

EXTENDED GENOME REPORT

Open Access

Genome sequence and description of the mosquitocidal and heavy metal tolerant strain *Lysinibacillus sphaericus* CBAM5

Tito David Peña-Montenegro, Lucía Lozano and Jenny Dussán*

Abstract

Lysinibacillus sphaericus CBAM5, was isolated from subsurface soil of oil well explorations in the Easter Planes of Colombia. This strain has potential in bioremediation of heavy-metal polluted environments and biological control of *Culex quinquefasciatus*. According to the phylogenetic analysis of 16S rRNA gene sequences, the strain CBAM5 was assigned to the *Lysinibacillus sphaericus* taxonomic group 1 that comprises mosquito pathogenic strains. After a combination assembly-integration, alignment and gap-filling steps, we propose a 4,610,292 bp chromosomal scaffold. The whole genome (consisting of 5,146,656 bp long, 60 contigs and 5,209 predicted-coding sequences) revealed strong functional and syntenial similarities to the *L. sphaericus* C3-41 genome. Mosquitocidal (Mtx), binary (Bin) toxins, cereolysin O, and heavy metal resistance clusters from *nik*, *ars*, *czc*, *mnt*, *ter*, *cop*, *cad*, and *znu* operons were identified.

Keywords: *Lysinibacillus sphaericus* CBAM5, DNA homology, Binary toxins, Mosquitocidal toxins, S-layer proteins, Heavy metal tolerance

Introduction

Lysinibacillus sphaericus is one of the bacteria used as a bio-insecticide as part of vector control programs against tropical diseases, such as malaria, filariasis, yellow fever, dengue fever and West Nile virus [1]. *L. sphaericus* isolates may be classified according to their larvicidal activity into high and low toxicity strains. High- and low-toxicity strains synthesize mosquitocidal toxins (Mtx) in vegetative growth cells [2]. Highly toxic strains produce a binary toxin coded by *binA* and *binB* genes in sporulating stages [3]. In addition, *L. sphaericus* larvicidal toxicity may be explained due to expression of Cry48/Cry49 toxin [4] and the S-layer protein [5]. Vegetative and sporulated cells of *L. sphaericus* CBAM5 are pathogenic towards *Culex quinquefasciatus* larvae [6]. LC50 (50% lethal concentration) toward *C. quinquefasciatus* larvae of strain CBAM5 is 1400 cells/mL from sporulated cultures, being this isolate assigned as a high-toxicity strain [6].

The biotechnological application of *L. sphaericus* is not limited to biological control. *L. sphaericus* biomass has

been applied in the bioremediation of heavy metals, such as cobalt, copper, chromium and lead [7] with specific metal binding in the cell surface [8]. Native Colombian isolates *L. sphaericus* OT4b.31 and IV(4)10 showed heavy metal bio-sorption in living and dead biomass, both strains expressing the S-layer proteins [9]. *L. sphaericus* strain CBAM5, along with other 24 native isolates, shown effective growth in arsenate, hexavalent chromium and/or lead [6,10].

Considering that *Lysinibacillus sphaericus* CBAM5 is a relevant native strain, not only by its highly toxic larvicidal activity but also by its heavy metal tolerance, we have chosen this strain to analyze its genomic sequence. In this report, we present a summary classification, and set of general features for *Lysinibacillus sphaericus* strain CBAM5 including previously unreported aspects of its phenotype, together with the description of its genome sequence and annotation.

Organism information

Lysinibacillus sphaericus is an aerobic, mesophilic, spore-forming and Gram-positive bacterium, commonly isolated from soil and water [11]. Formerly known as *Bacillus sphaericus*, the species was later reassigned to the genus

* Correspondence: jdussan@uniandes.edu.co
Centro de Investigaciones Microbiológicas – CIMIC, Universidad de los Andes, Bogotá, Colombia

Lysinibacillus because of its distinctive peptidoglycan membrane composition, and physiological features [12,13]. *Lysinibacillus sphaericus* strains have been classified into five DNA homology groups, where mosquito larvicidal strains were placed into DNA subgroup IIA [14] while the subgroup IIB was reclassified as *Lysinibacillus fusiformis* [15]. Later, according to 16S rDNA and lipid profile comparisons, *Lysinibacillus sphaericus* strains were classified into seven similarity subgroups, of which only four retained the previous description by Krych et al. [15]. Groups VI and VII were later reclassified as new species [16]. Because of the phenotypic and genetic diversity summarized above, most of the groups remain designated as *Lysinibacillus sphaericus sensu lato*.

Partial 16S rRNA gene sequences (1,421 bp) were aligned to establish the phylogenetic neighborhood of *Lysinibacillus sphaericus* CBAM5 (Figure 1). The phylogenetic tree was constructed by using the Maximum Likelihood method on the Tamura-Nei model [17]. Initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA6 [18]. The stability of relationships was assessed by bootstrap analysis based on 1,000 resamplings for the tree topology. *L. sphaericus* CBAM5 was assigned to the DNA similarity group 1 (formerly known as DNA homology group IIA), in line with a previous classification of mosquito pathogenic native strains [6].

Lysinibacillus sphaericus CBAM5 was isolated from drilling mineral base oil samples (CBAM by its acronym in Spanish), collected in the Eastern Planes region in Colombia. The strain was described as an aerobic, motile, catalase positive, Gram variable rod [10]. *L. sphaericus* CBAM5 is able to grow in acetate as sole carbon source, but not in glucose (Table 1, Additional file 1: Table S1). Spherical terminal spores within swollen sporangia were observed under light microscopy (Additional file 2: Figure S1). By scanning electron microscopy, *L. sphaericus* CBAM5 is estimated to measure 0.52 to 0.60 μm in width and 2.12 to 3.11 μm long (Additional file 3: Figure S2). Cultures grow at 15 to 40°C over a pH range of 6.0 to 9.0. Antibiotic resistance was evaluated separately by adding filter sterilized antibiotic solutions in Luria-Bertani broths and checking turbidity after 15 hours of growth. *L. sphaericus* CBAM5 is sensitive to kanamycin (12.5 $\mu\text{g}/\text{mL}$), chloramphenicol (30 $\mu\text{g}/\text{mL}$), erythromycin (25 $\mu\text{g}/\text{mL}$), and gentamicin (15 $\mu\text{g}/\text{mL}$), while it showed resistance to trimethoprim/sulfamethoxazol up to 50 $\mu\text{g}/\text{mL}/250 \mu\text{g}/\text{mL}$.

