Data in Brief

Complete genome sequencing and comparative genomic analysis of functionally diverse *Lysinibacillus sphaericus* III(3)7

Andrés Rey, Laura Silva-Quintero, Jenny Dussán *

Centro de Investigaciones Microbiológicas (CIMIC), Universidad de los Andes, Bogotá D.C., Cundinamarca, Colombia

**Abstract**

*Lysinibacillus sphaericus* III(3)7 is a native Colombian strain, the first one isolated from soil samples. This strain has shown high levels of pathogenic activity against *Culex quinquefasciatus* larvae in laboratory assays compared to other members of the same species. Using Pacific Biosciences sequencing technology we sequenced, annotated (de novo) and described the genome of strain III(3)7, achieving a complete genome sequence status. We then performed a comparative analysis between the newly sequenced genome and the ones previously reported for Colombian isolates *L. sphaericus* OT4b.31, CBAM5 and OT4b.25, with the inclusion of *L. sphaericus* C3-41 that has been used as a reference genome for most of previous genome sequencing projects. We concluded that *L. sphaericus* III(3)7 is highly similar with strain OT4b.25 and shares high levels of synteny with isolates CBAM5 and C3-41.

**Keywords:**

*Lysinibacillus sphaericus*  
Complete genome sequencing  
Putative extrachromosomal elements  
Comparative genomics

1. Introduction

*Lysinibacillus sphaericus* is an aerobic, gram positive, spore-forming bacterium, widely used in biological control of vector-borne diseases like Malaria and Dengue, due to its highly lethal larvicidal action [1,2]. However, *L. sphaericus* is a versatile microorganism, which also has been described as either tolerant or resistant to several toxic metals such as arsenic, hexavalent chromium and lead. These toxic metals have been largely associated with oily sludge, the latter being a contamination problem of water sources and soils in developing countries like Colombia, and in general in countries where oil exploitation has a huge environmental impact [3].

Some of the strains have been reported to be highly toxic against some mosquito species like *Culex* sp., *Anopheles* sp. and *Aedes* sp. [4]. the larvicidal activity of *L. sphaericus* focuses on second and third instar larvae. Also there are some other insect species targeted by this action, including nematodes, grass shrimps, cockroaches, cutworms and hemipterans [2], nevertheless it has been reported that there are some species that are not affected by *L. sphaericus*. The first examples of insects resistant to *L. sphaericus* are honey bees, in which adult bees longevity and reproduction are not affected by its insecticidal effects [5]. Resistance to *L. sphaericus* is also found in beneficial species from sewage treatment plants [6], and toxic or pathogenic effects have been reported negative in eukaryotes like shrimps, fishes, birds and mammals [7,8]. The fact that *L. sphaericus* pathogenic effects are limited against insects such as *Culex* sp. and *Aedes* sp. is of major interest in biological control because it implies both ecological, environmental and public health safety in the widespread usage of *L. sphaericus* as an effective controller of vector borne diseases, specially in tropical countries like Colombia where endemic diseases such as Yellow fever, Dengue, Chikungunya and Zika represent a considerable public health issue [9].

There have been reports on multiple mechanisms that allow the larvicidal action in *L. sphaericus*, including several mosquitocidal and specifically larvicidal toxins expressed in vegetative or sporulation phases, at vegetative growth phase proteins like toxins from the Mtx1 and Mtx2 family, comprising the toxins Mtx2, Mtx3 and Mtx4 [2,10], also binary toxins BinA and BinB [2]. In addition the larvicidal activity of *L. sphaericus* has been reported when vegetative cells, spores and S-layer proteins are administered to larvae [11,12].

During sporulation, highly toxic strains produce a binary toxin composed of proteins BinA and BinB. First BinB binds to a receptor in epithelial midgut cells that allows BinA to enter the cell in order to cause cellular lysis [11]. On the other hand in vegetative cells, both high and low-toxicity strains produce the Mtx1, Mtx2 and Mtx3 toxins, however Mtx1 and Mtx2 proteins are degraded by proteases during the stationary growth phase, hence these proteins are not detectable when cultures undergo sporulation [13].

