Genome Sequence of *Novoherbaspirillum* sp. UKPF54, a Plant Growth-Promoting Rhizobacterial Strain with $N_2O$-Mitigating Abilities, Isolated from Paddy Soil

Nan Gao, Chaowei Zhou, Weishou Shen, Sayuri Ota, Yutaka Shiratori, Tomoyasu Nishizawa, Kazuo Isobe, Xinhua He, Hanjie Ying, Keishi Senoo

National Engineering Research Center for Biotechnology and School of Biological and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, China
Jiangsu Key Laboratory of Atmospheric Environment Monitoring and Pollution Control, Collaborative Innovation Center of Atmospheric Environment and Equipment Technology, and School of Environmental Science and Engineering, Nanjing University of Information Science and Technology, Nanjing, China
Niigata Agricultural Research Institute, Niigata, Japan
Department of Resource Science, College of Agriculture, Ibaraki University, Ibaraki, Japan
Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
Centre of Excellence for Soil Biology, College of Resources and Engineering, Southwest University, Chongqing, China
Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Tokyo, Japan

**ABSTRACT** *Novoherbaspirillum* sp. strain UKPF54, a plant growth-promoting rhizobacterium with the ability to mitigate nitrous oxide emission from agriculture soils, has been successfully isolated from paddy soil in Kumamoto, Japan. Here, we report the whole-genome sequence of this strain.

*Herbaspirillum* spp. are endophytic diazotrophs which can colonize sugarcane, rice, maize, sorghum, red clover, and other crops (1–3). Some *Herbaspirillum* sp. strains and their close relatives, such as *Novoherbaspirillum* sp. strains, are known to promote plant growth, suggesting that they are important resource species for the development of biofertilizers (1–3). *Novoherbaspirillum* sp. strain UKPF54, previously called *Herbaspirillum* sp. strain UKPF54, was isolated from the rhizosphere of paddy soil in Kumamoto, Japan (4). It simultaneously promotes the growth of pasture plants and mitigates nitrous oxide emissions from soils (3, 5).

*Novoherbaspirillum* sp. UKPF54 was grown in 5 ml NBNS culture medium (briefly, 5 g liter$^{-1}$ peptone and 3 g liter$^{-1}$ beef extract containing 0.3 mM NaNO$_3$ and 4 mM sodium succinate [pH 7.0]) at 28°C and 220 rpm. The genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Germany). A SMRTbell library with a 20-kb insert size was constructed with the template prep kit 1.0 and the BluePippin size selection system, according to the manual. The genome was sequenced with the PacBio RS II DNA sequencing system using SMRT Cell 8Pac v3 and DNA polymerase binding kit P6 reagents. To remove the PacBio short reads, the RS HGAP assembly software (v3.0) was applied, with default parameters (6). When 5' and 3' ends were connected, the contig was assembled into a single circular DNA molecule. The circular DNA molecule had a mean subread length of 8,279 bp, an $N_{50}$ value of raw sequences of 11,260 bp, and a total of 1,453,750,364 bases and 175,579 reads. The sequencing depth reached 198×.

Genes were predicted using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP, revision 4.8) with the best-placed reference protein set (GeneMarkS2+) (7, 8). In addition, BlastKOALA (9) against the "species_prokaryotes" database was used for functional annotation and KEGG pathway mapping. A total of 4,307 protein-coding sequences, 56 tRNAs, 9 rRNAs, 4 noncoding RNAs (ncRNAs), and 50 pseudogenes were
discovered. The predicted functional genes consisted of 11 genes of the ABC transporters, 10 genes of the two-component system, and 5 genes of bacterial chemotaxis. The functional genes contained candidate genes associated with nitrogen metabolism and plant growth promotion (Table 1).

We analyzed the secondary metabolism cluster of the complete genome with antiSMASH v5.0.0 (10). The results showed that four secondary metabolite gene clusters relevant to the production of active substances were predicted. Moreover, two nonribosomal peptide synthetase (NRPS)-like fragment gene clusters and one beta-lactone-containing protease inhibitor (betalactone) gene cluster were predicted.

Overall, the whole-genome sequence is of critical importance to reveal the molecular mechanisms for the promotion of plant growth and the mitigation of nitrous oxide from agricultural soils by *Novoherbaspirillum* sp. UKPF54, thereby providing fundamental support to develop biofertilizer applications with this strain.

**Data availability.** The whole-genome sequence of *Novoherbaspirillum* sp. UKPF54 has been deposited in GenBank under the accession number CP040128. The raw reads have been deposited in the Sequence Read Archive (SRA) under the accession number SRR8943564.

**ACKNOWLEDGMENTS**

This study was financially supported by the National Natural Science Foundation of China (grants 41771291 and 31972503), the Natural Science Foundation of the Higher Education Institutions of Jiangsu Province (grant 18KJB210007), and the Jiangsu Synergistic Innovation Center for Advanced Bio-Manufacture (grant XTE1828), China, and

