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Complete Genome Sequences of Two Bacillus pumilus Strains from Cuatrociénegas, Coahuila, Mexico

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ABSTRACT We assembled the complete genome sequences of Bacillus pumilus strains 145 and 150a from Cuatrociénegas, Mexico. We detected genes codifying for proteins potentially involved in antagonism (bacteriocins) and defense mechanisms (abortive infection bacteriophage proteins and 4-azaleucine resistance). Both strains harbored prophage sequences. Our results provide insights into understanding the establishment of microbial interactions.

acillus pumilus is a Gram-positive, spore-forming bacterium residing in soil and water and in some extreme environments. Some strains are resistant to high levels of radiation and oxidizing agents [\(1\)](#page-1-0), and some antagonize other bacteria and disturb the structure and architecture of bacterial biofilms (e.g., Vibrio, Bacillus, and Pseudomo-nas [\[2](#page-1-1)-[4\]](#page-1-3)). Here, we report the complete genome sequence of B. pumilus 145 and B. pumilus 150a from the shallow water system of Churince in Cuatrociénegas, Coahuila, the detection of genes potentially involved in antagonism of other coinhabiting species, and the complete genome sequence of the Bacillus phage EZ-2018a. These findings will give insights into understanding the establishment of biological interactions. Total genomic DNA was sequenced with the PacBio RS II system, assembled with Canu v.1.3 [\(5\)](#page-1-4), and circularized with Circlator v.1.3 [\(6\)](#page-1-5). The B. pumilus 145 assembly resulted in two contigs representing the chromosome (3,937,399 bp, 265.81 \times mean coverage) and the Bacillus phage EZ-2018a (118,485 bp, 486.825 \times mean coverage), whereas the B. pumilus 150a assembly resulted in one contig (3,747,740 bp, 269.37 \times mean coverage). In the B. pumilus clade, the gene $qvrB$ is the most informative to distinguish between closely related species [\(7\)](#page-1-6). NCBI BLAST alignments [\(8\)](#page-1-7) of gyrB showed 98% similarity between B. pumilus BAT47140 and B. pumilus 145 and 100% identity between B. pumilus WP_041816108 and B. pumilus 150a. We used Rapid Annotations using Subsystems Technology (RAST) [\(5\)](#page-1-4) for genome annotation. Eight rRNA copies, 81 tRNAs, and 4,211 protein-coding sequences in 459 functional subsystems were identified in the *B. pumilus* 145 genome, and eight rRNA copies, 82 tRNAs, and 3,827 protein coding sequences in 466 subsystems in the B. pumilus 150a genome. There are 59 genes involved in virulence, disease, and defense in B. pumilus 145, and 61 in B. pumilus 150a. Twelve genes related to the bacteriocin stress response and one conferring tolerance to colicin E2 were annotated in both strains. Both strains harbored prophage sequences, as indicated by PHAST [\(9\)](#page-1-8). B. pumilus 145 contained four intact prophage regions in the chromosome and the Bacillus phage EZ-2018a, whereas B. pumilus 150a harbored two intact prophage regions in its chromosome. A gene coding for an abortive infection bacteriophage resistance protein (Abi) belonging to the Abi_2 family was detected in one of the prophage regions of B. pumilus 145. This defense mechanism is a cell death system activated by phage infection and provides protection to the bacterial population by limiting viral replication. Top hits in a PSI-BLAST search

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SOCIETY FOR MICROBIOLOGY [\(10\)](#page-1-9) indicated that a similar gene (91% amino acid identity) was found in Bacillus safensis and in Bacillus subtilis (58% amino acid identity), but it was not present in any other B. pumilus sequenced genome, suggesting that B. pumilus 145 acquired Abi through a mobile genetic element. On the other hand, B. pumilus 150a contained AzlC and AzlD genes that codify for branched-chain amino acid transport proteins possibly involved in conferring resistance to 4-azaleucine—previously detected in other Bacillus genomes, but not in any other B. pumilus [\(11,](#page-1-10) [12\)](#page-1-11). This leucine analogue is a potent growth inhibitor in several bacteria due to its incorporation into proteins in place of leucine [\(13,](#page-1-12) [14\)](#page-1-13). 4-Azaleucine is produced by Streptomyces [\(15\)](#page-1-14), one of the most abundant Actinobacteria in Cuatrociénegas [\(16\)](#page-1-15).

Accession number(s). These genomes have been deposited in GenBank under the accession numbers [CP027034,](https://www.ncbi.nlm.nih.gov/nuccore/CP027034) [CP027116,](https://www.ncbi.nlm.nih.gov/nuccore/CP027116) and [CP027117.](https://www.ncbi.nlm.nih.gov/nuccore/CP027117) The versions described in this paper are the first versions.

