




Draft Genome Sequences of *Pseudomonas koreensis* Strain UASWS1668, *Bacillus megaterium* Strain UASWS1667, and *Paenibacillus* sp. Strain UASWS1643, Considered Potential Plant Growth-Promoting Rhizobacteria

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ABSTRACT Plant growth-promoting rhizobacteria (PGPR) include species in the genera *Bacillus*, *Paenibacillus*, and *Pseudomonas*. We report here the draft genome sequences of the strains *Pseudomonas koreensis* UASWS1668 and *Bacillus megaterium* UASWS1667, isolated from a horse chestnut tree, and *Paenibacillus* sp. strain UASWS1643, isolated from a tomato stem. Auxin production and phosphate solubilization were biochemically confirmed.

Pseudomonas koreensis strain UASWS1668 and *Bacillus megaterium* strain UASWS1667, isolated from a horse chestnut tree, and *Paenibacillus* sp. strain UASWS1643, isolated from a tomato stem, were identified by 16S rRNA gene sequencing and later confirmed by whole 16S rRNA gene sequences extracted from their genome assemblies. A whole-genome sequencing strategy was chosen to confirm the potential of these bacteria as biostimulants. *Paenibacillus* sp. strain UASWS1643 showed 97.52% shared identity with the closest NCBI-referenced strain, *Paenibacillus xylanexedens* strain PAMC 22703 (1). *P. koreensis* has shown antifungal activities and plant growth-promoting rhizobacterium (PGPR) properties (2), particularly in heavy metal-contaminated soils (3) and under high-salt conditions (4–6), increasing plant fresh weight and root development. *Bacillus megaterium* is known for plant growth induction (7, 8), its antifungal properties, its resistance to heavy metals (8, 9), and adaption to acidic stress (10). The genus *Paenibacillus*, widely present in the soils and rhizospheres of many plants, commonly hosts PGPR species (11, 12), with nitrogen fixation, phosphate solubilization, auxin production, and siderophore secretion ability (11, 12).

Bacteria were isolated from the diseased tissues of horse chestnut or tomato according to a published method (13). DNA was extracted from pure cultures grown exponentially from a single colony in LB broth, with a modified cetyltrimethylammonium bromide protocol (14). Sequenced 16S amplicons produced with primers 27F and 1482 R (15) were identified by BLAST (16). Sequencing libraries were produced with the TruSeq Nano DNA library preparation kit (Illumina, USA). Whole-genome sequencing was performed with a MiniSeq high-output kit, in one Illumina MiniSeq run in 2 × 151-bp paired-end read format. The read quality was controlled with FastQC version 0.11.5 (17). Genome assemblies were computed with the SPAdes version 3.13.0 genome assembler (18), set to “paired-end” assembly and “careful” mode. The resulting contigs were ordered with BioEdit version 7.0.5 (19) and analyzed with QUASt version 4.6.3 (20), with the setting “QUASt: skip contigs shorter than 200 bp.” Automated gene annotations were carried out by the Prokaryotic Genome Annotation Pipeline (PGAP) version 4.1 (21) and RAST version 2.0 (22), with the ClassicRAST annotation scheme. Indole-3-acetic acid (IAA) production and phosphate solubilization were estimated according to published protocols (23, 24).

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TABLE 1 Genome sequence statistics summary and characteristics for *Pseudomonas koreensis* strain UASWS1668, *Bacillus megaterium* strain UASWS1667, and *Paenibacillus* sp. strain UASWS1643

Characteristic	Data for strain:		
	<i>Paenibacillus</i> sp. UASWS1643	<i>Bacillus megaterium</i> UASWS1667	<i>Pseudomonas koreensis</i> UASWS1668
Total length (bp)	7,354,805	5,171,380	6,177,111
GC content (%)	45.88	38.04	60.27
No. of CDSs ^a (PGAP)	6,422	5,232	5,660
No. of CDSs (RAST)	6,887	5,600	5,440
No. of tRNAs	63	70	59
No. of rRNAs	18	13	6
No. of phages	1	0	2
No. of plasmids	0	7	0
No. of auxin genes	4	4	4
No. of phosphorus metabolism genes	52	26	38
No. of nitrogen metabolism genes	34	12	14
Sequencing yield (Mbp)	445.96	550.32	574.37
Final coverage (×)	60.6	106.4	93
SRA accession no. for raw reads	SRX6863606	SRX6863642	SRX6863625
No. of reads	2,953,376	3,644,512	3,803,796
No. of scaffolds	117	113	70
Scaffold N ₅₀ (bp)	253,767	1,095,828	663,749
GenBank accession no. for assembled genome	VXKZ01000000	VXLA01000000	VXLB01000000
BioProject accession no.	PRJNA543413	PRJNA543415	PRJNA543411
BioSample accession no.	SAMN11658785	SAMN11658787	SAMN11658786

^aCDSs, coding DNA sequences.

The sequence details are given in Table 1. Using PlasmidFinder version 1.3 (25) and plasmidSPAdes (26), with default settings, no plasmids were found in *Pseudomonas koreensis* UASWS1668 or *Paenibacillus* sp. strain UASWS1643, while *Bacillus megaterium* UASWS1667 displayed 7 plasmids. No complete transposons or phages were found in *Bacillus megaterium* UASWS1667, whereas *Paenibacillus* sp. strain UASWS1643 harbored 1 phage on contig 116, and *Pseudomonas koreensis* UASWS1668 had 2 phages in contigs 68 and 70. Toxins and superantigens as well as virulence and disease genes were absent from all 3 strains, allowing these bacteria to be considered for agronomical use. All strains were predicted to synthesize antibiotics of the bacitracin type and to resist antibiotics and heavy metals, with 54 genes for *Pseudomonas koreensis* UASWS1668, 37 genes for *Bacillus megaterium* UASWS1667, and 47 genes for *Paenibacillus* sp. strain UASWS1643. Biochemical tests confirmed that the three strains produce auxin and solubilize phosphate, as predicted by the genome sequences.

