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Draft genome sequence of *Bacillus cereus* CITVM-11.1, a strain exhibiting interesting antifungal activities.

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Abstract

Bacillus cereus is a Gram-positive spore-forming bacterium possessing an important and historical record as human-pathogenic bacterium. However, several strains of this species exhibit an interesting potential to be used as plant-growth promoting rhizobacteria. Here, we report the draft genome sequence of *Bacillus cereus* strain CITVM-11.1, which consists of 37 contig sequences accounting for 5,746,486 bp, with a GC content of 34.8% and 5,752 predicted protein-coding sequences. Several of them could potentially be involved in plant-bacterium interactions and may contribute to the strong antagonistic activity shown by this strain against the charcoal rot fungus *Macrophomina phaseolina*. This genomic sequence also showed a number of genes that may confer to this strain resistance against several polluting heavy metals and for the bioconversion of mycotoxins.

Bacillus cereus is a Gram-positive and ubiquitous spore-forming bacterium that has been isolated from a wide range of ecosystems including water, dead insects, soil samples, the rhizosphere, the gut of several animals but is also associated with food poisoning by consumption of rice-based dishes [Krawczyk et al., 2015]. This bacterium is causing, after *Salmonella* and *Staphylococcus aureus*, the highest number of collective food poisoning outbreaks in Europe [Ramarao and Sanchis, 2013]. *B. cereus* food poisoning causes gastroenteritis which can be manifested in two different types of illness, one vomiting (emetic) form that resembles *S. aureus* infections and the diarrhoeal form, with a similar symptomatology to the infections caused by *Clostridium perfringens* [Ramarao and Sanchis, 2013].

Nevertheless, several strains of this species have demonstrated potential to be used as plant growth promoting rhizobacteria (PGPR) since they are capable of

exhibiting antagonistic activities against several phytopathogenic microorganisms [Kumar et al., 2014b] and inducing plant-systemic resistance against phytopathogenic bacteria such as *Pseudomona syringae* [Niu et al., 2011].

In this work, we report the draft genome sequence of *Bacillus cereus* strain CITVM-11.1, which was isolated from a soil sample obtained in a field of alfalfa plants (*Medicago sativa* L.) in the province of Córdoba, Argentina [Felipe et al., 2016]. This strain exhibited strong antagonistic activity *in vitro*, against the charcoal rot fungus *Macrophomina phaseolina* by causing inhibition of hyphal development and impaired formation of sclerotia [Felipe et al., 2016]. This finding was consistent with other *B. cereus* strains that have demonstrated their potential for the biocontrol of some phytopathogenic fungi, bacteria, and plant-parasitic nematodes both in *in vitro* assays and through *in vivo* trials [Kumar et al., 2014b; Martinez-Alvarez et al., 2016].

Purified total DNA from *B. cereus* CITVM-11.1 was obtained using the Wizard genomic DNA purification kit (Promega), following the instructions for the isolation of DNA from Gram-positive bacteria. Total DNA, which in some strains may be composed of the bacterial chromosome and a variable number of plasmids, was electrophoresed in 1% agarose gels stained with SYBR Safe (Thermo Fisher Scientific).

Genome sequencing was performed at Stabvida (Portugal) by using high-throughput Illumina sequencing technology with a genomic coverage of 1000×. Genome assembly was performed by assembling (*de novo*) the Illumina reads with Geneious R10 (Biomatters) into 37 contigs totalling 5,746,486 bp, with a maximum contig size of 695,448 bp and a G+C content of 34.8 %. Genome annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (2017 release), although it was also analysed with RAST [Aziz et al., 2008], which produced a total of 5,752 protein-coding sequences (CDs) plus 71 RNA genes (rRNAs and tRNAS) and 5

76 non-coding RNAs.

77 Phylogenetic analysis using *gyrB* gene sequence and following the methodology
78 described by Bavykin et al. (2004), showed that *B. cereus* strain CITVM-11.1 belongs
79 to Cereus B subgroup located at Cluster I inside the *Bacillus cereus* group [Bavykin et
80 al., 2004] (Figure S1, supplementary material).