*Bacillus sphaericus* was reassigned to the genus *Lysinibacillus* due to both phylogenetic analyses and physiological differences [14]. *L. sphaericus* is a functionally heterogeneous species, being divided into five DNA homology groups. Pathogenic (mosquitocidal) strains are found in subgroup IIA, nevertheless this homology group also contains non-pathogenic isolates. Subgroup IIB has been allocated to
2. Materials and Methods

2.1. Bacterial strains and culture conditions

The L. sphaericus strain III(3)7 used in this study was previously isolated from soil samples in an oak forest near Bogotá D.C., Colombia, and belonged to the CIMIC Culture Collection, (Table 1)[28]. For this isolate we started from previously cultured nutritive agar plates, then it was incubated in nutrient broth at 30 °C, 150 rpm, until absorbance at 600 nm reached 0.9, which is equivalent to 1 × 10^9 UFC/mL (data not shown). This strain was chosen due to its considerably high levels of pathogenic activity in Culicidae larvae and its potential in toxic metal bioremediation processes [12,20].

2.2. DNA sample preparation

Genomic DNA was extracted and purified using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, K0721), using the standard protocol for Gram-Positive Bacteria Genomic DNA Purification with modifications in the lysis procedure extending incubation time with lysis buffer to 1 h and doubling the recommended lysozyme concentration. Identity of the DNA samples was confirmed by amplification of the 16S rRNA gene, then sequenced and compared to Ribosomal Database Project RDP [33] and NCBI databases. DNA samples were quantified using Qubit 2.0 fluorometer (Thermo Scientific) and Nanodrop 2000 spectrophotometer (Thermo Scientific) in order to fulfill sample quality requirements (quantity 10 μg, concentration: >50 ng/μg, <200 ng/μg).

2.3. Genome sequencing and assembly

DNA samples that met the quality requirements were sent to Genome Quebec (Montreal, Canada). Genomic DNA samples were sequenced using an exclusively PacBio based sequencing strategy (Pacific Biosciences RS II) and as we can observe in Table 2 a Large insert library strategy was used, this strategy targets 20 kb fragments which affects detection of small plasmids, in this case reported plasmids in L. sphaericus are high molecular weight, hence the effect should not be that drastic as to completely avoid plasmid detection.

### Table 1

| MIGS-ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| MIGS-1  | Habitat  | Coleopteran (beetle) larvae | TAS |
| MIGS-3  | Salinity | Growth in Luria-Bertani broth (5% NaCl) | TAS |
| MIGS-22 | Oxygen requirement | Aerobic | TAS |
| MIGS-15 | Biotic relationship | Free living | TAS |
| MIGS-14 | Pathogenicity | Known, Coleopteran and Dipteron larvae | TAS |
| MIGS-4  | Geographic location | Chicaque Natural Reserve, Cundinamarca, Colombia | TAS |
| MIGS-5  | Sample collection time | 1995 | TAS |
| MIGS-4.1| Latitude | 4.607037 | TAS |
| MIGS-4.2| Longitude | −74.303202 | TAS |
| MIGS-4.3| Depth | 20–40 cm | TAS |
| MIGS-4.4| Altitude | 2583 m above sea level | TAS |

* Evidence 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 [21].

Lysinibacillus fusiformis [15], Nakamura classified L. sphaericus sensu lato into seven similarity groups using their 16S rRNA sequence. These similarity groups are in accordance with whole-cell fatty acid profiles, four of the phylogenetic groups correspond to the DNA hybridization groups.

In this study we present the complete genome analysis of L. sphaericus III(3)7, sequenced using exclusively Pacific Biosciences sequencing technology (PacBio RS II). We then performed a comparative genomic analysis of the sequenced strain with the 3 previously reported genomes for Colombian L. sphaericus isolates [16,17,18] and with their respective reference genome L. sphaericus C3-41 [19].

Classiﬁcation and general features of Lysinibacillus sphaericus III(3)7 according to the MIGS recommendations.
Genomic assembly was done using Hierarchical Genome Assembly Protocol (HGAP) workflow [34], the outcome was a de novo assembly that was compared to genomes previously reported on databases using Mega BLAST (NCBI), which uses an algorithm capable of aligning sequences that differ slightly as a result of sequencing or other similar “errors” (Data not shown).

