genomeA
Journals.ASM.org

Draft Genome Sequence of *Bacillus pumilus* Fairview, an Isolate Recovered from a Microbial Methanogenic Enrichment of Coal Seam Gas Formation Water from Queensland, Australia

Cassandra J. Vockler,[a] Paul Greenfield,[b] Nai Tran-Dinh,[a] David J. Midgley[a]

CSIRO Animal, Food and Health Sciences, North Ryde, NSW, Australia[a]; CSIRO Computational Informatics, North Ryde, NSW, Australia[b]

Despite its global abundance, *Bacillus pumilus* is poorly studied. The Fairview strain was obtained from a methanogenic anaerobic coal digester. The draft genome sequence was 3.8 Mbp long and contained 3,890 protein-coding genes. Like the SAFR-032 strain, it includes *B. pumilus*-specific proteins that likely confer enhanced resistance to environmental stresses.

Received 19 March 2014 **Accepted** 3 April 2014 **Published** 17 April 2014

Citation Vockler CJ, Greenfield P, Tran-Dinh N, Midgley DJ. 2014. Draft genome sequence of *Bacillus pumilus* Fairview, an isolate recovered from a microbial methanogenic enrichment of coal seam gas formation water from Queensland, Australia. Genome Announc. 2(2):e00279-14. doi:10.1128/genomeA.00279-14.

Address correspondence to Cassandra J. Vockler, cassandra.vockler@csiro.au.

Bacillus pumilus Fairview was isolated from a 45°C methanogenic anaerobic digester sample obtained from a coal seam gas formation water sample growing with a highly volatile bituminous Bowen Basin coal as the feedstock. The formation water was sourced from the Fairview coal seam gas field near Injune, Queensland, Australia (S25°51; E148°34) from a borehole intersecting a coal seam at approximately 600 m subsurface. On collection, the water was 45°C (pH 8.9) and had an electrical conductivity (EC) of 1,400 μS cm^{-1}. The digester was incubated at 45°C for 8 weeks before attempts were made to culture microbes from the consortium. These efforts included dilution on a range of media, including peptone, yeast-extract, and glucose (PYG) (all 1 g liter^{-1}) agar (1), followed by incubation to isolate facultative aerobes. One of the colonies isolated was the *B. pumilus* Fairview strain described. The pure culture was grown in liquid PYG, DNA was extracted, and Illumina (HiSeq, 100-bp paired-end [PE] library) sequencing was undertaken. Of the reads, 99.95% were assembled using Velvet 1/2/07 ($k = 41$) into the draft genome.

The draft genome of Fairview was 3,838,013 bp long and had coverage of ~200×. The genome was composed of 67 large contigs (>200 bp). Excluding the short contigs, mean and median contig lengths were 57,284 bp and 18,358 bp, respectively, with a maximum contig length of 568,093 bp. (The short contigs [<200 bp] are available at http://dx.doi.org/10.4225/08/531CF5598D431.) Genome annotation was undertaken with Integrated Microbial Genomes Expert Review (IMG ER) (2), which predicted 3,890 protein-coding genes. Based on coverage, we estimated that *B. pumilus* Fairview had five copies of the 16S gene. This 16S sequence has 99 to 100% identity with an array of *B. pumilus*, including the well-known SAFR-032 strain (3). Genomic comparisons of *B. pumilus* genomes available to date indicate that the Fairview strain is closely related to the CCMA-560 strain isolated from an oil-affected sediment (4) with which it shares ~89% of its genome. Further analyses of the genome using the SignalP (5) and dbCAN (6) pipelines revealed a small number of proteins which probably were extracellular β-glucosidase, pectin, or pectate lyase enzymes. Despite its presence in the digester, with coal as a sole source of carbon, no polyphenol oxidase or laccases were detected; however, a vanillyl-alcohol oxidase was identified and may be involved in some aromatic degradative processes.