81 From the 5,752 predicted protein-coding sequences, several of them could be
82 potentially involved in plant-bacterium interactions (e.g. auxin biosynthesis) and the
83 previously reported antagonistic activity against *M. phaseolina* (Figure 1).

84 Genes potentially involved in the biosynthesis of thiopeptides or thiazolyl
85 peptides have been found in the genome. The thiopeptide cyclothiazomycin B1 (CTB1)
86 is an antifungal cyclic thiopeptide isolated from a *Streptomyces* sp. that produces
87 growth inhibition and morphological changes of hyphae and induces fragility of the
88 fungal cell wall by binding chitin [Mizuhara et al., 2011] and capable of producing
89 growth inhibition of fungal species such as *Fusarium*, *Aspergillus* and *Penicillium* spp
90 [Mizuhara et al., 2011]. A similar impaired growth has been produced in the charcoal-
91 root fungus *M. phaseolina* on exposure to *B. cereus* strain CITVM-11.1, as previously
92 reported (Felipe et al., 2016). Wang et. al (2010) analysed the biosynthetic gene cluster
93 responsible of the production of cyclothiazomycin thiopeptide in *Streptomyces*
94 *hygroscopicus* 10-22 [Wang et al., 2010] and described a gene cluster model for the
95 biosynthesis of cyclothiazomycin that involves several genes encoding putative
96 functional enzymes, namely: Ser and Thr dehydratases, enzymes producing the thertiary
97 thioether and an epoxide hydrolase [Wang et al., 2010]. Homologous genes to those
98 described by Wang wet al. (2010) have been found at the genome of *B. cereus* CITVM-
99 11.1 at contig No.12 (Thr-dehydratase, L-serine dehydratase, thioestearase) and contig
100 No. 23 (epoxide hydrolase, thioestearase and a thioazol kinase), even though they are

not organized in a biosynthetic gene cluster. Some *B. cereus* strains have been also described as thiopeptide producing strains showing growth inhibition of *Aspergillus flavus* and *Fusarium oxysporum*, although genes responsible of this thiopeptide production have not been yet described [Kumar et al., 2014a; Kumar et al., 2014b]. Other genes showing significant similarity with chitinase enzymes and surfactins were also found in the genome and may be contributing to the antifungal activity exhibited by this *B. cereus* strain.

Gene cluster analysis using anti SMASH (antibiotics & secondary metabolites analysis shell) [Weber et al., 2015] showed that this strain potentially harbours 50 biosynthetic gene clusters. From them, best predicted gene clusters may be potentially involved in i) the synthesis and accumulation of polyhydroxyalkanoates with 100 % of the genes exhibiting similarity, ii) the production of the non-ribosomal peptide bacilibactin (siderophore) with 46 % of the genes exhibiting similarity, iii) synthesis of the non-ribosomal peptide bacitracin (antibiotic) with 100 % of the genes exhibiting similarity, iv) the synthesis of the bacteriocin Thuricin H with 60 % of the genes exhibiting similarity and v) the production of the siderophore petrobactin with 100 % of the genes exhibiting similarity (Figure S2, supplementary material).

Contig 27 was automatically circularized by Geneious R10 as a putative plasmid of 10,741 bp in size. Circularization of contigs occurs when running Geneious R10 *de novo* assembly tool and a pair of reads of each end of the contig match and also such reads must not intersect with each other in any other part of the contig. Accordingly, agarose gel electrophoresis of total DNA showed an additional band consistent with the presence of a plasmid (Fig 2A)". We have named this plasmid pBC11.1. Two RAST annotated genes on the plasmid might be related to the mobilization (horizontal transfer by conjugation) of the plasmid whereas two others, have been annotated by RAST as a

126 macrolide-efflux protein and a putative mercury resistance protein (Figure 2B).
127 Acquisition of antimicrobial-resistance genes in bacteria can occur by means of self-
128 transmissible plasmids (conjugative plasmids). These plasmids usually harbour all the
129 genes involved in mating-pore formation as well as the essential *mob* gene (encoding
130 DNA relaxase) and the recognition sequence commonly known as origin of transfer
131 (*oriT*) [Ramsay et al., 2016]. Despite the *mob* gene was found in pBC11.1 plasmid, we
132 could not effectively predict any known putative *oriT* sequence in this plasmid.