2.4. Genome annotation

The genome sequence was annotated using the automated prokaryotic annotation server: Rapid Annotations using Subsystem Technology (RAST) [35], then in order to obtain more information on the predicted coding regions we performed a Blast2Go [36] annotation, through the usage of this tool we obtained information on coding sequences that were not included in RAST subsystem calculations. We also used the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) [37]. The possible orthologs present in the chromosomal contig of both strains, were identified based on the COG database and classified accordingly [38].

2.5. Comparative genomic analysis

2.5.1. Multiple genome alignment

In order to compare the newly sequenced genome to the previously reported of L. sphaericus C3–41 and Colombian isolates OT4b.25, CBAM5 and OT4b.31 we used MAUVE [39], as a tool to check for synteny amongst large blocks of genomic sequences. We performed a multiple genome comparing strain III(3)7 against L. sphaericus OT4b.25, CBAM5, OT4b.31 and C3–41. We also executed the same analysis with L. sphaericus genome comparing strain III(3)7 against previously reported strain OT4b.25, CBAM5 and OT4b.31). We also made the same comparison with genes related bio-remediation of toxic metals, such as nickel, cobalt, arsenic and zinc.

2.5.2. Whole genome alignment

We used MUMmer [40] to run the global nucleotide based alignments to check for synteny amongst the sequences, we aligned strain by strain to analyze specific synthenial rearrangements in a case by case scenario. We performed the same analysis on the plasmid sequences separately.

2.5.3. Whole genome comparative visualization

BLAST Ring Image Generator (BRIG) [41] was used to show a genome wide visualization of coding sequences identity between L. sphaericus III(3)7 and those genomes of the strains mentioned above (L. sphaericus C3–41, OT4b.25, CBAM5 and OT4b.31).

2.5.4. Multi-Fasta comparative analysis

Using the Multi-Fasta reference option within BRIG [41] we compared the genes associated with multiple functions shown in laboratory assays with bioprospection importance. First we compared a set of genes related with larvicidal activity of L. sphaericus against larvae of vector-borne diseases. This analysis included sequences of genes such as: binary toxin genes (binA, binB), S-Layer proteins, hemolysin-D, chitin-binding proteins and chitin deacetylases. Secondly we compared genes directly involved in the nitrogen cycle, such as nitroreductases, regulatory proteins, transporters and proteins involved in nitric oxide synthesis. Finally we made the same comparison with genes related bio-remediation of toxic metals, such as nickel, cobalt, arsenic and zinc.

3. Results and discussion

3.1. Genome sequencing and assembly

Summary and statistics for the genome-sequencing project can be observed in Tables 2, 3 and 4, after assembly we obtained two contigs in both strains. Sequencing coverage averaged 207×. As a result of the HGAP assembly process L. sphaericus III(3)7 genome resulted in 2 contigs, both contigs were aligned via Megablast, to L. sphaericus strain C3–41 with a similarity percentage over 99%. Contig 1 of 4.66 Mbp aligned with the chromosomal sequences of strain C3–41 and contig 2 of 173 kpb aligned with its plasmid (pBpsh), suggesting that contig 2 might be a plasmid itself. GC content along the genome averaged 37.16%. Circular visualizations of the genome generated by DNAPlotter [42] can be observed in Fig. 1.

3.2. Genome annotation

We can observe in Table 4 that after annotation L. sphaericus III(3)7 has 4485 coding sequences, 149 RNA coding genes and 87 pseudogenes. Table 5 contains the COG functional annotation performed on the chromosomal contig.

The genome show a wide repertoire of potential protein encoding sequences in terms of mosquitocidal toxins and genes of crucial in the high levels of larvicidal activity that L. sphaericus III(3)7 has shown in laboratory assays. This activity has been previously reported in laboratory experiments determining the LC50 of this strain against Culex sp. [3,12] and Aedes sp. (Data not shown).

Protein encoding sequences for both binA and binB, previously reported as larvicidal toxins present in L. sphaericus, are also found in the chromosomal contig as contiguous open reading frames [17].