It remains unclear whether the taxon was growing in the digestor or was present as a spore; genes for respiratory nitrate reduction were not detected. Furthermore, spores of *B. pumilus* have been shown to have extreme resistance to a range of stressors (7–9) and this may be conferred by a range of repair proteins unique to *B. pumilus* (3). Like SAFR-032, the Fairview strain possesses analogs of a *B. pumilus*-specific DNA photo-lyase enzyme (csirobg2_00392) that may confer resistance properties to this strain. Despite occurring in many environments, *B. pumilus* is relatively poorly studied compared to its relative *B. subtilis*. The ability of *B. pumilus* to survive in extreme environments, host unusual genes, and produce a range of metabolites warrants further examination.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JFBY00000000. The version described in this paper is the first version, JFBY01000000.

ACKNOWLEDGMENTS

This study was funded by CSIRO Energy Flagship under a research sponsorship agreement with Santos Ltd., AGL and Origin Energy Resources.

We thank Stephen Kelemen and Steve Taylor and Santos Ltd. for collection of the coal and formation water sample from which Fairview was isolated.

REFERENCES

1. **Atlas RM.** 2004. Handbook of microbiological media, 3rd ed, vol 1. CRC Press, Boca Raton, FL.
2. **Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC.** 2012. IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res. **40:**D115–D122. http://dx.doi.org/10.1093/nar/gks596.
3. **Gioia J, Yerrapragada S, Qin X, Jiang H, Igboeli OC, Muzny D, Dugan-Rocha S, Ding Y, Hawes A, Liu W, Perez L, Kovar C, Dinh H, Lee S, Nazareth L, Blyth P, Holder M, Buhay C, Tirumalai MR, Liu Y, Das-**

gupta I, Bokhetache L, Fujita M, Karouia F, Eswara Moorthy P, Siefert J, Uzman A, Buzumbo P, Verma A, Zwiya H, McWilliams BD, Olowu A, Clinkenbeard KD, Newcombe D, Golebiewski L, Petrosino JF, Nicholson WL, Fox GE, Venkateswaran K, Highlander SK, Weinstock GM. 2007. Paradoxical DNA repair and peroxide resistance gene conservation in *Bacillus pumilus* SAFR-032. PLoS One **2:**e928. http://dx.doi.org/10.1371/journal.pone.0000928.

4. **Domingos DF, Dellagnezze BM, Greenfield P, Reyes LR, Melo IS, Midgley DJ, Oliveira VM.** 2013. Draft genome sequence of *Bacillus pumilus* CCMA-560, isolated from an oil-contaminated mangrove swamp. Genome Announc. **1**(5):e00707-13. http://dx.doi.org/10.1128/genomeA.00707-13.
5. **Petersen TN, Brunak S, von Heijne G, Nielsen H.** 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat. Methods **8:**785–786. http://dx.doi.org/10.1038/nmeth.1701.
6. **Yin Y, Mao X, Yang J, Chen X, Mao F, Xu Y.** 2012. dbCAN: a web resource for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. **40:**W445–W451. http://dx.doi.org/10.1093/nar/gks479.
7. **Kempf MJ, Chen F, Kern R, Venkateswaran K.** 2005. Resistant spores of *Bacillus pumilus* from a spacecraft assembly facility. Astrobiology **5:**391–405. http://dx.doi.org/10.1089/ast.2005.5.391.
8. **Setlow P.** 2006. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. J. Appl. Microbiol. **101:**514–525. http://dx.doi.org/10.1111/j.1365-2672.2005.02736.x.
9. **Newcombe DA, Schuerger AC, James N, Dickinson D, Tanner R, Newcombe DA, Schuerger AC, Benardini JN, Dickinson D, Tanner R, Venkateswaran K.** 2005. Survival of spacecraft-associated microorganisms under simulated Martian UV irradiation. Appl. Environ. Microbiol. **71:**8147–8156. http://dx.doi.org/10.1128/AEM.71.12.8147-8156.2005.