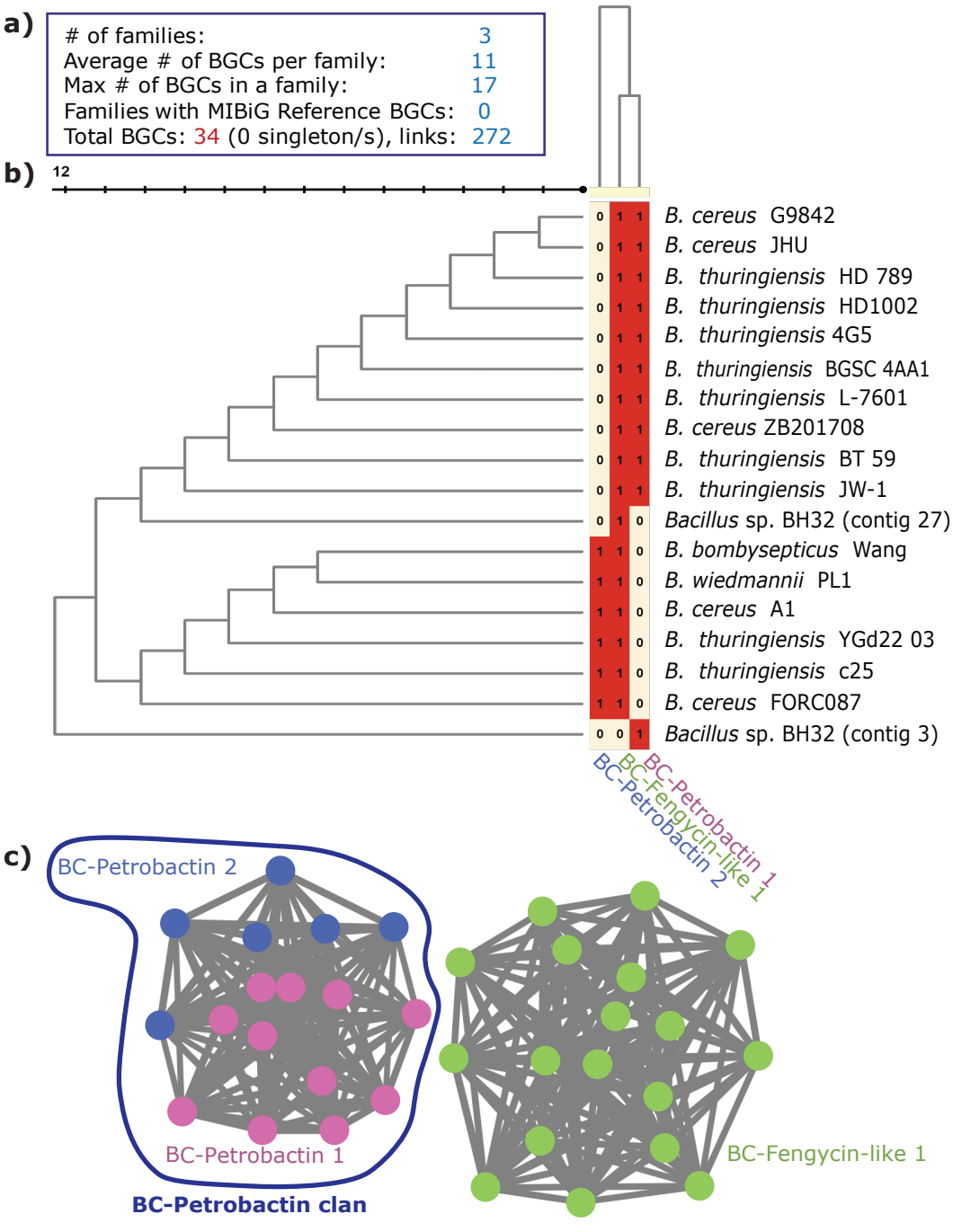
Pfam domains

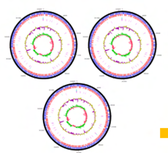
PF08445 FR47-like protein

Clade I

Clade II

PF00583 Acetyltransferase (GNAT) family
PF14698 Argininosuccinate lyase C-terminal
PF00206 Lyase
PF13354 Beta-lactamase enzyme family
PF01156 Inosine-uridine preferring nucleoside hydrolase
PF00474 Sodium:solute symporter family
PF06196 Protein of unknown function (DUF997)
PF00171 Aldehyde dehydrogenase family
PF13561 Enoyl-(Acyl carrier protein) reductase
PF14151 YfhD-like protein
PF13243 Squalene-hopene cyclase C-terminal domain
PF13249 Squalene-hopene cyclase N-terminal domain
PF02681 Divergent PAP2 family
PF00892 EamA-like transporter family
PF06961 Protein of unknown function (DUF1294)
PF10609 NUBPL iron-transfer P-loop NTPase
PF01883 Iron-sulfur cluster assembly protein
PF02597 ThiS family
PF02391 MoaE protein
PF03454 MoeA C-terminal region (domain IV)
PF00994 Probable molybdopterin binding domain
PF03453 MoeA N-terminal region (domain I and II)
PF00899 ThiF family
PF01226 Formate/nitrite transporter
PF06463 Molybdenum Cofactor Synthesis C
PF04055 Radical SAM superfamily
PF02634 FdhD/NarQ family

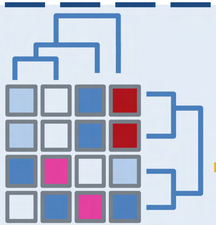

a)
# of families: 3
Average # of BGCs per family: 11
Max # of BGCs in a family: 17
