

Genome Sequence of *Leucobacter* sp. 4J7B1, a Plant-Osmoprotectant Soil Microorganism

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We report the first genome sequence for *Leucobacter* sp. 4J7B1, a newly described desiccation-tolerant strain. The complete genome sequence of *Leucobacter* sp. 4J7B1 has been sequenced and is estimated to be around 3.5 Mb in size, with an average GC content of 62.18%. We predict 2,953 protein-coding sequences.

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The genus *Leucobacter* belongs to the class of high-GC-content, Gram-positive, nonsporulating Actinobacteria. Species of *Leucobacter* have been recovered from diverse ecological niches, including plants' phyllosphere (1). *Leucobacter* sp. 4J7B1 is a desiccation-tolerant bacterium isolated from a *Nerium oleander* rhizosphere subjected to severe drought (2). Isolation of other desiccation-tolerant microorganisms from this environment, including the new species *Arthrobacter siccitolerans* 4J27, has been reported recently (3, 4). The production of xeroprotectants, such as glycerol, by many desiccation-tolerant microorganisms protects themselves against damage caused by drought and salts (5–11) and other stressors (12). Thus, the combination of soy plants and *Leucobacter* sp. 4J7B1 in the presence of 200-mM NaCl results in significant protection of the plant by the microorganism. This protection effect might be the result of glycerol production, a well-known osmoprotectant. To our knowledge, the complete genome sequence of the genus *Leucobacter* sp. 4J7B1 has not been deposited in the DDBJ/EMBL/GenBank databases. In this research, we determine the whole-genome sequence of *Leucobacter* sp. 4J7B1 with pyrosequencing technology as implemented at the 454 Life Science-Roche platform with a combined approach of shotgun and 8-Kb mate-pair sequencing (12).

A total of 150,575 reads were produced, with an average read length of 603 bases for the shotgun and 123,838 sequences with an average read length of 389,53 bases for the mate-pair sequencing strategy. The total number of sequenced bases is 129,339,181, representing a sequencing depth of around 38×. *De novo* assembly was performed with default parameters by using the Newbler version 2.6 assembler. The assembly resulted in 432 contigs, 149 of which were larger than 500 bp. The N_{50} of the contig assembly was 871,355 bp, and the largest contig was 1,030,920 bp. Most of these contigs were ordered in two scaffolds (based on mate-pair information), where the largest scaffold was 3,069,722 bp. This combination of scaffolds and contigs resulted in an estimated genome size of 3.5 Mb. Gap closure was attempted using gap-spanning clones and PCR products. Putative coding sequences were predicted and genes were annotated with a pipeline implemented at Lifesequencing S.L. (Valencia, Spain). Briefly, protein-coding se-

quences were predicted by the combined use of Glimmer (13–15), RNAmmer (16), tRNAScan (17, 18), and BLAST (19, 20). The complete genomic information for *Leucobacter* sp. 4J7B1 was found to contain 2,953 protein-coding genes, 5 rRNA operons, and 49 tRNA genes, with an average GC content for that chromosome of 62.18%.

Analysis of this genome sequence data led to propose the presence of several genes involved in glycerol metabolism in bacteria, such as *tagD*, *glpF*, or *glpQ1*, among others. This knowledge can lead to advance biotechnological applications in osmoprotection engineering (6, 8, 21).

In summary, the complete genome sequence of *Leucobacter* sp. 4J7B1 will contribute to improving the knowledge of plants' osmoprotection by microorganisms.

Nucleotide sequence accession numbers. The complete genome sequence of *Leucobacter* sp. 4J7B1 has been deposited in the TBL/EMBL/GenBank databases under accession numbers CDWJ01000001 to CDWJ01000432.

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REFERENCES

- Behrendt U, Ulrich A, Schumann P. 2008. *Leucobacter tardus* sp. nov., isolated from the phyllosphere of *Solanum tuberosum* L. 2008. *Int J Syst Evol Microbiol* 58:2574–2578. <http://dx.doi.org/10.1099/ijs.0.2008/001065-0>.
- Narváez-Reinaldo JJ, Barba I, González-López J, Tunnacliffe A, Manzanera M. 2010. Rapid method for isolation of desiccation-tolerant strains and xeroprotectants. *Appl Environ Microbiol* 76:5254–5262. <http://dx.doi.org/10.1128/AEM.00855-10>.
- Manzanera M, Santa-Cruz-Calvo L, Vilchez JI, García-Fontana C, Silva-Castro GA, Calvo C, González-López J. 2014. Genome sequence of *Arthrobacter siccitolerans* 4J27, a xeroprotectant-producing desiccation-tolerant microorganism. *Genome Announc* 2(3): e00526-14. <http://dx.doi.org/10.1128/genomeA.00526-14>.