Genome sequencing information

Genome project history

The genome sequencing of *Lysinibacillus sphaericus* CBAM5 was supported by the CIMIC (Centro de

Investigaciones Microbiológicas) laboratory at the University of Los Andes within the Grant (1204-452-21129) of the Instituto Colombiano para el Fomento de la Investigación Francisco José de Caldas. Whole genomic DNA extraction and bioinformatics analysis was performed at CIMIC laboratory, whereas libraries construction and whole shotgun sequencing at the Beijing Genome Institute (BGI) Americas Laboratory (Tai Po, Hong Kong). The applied pipeline included quality check of reads, de novo assembly, a gap-filling step and mapping against a reference genome. This whole genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession AYKQ00000000. The version described in this paper is the first version, AYKQ01000000. A summary of the project information is shown in Table 2.

Growth conditions and DNA isolation

Lysinibacillus sphaericus strain CBAM5 was grown in nutrient broth for 16 hours at 30°C and 150 rev/min. High molecular weight DNA was isolated using the EasyDNA® Kit (Carlsbad, CA, USA. Invitrogen) as indicated by the manufacturer. DNA purity and concentration were determined in a NanoDrop spectrophotometer (Wilmington, DE, USA. Thermo Scientific).

Genome sequencing and assembly

After DNA extraction, samples were sent to the Beijing Genome Institute (BGI) Americas Laboratory (Tai Po, Hong Kong). Purified DNA sample was first sheared into smaller fragments with a desired size by a Covaris E210 ultrasonicator. Then the overhangs resulting from fragmentation were converted into blunt ends by using T4 DNA polymerase, Klenow Fragment and T4 polynucleotide kinase. After adding an "A" base to the 3' end of the blunt phosphorylated DNA fragments, adapters were ligated to the ends of the DNA fragments. The desired fragments were purified through gel-electrophoresis, then selectively enriched and amplified by PCR. The index tag was introduced into the adapter at the PCR stage as appropriate, and a library quality test was performed. Lastly, qualified, short, paired-ends of 90:90 bp length with 500 bp insert libraries were used to cluster preparation and to conduct whole-shotgun sequencing in Illumina Hi-Seq 2000 sequencers.

Using the FASTX-Toolkit version 0.6.1 [31] and clean_reads version 0.2.3 from the ngs_backbone pipeline [32] reads were trimmed and quality filtered. Four preliminary assemblies were obtained by using: SOAPdenovo version 2.04 [33], Velvet version 1.2.10 [34], ABySS version 1.3.7 [35], and CLC Assembly Cell version 4.0.10 [36]. Those assemblies were integrated in the CISA pipeline resulting in a consensus assembly [37]. SOAPdenovo and CLC Assembly Cell packages included automatic scaffolding and k-mer/overlapping

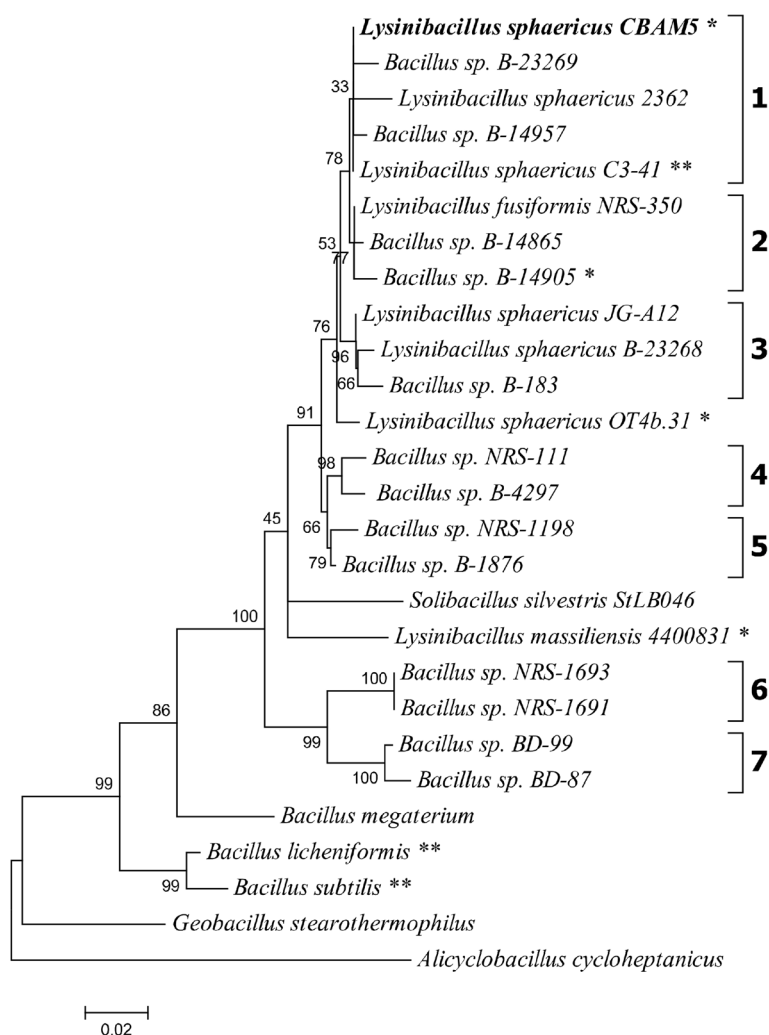


Figure 1 Phylogenetic tree highlighting the position of *Lysinibacillus sphaericus CBAM5*. Phylogenetic analyses included available type strains and other non-assigned species within the families *Alicyclobacillaceae* and *Bacillaceae*. Right brackets encompass each homology group (1–7) according to Nakamura’s benchmarks [15]. Nucleotide sequences obtained from GenBank and used in the phylogenetic analyses were as follows: *Alicyclobacillus cycloheptanicus* 1457 (X51928), *Geobacillus stearothermophilus* 10 (X57309), *Bacillus subtilis* 168^T (X60646), *Bacillus licheniformis* DSM 13^T (X68416), *Bacillus megaterium* IAM 13418^T (D16273), *Bacillus sp. BD-87* (AF169520), *Bacillus sp. BD-99* (AF169525), *Bacillus sp. NRS-1691* (AF169531), *Bacillus sp. NRS-1693* (AF169533), *Solibacillus silvestris* StLB046 (NR_074954), *Lysinibacillus massiliensis* 4400831 (NR_043092), *Bacillus sp. B-1876* (AF169494), *Bacillus sp. NRS-1198* (AF169528), *Bacillus sp. B-4297* (AF169507), *Bacillus sp. NRS-111* (AF169526), *Lysinibacillus sphaericus* OT4b.31 (JQ744623), *Lysinibacillus sphaericus* B-23268^T (AF169495), *Bacillus sp. B-183* (AF169493), *Lysinibacillus sphaericus* JG-A12 (AM292655), *Bacillus sp. B-14905* (AF169491), *Bacillus sp. B-14865* (AF169490), *Lysinibacillus fusiformis* NRS-350 (AJ310083), *Lysinibacillus sphaericus* C3-41 (CP000817:16818–18361), *Bacillus sp. B-14957* (AF169492), *Lysinibacillus sphaericus* 2362 (L14011), *Bacillus sp. B-23269* (AF169496), *Lysinibacillus sphaericus* CBAM5 (KK037167:893906–895445). The tree with the highest log likelihood (−6732.2703) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Lineages with type strain genome sequencing projects registered in GOLD [57] are labeled with one asterisk, those also listed as ‘Complete and Published’ with two asterisks.