RAST Annotation revealed a set of subsystems with coding sequences for several metabolic processes of environmental importance, such as 21 subsystems dedicated to nitrogen cycling including denitrification, ammonia assimilation and nitric oxide synthesis. It also revealed 12 subsystems related with aromatic compound metabolism, including toluene, benzoate and catechol degradation pathways. Finally RAST

| Label                   | Size (bp)  | Topology |
|-------------------------|------------|----------|
| Chromosomal contig      | 4,663,526  | Circular |
| Extrachromosomal element| 173,793    | Circular |

| Attribute               | Value     |
|-------------------------|-----------|
| Chromosomal size (bp)   | 4,663,526 |
| DNA GC content (bp)     | 1,732,966 (37.16%) |
| Number of replicons     | 1         |
| Extrachromosomal        | 1         |
| Total genes             | 4485      |
| tRNA genes              | 149       |
| ncRNA genes             | 107       |
| Pseudogenes             | 87        |
| CRISPR arrays           | 1         |
| Genes assigned to COGs  | 2468      |
The genome *L. sphaericus* III(3)7 only shows a coding sequence for a Haemolysin-D which activity might have potential implications in *L. sphaericus* III(3)7 pathogenic activity in larvae. We can confirm the presence of a coding sequences for chitin deacetylases, these proteins are directly involved in degradation processes of chitin in a water environment into chitosan and acetone, a chitin deacetylase coding sequence has previously been reported in the genome of native Colombian strain of *L. sphaericus* CBAM5, having a putative domain of the protein NodB. Complementing the presence of the chitin deacetylase downstream along the genome there are two genes encoding chitin binding proteins with a 100% identity with the same protein reported on the genome of both the reference strain *L. sphaericus* C3-41 and Colombian isolate strain CBAM5. [17] These two genes compose an interesting metabolic pathway that may be involved in the process of inhibiting cuticle synthesis when larvae are undergoing instar switching.

Additionally the genome *L. sphaericus* III(3)7 shows 13 coding sequences for S-Layer and S-Layer like proteins, proteins that have shown a direct involvement in larvicidal activity. These sequences are coherent on what has been reported for all *L. sphaericus* strains both experimentally and through genome sequencing and annotation [12]. As a result of sequencing, assembly and annotation, we propose a potential extrachromosomal element, taking into account most of the proteins encoded by contig 2 correlates with the presence of a plasmid.

Annotation of contig 2 showed a coding sequence for protein TraG highly involved in conjugation processes in F and F-like plasmids. There is a 100% identity with the conjugal transfer protein TraG of *L. sphaericus* C3-41, and the plasmid replication protein involved in plasmid replication of this same strain with a 100% identity. Using Blast-domain this protein showed a domain similar to FtsZ that a protein considered the prokaryotic homologue to tubulin and is mainly involved in cell division. Also two protein-encoding sequences were found for site-specific recombinases like XerS that is also present on *L. sphaericus* C3-41 plasmid and DNA repair proteins like RadC. We also found sequences that belong to a Type I—C CRISPR array that includes three proteins Cas7/Csd2, Cas8c/Csd1 and Cas5, multiple DNA binding proteins, restriction endonucleases, helicases and reverse transcriptases.

All the protein coding sequences previously mentioned, can be deemed evidence of the potential of the presence of a plasmid in *L. sphaericus* III(3)7, furthermore during the annotation of this contig we came across the presence of multiple hypothetical proteins that are related with high levels of identity to those reported on plasmid pBsph that belongs to *L. sphaericus* C3-41, pBsph is the only high molecular weight plasmid reported in *L. sphaericus* [19].