133 In addition, the genomic sequence also exhibits other RAST annotated genes
134 that could be related to the metabolism of several heavy metals that pollute the
135 environment, namely: i) for arsenic (As), three arsenic efflux pump proteins, one
136 arsenical resistance operon repressor and two arsenical-resistance proteins; ii) for
137 copper (Cu), a membrane protein for copper uptake and a copper resistance protein D;
138 ii) for cobalt (Co), zinc (Zn) and cadmium (Cd); three cobalt-zinc-cadmium resistance
139 proteins; iv) for mercury (Hg), one predicted gene, located at the plasmid pBC11.1,
140 potentially encode for a mercury resistance protein; v) for aluminium (Al), an
141 aluminium resistance protein and vi) for tellurium (Te), one tellurite-resistance protein
142 and three tellurium-resistance proteins. Some of the heavy metals mentioned above, e.g.
143 Zn, Cu, Ni, Co with chromium (Cr), are necessary as micronutrients, playing vital roles
144 in metabolic and physiological processes of microorganisms, plants and animals.
145 However, non-essential heavy metals such as silver (Ag), As, Cd, Pb and Hg are not
146 necessary for living organisms and their presence in soil and water sources pollute
147 ecosystems [Fashola et al., 2016].

148 The genomic sequence of *B. cereus* strain CITVM-11.1 also exhibits several
149 enzyme-coding genes that might be involved in the biotransformation of mycotoxins
150 [Loi et al., 2017]. Such genes, harboured at CITVM-11.1 strain, encode the following

enzymes: i) oxidases, peroxidases, reductases and manganese peroxidases (potential aflatoxin-degrading enzymes); ii) carboxylesterases, aminotransferases an esterase (potential fumonisin-degrading enzymes) and iii) cytochrome P450 and glycosyltransferases (potential trichothecenes-degrading enzymes) [Loi et al., 2017]. Thus, *B. cereus* CITVM-11.1 could be a good source of enzymes for reducing mycotoxin accumulation of staple food commodities [Loi et al., 2017], although it has been shown to be a β-hemolytic strain (data not shown) that contains genes coding known enterotoxins and their elimination may be necessary.

In this work, we report the draft genome sequence of *B. cereus* CITVM-11.1, which showed a strong antagonistic activity against the charcoal rot fungus *M. phaseolina*. This draft genome sequence provides an overview of the genes that could be involved in plant-microbe interactions and the development of antagonistic activities against phythopathogenic fungi, as well as indicating the potential of this strain to tolerate the toxic activity of a number of heavy metals. The preliminary results presented in this work encourage us to perform deeper studies, in order to elucidate both the biocontrol and bioremediation potential of this strain, which deserves to be further investigated.

This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number MVFX00000000. The version described in this paper is the first version, MVFX01000000.

Acknowledgements

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177

178 **Disclosure Statement**

179 The authors declare no conflict of interests**.**

References

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. The RAST server: rapid annotations using subsystems technology. BMC Genomics 2008; 9:1-15.

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

Fashola MO, Ngole-Jeme VM, Babalola OO. Heavy Metal Pollution from Gold Mines: Environmental Effects and Bacterial Strategies for Resistance. Int J Environ Res Public Health 2016; 13.

Felipe V, Palma L, Yaryura P. Antagonistic activity of *Bacillus* sp. strain isolated in Córdoba, Argentina against *Macrophomina phaseolina* (Tassi) Goid. . Rev Argent Microbiol 2016; http://dx.doi.org/10.1016/j.ram.2016.08.008.

Krawczyk AO, de Jong A, Eijlander RT, Berendsen EM, Holsappel S, Wells-Bennik MH, Kuipers OP. Next-Generation Whole-Genome Sequencing of Eight Strains of *Bacillus cereus*, Isolated from Food. Genome Announc 2015; 3.

Kumar SN, Nambisan B, Mohandas C. Purification and identification of two antifungal cyclic dipeptides from *Bacillus cereus* subsp. *thuringiensis* associated with a rhabditid entomopathogenic nematode especially against *Fusarium oxysporum*. J Enzyme Inhib Med Chem 2014a; 29:190-197.