Families with MIBiG Reference BGCs: 0
Total BGCs: 34 (0 singleton/s), links: 272
b)
12
B. cereus G9842
B. cereus JHU
B. thuringiensis HD 789
B. thuringiensis HD1002
B. thuringiensis 4G5
B. thuringiensis BGSC 4AA1
B. thuringiensis L-7601
B. cereus ZB201708
B. thuringiensis BT 59
B. thuringiensis JW-1
Bacillus sp. BH32 (contig 27)
B. bombysepticus Wang
B. wiedmannii PL1
B. cereus A1
B. thuringiensis YGd22 03
B. thuringiensis c25
B. cereus FORC087
Bacillus sp. BH32 (contig 3)
BC-Petrobactin 1
BC-Fengycin-like 1
BC-Petrobactin 2
c)
BC-Petrobactin 2
BC-Petrobactin 1
BC-Petrobactin clan
BC-Fengycin-like 1

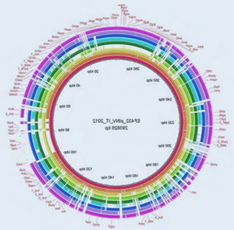


B. cereus s.l
genomes

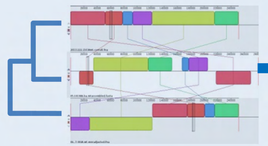
Prokka
+
antiSMASH

Manual approach



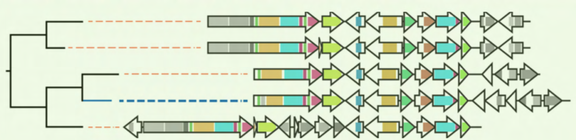


BRIG

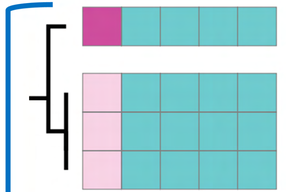


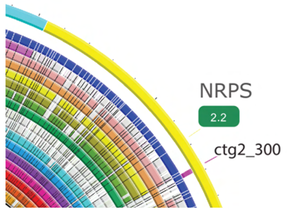
Phylogeny + Mauve

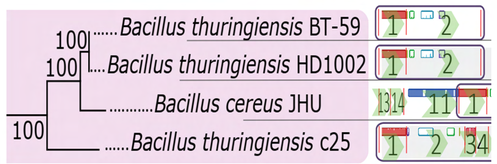
Automatic approach



BIGSCAPE CORASON

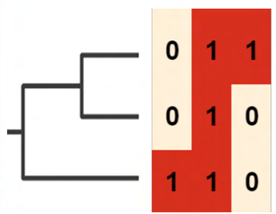


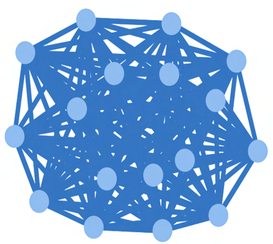


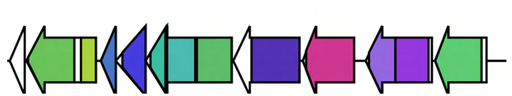


Output

- BGC counts/distribution
- BGC gene identity
- BGC conservation/ synteny







Output

- BGC families distribution
- BGC families networks
- BGC Pfam domains conservation