The presence of an extrachromosomal element in *L. sphaericus* III(3)7 is yet to be demonstrated by *in vitro* assays, but this evidence can be an initial step to describing a plasmid similar to the one found in *L. sphaericus* C3-41, perhaps due to low sequence representation in the sequenced samples we were not able to describe more proteins related with the presence of a plasmid this strain, nevertheless NCBI classifies contig 2 of both strains as plasmids (Accession numbers:

![Diagram A](image1.png)  ![Diagram B](image2.png)

**Fig. 1.** Circular visualization of: A) *Lysinibacillus sphaericus* III(3)7 chromosomal contig, B) *L. sphaericus* III(3)7 putative extrachromosomal element. The inner circle represents the outer and second circles represent predicted coding regions on the forward (clockwise) and reverse (counterclockwise) DNA strands respectively. The third circle shows the GC content of the sequence, the final circle show the GC skew calculated as \((G − C) / (G + C)\). The numbers on the outside of these circles indicate locations within the genomic contig. Image generated by DNAPlotter.

| Code | Value | % age | Description |
|------|-------|-------|-------------|
| J    | 112   | 4.53  | Translation |
| K    | 171   | 6.91  | Transcription |
| L    | 77    | 3.13  | Replication, recombination and repair |
| B    | 1     | 0.04  | Chromatin structure and dynamics |
| D    | 39    | 1.59  | Cell cycle control, mitosis and meiosis |
| V    | 79    | 3.21  | Defense mechanisms |
| T    | 224   | 9.08  | Signal transduction mechanisms |
| M    | 106   | 4.27  | Cell wall/membrane biogenesis |
| N    | 28    | 1.14  | Cell motility |
| W    | 3     | 0.12  | Extracellular structures |
| U    | 23    | 0.92  | Intracellular trafficking and secretion |
| O    | 121   | 4.89  | Posttranslational modification, protein turnover, chaperones |
| X    | 11    | 0.43  | Phage derived proteins, transposases, mobilome components |
| C    | 101   | 4.09  | Energy production and conversion |
| G    | 101   | 4.09  | Carbohydrate transport and metabolism |
| E    | 274   | 11.10 | Amino acid transport and metabolism |
| F    | 35    | 1.41  | Nucleotide transport and metabolism |
| H    | 96    | 3.88  | Coenzyme transport and metabolism |
| I    | 47    | 1.91  | Lipid transport and metabolism |
| P    | 339   | 13.71 | Inorganic ion transport and metabolism |
| Q    | 73    | 2.93  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 343   | 13.90 | General function prediction only |
| S    | 64    | 2.59  | Function unknown |
| –    | 2168  | 46.76 | Not in COGs |

* The total is based on the total number of protein coding genes in the annotated genome.
these analyzes can be observed in Fig. 2. We used MAUVE for multiple genome alignments, the results of this study requires further characterization.

3.3. Comparative genomics

3.3.1. Multiple genome alignments using MAUVE

It was of our interest to compare the genome sequenced in this study to those previously sequenced of Colombian strains and the reference genome used for L. sphaericus genome sequencing projects to this date. We used MAUVE for multiple genome alignments, the results of these analyzes can be observed in Fig. 2.

As it can be observed in Fig. 2A there is a high level of synteny amongst strains OT4b.25, C3-41, CBAM5 and the strain sequenced in this study, as a result from the multiple genome alignment there are 5 homologous genomic blocks that are present in all strains in some cases with inversions and different positioning within each chromosome.

It is important to take into account that L. sphaericus C3-41 was the first genome to be sequenced of the species and has been used as a reference genome for assembly on most subsequent sequencing projects, including Colombian isolate CBAM5.

In Fig. 2B we can see the results of the same multiple alignments including L. sphaericus OT4b.31 sequenced at CIMIC by Peña-Montenegro and Dussán [16]. After the inclusion of this genomic sequence we can observe that the multiple alignment changes considerably. Instead of showing high levels of synteny amongst strains we see a divergence that can be reflected in over 30 homologous blocks scattered all over the genomic sequences. We can infer an important divergence between the genome of L. sphaericus OT4b.31 and the other strains included in the analysis, this coincides with the fact that out of the 5 genomes strain OT4b.31 is the only one with a de novo assembly approach and sequenced by using Illumina sequencing technology.

Fig. 2C shows the same analysis for pBSph and the putative extrachromosomal elements found in L. sphaericus III(3)7 and previously reported OT4b.25. Again we can observe a high level of synteny amongst the analyzed sequences which further supports the claim that strain III(3)7 possesses an extrachromosomal element.

