

Complete Genome Sequence of the Broad-Host-Range *Paenibacillus larvae* Phage phiIBB_P123

Ana Oliveira,^a Luís D. R. Melo,^a Andrew M. Kropinski,^{b,c} Joana Azeredo^a

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga, Portugal^a; Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, ON, Canada^b; Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada^c

***Paenibacillus larvae* is a Gram-positive bacterium that causes American foulbrood, an important disease in apiculture. We report the first complete genome sequence of a *P. larvae* phage, phiIBB_P123, isolated from a hive in northern Portugal. This phage belongs to the family Siphoviridae.**

Received 21 May 2013 Accepted 7 August 2013 Published 5 September 2013

Citation Oliveira A, Melo LDR, Kropinski AM, Azeredo J. 2013. Complete genome sequence of the broad-host-range *Paenibacillus larvae* phage phiIBB_P123. *Genome Announc* 1(5):e00438-13. doi:10.1128/genomeA.00438-13.

Copyright © 2013 Oliveira et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Joana Azeredo, jazeredo@deb.uminho.pt.

American foulbrood is a bacterial disease caused by *Paenibacillus larvae*, a Gram-positive bacterium wherein the spore is the infectious form. *P. larvae* causes hive destruction and consequently important economic losses in apiculture worldwide (1). Moreover, a European Community regulation (no. 2377/90) limits the presence of antibiotics in honey, excluding its use for therapy. Therefore, the development of alternative antimicrobial methods is of utmost importance. Bacteriophages, viruses that infect and lyse bacteria, have shown great efficacy in controlling bacterial diseases in animal production (2–5).

The phage phiIBB_P123 was isolated after culturing of strain H23 (host) found in honeybee larvae from a Portuguese hive. This phage formed plaques on most of the *P. larvae* strains tested. Morphologically, it is from the Siphoviridae family.

PhiIBB_P123 was propagated in H23 under incubation at 37°C with 5% CO₂, and afterward the phage DNA was extracted. The genome was sequenced using Roche/454-recommended procedures at the Plateforme d'analyses génomiques of the Institut de Biologie Intégrative et des Systèmes (Laval University, Québec, Canada). Shotgun reads were assembled using the gsAssembler module of Newbler v 2.5.3. Potential open reading frames (ORFs) were annotated using myRAST (6). The presence of Shine-Dalgarno sequences upstream of each ORF and the search for additional ORFs were checked manually in Kodon (Applied Maths, Austin, TX). Putative protein functions were assigned using BLASTP (7) and Pfam (8) with databases available on April 2013. Transmembrane domains were predicted using Phobius (9) and TMHMM (10). Putative host-dependent (SigA) promoters were discovered with their consensus sequence TTGACA-N14-tgnTATAAT (11). The Rho-independent terminators and calculations of the free energy of their secondary structures were predicted using ARNold (12) and Mfold (13), respectively. tRNAscan-SE (14) and ARAGORN (15) were used for tRNA detection.

The genomic double-stranded DNA of phiIBB_P123 consists of 41,294 bp, with a GC content of 40.9%. The latter value is less than that for the host at 44 GC mol%. The genome was scanned

for coding DNA sequences (CDS) resulting in 68 CDS, ranging from 117 bp (39 codons) to 2,928 bp (976 codons). The initiation codon of 81% of the predicted CDS is ATG, while 10% of the CDS start with GTG and 9% with TTG. Based on BLAST and Pfam analysis, 51% of the proteins have been assigned functions; 18% were defined as conserved hypothetical proteins and 31% are unique. The presence of a serine recombinase/resolvase (gp30), repressor (gp31), and antirepressor (gp39) indicates that this is a temperate phage (16). The endolysin of this phage is an N-acetylmuramoyl-L-alanine amidase, identified as an amidase_2 domain, common to other *Bacillus* spp. (17). It also encodes a 975-amino-acid protein (gp26) possessing several ricin-type beta-trefoil lectin domain-like domains (pfam14200) identical to *Paenibacillus larvae* toxin 1 (AGJ74029), suggesting that like many temperate phages, phiIBB_P123 is capable of lysogenic conversion (18, 19).