Kumar SN, Sreekala SR, Chandrasekaran D, Nambisan B, Anto RJ. Biocontrol of *Aspergillus* species on peanut kernels by antifungal diketopiperazine producing *Bacillus cereus* associated with entomopathogenic nematode. PLoS One 2014b; 9:e106041.

Loi M, Fanelli F, Liuzzi VC, Logrieco AF, Mule G. Mycotoxin Biotransformation by Native and Commercial Enzymes: Present and Future Perspectives. Toxins (Basel) 2017; 9.

Martinez-Alvarez JC, Castro-Martinez C, Sanchez-Pena P, Gutierrez-Dorado R, Maldonado-Mendoza IE. Development of a powder formulation based on *Bacillus cereus* sensu lato strain B25 spores for biological control of *Fusarium verticillioides* in maize plants. World J Microbiol Biotechnol 2016; 32:75.

Mizuhara N, Kuroda M, Ogita A, Tanaka T, Usuki Y, Fujita K. Antifungal thiopeptide cyclothiazomycin B1 exhibits growth inhibition accompanying morphological changes via binding to fungal cell wall chitin. Bioorg Med Chem 2011; 19:5300-5310.

Niu DD, Liu HX, Jiang CH, Wang YP, Wang QY, Jin HL, Guo JH. The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. Mol Plant Microbe Interact 2011; 24:533-542.

Ramarao N, Sanchis V. The pore-forming haemolysins of bacillus cereus: a review. Toxins (Basel) 2013; 5:1119-1139.

Ramsay JP, Kwong SM, Murphy RJ, Yui Eto K, Price KJ, Nguyen QT, O'Brien FG, Grubb WB, Coombs GW, Firth N. An updated view of plasmid conjugation and mobilization in *Staphylococcus*. Mob Genet Elements 2016; 6:e1208317.

Wang J, Yu Y, Tang K, Liu W, He X, Huang X, Deng Z. Identification and analysis of the biosynthetic gene cluster encoding the thiopeptide antibiotic cyclothiazomycin in *Streptomyces hygroscopicus* 10-22. Appl Environ Microbiol 2010; 76:2335-2344.

Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Muller R, Wohlleben W, Breitling R, Takano E, Medema MH. antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015; 43:W237-243.

Figure captions

Figure 1: Potential plant-bacterium interaction and PGPR related features predicted and annotated by RAST are highlighted in red.

Figure 2: A) Agarose gel electrophoresis of total DNA showing the genomic and plasmid DNA (MM: molecular marker). B) Map of the circularized contig sequence (contig 27).

