Short Communication

Journal of Molecular Microbiology and Biotechnology

J Mol Microbiol Biotechnol 2018;28:47–51
DOI: 10.1159/000487597

Received: July 11, 2017
Accepted: February 10, 2018
Published online: April 25, 2018

Draft Genome Sequence of *Bacillus cereus* CITVM-11.1, a Strain Exhibiting Interesting Antifungal Activities

Javier Caballero[a, b] Cecilia Peralta[c, d] Antonella Molla[d] Eleodoro E. Del Valle[e] Primitivo Caballero[a, b] Colin Berry[f] Verónica Felipe[d] Pablo Yaryura[c, d] Leopoldo Palma[c, d]

[a]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Mutilva Baja, Spain; [b]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; [c]Centro de Investigaciones y Transferencia de Villa María (CIT-VM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Villa María, Villa María, Argentina; [d]Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas (IAPCByA), Universidad Nacional de Villa María (UNVM), Villa María, Argentina; [e]Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Esperanza, Argentina; [f]Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Keywords
Bacillus cereus · Draft genome sequence · Antifungal activity

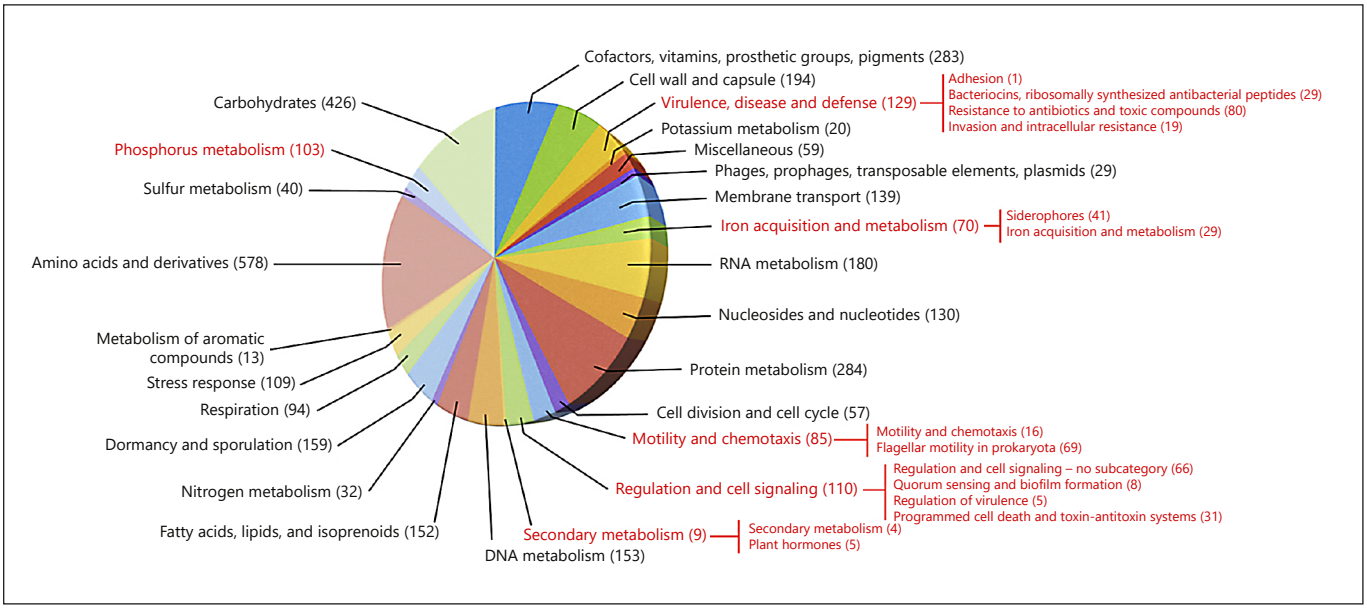
Abstract
Bacillus cereus is a gram-positive, spore-forming bacterium possessing an important and historical record as a human-pathogenic bacterium. However, several strains of this species exhibit interesting potential to be used as plant growth-promoting rhizobacteria. Here, we report the draft genome sequence of *B. 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 root rot fungus, *Macrophomina phaseolina*. This genomic sequence also showed a number of genes that may confer 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, and the gut of several animals, but is also associated with food poisoning from rice-based dishes [Krawczyk et al., 2015]. It causes, 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 as 2 different types, a vomiting (emetic) form that resembles *S. aureus* infections, and a diarrheal form, with similar symptomatology to infections caused by *Clostridium perfringens* [Ramarao and Sanchis, 2013].

