Whole-Genome Sequence of *Paenibacillus polymyxa* Strain SRT9.1, a Promising Plant Growth-Promoting Bacterium

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ABSTRACT *Paenibacillus polymyxa* SRT9.1 is an epiphytic bacterium capable of inhibiting plant-pathogenic bacteria. The strain has potential for development as a biocontrol agent for use in agriculture. We report the whole-genome sequence of *Paenibacillus polymyxa* SRT9.1, consisting of 6,754,470 bp and 7,878 coding sequences, with an average G+C content of 45%.

*P*aenibacillus polymyxa, a Gram-positive bacterium, has long been used as a phytopathogen biological control agent and plant growth and development booster. The plant growth-promoting and biocontrol activity of *Paenibacillus polymyxa* against plant pathogens is attributed to antimicrobial compound production, nitrogen fixation, the secretion of hydrolytic enzymes, and siderophore production (1–4).

P. polymyxa SRT9.1 was isolated from the surface of fungicide-treated red sweet peppers sourced from the Agricultural Research Council—Vegetable and Ornamental Plants Institute (ARC-VOP), Roodeplaat, Pretoria, South Africa (25°59′S, 28°35′E; altitude, 1,200 m above sea level). Fresh peppers, grown in open soil conditions, were collected in sterile, sealed plastic bags (5). Bacterial biofilms on the surfaces of the pepper fruits were recovered using sterile cotton swabs soaked in a solution containing 0.15 M NaCl and 0.1% vol/vol Tween 20 (6). The swabs were vortexed in sterile Eppendorf tubes containing saline solution (0.85% [vol/vol] NaCl). The supernatant was serially diluted, plated onto Trypticase soy agar (TSA), and incubated for 48 h at 30°C under aerobic conditions. Distinct colonies were streaked onto TSA. *P. polymyxa* SRT9.1 was taxonomically identified as in references 7 and 8. The strain tested positive for *in vitro* plant growth-promoting abilities such as siderophore production and phosphate solubilization (3). The pure strain was subcultured in Trypticase soy broth (TSB), incubated at 30°C for 48 h, and stored in 50% (vol/vol) glycerol at −80°C. Revived *P. polymyxa* SRT9.1 was then subcultured on TSA and the biomass used for DNA extraction.

Genomic DNA of the SRT9.1 strain was extracted using the Quick-DNA fungal/bacterial miniprep kit (Zymo Research, Irvine, CA; catalog number D6005) and sequenced on the Illumina NextSeq platform at Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). The NEBNext Ultra II FS DNA library prep kit (New England Biolabs, Ipswich, MA) was used for DNA library preparation. The libraries were then sequenced on the Illumina NextSeq 550 platform, and a total of 2,224,054 reads generated from each sample (2 × 150-bp paired-end reads) were visualized using the KBase platform (9). The quality of the reads was evaluated using FastQC v0.11.5 (10), while low-quality reads and sequence adaptors were removed using Trimmomatic v0.36 (11). The genome assembly was performed using SPAdes v3.13.0 (12) with default parameters, yielding 3,172 contigs with a coverage of 100×. The assembly yielded a genome

seqeuence of 233 Mbp, 6,754,470 bp long, with a G+C content of 45.0%. The contigs had N_{50} and L_{50} values of 219,142 bp and 9, respectively. The contigs were annotated using the Rapid Annotations Subsystems Technology v1.073 toolkit (13) and the publicly available NCBI Prokaryotic Genome Annotation Pipeline (14), which identified 7,878 protein-coding genes, 63 RNA genes, 12 rRNA genes, 46 tRNA genes, and 136 pseudogenes. Additionally, secondary metabolites for antagonism of *Fusarium graminearum* (e.g., polymyxin and fusaricidin) (15) were identified using antiSMASH v5.0.0 (16). A whole-genome-based phylogenic tree for SRT9.1 was constructed using the Type (Strain) Genome Server (17), and SRT9.1 showed close similarity with *Paenibacillus polymyxa* ATCC 842 (GenBank accession number AFOX01000032.1), with a digital DNA-DNA hybridization (dDDH) value of 99.9% to the type strain.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAJFEV000000000. The version described in this paper is the first version. The SRA accession number is SRR16351787, the BioProject accession number is PRJNA771517, and the BioSample accession number is SAMN22314579.

REFERENCES

1. Hao TY, Chen SF. 2017. Colonization of wheat, maize and cucumber by *Paenibacillus polymyxa* WLY78. PLoS One 12:e0169980. https://doi.org/10.1371/journal.pone.0169980.
2. Weselowski B, Nathoo N, Eastman AW, MacDonald J, Yuan ZC. 2016. Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. BMC Microbiol 16:244. https://doi.org/10.1186/s12866-016-0860-y.
3. Mamphogoro TP, Kamutando CN, Maboko MM, Aiyegoro OA, Babalola OO. 2021. Epiphytic bacteria from sweet pepper antagonistic in vitro to *Ralstonia solanacearum* BD 261, a causative agent of bacterial wilt. Microorganisms 9:1947. https://doi.org/10.3390/microorganisms9091947.
4. Adeniji AA, Ayangbenro AS, Babalola OO. 2021. Genomic exploration of *Bacillus thuringiensis* MORWBS1.1, candidate biocontrol agent, predicts genes for biosynthesis of zwittermicin, 4,5-DOPA dioxygenase extradiol, and quercetin 2,3-dioxygenase. Mol Plant Microbe Interact 34:602–605. https://doi.org/10.1094/MPMI-10-20-0272-SC.
5. Mamphogoro TP, Maboko MM, Babalola OO, Aiyegoro OA. 2020. Bacterial communities associated with the surface of fresh sweet pepper (*Capsicum annuum*) and their potential as biocontrol. Sci Rep 10:8560. https://doi.org/10.1038/s41598-020-65587-9.
6. Paulino LC, Tseng CH, Strober BE, Blaser MJ. 2006. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. J Clin Microbiol 44:2933–2941. https://doi.org/10.1128/JCM.00785-06.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
8. Saitou N, Nei M. 1987. The neighborjoining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.
9. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia J-M, Chia J-M, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, et al. 2018. KBase: the United States Department of Energy Systems Biology Knowledgebase. Nat Biotechnol 36:566–569. https://doi.org/10.1038/nbt.4163.
10. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Institute, Cambridge, UK. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
11. 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.
12. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.
13. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
14. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin 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.
15. Zalila-Kolsi I, Ben Mahmoud A, Ali H, Sellami S, Nasfi Z, Tounsi S, Jamoussi K. 2016. Antagonist effects of *Bacillus* spp. strains against *Fusarium graminearum* for protection of durum wheat (*Triticum turgidum* L. subsp. durum). Microbiol Res 192:148–158. https://doi.org/10.1016/j.micres.2016.06.012.
16. 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.
17. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3.