optimization steps. To obtain structural insight of a chromosomal scaffold, we used CONTIGuator.2 [38], using the *Lysinibacillus sphaericus* strain C3-41 chromosome (accession number: CP000817.1) as reference. Some gaps were successfully filled by using GapFiller [39]. Gap-filling steps were applied over each one of the preliminary assemblies and over the final consensus assembly. Quality assessment of the assembly was performed with iCORN

[40]. The error rate of the final assembly is less than 1 in 1,000,000 bp. We compared the chromosomal assembly of *L. sphaericus* CBAM5 with the chromosome sequences of *L. sphaericus* C3-41 and *L. sphaericus* OT4b.31 by maximal unique matching of translated sequences with PROmer [41], and a read mapping single nucleotide polymorphism (SNP) effect analysis with SnpEff package [42].

Table 1 Classification and general features of *Lysinibacillus sphaericus* CBAM5 according to the MIGS recommendations [19]

MIGS ID	Property	Term	Evidence code ^a
	Current classification	Domain <i>Bacteria</i>	TAS [20]
		Phylum <i>Firmicutes</i>	TAS [21-23]
		Class <i>Bacilli</i>	TAS [24,25]
		Order <i>Bacillales</i>	TAS [26,27]
		Family <i>Bacillaceae</i>	TAS [26,28]
		Genus <i>Lysinibacillus</i>	TAS [13,29]
		Species <i>Lysinibacillus sphaericus</i>	TAS [11,13]
		Strain CBAM5	TAS [10]
	Gram stain	Positive in vegetative cells, variable in sporulating stages	IDA
	Cell shape	Straight rods	IDA
	Motility	Motile	IDA
	Sporulation	Sporulating	IDA
	Temperature range	15 – 40°C	IDA
	Optimum temperature	30°C	IDA
	Carbon source	Complex carbohydrates	TAS [10]
	Energy metabolism	Heterotroph	TAS [10]
MIGS-6	Habitat	Subsurface soil	TAS [10]
MIGS-6.3	Salinity	Growth in Luria-Bertani broth (5% NaCl)	IDA
MIGS-22	Oxygen requirement	Aerobic	TAS [10]
MIGS-15	Biotic relationship	Free living	TAS [10]
MIGS-14	Pathogenicity	Pathogenic toward <i>Culex quinquefasciatus</i> larvae	TAS [6]
MIGS-4	Geographic location	Eastern Planes oil basins, Colombia	TAS [10]
MIGS-5	Sample collection time	January 2005	TAS [10]
MIGS-4.1	Latitude	5.0121944	TAS [10]
MIGS-4.2	Longitude	-72.7109167	TAS [10]
MIGS-4.3	Depth	20 m	TAS [10]
MIGS-4.4	Altitude	350 m above sea level	TAS [10]

^aEvidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [30].

Genome annotation

The Glimmer 3 gene finder was used to identify and extract sequences for potential coding regions. To achieve the functional annotation steps, the RAST server [43] and Blast2GO pipelines [44] were used. Blast2GO performed the blasting, GO-mapping and annotation steps; which included a description according to the ProDom,

Table 2 Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Improved high-quality draft
MIGS-28	Libraries used	One paired end tags 90:90 bp with 500 bp insert
MIGS-29	Sequencing platforms	Illumina Hi-Seq 2000
MIGS-31.2	Fold coverage	100x
MIGS-30	Assemblers	CISA version 1.3, SOAPdenovo version 2.04, Velvet version 1.2.10, ABySS version 1.3.7, CLC Assembly Cell version 4.0.10
MIGS-32	Gene calling method	Glimmer3, tRNAscan-SE, RNAmmer
	Genbank ID	AYKQ00000000
	Genbank Date of Release	February 1, 2014
	GOLD ID	GI0057485
	Project relevance	Biotechnology, metabolic pathway

FingerPRINTScan, PIR-PSD, Pfam, TIRGFam, PROSITE, ProDom, SMART, SuperFamily, Pattern, Gene3D, PANTHER, SignalIP and TM-HMM databases. The results were summarized with InterPro [45]. Additionally, a GO-EnzymeCode mapping step was used to retrieve KEGG pathway-maps. tRNA genes were identified by using tRNAscan-SE [46] and rRNA genes by using RNAmmer [47]. The possible orthologs of the genome were identified based on the COG database and classified accordingly [48]. Prophage region prediction was also conducted by using the PHAST tool [49].

Genome properties

The genome summary and statistics are provided in Tables 3 and 4, and Figure 2. The genome consists of 60 scaffolds in 5,146,656 bp total size with a GC content of 37.19%. A total of 19 scaffolds were successfully aligned to a reference sequence, comprising 4,610,292 bp of sequence and are represented by the red and blue bars within the outer ring of Figure 2. Of the 5,620 genes predicted, 5,209 were protein-coding genes and 207 RNAs were identified. Genes assigned a putative function comprised 57.37% of the protein-coding genes while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 5.