3.3.2. Whole genome alignments using MUMmer

MUMmer was used to perform whole genome alignments. In Fig. 3 we can observe MUMmer dot-plots resulting from the alignment of the chromosomal sequence of L. sphaericus III(3)7 with strains C3-41, CBAM5, OT4b.31 and OT4b.25. Fig. 4 shows whole sequence alignments between pBSph and plasmid sequence found in this study and strain OT4b.25.

As it can be observed in Fig. 3A, B and D, the same level of synteny shown between L. sphaericus III(3)7, OT4b.25, and CBAM5 is maintained and the same inversions against strains CBAM5 and C3-41 shown in the multiple alignment in Fig. 2 can be seen in these dot-plots, furthermore there seems to be a higher similarity between the strains sequenced in this study than when compared to genome sequences of the other isolates. Again strain OT4b.31 seems to be the most divergent of the five showing the same basic outline of the dot-plot but not being able to achieve whole segment alignments.

When we performed the same analysis for the extrachromosomal elements present in strains C3-41, OT4b.25 and III(3)7 we could observe the same levels of synteny and similarity shown in the multiple sequence alignment. In this case we can see that the sequences of the putative extrachromosomal elements of strain C3-41 has lower similarity when compared with pIII(3)7 and pOT4b.25, than the latter when compared amongst themselves (Fig. 4).

3.4. BLAST ring image generator (BRIG)

3.4.1. Whole genome comparison

We compared the genomes of L. sphaericus III(3)7, OT4b.25, CBAM5, OT4b.31 and C3-41, as it can be observed in Fig. 5, using as reference genome the strain sequenced in this study. We can see that the similarity showed by strains CBAM5, C3-41, OT4b.25 and III(3)7 is maintained even when compared in an analysis like the one performed with BRIG, in which every open reading frame that is present in the reference genome, but absent in the genomes compared, is represented as a blank.
space. Even though small or punctual differences between the most similar strains are not apparent in this kind of analysis, we can clearly see that strain OT4b.31 is again the most different amongst the strains analyzed.

3.4.2. Multi-FASTA reference gene analysis

Once we compared the whole genomes, we compared specific set of genes that are related with phenotypic characteristics in which *L. sphaericus* strains have excelled at and have been proven valuable for bioprospection purposes.

In the case of larvicidal activity (Fig. 6A and B) we can observe that when we compared the genes present in strain III(3)7 there is almost a perfect match (100% identity) with strains OT4b.25, CBAM5 and C3-41. However when compared with the genes present in strain OT4b.31 there is no match against fractions of the *binA* and *binB* genes, and some of the copies of S-layer protein are missing, as seems to be the case with both chitin deacetylase copies. These results go in accordance with the fact that *L. sphaericus* OT4b.31 is non-pathogenic and that in the annotation of its genome absence of larvicidal activity genes was recorded [16].

When comparing genes related with nitrogen cycling there seem to be a higher level of identity amongst all strains. However there in two cases there is a lower level of identity, a nitroreductase that only shows 50% identity and a NAD(P)H nitroreductase that has 70% identity with the reference strain.

Finally when comparing toxic metal remediation genes, strain OT4b.31 shows it is missing 3 genes important for arsenic resistance, including a transcriptional regulator ArsR from which it has another copy that presents 50% identity in its overall sequence. It is also missing an “arsenic resistance protein”. In this case we found a difference with *L. sphaericus* C3-41 in a nickel transporter that shows 70% identity. As reported by Peña-Montenegro, et al. in 2015 *L. sphaericus* CBAM5 showed presence of resistance genes for both arsenic and cobalt.

Overall this Multi-FASTA analysis shows really low levels of genetic diversity within *L. sphaericus* strains.
4. Conclusions

We sequenced, annotated and described the genome of native Colombian strain L. sphaericus III(3)7. When compared with its closest genome sequences also Colombian isolate L. sphaericus CBAM5, OT4b.25 and L. sphaericus C3-41, it shows similar regions with few synthenial arrangements, nevertheless when compared with Colombian strain OT4b.31 the assembled and annotated genome shows few similar regions and many synthenial rearrangements.