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REFERENCES

- 1. Stepanov VG, Tirumalai MR, Montazari S, Checinska A, Venkateswaran K, Fox GE. 2016. Bacillus pumilus SAFR-032 genome revisited: sequence update and re-annotation. PLoS One 11:e0157331. [https://doi.org/10](https://doi.org/10.1371/journal.pone.0157331) [.1371/journal.pone.0157331.](https://doi.org/10.1371/journal.pone.0157331)
- 2. Hill JE, Baiano JCF, Barnes AC. 2009. Isolation of a novel strain of Bacillus pumilus from penaeid shrimp that is inhibitory against marine pathogens. J Fish Dis 32:1007–1016. [https://doi.org/10.1111/j.1365-2761.2009](https://doi.org/10.1111/j.1365-2761.2009.01084.x) [.01084.x.](https://doi.org/10.1111/j.1365-2761.2009.01084.x)
- 3. Pérez-Gutiérrez R-A, López-Ramírez V, Islas Á, Alcaraz LD, Hernández-González I, Olivera BCL, Santillán M, Eguiarte LE, Souza V, Travisano M, Olmedo-Alvarez G. 2013. Antagonism influences assembly of a Bacillus guild in a local community and is depicted as a food-chain network. ISME J 7:487– 497. [https://doi.org/10.1038/ismej.2012.119.](https://doi.org/10.1038/ismej.2012.119)
- 4. Nithya C, Aravindraja C, Pandian SK. 2010. Bacillus pumilus of Palk Bay origin inhibits quorum-sensing-mediated virulence factors in gramnegative bacteria. Res Microbiol 161:293–304. [https://doi.org/10.1016/j](https://doi.org/10.1016/j.resmic.2010.03.002) [.resmic.2010.03.002.](https://doi.org/10.1016/j.resmic.2010.03.002)
- 5. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722-736. [https://doi](https://doi.org/10.1101/gr.215087.116) [.org/10.1101/gr.215087.116.](https://doi.org/10.1101/gr.215087.116)
- 6. Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16:294. [https://doi.org/10.1186/s13059-015](https://doi.org/10.1186/s13059-015-0849-0) [-0849-0.](https://doi.org/10.1186/s13059-015-0849-0)
- 7. Liu Y, Lai Q, Dong C, Sun F, Wang L, Li G, Shao Z. 2013. Phylogenetic diversity of the Bacillus pumilus group and the marine ecotype revealed by multilocus sequence analysis. PLoS One 8:e80097. [https://doi.org/10](https://doi.org/10.1371/journal.pone.0080097) [.1371/journal.pone.0080097.](https://doi.org/10.1371/journal.pone.0080097)
- 8. NCBI Resource Coordinators. 2016. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 41:D8 –D20. [https://doi.org/10.1093/nar/gks1189.](https://doi.org/10.1093/nar/gks1189)
- 9. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. [https://doi.org/10](https://doi.org/10.1093/nar/gkr485) [.1093/nar/gkr485.](https://doi.org/10.1093/nar/gkr485)
- 10. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389 –3402. [https://doi](https://doi.org/10.1093/nar/25.17.3389) [.org/10.1093/nar/25.17.3389.](https://doi.org/10.1093/nar/25.17.3389)
- 11. Belitsky BR, Gustafsson MC, Sonenshein AL, Von Wachenfeldt C. 1997. An lrp-like gene of Bacillus subtilis involved in branched-chain amino acid transport. J Bacteriol 179:5448 –5457. [https://doi.org/10.1128/jb.179.17](https://doi.org/10.1128/jb.179.17.5448-5457.1997) [.5448-5457.1997.](https://doi.org/10.1128/jb.179.17.5448-5457.1997)
- 12. Belitsky BR. 2015. Role of branched-chain amino acid transport in Bacillus subtilis CodY activity. J Bacteriol 197:1330-1338. [https://doi.org/10](https://doi.org/10.1128/JB.02563-14) [.1128/JB.02563-14.](https://doi.org/10.1128/JB.02563-14)
- 13. Harrison LI, Christensen HN, Handlogten ME, Oxender DL, Quay SC. 1975. Transport of L-4-azaleucine in Escherichia coli. J Bacteriol 122:957–965.
- 14. Sabina J, Dover N, Templeton LJ, Smulski DR, Söll D, LaRossa RA. 2003. Interfering with different steps of protein synthesis explored by transcriptional profiling of Escherichia coli K-12. J Bacteriol 185:6158 – 6170. [https://doi.org/10.1128/JB.185.20.6158-6170.2003.](https://doi.org/10.1128/JB.185.20.6158-6170.2003)
- 15. Argoudelis AD, Herr RR, Mason DJ, Pyke TR, Zieserl JF. 1967. New amino acids from Streptomyces. Biochemistry 6:165–170. [https://doi.org/10](https://doi.org/10.1021/bi00853a027) [.1021/bi00853a027.](https://doi.org/10.1021/bi00853a027)
- 16. Arocha-Garza HF, Canales-Del Castillo R, Eguiarte LE, Souza V, De la Torre-Zavala S. 2017. High diversity and suggested endemicity of culturable Actinobacteria in an extremely oligotrophic desert oasis. PeerJ 5:e3247. [https://doi.org/10.7717/peerj.3247.](https://doi.org/10.7717/peerj.3247)