Table 3 Summary of genome

Label	Size (bp)	Topology	INSDC identifier
Chromosomal scaffold	4,610,292	Circular	KK037167.1
Extrachromosomal elements	536,364	Linear	KK037168.1-KK037224.1

Table 4 Nucleotide content and gene count levels of the genome

Attribute	Value	% of total ^a
Genome size (bp)	5,146,656	100.00
DNA GC content (bp)	1,913,947	37.19
DNA coding region (bp)	4,311,603	83.77
Number of replicons	1	
Total genes	5,620	100
RNA genes	207	3.68
tRNA genes	180	3.20
Protein-coding genes	5,209	92.69
Genes in paralog clusters	151	2.69
Genes assigned to COGs	3701	65.85
1 or more conserved domains	2,520	44.84
2 or more conserved domains	834	14.84
3 or more conserved domains	361	6.42
Genes with function prediction	3,224	57.37
Genes assigned Pfam domains	3,995	71.09
Genes with signal peptides	459	8.17
Genes with transmembrane helices	1,140	20.28
CRISPR repeats	1	

^aThe total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

Insights into the genome

We propose a 19-supercontig chromosomal scaffold of *Lysinibacillus sphaericus* CBAM5 with 4.61 Mbp in length, corresponding to a 99.4% of the reference chromosomal sequence. The remaining non-mapped or non-integrated contigs were aligned to plasmid reference sequences, leading to no significant coverage levels (data not shown). Then, we assigned those contigs as a set of potential extra-chromosomal elements. Chromosomal comparison from the PROmer analysis between *L. sphaericus* strains CBAM5 and C3-41 showed that most of the two chromosomes mapped onto each other, revealing large segments of high similarity (Figure 3). In contrast, the comparison between the native strains *L. sphaericus* CBAM5 and OT4b.31 revealed scattered regions across the dot-plot, corresponding to low coverage levels and different syntenial arrangements. Only variants with a phred-scaled quality and depth coverage scores greater than 100 were considered valid for the SNV analysis. We found 378 variants corresponding to 4531 effects being classified as follows: 170 insertions, 280 deletions, 2020 downstream effects, 182 frame shifts, 211 intergenic effects, 2 start losts, 2 stop losts and 2114 upstream effects. In addition, no transitions, transversions, missense or silent effects were identified. As per most of the variant effects, in comparison to the C3-41 strain, are allocated upstream and downstream of the gene operons, we suggest that *L. sphaericus* CBAM5 may enclose different regulatory elements or non-coding sequences.

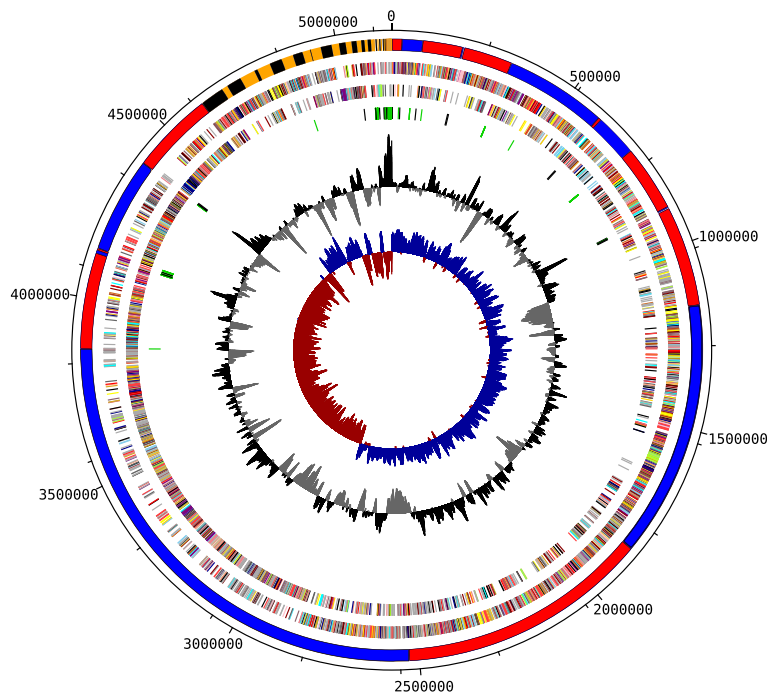


Figure 2 Graphical map of the genome. From outside to the center: Ordered and oriented scaffolds assigned to chromosome in blue and red, extrachromosomal scaffolds in orange and black, Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs gray), GC content and GC skew.

Table 5 Number of genes associated with the 25 general COG functional categories

Code	Value	% age ^a	Description
J	190	3.65	Translation
A	1	0.02	RNA processing and modification
K	337	6.47	Transcription
L	193	3.71	Replication, recombination and repair
B	2	0.04	Chromatin structure and dynamics
D	39	0.75	Cell cycle control, mitosis and meiosis
V	66	1.27	Defense mechanisms
T	191	3.67	Signal transduction mechanisms
M	153	2.94	Cell wall/membrane biogenesis
N	79	1.52	Cell motility
U	23	0.44	Intracellular trafficking and secretion
O	116	2.23	Posttranslational modification, protein turnover, chaperones
C	162	3.11	Energy production and conversion
G	154	2.96	Carbohydrate transport and metabolism
E	396	7.60	Amino acid transport and metabolism
F	111	2.13	Nucleotide transport and metabolism
H	167	3.21	Coenzyme transport and metabolism
I	141	2.71	Lipid transport and metabolism
P	222	4.26	Inorganic ion transport and metabolism
Q	37	0.71	Secondary metabolites biosynthesis, transport and catabolism
R	480	9.21	General function prediction only
S	441	8.47	Function unknown
-	1508	28.95	Not in COGs

^aThe total is based on the total number of protein coding genes in the annotated genome.

Chromosome structure

The origin of replication of the chromosome of *L. sphaericus* CBAM5 was estimated by similarities to several features of the corresponding regions in *L. sphaericus* C3-41, *Bacillus* sp. B-14905 and other close related bacteria, including colocalization of the genes: *dnaA*, *dnaN*, *dnaX*, *recR* and *recF*; and GC nucleotide skew $[(G-C)/(G+C)]$ analysis. In the contig 19 (EWH31640:EWH31645) we found a typical cluster consisting of *dnaA*, *dnaN*, *recF*, *gyrA* and *gyrB* boxes. The predicted genes *dnaB*, *dnaI*, *dnaG*, *dnaE*, *holA*, *holB*, *priAB*, *polA* and *recA* were also found spread in the chromosomal and extrachromosomal sequences. The replication termination site of the chromosomal scaffold is believed to be localized near 2.92 Mbp in the contig 14. According to GC skew analysis, the coding bias for the two strands of the chromosome is for the majority of CDSs to be on the outer strand from 0 to ~2.92 Mbp, and on the inner strand from ~2.92 Mbp to the end of the chromosomal scaffold (contig 19, Figure 2). This was also confirmed by the presence of *parC* (EWH32537)

and *parE* (EWH32538), which encode the subunits of the chromosome-partitioning enzyme topoisomerase IV. Similar to previous reports [50,51], we did not find the homolog of *rtp* (replication terminator protein-encoding gene) in the chromosomal assembly of CBAM5.