Figure 1

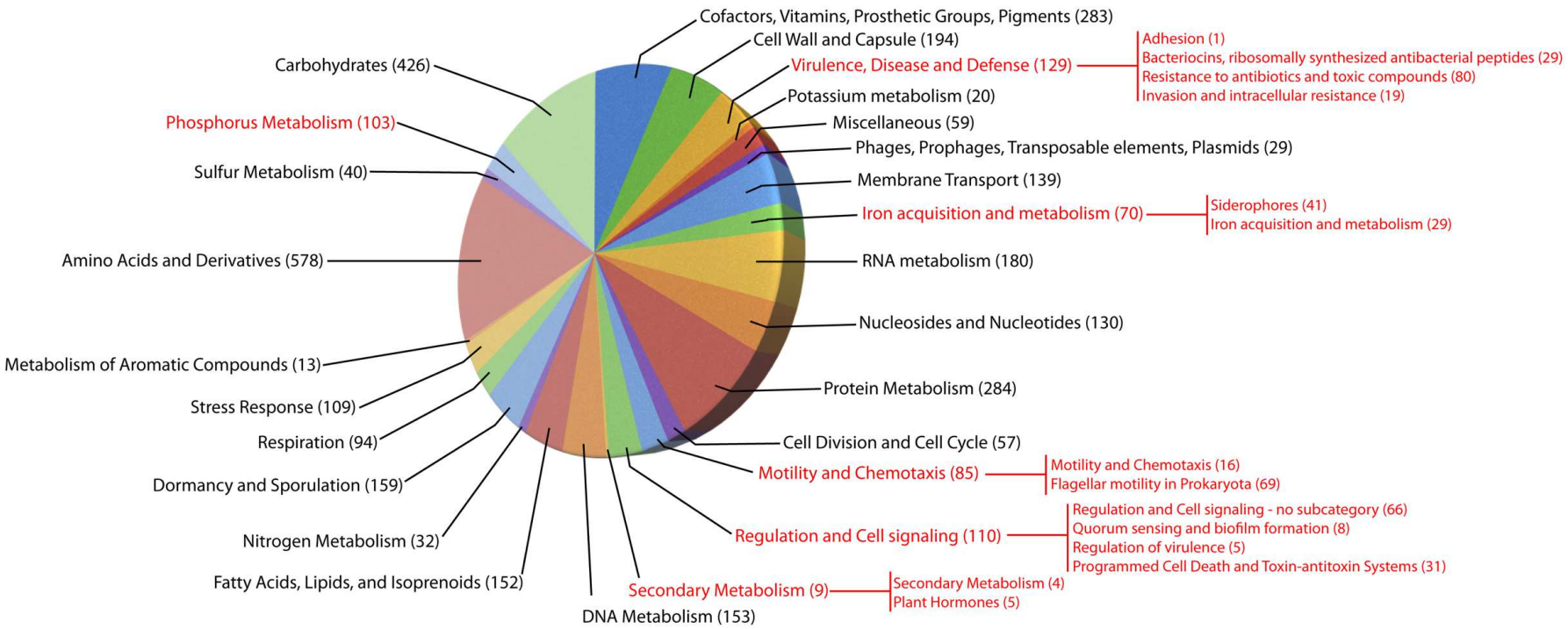
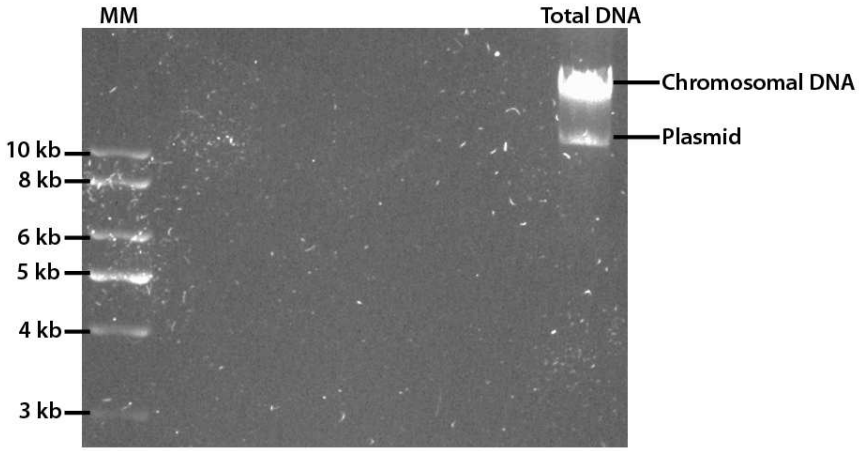
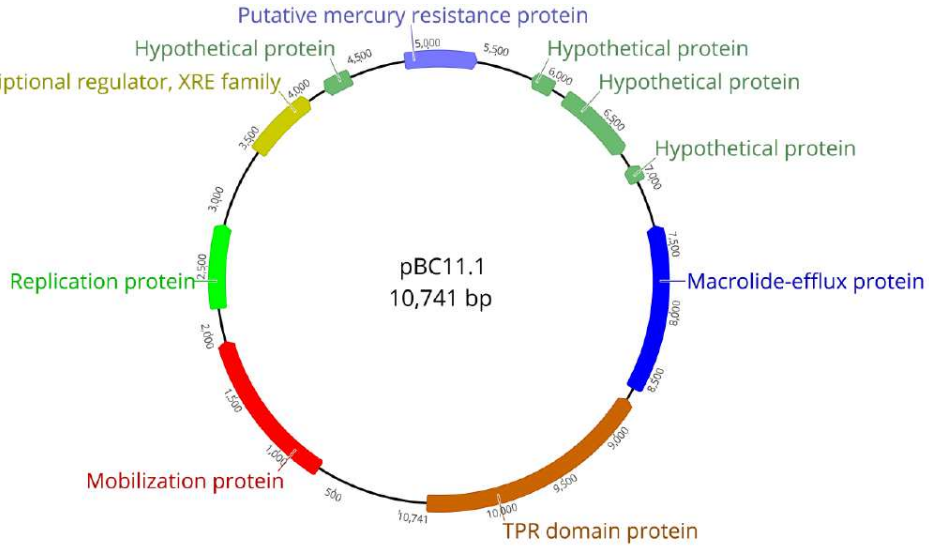