Javier Caballero and Cecilia Peralta contributed equally to this work. This whole-genome shotgun project was deposited in DDBJ/ENA/GenBank under the accession No. MVFX00000000 (this paper is the first version: MVFX01000000).

E-Mail karger@karger.com
www.karger.com/mmb

Leopoldo Palma
Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas
Universidad Nacional de Villa María, Avenida Arturo Jauretche 1555
ES–5900 Villa María, Córdoba (Argentina)
E-Mail palma.leopoldo@gmail.com



Color version available online

Fig. 1. Potential plant-bacterium interaction. Plant growth-promoting rhizobacteria-related features predicted and annotated by RAST are highlighted in red.

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 *Pseudomonas syringae* [Niu et al., 2011].

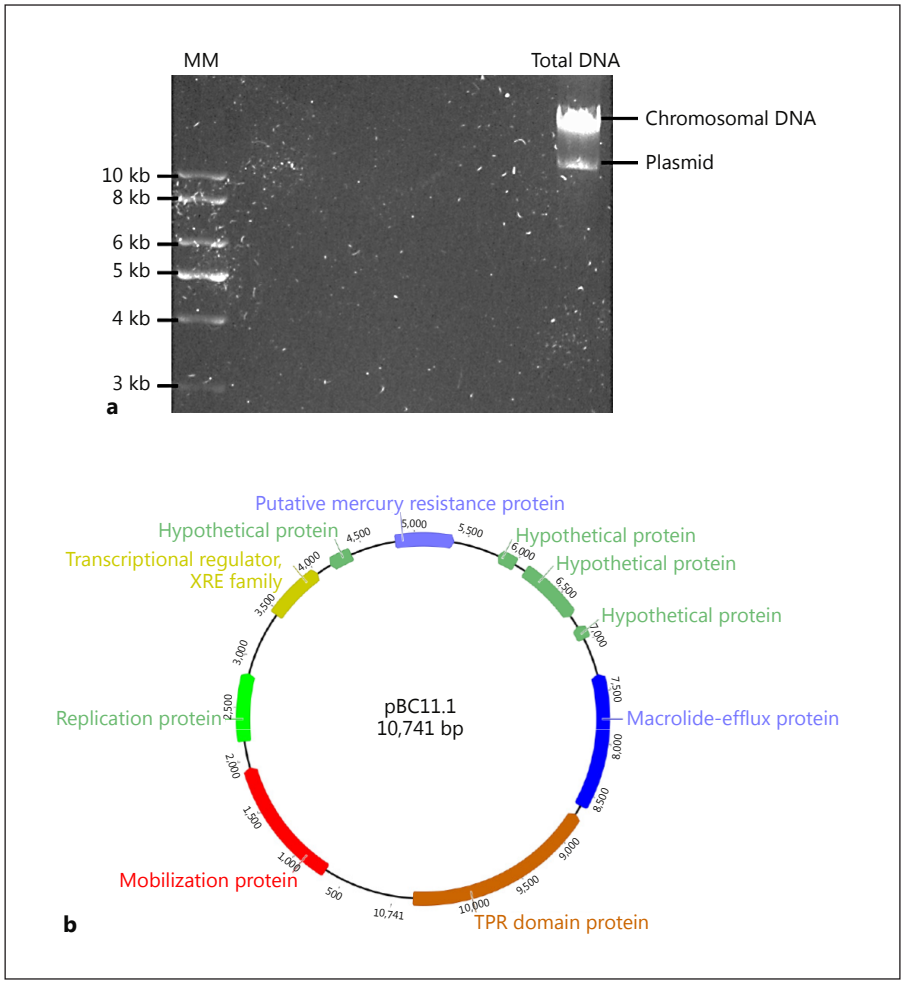
In this work, we report the draft genome sequence of *B. cereus* strain CITVM-11.1, which was isolated from a soil sample obtained in a field of alfalfa plants (*Medicago sativa* L.) in the province of Córdoba, Argentina [Felipe et al., 2017]. This strain exhibited strong antagonistic activity in vitro to the charcoal root rot fungus *Macrophomina phaseolina*, by causing the inhibition of hyphal development and impaired the formation of sclerotia [Felipe et al., 2017]. This finding was consistent with those about other *B. cereus* strains that have demonstrated their potential for the biocontrol of some phytopathogenic fungi, bacteria, and plant-parasitic nematodes, in both in vitro assays and 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) and 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 gel 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 1,000×. Genome assembly was performed by assembling (de novo) the Illumina reads with Geneious R10 (Biomatters) into 37 contigs, totaling 5,746,486 bp, with a maximum contig size of 695,448 bp and a GC content of 34.8%. Genome annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (2017 release), but was also analyzed with RAST [Aziz et al., 2008], and produced a total of 5,752 protein-coding sequences, plus 71 RNA genes (rRNAs and tRNAS), and 5 noncoding RNAs.