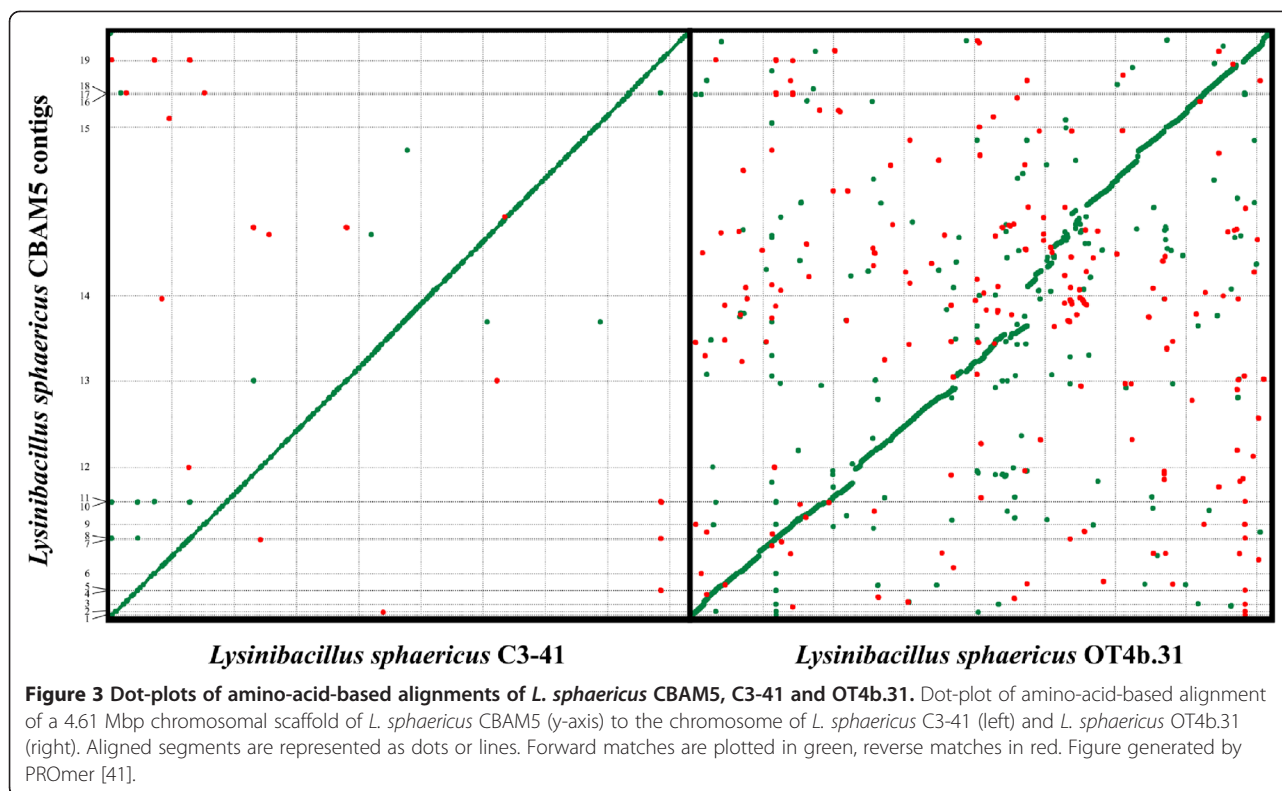
Mobile elements

Lysinibacillus sphaericus CBAM5 displays 28 CDSs annotated as transposases, including three allocated in the extrachromosomal sequences. The most frequent families are IS1182, IS3 and IS4. In addition, four incomplete prophage regions were identified as follows: *Thermus* phage ϕ OH2 (contig 12), *Burkholderia* phage ST79 (contig 14), and two regions comprising the *Clostridium* phage ϕ SM101 (contigs 14 and 28). Prophage regions ϕ OH2 and ST79 included putative encoding sequences for tail, lysis and baseplate proteins. None of the reported phages has been described in the Colombian strain *L. sphaericus* OT4b.31 [50].

Larvicidal toxins

The genome of *L. sphaericus* CBAM5 shows a wide repertoire of potential encoding sequences in terms of mosquito-cidal toxins. In the contig 11, we found Mtx1 (EWH35097) and Mtx2 (EWH35034) CDSs located in an identical cluster as Hu et al. [51] described in the genome of *L. sphaericus* C3-41. This cluster includes two insertion sequences, one of them consisting of a disrupted transposase between the *mtx1* and *mtx2*, as well. One Mtx3 CDSs (EWH32377) was found in contig 14. Upstream of this sequence, we could identify some IS3 family mobile elements and putative DeoR family transcriptional regulators. In addition, we found one hypothetical toxin from the family Mtx2 (PFam PF03318) in contig 11 (EWH35106) and a putative cereolysin O CDS (EWH31995) being described to be active against the German cockroach *Blattella germanica* [52] in contig 15.

The binary toxin genes *binA* (EWH32662) and *binB* (EWH32663) of *L. sphaericus* CBAM5, which are the main source of its larvicidal activity [51], were found in the contig 14 following a similar arrangement as the 35-kb duplicate cluster of *L. sphaericus* C3-41 (Figure 4). Nearby the *binA* and *binB* genes, we found a putative Mtx2/3 toxin (EWH32665), two CDSs for phage integrases in the 5' start of the 35-kb fragment. *L. sphaericus* CBAM5 also share a germination gene cluster equivalent to the *B. anthracis* plasmid pXO1 and the BinA/BinB cluster of *L. sphaericus* C3-41, having a GerXB-KA-XC gene cluster upstream of a transposase [51,53]. Comparing the region comprised between the germination operon and the *binA-binB* genes across the sequences of *L. sphaericus* CBAM5, C3-41 and 2297, we found an equivalent homology of putative transposases with different length and disruption points. The strain CBAM5 has two