### TABLE 1 Predicted genes associated with nitrogen metabolism and plant growth promotion in the *Novoherbaspirillum* sp. UKPF54 genome

| Gene function              | Gene name | Product | Protein accession no. | Inference amino acid sequence ID |
|---------------------------|-----------|---------|-----------------------|----------------------------------|
| Nitrogen fixation         | *fdx*     | ISC system 2Fe-2S type ferredoxin | QDZ27364 WP_012079114 |
|                           | *fdxH*    | Formate dehydrogenase subunit beta | QDZ27883 WP_018604908 |
|                           | *fixA*    | Electron transfer flavoprotein subunit beta/FixA family protein | QDZ26876 WP_015436355 |
|                           | *fixB*    | Electron transfer flavoprotein subunit alpha/FixB family protein | QDZ26877 WP_008445988 |
|                           | *nifU*    | SUF system NifU family Fe-S cluster assembly protein | QDZ29484 WP_018310943 |
| Nitrate utilization       | *napA*    | Nitrate reductase catalytic subunit NapA | QDZ28521 YP_006896445 |
|                           | *napE*    | Nitrate reductase | QDZ28519 WP_006394233 |
|                           | *narG*    | Nitrate reductase subunit alpha | QDZ28003 WP_018151853 |
|                           | *narH*    | Nitrate reductase subunit beta | QDZ28002 WP_011871146 |
|                           | *narl*    | Respiratory nitrate reductase subunit gamma | QDZ28000 WP_011871144 |
|                           | *narJ*    | Nitrate reductase molybdenum cofactor assembly chaperone | QDZ28001 WP_008953006 |
| Nitrite utilization       | *nirB*    | Nitrite reductase large subunit | QDZ29679 WP_017876120 |
|                           | *nirD*    | Nitrite reductase small subunit NirD | QDZ26547 WP_011829911 |
|                           | *nirK*    | Nitrite reductase, copper containing | QDZ28940 WP_013213801 |
| Nitrous oxide reduction   | *nosD*    | Nitrous oxide reductase family maturation protein NosD | QDZ30537 WP_017879062 |
|                           | *nosL*    | Nitrous oxide reductase accessory protein NosL | QDZ30535 WP_018077130 |
|                           | *nosZ*    | Nitrous oxide reductase | QDZ28019 WP_019898632 |
| Nitrogen regulation       | *fnr*     | Fumarate/nitrate reduction transcriptional regulator Fnr | QDZ29797 WP_011871123 |
|                           | *ntrC*    | Nitrogen regulation protein NRII | QDZ28461 WP_019141540 |
| Indole acetic acid synthesis | *trpC*  | Indole-3-glycerol phosphate synthase TrpC | QDZ30261 WP_005671473 |
| Phosphate solubilization  | *pqqB*    | Pyrroloquinoline quinone biosynthesis protein | QDZ26968 WP_013691667 |
| Acetolactate synthesis    | *ilvB*    | Acetolactate synthase 3 catalytic subunit | QDZ28257 WP_016832326 |
|                           | *ilvC*    | Ketol-acid reductoisomerase | QDZ28253 WP_003269193 |
|                           | *ilvD*    | Dihydroxy-acid dehydratase | QDZ28620 WP_007877043 |
|                           | *ilvN*    | Acetolactate synthase small subunit | QDZ28256 WP_004630835 |

---

*ISC, iron-sulfur cluster; SUF, sulfur assimilation.
NCBI accession number of most similar protein sequence from which function is inferred. ID, identification.*
the Japan Society for the Promotion of Science through a postdoctoral fellowship (grant 14F04390), JSPS KAKENHI (grant JP15KT0024), the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (grant 26037B), and the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and Food Industry (grant 27004C), Japan.

REFERENCES

1. Choudhury ATMA, Kennedy IR. 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol Fert Soils 39:219–227. https://doi.org/10.1007/s00374-003-0706-2.

2. Chubatsu LS, Monteiro RA, de Souza EM, de Oliveira MAS, Yates MG, Wassem R, Bonatto AC, Huergo LF, Steffens MBR, Rigo LU, Pedrosa FD. 2012. Nitrogen fixation control in Herbaspirillum seropedicae. Plant Soil 356:197–207. https://doi.org/10.1007/s11104-011-0819-6.

3. Gao N, Shen W, Kakuta H, Tanaka N, Fujiwara T, Nishizawa T, Takaya N, Nagamine T, Itoke K, Otsuka S, Senoo K. 2016. Inoculation with nitrous oxide (N_{2}O)-reducing denitrifier strains simultaneously mitigates N_{2}O emission from pasture soil and promotes growth of pasture plants. Soil Biol Biochem 97:83–91. https://doi.org/10.1016/j.soilbio.2016.03.004.

4. Ashida N, Ishii S, Hayano S, Tago K, Tsuji T, Yoshimura Y, Otsuka S, Senoo K. 2010. Isolation of functional single cells from environments using a micromanipulator: application to study denitrifying bacteria. Appl Microbiol Biotechnol 85:1211–1217. https://doi.org/10.1007/s00253-009-2330-z.

5. Gao N, Shen WS, Camargo E, Shiratori Y, Nishizawa T, Itoke K, He XH, Senoo K. 2017. Nitrous oxide (N_{2}O)-reducing denitrifier-inoculated organic fertilizer mitigates N_{2}O emissions from agricultural soils. Biol Fertil Soils 53:885–898. https://doi.org/10.1007/s00374-017-1231-z.

6. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korfich J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.

7. Tatusova T, DiCuccio M, Badretdin A, Chevervinn V, Navrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovksy M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

8. Haft DH, DiCuccio M, Badretdin A, Brover V, Chevervinn V, O’Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res 46:D851–D860. https://doi.org/10.1093/nar/gkx1068.

9. Kanehisa M, Sato Y, Morishima K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006.

10. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10.1093/nar/gkz310.