Data availability. This genome sequencing project has been deposited in the NCBI Sequence Read Archive (SRA) and genome databases. The genome, SRA, BioProject, and BioSample accession numbers and statistics for the three individual strains are given in Table 1.

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REFERENCES

- Hwang J, Shin SC, Han JW, Hong SP, Min W-K, Chung D, Kim HJ. Complete genome sequence of *Paenibacillus xylanexedens* PAMC 22703, a xylan-degrading bacterium. *Mar Genom* 100788. <https://doi.org/10.1016/j.margen.2020.100788>.
- Gu Y, Ma Y-N, Wang J, Xia Z, Wei H-L. 2020. Genomic insights into a plant growth-promoting *Pseudomonas koreensis* strain with cyclic lipopeptide-mediated antifungal activity. *Microbiologyopen* e1092. <https://doi.org/10.1002/mbo3.1092>.
- Giridhar Babu A, Shea PJ, Sudhakar D, Jung I-B, Oh B-T. 2015. Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil. *J Environ Manage* 151:160–166. <https://doi.org/10.1016/j.jenvman.2014.12.045>.
- Krishnamoorthy R, Kim K, Subramanian P, Senthilkumar M, Anandham R, Sa T. 2016. Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. *Agric Ecosyst Environ* 231:233–239. <https://doi.org/10.1016/j.agee.2016.05.037>.
- Selvakumar G, Shagol CC, Kim K, Han S, Sa T. 2018. Spore associated bacteria regulates maize root K⁺/Na⁺ ion homeostasis to promote salinity tolerance during arbuscular mycorrhizal symbiosis. *BMC Plant Biol* 18:109. <https://doi.org/10.1186/s12870-018-1317-2>.

6. Kasotia A, Varma A, Choudhary DK. 2015. *Pseudomonas*-mediated mitigation of salt stress and growth promotion in *Glycine max*. *Agric Res* 4:31–41. <https://doi.org/10.1007/s40003-014-0139-1>.
7. López-Bucio J, Campos-Cuevas JC, Hernández-Calderón E, Velásquez-Becerra C, Fariás-Rodríguez R, Macías-Rodríguez LI, Valencia-Cantero E. 2007. *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 20:207–217. <https://doi.org/10.1094/MPMI-20-2-0207>.
8. Nascimento FX, Hernández AG, Glick BR, Rossi MJ. 2020. Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest. *Biotechnol Rep (Amst)* 25:e00406. <https://doi.org/10.1016/j.btre.2019.e00406>.
9. Cai X, Zheng X, Zhang D, Iqbal W, Liu C, Yang B, Zhao X, Lu X, Mao Y. 2019. Microbial characterization of heavy metal resistant bacterial strains isolated from an electroplating wastewater treatment plant. *Ecotoxicol Environ Saf* 181:472–480. <https://doi.org/10.1016/j.ecoenv.2019.06.036>.
10. Goswami G, Panda D, Samanta R, Boro RC, Modi MK, Bujarbaruah KM, Barooah M. 2018. *Bacillus megaterium* adapts to acid stress condition through a network of genes: insight from a genome-wide transcriptome analysis. *Sci Rep* 8:16105. <https://doi.org/10.1038/s41598-018-34221-0>.
11. Govindasamy V, Senthilkumar M, Magheshwaran V, Kumar U, Bose P, Sharma V, Annapurna K. 2011. *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture, p 333–364. In Maheshwari DK (ed), *Plant growth and health promoting bacteria*. Springer-Verlag, Berlin, Germany.
12. Grady EN, MacDonald J, Liu L, Richman A, Yuan Z-C. 2016. Current knowledge and perspectives of *Paenibacillus*: a review. *Microb Cell Fact* 15:203. <https://doi.org/10.1186/s12934-016-0603-7>.
13. Lefort F, Crovadore J, Gilodi R, Calmin F, Oszako T, Nowakowska JA. 2009. An unknown trees die back caused by *Pseudomonas* species in Switzerland. *Folia For Pol Ser A For* 51:171–175.
14. Lefort F, Douglas GC. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Ann For Sci* 56:259–263. <https://doi.org/10.1051/forest/19990308>.
15. Lane DJ. 1991. 16S/23S rRNA sequencing, p 115–175. In Stackebrandt E, Goodfellow M (ed), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, New York, NY.
16. 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](https://doi.org/10.1016/S0022-2836(05)80360-2).
17. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
18. 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>.
19. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser (Oxf)* 41:95–98.
20. 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>.
21. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
22. 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>.
23. Gordon SA, Weber RP. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol* 26:192–195. <https://doi.org/10.1104/pp.26.1.192>.
24. Liu Z, Li YC, Zhang S, Fu Y, Fan X, Patel JS, Zhang M. 2015. Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. *Appl Soil Ecol* 96:217–224. <https://doi.org/10.1016/j.apsoil.2015.08.003>.
25. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
26. Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner PA. 2016. plasmidSPAdes: assembling plasmids from whole genome sequencing data. *Bioinformatics* 32:3380–3387. <https://doi.org/10.1093/bioinformatics/btw493>.