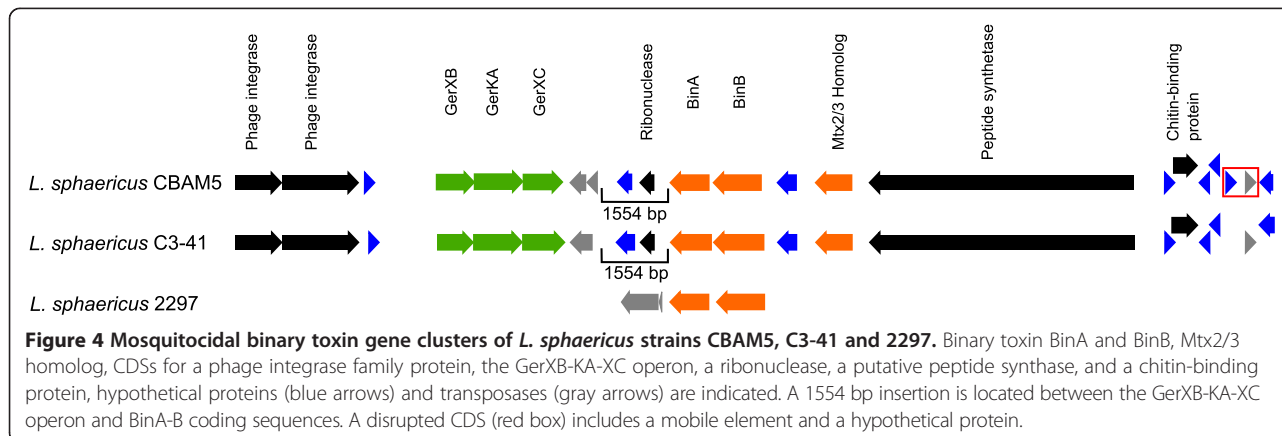


mobile elements of 459 and 312 bp in length, which is similar to strains 2297 and CBAM5 showing a probable transposase pseudogene with 1,110 bp and 591 bp in length, respectively (Figure 4). As a final remark, in the 3' end of the 35-kb fragment we found an incomplete encoding sequence for β -carotene 15,15'-monooxygenase probably disrupted by a mobile element (depicted with a red box in Figure 4). Hu et al. [51] hypothesized that the conserved 35-kb sequence, including the BinA, BinB, and the two phage integrase family proteins, are probably unique to the taxonomic *L. sphaericus* group 1 (formerly known as group IIA) being the remnant of a potential phage infection. Even though we cannot confirm the presence of

additional BinA-B CDS sequences in the genome of *L. sphaericus* CBAM5, we suggest further research to confirm the participation of phage infections on the evolution of larvicidal toxins in the strain CBAM5.

Surface (S) layer proteins and toxic metal resistances

L. sphaericus CBAM5 exhibits 21 CDSs described as surface (S) layer proteins or S-layer homologs in its genome. The fragment covered from EWH35069 to EWH35072 includes four CDSs encoding for a variable protein, a putative S-layer associated protein, a P60 invasion-associated protein and a N-acetylmuramoyl-L-alanine amidase. Probably the genes located in this fragment may participate in



the larvicidal activity of the strain CBAM5, given that the same genes have been described as differentially expressed in virulent infections of *Lysteria monocytogenes* [54]. A total of 14 CDSs show three SLHs motifs near to the N terminal region, similarly to the *slpC* gene previously described in native strains [5]. In addition, we found two S-layer surface array proteins in the chromosomal scaffold and another in extrachromosomal sequences.

A total of 64 CDSs corresponded to encoding sequences involved in responses against toxic metal(oid)s. Among those coding sequences, we found the following operons: *arsRBCDA*, *arsRBC*, *cadCA*, *mntABCD*, *nikABCDE*, *terD-terD-terD*, *zurR-znuBC*, and *czrA-czcD-csoR-copZA*. We could identify various genes probably involved in metal (loid) resistances spread across the genome (Additional file 4: Table S2). The *chrA* gene seems to be the only representative of the *chr* operon in the genome of *L. sphaericus* CBAM5. Previous reports have shown that microorganisms bearing *chrA* homologues display highly variable resistance levels against Cr(VI) [55]. However, two superoxide dismutase putative proteins (EWH33050, EWH30224) and several CDS ascribed as flavin reductases (EC 1.5.1.29), nitroreductases (EC 1.5.1.34) and quinone reductases (EC 1.1.5.4) could cooperate in the Cr(VI) resistance, in agreement with previous reports [55,56].

Given the heavy metal resistance of *L. sphaericus* CBAM5 in polluted environments, and supported by the identification of genes in Additional file 4: Table S2, we could expect the assistance of efflux pump systems and heavy-metal resistance proteins specific to As, Sb, Ni, Zn, Cu, Cd, Te, Cr, Mn and Co. By the evaluation of coalescent models, Villegas-Torres et al. [10] proposed that *L. sphaericus* CBAM5 may have acquired the *arsC* gene through recent events of horizontal gene transfer as a possible adaptation to polluted environments. However, we found highly similar homologues of heavy metal resistance proteins of the CBAM5 strain in microorganisms isolated from non-polluted environments (i.e. *czrA-czcD-csoR-copZA*, *cadCA*, and *arsRBC* in *L. sphaericus* OT4B.31 [50]). Further analysis on plasmids, prophage content, or conjugation factors may clarify the origin of resistance (as well as larvicidal) genes. Finally, based in the KEGG analysis, some predicted proteins might participate in peripheral pathways for the degradation of geraniol, chlorocyclohexane, chlorobenzene, benzoate, bisphenol, fluorobenzoate, toluene, chloroalkane, chloroalkene, naphthalene, aminobenzoate, styrene, atrazine, limonene and pinene.

Conclusions

Lysinibacillus sphaericus CBAM5 was isolated from drilling mineral base oil samples at the subsurface soil level. By comparing the chromosomal sequences between *L. sphaericus* strains CBAM5 and C3-41, we identified

distinctive similarities of the DNA homology group IIA. The evidence of the binary toxins allocated in a conserved cluster delimited by mobile elements, resembles a probable phage invasion in the DNA subgroup IIA of the *Lysinibacillus sphaericus* species. Along with the biological control potential given by the Mtx, Bin and cerolysin toxins, *L. sphaericus* CBAM5 displays encoding sequences for S-layer proteins and heavy-metal efflux pumps, which may confer resistance to As, Sb, Ni, Zn, Cu, Cd, Te, Cr, Mn and Co in polluted environments.

Additional files

Additional file 1: Table S1. Associated record according to the MIGS recommendations.

Additional file 2: Figure S1. Light microscopy of *Lysinibacillus sphaericus* CBAM5 growth in acetate broth. (A) Gram staining of vegetative cells after 6 hours of growth. (B) Schaeffer-Fulton staining of sporulating culture after 24 hours of growth.

Additional file 3: Figure S2. Scanning electron micrograph of *Lysinibacillus sphaericus* CBAM5. The micrograph was obtained on a JEOL JSM-5800LV (Japan) scanning electron microscope at an operating voltage of 20 kV and 10000x magnifications.

Additional file 4: Table S2. Genes possibly involved in metal(loid) resistances identified in the genome sequence of *Lysinibacillus sphaericus* CBAM5.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LL performed the DNA and sequencing experiments. TDP performed antibiotics, microscopy and bioinformatics analysis. All authors drafted, read and approved the final manuscript.

