



Genome Sequence of *Microbacterium* sp. Strain 3J1, a Highly Desiccation-Tolerant Bacterium That Promotes Plant Growth

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The genome sequence for *Microbacterium* sp. strain 3J1, a desiccation-tolerant organism isolated from the *Nerium oleander* rhizosphere, is reported here. The genome is estimated to be approximately 3.5 Mb in size, with an average G+C content of 67.7% and a predicted number of protein-coding sequences of 3,310.

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icrobacterium sp. strain 3J1 is a highly desiccation-tolerant Gram-positive bacterium belonging to the Actinobacteria phylum and the Microbacteriaceae family, and it is isolated from the Nerium oleander rhizosphere (1). The genome sequences of other desiccation-tolerant microorganisms have been reported (2-5), including that of the recently described new species, Arthrobacter siccitolerant (strain 4J27) (6). In response to changes in osmotic conditions and water activity, these microorganisms produce different compounds (1) known as xeroprotectants (7). These compounds, which are produced to protect essential biomolecules and cell integrity, allow the cell to tolerate extremely low concentrations of water and other chemical insults (8-10), including reactive oxygen species (11). The major water-soluble antioxidants found to date are glutathione (γ -glutamylcysteinylglycine [GSH]) and ascorbic acid (12), and the main lipid-soluble antioxidants are tocopherols and carotenes (13), although other antioxidant molecules have been found with important roles in desiccation tolerance (11).

Here, the whole-genome sequence of Microbacterium sp. 3J1 is reported based on pyrosequencing technology implemented in the 454 Life Sciences-Roche platform with a combined approach based on 8-kb mate pair and shotgun sequencing (Lifesequencing SL, Valencia, Spain) (14). This technology was used to obtain a total of 109,001 sequences with the mate pair sequencing, rendering an average read length of 286 nucleotides and a total of 128,699 sequences, yielding an average length of 595 nucleotides with the shotgun sequencing strategy. The total number of sequenced bases was 107,758,549, representing a sequencing depth of around 29×. For de novo assembly, Newbler Assembler version 2.6 was used, with default parameters. This assembly yielded 30 contigs, of which 15 were >500 bp. The N_{50} of the contig assembly was 326,731 bp, and the largest contig was 1,103,902 bp. Mate pair information indicated that most of these contigs were ordered in two scaffolds, the largest comprising 3,402,533 bp. The estimated genome size of 3.5 Mb was deduced from this combination of scaffolds and contigs. Gap-spanning clones and PCR products were used to attempt gap closure, and putative coding sequences

were predicted. Genes were annotated with a pipeline implemented at Lifesequencing, and protein-coding sequences (CDSs) were predicted with Glimmer (15–17), RNAmmer (18), tRNAscan (19, 20), and BLAST (21, 22) in combination. Most of the contigs used to obtain complete genomic information for *Microbacterium* sp. 3J1 are contained in two scaffolds, with an average G+C content of 67.7%. The genome was found to contain 3,310 protein-coding genes, 4 rRNA operons, and 44 tRNA genes.

On the basis of this genome sequence, we propose the presence of pathways for the biosynthesis of antioxidants, including glutathione, ascorbic acid, tocopherols, and α -, β -, δ -, ε -, γ -, and ζ -carotene, among many others.

The complete genome sequence of *Microbacterium* sp. 3J1 will contribute to the development of biotechnological applications in the field of anhydrobiotic engineering (23).

Nucleotide sequence accession numbers. The complete genome sequence of *Microbacterium* sp. 3J1 has been deposited in the TBL/EMBL/GenBank databases under the BioProject number PRJEB8445 and accession numbers CDWI01000001 to CDWI01000030.

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REFERENCES

- 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, Vílchez JI, García-Fontana C, Calvo C, González-López J. 2015. Genome sequence of *Leucobacter* sp. 4J7B1, a plantosmoprotectant soil microorganism. Genome Announc 3(3):e00398-15. http://dx.doi.org/10.1128/genomeA.00398-15.

- Manzanera M, Santa-Cruz-Calvo L, Vílchez JI, García-Fontana C, Silva-Castro GA, Calvo C, González-López J. 2014. Genome sequence of *Arthrobacter siccitolerans* 4J27, a xeroprotectant-producing desiccationtolerant microorganism. Genome Announc 2(3):e00526-14. http:// dx.doi.org/10.1128/genomeA.00526-14.
- Manzanera M, García-Fontana C, Vílchez JI, González-López J. 2015. Genome sequence of *Rhodococcus* sp. 4J2A2, a desiccation-tolerant bacterium involved in biodegradation of aromatic hydrocarbons. Genome Announc 3(3):e00592-15. http://dx.doi.org/10.1128/genomeA.00592-15.
- Manzanera M, Narváez-Reinaldo JJ, García-Fontana C, Vílchez JI, González-López J. 2015. Genome sequence of Arthrobacter koreensis 5J12A, a plant growth-promoting and desiccation-tolerant strain. Genome Announc 3:e00648-15. http://dx.doi.org/10.1128/genomeA.00648 -15.
- SantaCruz-Calvo L, González-López J, Manzanera M. 2013. Arthrobacter siccitolerans sp. nov., a highly desiccation-tolerant, xeroprotectantproducing strain isolated from dry soil. Int J Syst Evol Microbiol 63: 4174–4180. http://dx.doi.org/10.1099/ijs.0.052902-0.
- 7. 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.
- Vilchez S, Manzanera M. 2011. Biotechnological uses of desiccationtolerant 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.
- 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.
- Vílchez S, Tunnacliffe A, Manzanera M. 2008. Tolerance of plasticencapsulated *Pseudomonas putida* KT2440 to chemical stress. Extremophiles 12:297–299. http://dx.doi.org/10.1007/s00792-007-0123-9.
- Kranner I, Birtic S. 2005. A modulating role for antioxidants in desiccation tolerance. Integr Comp Biol 45:734–740. http://dx.doi.org/10.1093/ icb/45.5.734.
- Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49: 249–279.

- Munné-Bosch S, Alegre L. 2002. The function of tocopherols and tocotrienols in plants. Crit Rev Plant Sci 21:31–57. http://dx.doi.org/10.1080/ 0735-260291044179.
- 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.
- 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.
- 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.
- 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.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. http://dx.doi.org/10.1093/ nar/gkm160.
- 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. http://dx.doi.org/10.1093/nar/gki366.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a 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.
- 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.
- 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.
- Tunnacliffe A, García de Castro A, Manzanera M. 2001. Anhydrobiotic engineering of bacterial and mammalian cells: is intracellular trehalose sufficient? Cryobiology 43:124–132. http://dx.doi.org/10.1006/ cryo.2001.2356.