



Complete Genome Sequence of *Kosakonia sacchari* Strain BO-1, an Endophytic Diazotroph Isolated from a Sweet Potato

Rina Shinjo,ª Kazuma Uesaka,ª Kunio Ihara,^b Kseniia Loshakova,ª Yuri Mizuno,ª Katsuya Yano,ª Aiko Tanakaª

Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Japana; Center for Gene Research, Nagoya University, Chikusa, Nagoya, Japana

The complete genome sequence of the endophytic diazotroph *Kosakonia sacchari*, isolated from a sweet potato, was analyzed. The 4,902,106-bp genome with 53.7% G+C content comprises 4,638 open reading frames, including *nif* genes, 84 tRNAs, and seven complete rRNAs in a circular chromosome.

Received 5 July 2016 Accepted 18 July 2016 Published 8 September 2016
Citation Shinjo R, Uesaka K, Ihara K, Loshakova K, Mizuno Y, Yano K, Tanaka A. 2016. Complete genome sequence of Kosakonia sacchari strain BO-1, an endophytic diazotroph
isolated from a sweet potato. Genome Announc 4(5):e00868-16. doi:10.1128/genomeA.00868-16.
Copyright © 2016 Shinjo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.
Address correspondence to Aiko Tanaka, aikotana@agr.nagoya-u.ac.jp.

The endophytic bacterial strain BO-1 was originally isolated from the surface-sterilized stem of a sweet potato (*Ipomoea batatas*, cultivar "Beniotome") from Miyakonojo, Miyazaki, Japan, and identified as *Klebsiella oxytoca* based on a partial sequence of the 16s rRNA (1). The strain grew diazotrophically in a free-living state under nitrogen-deficient conditions and showed a positive reaction in the acetylene reduction activity test (1). Whole-genome sequencing analysis revealed that the genome of strain BO-1 is most similar to *Kosakonia sacchari* strain SP1, isolated from sugarcane (2); therefore, this strain likely belongs to *Kosakonia sacchari*, which is a Gram-negative, aerobic, nonspore-forming, motile rod-shaped species, recently reclassified from *Enterobacter sacchari* (3).

The bacterial strain BO-1 (MAFF 211344) was obtained from the National Institute of Agrobiological Science (NIAS, Japan). Genomic DNA was extracted using a genomic DNA purification kit (Promega, USA). Paired-end DNA libraries were prepared for sequencing with an Illumina MiSeq platform. A total of 12,874,059 high-quality paired-end reads were generated, with 788-fold coverage. *De novo* assembly was performed using SPAdes genome assembler version 3.6.2 (4). Removal of short contigs resulted in 916 contigs with an N_{50} of 828,908 bp. Gaps were closed by PCR amplification and Sanger sequencing of the PCR products. The genome of *K. sacchari* BO-1 consists of a circular chromosome (4,902,106 bp; 53.7% G+C content).

The strain BO-1 is most closely related to *K. sacchari* strain SP1, with which it shares 98.67% average nucleotide identity (ANI) (5). Since two genomes with an ANI >95% are considered to be from the same species, the strain BO-1 is reidentified as *Kosakonia sacchari*. The chromosome of *K. sacchari* strain SP1 had the highest sequence similarities to the chromosome of *Enterobacter* sp. strain R4-368, an endophytic nitrogen-fixing bacterium isolated from *Jatropha curcas* (2, 6). The ANIs of *Enterobacter* sp. strain R4-368 with *K. sacchari* strain BO-1 and strain SP1 were 94.06% and 94.01%, respectively.

The genomic sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok). The chromosome has

4,448 coding sequences, seven sets of rRNA genes, and 84 tRNA genes. The genome annotation also confirmed the presence of the *nif* gene locus for nitrogen fixation.

Accession number(s). The genome sequence of *K. sacchari* strain BO-1 was deposited in DDBJ/EMBL/GenBank under the accession number CP016337.

ACKNOWLEDGMENTS

We thank Katsuki Adachi (Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Japan), Motohiko Kondo (Nagoya University, Japan), and Joyce Cartagena (Nagoya University, Japan) for comments on the manuscript. We also thank Hiroyuki Sawada (Genetic Resources Center, National Agriculture and Food Research Organization, Japan) for advice on classifying bacterial strains.

FUNDING INFORMATION

This work, including the efforts of Aiko Tanaka, was funded by Ministry of Education, Culture, Sports, Science, and Technology (MEXT) (15K07839 and 16J40044). This work, including the efforts of Aiko Tanaka, was funded by Naito Foundation.

REFERENCES

- Adachi K, Nakatani M, Mochida H. 2002. Isolation of an endophytic diazotroph, *Klebsiella oxytoca*, from sweet potato stems in Japan. Soil Sci Plant Nutr 48:889–895. http://dx.doi.org/10.1080/00380768.2002.10408717.
- Chen M, Zhu B, Lin L, Yang L, Li Y, An Q. 2014. Complete genome sequence of *Kosakonia sacchari* type strain SP1(T). Stand Genomic Sci 9:1311–1318. http://dx.doi.org/10.4056/sigs.5779977.
- 3. Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. 2013. Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): proposal to reclassify E. nimipressuralis and E. amnigenus into Lelliottia gen. nov. as Lelliottia nimipressuralis comb. nov. and Lelliottia amnigena comb. nov., respectively, E. gergoviae and E. pyrinus into Pluralibacter gen. nov. as Pluralibacter gergoviae comb. nov. and Pluralibacter pyrinus comb. nov., respectively, E. cowanii, E. radicincitans, E. oryzae and E. arachidis into Kosakonia gen. nov. as Kosakonia cowanii comb. nov., Kosakonia radicincitans comb. nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and E. turicensis, R. nov. Cronobacter helveticus comb. nov.

and emended description of the genera *Enterobacter* and *Cronobacter*. Syst Appl Microbiol **36:**309–319. http://dx.doi.org/10.1016/ j.syapm.2013.03.005.

- 4. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/cmb.2012.0021.
- Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. http://dx.doi.org/10.1099/ijs.0.64483-0.
- Madhaiyan M, Peng N, Ji L. 2013. Complete genome sequence of *Entero-bacter* sp. strain R4-368, an endophytic N-fixing gammaproteobacterium isolated from surface-sterilized roots of *Jatropha curcas* L. Genome Announc 1(4):e00544-13. http://dx.doi.org/10.1128/genomeA.00544-13.