Acknowledgements

This work was performed under the auspices of the Grant 1204-452-21129 from the Instituto Colombiano para el fomento de la Investigación Francisco José de Caldas – Colciencias, the Research Fund from the Faculty of Sciences at Universidad de los Andes, and the Centro de Investigaciones Microbiológicas (CIMIC).

Received: 12 June 2014 Accepted: 21 November 2014

Published: 20 January 2015

References

1. Berry C. The bacterium, *Lysinibacillus sphaericus*, as an insect pathogen. *J Invertebr Pathol.* 2012; **109**:1–10.
2. Thanabalu T, Porter AG. Efficient expression of a 100-kilodalton mosquitocidal toxin in protease-deficient recombinant *Bacillus sphaericus*. *Appl Environ Microbiol.* 1995; **61**:4031–6.
3. Baumann P, Clark MA, Baumann L, Broadwell AH. *Bacillus sphaericus* as a mosquito pathogen: properties of the organism and its toxins. *Microbiol Rev.* 1991; **55**:425–36.
4. Jones GW, Nielsen-LeRoux C, Yang Y, Yuan Z, Dumas VF, Monnerat RG, Berry C. A new Cry toxin with a unique two-component dependency from *Bacillus sphaericus*. *FASEB J.* 2007; **21**:4112–20.
5. Lozano LC, Ayala JA, Dussán J. *Lysinibacillus sphaericus* S-layer protein toxicity against *Culex quinquefasciatus*. *Biotechnol Lett.* 2011; **33**:2037–41.
6. Lozano LC, Dussán J. Metal tolerance and larvicidal activity of *Lysinibacillus sphaericus*. *World J Microbiol Biotechnol.* 2013; **29**:1383–9.
7. Tuzen M, Uluozlu OD, Usta C, Soylak M. Biosorption of copper(II), lead(II), iron(III) and cobalt(II) on *Bacillus sphaericus*-loaded Diaion SP-850 resin. *Anal Chim Acta.* 2007; **581**:241–6.