4. SantaCruz-Calvo L, González-López J, Manzanera M. 2013. *Arthrobacter siccitolerans* sp. nov., a highly desiccation-tolerant, xeroprotectant-producing strain isolated from dry soil. *Int J Syst Evol Microbiol* 63: 4174–4180. <http://dx.doi.org/10.1099/ijss.0.052902-0>.
5. Vilchez S, Manzanera M. 2011. Biotechnological uses of desiccation-tolerant microorganisms for the rhizoremediation of soils subjected to seasonal drought. *Appl Microbiol Biotechnol* 91:1297–1304. <http://dx.doi.org/10.1007/s00253-011-3461-6>.
6. Manzanera M, García de Castro A, Tøndervik A, Rayner-Brandes M, Strøm AR, Tunnacliffe A. 2002. Hydroxyectoine is superior to trehalose for anhydrobiotic engineering of *Pseudomonas putida* KT2440. *Appl Environ Microbiol* 68:4328–4333. <http://dx.doi.org/10.1128/AEM.68.9.4328-4333.2002>.
7. Manzanera M, Vilchez S, Tunnacliffe A. 2004. High survival and stability rates of *Escherichia coli* dried in hydroxyectoine. *FEMS Microbiol Lett* 233:347–352. <http://dx.doi.org/10.1016/j.femsle.2004.03.005>.
8. Julca I, Alaminos M, González-López J, Manzanera M. 2012. Xeroprotectants for the stabilization of biomaterials. *Biotechnol Adv* 30: 1641–1654. <http://dx.doi.org/10.1016/j.biotechadv.2012.07.002>.
9. Manzanera M, Vilchez S, Tunnacliffe A. 2004. Plastic encapsulation of stabilized *Escherichia coli* and *Pseudomonas putida*. *Appl Environ Microbiol* 70:3143–3145. <http://dx.doi.org/10.1128/AEM.70.5.3143-3145.2004>.
10. Billi D, Wright DJ, Helm RF, Prickett T, Potts M, Crowe JH. 2000. Engineering desiccation tolerance in *Escherichia coli*. *Appl Environ Microbiol* 66:1680–1684. <http://dx.doi.org/10.1128/AEM.66.4.1680-1684.2000>.
11. Crowe JH, Crowe L, Carpenter JF, Wistrom A. 1987. Stabilization of dry phospholipid-bilayers and proteins by sugars. *Biochem J* 242:1–10.
12. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Ho CH, Irzyk GP. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. <http://dx.doi.org/10.1038/nature03959>.
13. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res* 27: 4636–4641. <http://dx.doi.org/10.1093/nar/27.23.4636>.
14. Salzberg SL, Delcher AL, Kasif S, White O. 1998. Microbial gene identification using interpolated Markov models. *Nucleic Acids Res* 26: 544–548. <http://dx.doi.org/10.1093/nar/26.2.544>.
15. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. *Bioinformatics* 23:673–679. <http://dx.doi.org/10.1093/bioinformatics/btm009>.
16. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of rRNA genes. *Nucleic Acids Res* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
17. Schattner P, Brooks AN, Lowe TM. 2005. The tRNA scan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res* 33:W686–W689. <http://dx.doi.org/10.1093/nar/gki366>.
18. Lowe TM, Eddy SR. 1997. Program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955–964. <http://dx.doi.org/10.1093/nar/25.5.0955>.
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2).
20. Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schäffer AA. 2008. Database indexing for production MegaBLAST searches. *Bioinformatics* 24:1757–1764. <http://dx.doi.org/10.1093/bioinformatics/btn322>.
21. Vilchez S, Tunnacliffe A, Manzanera M. 2008. Tolerance of plastic-encapsulated *Pseudomonas putida* KT2440 to chemical stress. *Extremophiles* 12:297–299. <http://dx.doi.org/10.1007/s00792-007-0123-9>.