We found evidence that suggest that L. sphaericus III(3)7 have a plasmid similar to the one reported in L. sphaericus C3-41, however this fact still needs to be supported by in vitro evidence, the same case as in previously reported L. sphaericus OT4b.25.

After whole genome BLAST comparison and Multi-FASTA reference comparative analysis, we conclude that the genetic diversity amongst compared L. sphaericus strains is low, with the exception of L. sphaericus OT4b.31.

Acknowledgements

We acknowledge and appreciate the contribution of Frederick Robidoux, Genevieve Geneau and Alfredo Staffa at McGill University and Genome Québec Innovation Center who largely contributed to this study. This study was performed under the auspices of the Centro de Investigaciones Microbiológicas (CIMIC) and the Sciences faculty at the Universidad de los Andes. Special thanks to Tito Peña-Montenegro for his comments and suggestions.

References

[1] P. Baumann, M.A. Clark, L. Baumann, A.H. Broadwell, Bacillus sphaericus as a mosquito pathogen: properties of the organism and its toxins. Microbiol. Rev. 55 (3) (1991) 425–436.
[2] C. Berry, The bacterium, Lysinibacillus sphaericus, as an insect pathogen. J. Invertebr. Pathol. 109 (1) (2012) 1–10, http://dx.doi.org/10.1016/j.jip.2011.11.008.
[3] L.C. Lozano, J. Dussan, Metal tolerance and larvicidal activity of Lysinibacillus sphaericus. World J. Microbiol. Biotechnol. 29 (8) (2013) 1383–1389, http://dx.doi.org/10.1007/s11274-013-1301-9.
[4] Y. Zhang, E. Liu, C. Cai, Z. Chen, Isolation of two highly toxic Bacillus sphaericus strains. Insecticidal Microorg. 1 (1987) 98–99.
[5] E.W. Davidson, H.L. Morton, J.O. Moffett, S. Singer, Effect of Bacillus sphaericus strain SSII-1 on honey bees, Apis mellifera. J. Invertebr. Pathol. 29 (3) (1977) 344–346, http://dx.doi.org/10.1016/0022-2011(77)90041-4.
[6] N.S. Tietze, M.A. Olson, P.C. Hester, J.J. Moore, Tolerance of sewage treatment plant microorganisms to mosquitocides. J. Am. Mosq. Control Assoc. 9 (4) (1993) 477–479.
[7] M.D. Brown, T.M. Watson, J. Carter, D.M. Purdie, B.H. Kay, T.M. Watson, B.H. Kay, Toxicity of VectoLex (Bacillus sphaericus) products to selected Australian mosquito and nontarget species. J. Econ. Entomol. 97 (1) (2004) 51–58, http://dx.doi.org/10.1603/0022-0493-97.1.51.
[8] L.A. Lacey, Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control. J. Am. Mosq. Control Assoc. 23 (2 Suppl.) (2007) 133–163, http://dx.doi.org/10.2987/8756-971x(2007)23[133:beslab2.0.co;2.
[9] M. Fischer, Dengue, chikungunya, and other Aedes mosquito-borne diseases. http://www.cdc.gov/dengue/grandrounds/pdf/archives/2015/may2015.pdf:CDC2015.
[10] A.C. Porter, E.W. Davidson, J.W. Liu, Mosquitocidal toxins of Bacilli and their genetic manipulation for effective biological control of mosquitoes. Microbiol. Rev. 57 (4) (1993) 838–861.
[11] M.C. Allievi, M.M. Palomino, M. Prado Acosta, L. Lanati, S.M. Rozal, C. Sánchez-Rivas, C. Sánchez-Rivas, Contribution of S-layer proteins to the mosquitocidal activity of Lysinibacillus sphaericus. PLoS One 9 (10) (2014), e111114 http://dx.doi.org/10.1371/journal.pone.0111114.
[12] L.C. Lozano, J.A. Ayala, J. Dussan, Lysinibacillus sphaericus S-layer protein toxicity against Culex quinquefasciatus. Biotechnol. Lett. 33 (10) (2011) 2037–2041, http://dx.doi.org/10.1007/s10529-011-0666-9.