8. Pollmann K, Raff J, Merroun M, Fahmy K, Selenska-Pobell S. **Metal binding by bacteria from uranium mining waste piles and its technological applications.** *Biotechnol Adv.* 2006; **24**:58–68.
9. Velásquez L, Dussan J. **Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*.** *J Hazard Mater.* 2009; **167**:713–6.
10. Villegas-Torres MF, Bedoya-Reina OC, Salazar C, Vives-Florez MJ, Dussan J. **Horizontal *arsC* gene transfer among microorganisms isolated from arsenic polluted soil.** *Int Biodeterior Biodegradation.* 2011; **65**:147–52.
11. Claus D, Berkeley RCW. **Genus *Bacillus* Cohn 1872, 174AL.** In: Sneath PHA, Mair NS, Sharpe ME, Holt JG, editors. *Bergey's Man Syst Bacteriol. Volume 2.* Baltimore: The Williams and Wilkins Co; 1986: p. 1105–39.
12. White PJ, Lotay HK. **Minimal nutritional requirements of *Bacillus sphaericus* NCTC9602 and 26 other strains of this species: the majority grow and sporulate with acetate as sole major source of carbon.** *J Gen Microbiol.* 1980; **118**:13–9.
13. Ahmed I, Yokota A, Yamazoe A, Fujiwara T. **Proposal of *Lysinibacillus boronitolersans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov.** *Int J Syst Evol Microbiol.* 2007; **57**:1117–25.
14. Krych VK, Johnson JL, Yousten AA. **Deoxyribonucleic acid homologies among strains of *Bacillus sphaericus*.** *Int J Syst Bacteriol.* 1980; **30**:476–84.
15. Nakamura LK. **Phylogeny of *Bacillus sphaericus*-like organisms.** *Int J Syst Evol Microbiol.* 2000; **50**:1715–22.
16. Nakamura LK, Shida O, Takagi H, Komagata K. ***Bacillus pycnus* sp. nov. and *Bacillus neidei* sp. nov., round-spored bacteria from soil.** *Int J Syst Evol Microbiol.* 2002; **52**(Pt 2):501–5.
17. Tamura K, Nei M. **Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees.** *Mol Biol Evol.* 1993; **10**:512–26.
18. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. **MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.** *Mol Biol Evol.* 2013; **30**:2725–9.
19. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, Ashburner M, Axelrod N, Baldauf S, Ballard S, Boore J, Cochrane G, Cole J, Dawyndt P, De Vos P, DePamphilis C, Edwards R, Faruque N, Feldman R, Gilbert J, Gilna P, Glöckner FO, Goldstein P, Guralnick R, Haft D, Hancock D, et al. **The minimum information about a genome sequence (MIGS) specification.** *Nat Biotechnol.* 2008; **26**:541–7.
20. Woese CR, Kandler O, Wheelis ML. **Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya.** *Proc Natl Acad Sci.* 1990; **87**:4576–9.
21. Gibbons NE, Murray RGE. **Proposals Concerning the Higher Taxa of Bacteria.** *Int J Syst Bacteriol.* 1978; **28**:1–6.
22. Garrity GM, Holt JG. **The Road Map to the Manual.** In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's Man Syst Bacteriol. Volume 1.* Second ed. New York: Springer; 2001: p. 119–69.
23. Murray RGE. **The Higher Taxa, or, a Place for Everything...?** In: Holt JG, editor. *Bergey's Man Syst Bacteriol. Volume 1.* First ed. Baltimore: The Williams and Wilkins Co; 1984: p. 31–4.
24. Ludwig W, Schleifer KH, Whitman WB, Krieg NR, Ludwig W, Rainey EA, Schleifer KH, Withman WB. **Class I. Bacilli Class Nov.** In: De Vos P, Garrity GM, Jones D, editors. *Bergey's Manual of Systematic Bacteriology. In: Vol 3 (The Firmicutes). Second. Volume 3.* Dordrecht, Heidelberg, London, New York: Springer; 2009: p. 19–20.
25. **List of new names and new combinations previously effectively, but not validly, published.** *Int J Syst Evol Microbiol.* 2010; **60**:469–72.
26. Skerman VBD, McGowan V, Sneath PHA. **Approved Lists of Bacterial Names.** *Int J Syst Bacteriol.* 1980; **30**:225–420.
27. Prévot AR. *Hauduroy P, Ehringer G, Guillot G, Magrou J, Prévot AR, Rosset, Urbain A (eds) Dictionnaire Des Bactéries Pathogènes.* 2nd ed. Paris: Masson; 1953: p. 1–692.
28. Fischer A. **Untersuchungen über bakterien.** *Jahrbücher für Wissenschaftliche Bot.* 1895; **27**:1–163.
29. Jung MY, Kim J-S, Paek WK, Styrak I, Park I-S, Sin Y, Paek J, Park KA, Kim H, Kim HL, Chang Y-H. **Description of *Lysinibacillus sinduriensis* sp. nov., and transfer of *Bacillus massiliensis* and *Bacillus odyseeyi* to the genus *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odyseeyi* co.** *Int J Syst Evol Microbiol.* 2012; **62**:2347–55.
30. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Isel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. **Gene Ontology: tool for the unification of biology.** *Nat Genet.* 2000; **25**:25–9.
31. **FASTX-Toolkit.** http://hannonlab.cshl.edu/fastx_toolkit/.
32. Blanca JM, Pascual L, Ziarolo P, Nuez F, Cañizares J. **ngs_backbone: a pipeline for read cleaning, mapping and SNP calling using Next Generation Sequence.** *BMC Genomics.* 2011; **12**.
33. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. **De novo assembly of human genomes with massively parallel short read sequencing.** *Genome Res.* 2010; **20**:265–72.
34. Zerbino DR, Birney E. **Velvet: Algorithms for de novo short read assembly using de Bruijn graphs.** *Genome Res.* 2008; **18**:821–9.
35. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. **ABYSS: A parallel assembler for short read sequence data.** *Genome Res.* 2009; **19**:1117–23.
36. **CLC Genomics Workbench.** <http://www.clcbio.com/products/clc-genomics-workbench/>.
37. Lin S-H, Liao Y-C. **CISA: contig integrator for sequence assembly of bacterial genomes.** *PLoS One.* 2013; **8**:e60843.
38. Galardini M, Biondi EG, Bazzicalupo M, Mengoni A. **CONTIGuator: a bacterial genomes finishing tool for structural insights on draft genomes.** *Source Code Biol Med.* 2011; **6**.
39. Nadalin F, Vezzi F, Policriti A. **GapFiller: a de novo assembly approach to fill the gap within paired reads.** *BMC Bioinformatics.* 2012; **13**(Suppl 14):S8.
40. Otto TD, Sanders M, Berriman M, Newbold C. **Iterative Correction of Reference Nucleotides (iCORN) using second generation sequencing technology.** *Bioinformatics.* 2010; **26**:1704–7.
41. Delcher AL, Phillippy A, Carlton J, Salzberg SL. **Fast algorithms for large-scale genome alignment and comparison.** *Nucleic Acids Res.* 2002; **30**:2478–83.
42. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. **A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3.** *Fly.* 2012; **6**:80–92.
43. Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formsma K, Gerdes S, Glass E, Kubal M, Meyer F, Olsen G, Olson R, Osterman A, Overbeek R, McNeil L, Paarmann D, Paczian T, Parrello B, Pusch G, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. **The RAST Server: Rapid Annotations using Subsystems Technology.** *BMC Genomics.* 2008; **9**.
44. Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A. **High-throughput functional annotation and data mining with the Blast2GO suite.** *Nucleic Acids Res.* 2008; **36**:3420–35.
45. Apweiler R, Attwood TK, Bairoch A, Bateman A, Birney E, Biswas M, Bucher P, Cerutti L, Corpet F, Croning MDR, Durbin R, Falquet L, Fleischmann W, Gouzy J, Hermjakob H, Hulo N, Jonassen I, Kahn D, Kanapin A, Karavidopoulou Y, Lopez R, Marx B, Mulder NJ, Oinn TM, Pagni M, Servant F, Sigrist CJA, Zdobnov EM. **The InterPro database, an integrated nucleotide resource for protein families, domains and functional sites.** *Nucleic Acids Res.* 2001; **29**:37–40.
46. Lowe TM, Eddy SR. **tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence.** *Nucleic Acids Res.* 1997; **25**:955–64.
47. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. **RNAmmer: consistent and rapid annotation of ribosomal RNA genes.** *Nucleic Acids Res.* 2007; **35**:3100–8.
48. Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV. **The COG database: new developments in phylogenetic classification of proteins from complete genomes.** *Nucleic Acids Res.* 2001; **29**:22–8.
49. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. **PHAST: A Fast Phase Search Tool.** *Nucleic Acids Res.* 2011.
50. Peña-Montenegro TD, Dussan J. **Genome sequence and description of the heavy metal tolerant bacterium *Lysinibacillus sphaericus* strain OT4b.31.** *Stand Genomic Sci.* 2013; **9**:42–56.
51. Hu X, Fan W, Han B, Liu H, Zheng D, Li Q, Dong W, Yan J, Gao M, Berry C, Yuan Z. **Complete genome sequence of the mosquitocidal bacterium *Bacillus sphaericus* C3-41 and comparison with those of closely related *Bacillus* species.** *J Bacteriol.* 2008; **190**:2892–902.
52. Nishiwaki H, Nakashima K, Ishida C, Kawamura T, Matsuda K. **Cloning, functional characterization, and mode of action of a novel insecticidal pore-forming toxin, sphaericolysin, produced by *Bacillus sphaericus*.** *Appl Environ Microbiol.* 2007; **73**:3404–11.
53. Okinaka RT, Cloud K, Hampton O, Hoffmaster AR, Hill KK, Keim P, Koehler TM, Lamke G, Kumano S, Mahillon J, Manter D, Martinez Y, Ricke D,

- Svensson R, Jackson PJ. Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. *J Bacteriol.* 1999; **181**:6509–15.
54. Camejo A, Buchrieser C, Couvé E, Carvalho F, Reis O, Ferreira P, Sousa S, Cossart P, Cabanes D. In vivo transcriptional profiling of *Listeria monocytogenes* and mutagenesis identify new virulence factors involved in infection. *PLoS Pathog.* 2009; **5**:e1000449.
55. Viti C, Marchi E, Decorosi F, Giovannetti L. Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. *FEMS Microbiol Rev.* 2014; **38**:633–59.
56. He M, Li X, Liu H, Miller SJ, Wang G, Rensing C. Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain *Lysinibacillus fusiformis* ZC1. *J Hazard Mater.* 2011; **185**:682–8.
57. Liolios K, Chen I-MA, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, Kyrpides NC. The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res.* 2010; **38**(suppl 1):D346–54.

doi:10.1186/1944-3277-10-2

Cite this article as: Peña-Montenegro *et al.*: Genome sequence and description of the mosquitocidal and heavy metal tolerant strain *Lysinibacillus sphaericus* CBAM5. *Standards in Genomic Sciences* 2015 **10**:2.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