Phylogenetic analysis using the *gyrB* gene sequence and following the methodology described by Bavykin et al. [2004] showed that *B. cereus* strain CITVM-11.1 belongs to the *cereus* B subgroup located at cluster I inside the *Bacillus cereus* group (online suppl. Fig. S1; see www.karger.com/doi/10.1159/000487597 for all online suppl. material).

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



Color version available online

Fig. 2. a Agarose gel electrophoresis of total DNA showing the genomic and plasmid DNA. MM, molecular marker. **b** Map of the circularized sequence contig (contig 27).

Genes potentially involved in the biosynthesis of thiopeptides or thiazolyl peptides have been found in the genome. The thiopeptide cyclothiazomycin B1 (CTB1) is an antifungal cyclic thiopeptide isolated from a *Streptomyces* sp. that produces growth inhibition and morphological changes of the hyphae, induces fragility of the fungal cell wall by binding chitin [Mizuhara et al., 2011], and is capable of inhibiting the growth of fungal species such as *Fusarium*, *Aspergillus*, and *Penicillium* spp. [Mizuhara et al., 2011]. A similar impaired growth has been produced in the charcoal root rot fungus *M. phaseolina* on exposure to *B. cereus* strain CITVM-11.1, as previously reported [Felipe et al., 2017]. Wang et al. [2010] analyzed the biosynthetic gene cluster responsible for the production of cyclothiazomycin thiopeptide in *Streptomyces hygroscopicus* 10–22. They described a gene cluster model for the biosynthesis of cyclothiazomycin that involves several genes encoding putative functional enzymes, namely, Ser and Thr dehydratases, enzymes producing the thertiary thioether, and an epoxide hydrolase. Genes homologous with those described by Wang et al. [2010] have been found at the genome of *B. cereus* CITVM-11.1 at contig 12 (Thr-dehydratase, L-serine dehydratase, and thioestearase) and contig 23 (epoxide hydrolase, thioestearase, and a thioazol kinase), even though they are not organized in a biosynthetic gene cluster. Some *B. cereus* strains have also been described as thiopeptide-producing strains, and show growth inhibition of *Aspergillus flavus* and *Fusarium oxysporum*, although the genes responsible for this thiopeptide production have not been yet described [Kumar et al., 2014a, b]. Other genes showing significant similarity with chitinase enzymes and surfactins were also found in the genome, and may contribute to the antifungal activity exhibited by this *B. cereus* strain.

Gene cluster analysis using antiSMASH [Weber et al., 2015] showed that this strain potentially harbors 50 bio-

synthetic gene clusters. The best-predicted gene clusters may be involved in (i) the synthesis and accumulation of polyhydroxyalkanoates, with 100% of the genes exhibiting similarity; (ii) the production of the nonribosomal peptide bacilibactin (siderophore), with 46% of the genes exhibiting similarity; (iii) synthesis of the nonribosomal 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 (online suppl. Fig. S2).