Two putative promoters were identified as having homology with SigA *Bacillus* promoters and three rho-independent terminators but no tRNAs.

Nucleotide sequence accession number. This whole genome shotgun project has been deposited at GenBank under the accession number [KF010834](https://www.ncbi.nlm.nih.gov/nuccore/KF010834). The version described in this paper is the first version.

ACKNOWLEDGMENTS

A.O. and L.D.R.M. acknowledge the FCT (Fundação para a ciência e a tecnologia) grants SFRH/BPD/69356/2010 and SFRH/BD/66166/2009, respectively.

We also acknowledge Leon Kluskens for providing *P. larvae* strains.

REFERENCES

1. Genersch E, Forsgren E, Pentikäinen J, Ashiralieva A, Rauch S, Kilwiniski J, Fries I. 2006. Reclassification of *Paenibacillus larvae* subsp. *pulvificiens* and *Paenibacillus larvae* subsp. *larvae* as *Paenibacillus larvae* without subspecies differentiation. *Int. J. Syst. Evol. Microbiol.* 56:501–511.
2. Oliveira A, Sereno R, Azeredo J. 2010. *In vivo* efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses. *Vet. Microbiol.* 146:303–308.

3. Park SC, Nakai T. 2003. Bacteriophage control of *Pseudomonas plecoglossicida* infection in ayu *Plecoglossus altivelis*. *Dis. Aquat. Organ* 53:33–39.
4. Pouillot F, Chomton M, Blois H, Courroux C, Noelig J, Bidet P, Bingen E, Bonacorsi S. 2012. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b:H4-ST131 *Escherichia coli* strain producing CTX-M-15. *Antimicrob. Agents Chemother.* 56: 3568–3575.
5. Smith HW, Huggins MB. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J. Gen. Microbiol.* 129:2659–2675.
6. 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. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. doi:10.1186/1471-2164-9-75.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
8. Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Lu F, Marchler GH, Mullokandov M, Omelchenko MV, Robertson CL, Song JS, Thanki N, Yamashita RA, Zhang D, Zhang N, Zheng C, Bryant SH. 2011. CDD: a conserved domain database for the functional annotation of proteins. *Nucleic Acids Res.* 39:D225–D229.
9. Käll L, Krogh A, Sonnhammer EL. 2004. A combined transmembrane topology and signal peptide prediction method. *J. Mol. Biol.* 338: 1027–1036.
10. Käll L, Sonnhammer EL. 2002. Reliability of transmembrane predictions in whole-genome data. *FEBS Lett.* 532:415–418.
11. Sierro N, Makita Y, de Hoon M, Nakai K. 2008. DBTBS: a database of transcriptional regulation in *Bacillus subtilis* containing upstream intergenic conservation information. *Nucleic Acids Res.* 36:D93–D96.
12. Naville M, Ghuillot-Gaudeffroy A, Marchais A, Gautheret D. 2011. ARNold: a web tool for the prediction of Rho-independent transcription terminators. *RNA Biol.* 8:11–13.
13. Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31:3406–3415.
14. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res.* 33:W686–W689.
15. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.* 32:11–16.
16. Thorpe HM, Smith MC. 1998. *In vitro* site-specific integration of bacteriophage DNA catalyzed by a recombinase of the resolvase/invertase family. *Proc. Natl. Acad. Sci. U. S. A.* 95:5505–5510.
17. Oliveira H, Melo LD, Santos SB, Nóbrega FL, Ferreira EC, Cerca N, Azeredo J, Kluskens LD. 2013. Molecular aspects and comparative genomics of bacteriophage endolysins. *J. Virol.* 87:4558–4570.
18. Christie GE, Allison HE, Kuzio J, McShan WM, Waldor MK, Kropinski AM. 2012. Prophage-induced changes in cellular cytochemistry and virulence, p 33–60. *In* Hyman P, Abedon ST (ed), *Bacteriophages in health and disease*. CAB International, Wallingford, Oxon, United Kingdom.
19. Sabour PM, Griffiths MW (ed). 2010. *Bacteriophages in the control of food- and waterborne pathogens*. ASM Press, Washington, DC.