Genome Sequences of Novel Azospirillum sp. Strains B21 and Sh1, Isolated from Raised Sphagnum Bogs, and Type Strains Azospirillum lipoferum 59b and Azospirillum oryzae COC8

Denis S. Grouzdev,a Ekaterina N. Tikhonova,b Irina K. Kravchenko

aInstitute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia
bWinogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

ABSTRACT Here, we report the genomic sequences of the novel Azospirillum sp. strains B21 and Sh1, isolated from raised bogs, along with the genome sequences of Azospirillum lipoferum 59bT, the type species of the genus, and Azospirillum oryzae COC8T, which were analyzed to get more knowledge about the genus Azospirillum.

Bacteria of the genus Azospirillum, belonging to the order Rhodospirillales and the family Rhodospirillaceae, are well-characterized plant growth-promoting rhizobacteria (PGPR) due to their capacity for fixing atmospheric nitrogen and their ability to colonize roots of cereals and other grasses and to produce phytohormones. Recently Azospirillum palustre, a novel methylotrophic bacterium isolated from raised bog, was described (1).

Azospirillum sp. strains B21 and Sh1 were isolated from acidic Sphagnum-dominated peat soil in the Russian Federation. Strain B21 was isolated from the Sosvyatskoye peatland in the Tver region in 2000 (2). Strain Sh1 was isolated in 2018 from the upper 0- to 5-cm layer of a typical raised bog in the Shatura region, Moscow Oblast, which was afforested, i.e., drained and planted with trees. Pure cultures were obtained by serial 10-fold dilutions on N-free NFb agar medium (1.5%) (3). The plates were incubated at 29°C for 72 h, and the isolates were kept as pure cultures. DNA was purified from the cell biomass using the DNeasy PowerSoil kit (Qiagen, Germany) following the manufacturer’s instructions. The 16S rRNA gene was amplified with the 27F and 1492R primers (4), and purified PCR products were sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, USA). The 16S rRNA sequence analysis conducted using the online tool EzBioCloud (5) revealed that B21 shares 99.2% similarity with Azospirillum oryzae COC8T (6), and Sh1 shares 99.0% similarity with Azospirillum palustre B2T (1, 7). Both bacteria were assigned to clade L (Azospirillum lipoferum) (8).

Despite the fact that Azospirillum lipoferum 59bT (9) and Azospirillum oryzae COC8T (6) have been described for a long time, genome-wide studies for these organisms were absent, which prevented a detailed comparison with newly isolated azospirilla. The strains Azospirillum lipoferum 59bT (IBPPM 173 = VKM B-1519 = ATCC 29707) and Azospirillum oryzae COC8T (IBPPM 548 = LMG 23844 = IAM 15130) were provided by the Collection of Rhizosphere Microorganisms (collection.ibppm.ru) in the framework of scientific cooperation.

To perform whole-genome sequencing, all strains were cultivated on the NFb agar medium for 72 h at 29°C. Colonies were washed from the plate with liquid NFb medium and centrifuged. The genomic DNA was extracted using the DNeasy PowerSoil kit (Qiagen, Germany), following the manufacturer’s instructions. The libraries were constructed with the NEBNext DNA library prep reagent set for Illumina according to the protocol for the kit. The genomes were sequenced using the HiSeq 2500 platform.

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Address correspondence to Denis S. Grouzdev, denisgrouzdev@gmail.com.
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(Illumina, Inc., USA) with 150-bp (Azospirillum lipoferum 59bT, Azospirillum oryzae COC8T, and Azospirillum sp. strain Sh1) and 100-bp (Azospirillum sp. strain B21) paired-end reads. Low-quality reads were trimmed using Trimmomatic v. 0.36 (10) with the default settings for paired-end reads. Subsequently, the quality-filtered reads were de novo assembled with SPAdes v. 3.13.0 using the default settings (11). The resulting assemblies were quality assessed with QUAST v. 5.0 (12). Identification of protein-coding sequences and primary annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13). Summary statistics and characteristic features of the whole-genome sequencing, assembly, and annotation of the four strains are given in Table 1. These genome sequences provide valuable data to study the ecology, evolution, and physiology of Azospirillum species.