Cofactors, Vitamins, Prosthetic Groups, Pigments (283)
Cell Wall and Capsule (194)
Virulence, Disease and Defense (129)
Adhesion (1)
Bacteriocins, ribosomally synthesized antibacterial peptides (29)
Resistance to antibiotics and toxic compounds (80)
Invasion and intracellular resistance (19)
Potassium metabolism (20)
Miscellaneous (59)
Phages, Prophages, Transposable elements, Plasmids (29)
Membrane Transport (139)
Iron acquisition and metabolism (70)
Siderophores (41)
Iron acquisition and metabolism (29)
RNA metabolism (180)
Nucleosides and Nucleotides (130)
Protein Metabolism (284)
Cell Division and Cell Cycle (57)
Motility and Chemotaxis (85)
Motility and Chemotaxis (16)
Flagellar motility in Prokaryota (69)
Regulation and Cell signaling (110)
Regulation and Cell signaling - no subcategory (66)
Quorum sensing and biofilm formation (8)
Regulation of virulence (5)
Programmed Cell Death and Toxin-antitoxin Systems (31)
Secondary Metabolism (9)
Secondary Metabolism (4)
Plant Hormones (5)
DNA Metabolism (153)
Fatty Acids, Lipids, and Isoprenoids (152)
Nitrogen Metabolism (32)
Dormancy and Sporulation (159)
Respiration (94)
Stress Response (109)
Metabolism of Aromatic Compounds (13)
Amino Acids and Derivatives (578)
Sulfur Metabolism (40)
Phosphorus Metabolism (103)
Carbohydrates (426)

Figure 2


MM
Total DNA
Chromosomal DNA
Plasmid
10 kb
8 kb
6 kb
5 kb
4 kb
3 kb

A)


Putative mercury resistance protein
Hypothetical protein
Hypothetical protein
Transcriptional regulator, XRE family
Hypothetical protein
Hypothetical protein
pBC11.1
10,741 bp
Replication protein
Macrolide-efflux protein
Mobilization protein
TPR domain protein

B)