Contig 27 was automatically circularized by Geneious R10 as a putative plasmid of 10,741 bp in size. Circularization of contigs occurs when running the Geneious R10 de novo assembly tool and a pair of reads of each end of the contig match; 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 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, but 2 others have been annotated by RAST as a macrolide-efflux protein and a putative mercury resistance protein (Fig. 2b). The acquisition of antimicrobial-resistance genes in bacteria can occur by means of self-transmissible plasmids (conjugative plasmids). These plasmids usually harbor all the genes involved in mating-pore formation as well as the essential *mob* gene (encoding DNA relaxase), and the recognition sequence commonly known as origin of transfer *(oriT)* [Ramsay et al., 2016]. Despite the *mob* gene being found in the pBC11.1 plasmid, we could not effectively predict any known putative *oriT* sequence in this plasmid.

In addition, the genomic sequence exhibits other RAST-annotated genes that could be related to the metabolism of several heavy metals that pollute the environment, namely: (i) for arsenic (As), 3 As efflux pump proteins, 1 As resistance operon repressor, and 2 As resistance proteins; (ii) for copper (Cu), a membrane protein for Cu uptake and a Cu resistance protein D; (iii) for cobalt (Co), zinc (Zn), and cadmium (Cd), 3 Co-Zn-Cd resistance proteins; (iv) for mercury (Hg), 1 predicted gene located at the plasmid pBC11.1, potentially encoded for an Hg resistance protein; (v) for aluminium (Al), an Al resistance protein; and (vi) for tellurite (Te), 1 Te resistance protein and 3 Te resistance proteins. Some of the heavy metals mentioned above, e.g., Zn, Cu, Ni, and Co, along with chromium (Cr), are necessary as micronutrients, playing a vital role in the metabolic and physiological processes of microorganisms, plants, and animals. However, nonessential heavy metals such as silver (Ag), As, Cd, lead (Pb), and Hg are not necessary for living organisms, and their presence in soil and water sources pollute ecosystems [Fashola et al., 2016].

The genomic sequence of *B. cereus* strain CITVM-11.1 also exhibits several enzyme-coding genes that might be involved in the biotransformation of mycotoxins [Loi et al., 2017]. Such genes, harbored by the CITVM-11.1 strain, encode the following enzymes: (i) oxidases, peroxidases, reductases, and manganese peroxidases (potential aflatoxin-degrading enzymes); (ii) carboxylesterases, aminotransferases, and esterases (potential fumonisin-degrading enzymes); and (iii) cytochrome P450 and glycosyltransferases (potential trichothecene-degrading enzymes) [Loi et al., 2017]. Thus, *B. cereus* CITVM-11.1 could be a good source of enzymes for reducing mycotoxin accumulation in staple food commodities [Loi et al., 2017], although it has also been shown to be a β-hemolytic strain (data not shown) that contains genes coding known enterotoxins, so 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 root rot fungus *M. phaseolina*. This draft genome sequence provided an overview of the genes that could be involved in plant-microbe interactions and the development of antagonistic activities against phythopathogenic fungi, and also indicated the potential of this strain to tolerate the toxic activity of a number of heavy metals. The preliminary results presented in this work have encouraged us to perform more thorough studies, in order to elucidate both the biocontrol and bioremediation potential of this strain, which deserves to be investigated further.

Acknowledgements

This work was supported by a research grant awarded by the Universidad Nacional de Villa María (UNVM) and the Instituto de Investigación-UNVM (Disp. No. 03/2016, Anexo I). We also thank the Centro de Investigación y Transferencia Villa María (CIT-VM) for the support provided for this research project.

Disclosure Statement

The authors declare no conflict of interests.

References

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al: 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:E1047.

Felipe V, Palma L, Yaryura P: Antagonistic activity of *Bacillus* sp. strain isolated in Córdoba, Argentina against *Macrophomina phaseolina* (Tassi) Goid. Rev Argent Microbiol 2017;49:402–403.

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:e01480.

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:E111.

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–W243.