Data availability. These whole-genome projects have been deposited at DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The versions described in this paper are the first versions.

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REFERENCES

1. Tikhonova EN, Grouzdev DS, Kravchenko IK. 2019. Azospirillum palustre sp. nov., a methylotrophic nitrogen-fixing species isolated from raised bog. Int J Syst Evol Microbiol 69:2787–2793. https://doi.org/10.1099/ijsem.0.003560.

2. Doroshenko EV, Boulygina ES, Spiridonova EM, Tourova TP, Kravchenko IK. 2007. Isolation and characterization of nitrogen-fixing bacteria of the genus Azospirillum from the soil of a Sphagnum peat bog. Microbiology 76:93–101. https://doi.org/10.1134/S0026261707010134.

3. Dobereiner J. 1980. Forage grasses and grain crops, p 535–555. In Bergeresen JF (ed), Methods of evaluation of biological nitrogen fixation. John Wiley & Sons Inc., New York, NY.

4. Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A 82:6965–6969. https://doi.org/10.1073/pnas.82.20.6965.

5. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EBiCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755.

6. Xie C-H, Yokota A. 2005. Azospirillum oryzae sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant Oryza sativa. Int J Syst Evol Microbiol 55:1435–1438. https://doi.org/10.1099/ijs.0.63503-0.

7. Grouzdev DS, Tikhonova EN, Krutkina MS, Kravchenko IK. 2018. Genome sequence of the methylotrophic Azospirillum sp. strain B2 isolated from raised Sphagnum bog. Genome Announc 6:e00492-18. https://doi.org/10.1128/genomeA.00492-18.

8. Maroniche GA, García JE, Salcedo F, Cresu CM. 2017. Molecular identification of Azospirillum spp.: limitations of 16S rRNA and qualities of ppoD as genetic markers. Microbiol Res 195:1–10. https://doi.org/10.1016/j.micres.2016.11.009.

9. Tarrand JJ, Krieg NR, Dobereiner J. 1978. A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerinck) comb. nov. and Azospirillum brasilense sp. nov. Can J Microbiol 24:967–980. https://doi.org/10.1139/m78-160.

10. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

TABLE 1 Characteristics of draft genome sequences and accession numbers of Azospirillum oryzae COC8T, Azospirillum lipoferum 59bT, and Azospirillum sp. strains Sh1 and B21

| Characteristic | A. oryzae COC8T | A. lipoferum 59bT | Azospirillum sp. Sh1 | Azospirillum sp. B21 |
|---------------|----------------|----------------|----------------|----------------|
| BioProject no. | PRJNA563036 | PRJNA563039 | PRJNA562938 | PRJNA563053 |
| GenBank accession no. | VTTM00000000 | VTTM00000000 | VTTM00000000 | VTTM00000000 |
| BioSample no. | SAMN12661881 | SAMN12661906 | SAMN12660160 | SAMN12662157 |
| SRA no. | SRR10092265 | SRR10092045 | SRR10092256 | SRR10103333 |
| Genome size (bp) | 6,755,201 | 7,987,183 | 7,274,603 | 7,463,459 |
| G+C content (%) | 67.36 | 67.27 | 67.71 | 67.33 |
| No. of protein-coding genes | 5,859 | 6,903 | 6,319 | 6,534 |
| Total no. of genes | 6,077 | 7,152 | 6,561 | 6,781 |
| No. of pair-end reads | 4,606,976 | 4,476,896 | 4,770,146 | 23,572,400 |
| L50 value | 5 | 7 | 14 | 6 |
| N50 value (bp) | 405,582 | 372,840 | 158,668 | 465,063 |
| Coverage (x) | 154 | 127 | 141 | 236 |
| Total no. of scaffolds | 39 | 72 | 103 | 57 |
| No. of scaffolds | 405,582 | 372,840 | 158,668 | 465,063 |
| No. of RNAs | 74 | 76 | 74 | 76 |
11. 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. https://doi.org/10.1089/cmb.2012.0021.

12. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

13. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufo S, Li W. 2013. Prokaryotic Genome Annotation Pipeline. In The NCBI handbook, 2nd ed. NCBI, Bethesda, MD. https://doi.org/10.1093/nar/gkw569.