Supplementary Material

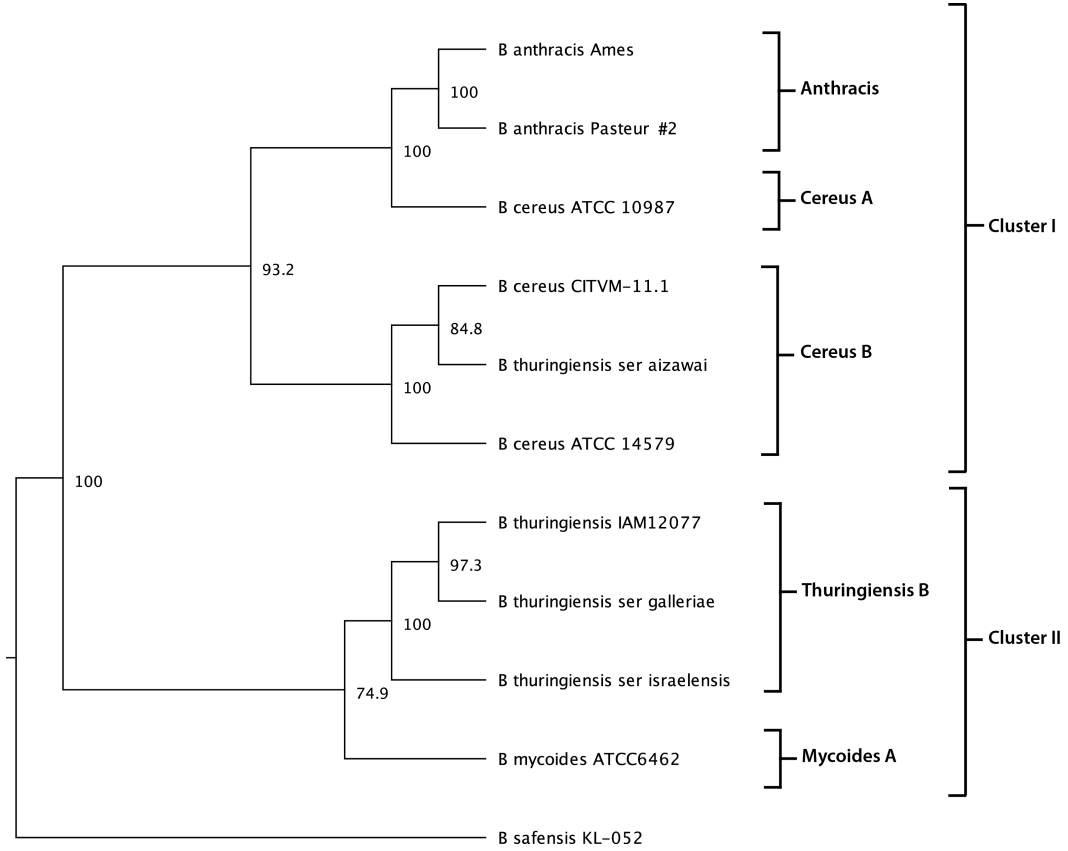


Figure S1: Genetic relationship of *B. cereus* CITVM-11.1 and other strains based on *gyrB* gene sequences [Bavykin et al., 2004].

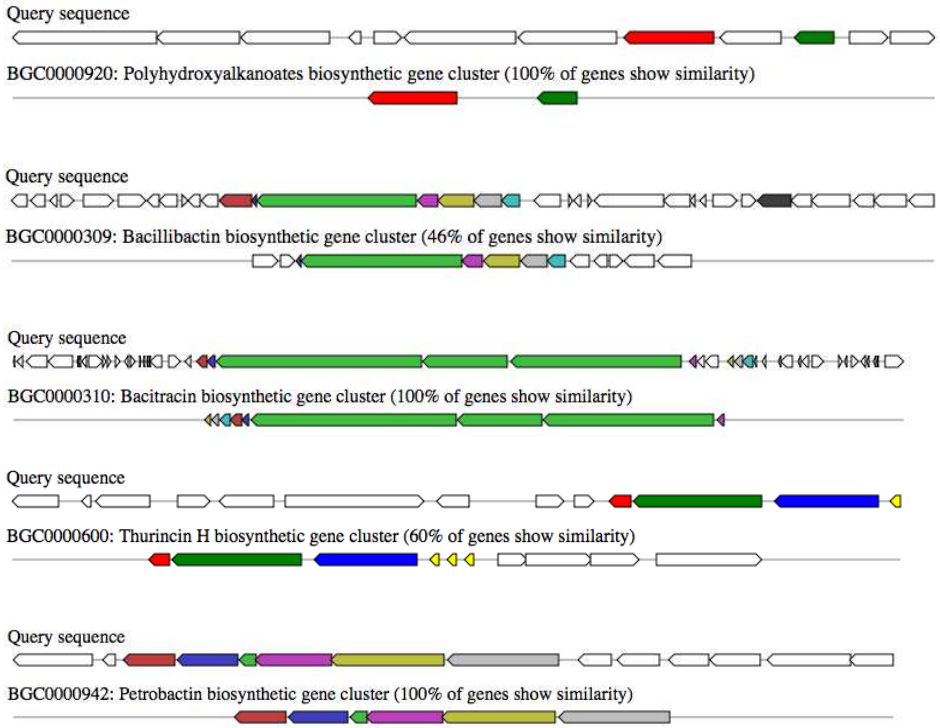


Figure S2: Potential biosynthetic gene clusters associated to secondary metabolites production identified with antiSMASH [Weber et al., 2015].