



Whole-Genome Sequence of *Pseudomonas graminis* Strain UASWS1507, a Potential Biological Control Agent and Biofertilizer Isolated in Switzerland

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We report here the whole-genome shotgun sequence of the strain UASWS1507 of the species *Pseudomonas graminis*, isolated in Switzerland from an apple tree. This is the first genome registered for this species, which is considered as a potential and valuable resource of biological control agents and biofertilizers for agriculture.

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seudomonas graminis is a nonfluorescent Gram-negative bacterium, described in 1999 (1). Initially isolated from the phyllosphere of grasses (2), it has also been found on fruits (3, 4), sugarcane (5), and apple (6). Some strains have been shown to reduce the growth of foodborne pathogens in fresh-cut apples (3, 4), melon (7), and fresh-cut lettuce (8), and to increase plant growth in sugarcane (5). A strain has proved to protect apple trees from the fire blight agent Erwinia amylovora (6,9) more efficiently than the commercial strains A506 of Pseudomonas fluorescens and C9-1 of Pantoea vagans. Pseudomonas graminis bacteria have a GC content of 60 to 61% (1), are aerobic, non-spore-forming, rodshaped (0.5 to 1.0 \times 3.5 to 5.0 μ m), and motile between 15 and 20°C. They grow in a temperature range of 4 to 40°C (optimum 25°C) and form yellow colonies. The strain UASWS1507 was isolated in Switzerland from a core sample made in the trunk of a Malus pumila tree. Initially identified as Pseudomonas graminis by 16S rDNA sequencing, it shared 99.9% identity with more than 20 *P. graminis* strains in the GenBank database (10).

Genomic DNA was extracted from a pure axenic culture following an adapted protocol (11). Libraries were prepared using the TruSeq DNA PCR-Free library preparation kits (Illumina, USA). Whole-genome shotgun sequencing was carried out within one Illumina MiniSeq run at 2×150 -bp paired-end read lengths, using a MiniSeq Mid Output kit, which provided a genome coverage of 128×. Quality control of the reads was assessed with FastQC (12). Genome assembly was computed with SPAdes Genome assembler 3.8.1 (13) yielding 71 contigs (\geq 200 bp.), which were arranged with BioEdit (14) and analyzed with QUAST (15). The genome total length was 5,957,886 bp, with a GC content of 60.25% and an N_{50} value of 344,532 bp. Automated gene annotation was carried out by the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (16) and RAST version 2.0 (17, 18). PlasmidFinder (19) and plasmidSPAdes (20) did not detect any plasmid. The annotation with RAST identified 5,149 coding sequence genes (CDSs) distributed in 529 subsystems, whereas

PGAAP identified 5,247 genes for 5,177 CDSs and 5,045 coding genes, 132 pseudogenes, and 70 RNA genes. No complete transposon or phages were found.

As annotations confirmed the absence of toxins, superantigens, and virulence and disease genes, this bacterium could be considered for agricultural and environmental uses. It is equipped with 139 genes for chemotaxis and flagellar motility, which assures dissemination in soils. The presence of four genes involved in plant auxin synthesis is promising of plant growth-promoting rhizobacteria (PGPR) properties (21). The bacterium features resistance genes against metals (arsenic, cadmium, cobalt, copper, and zinc) and a few antibiotics (penicillin, fluoroquinolones, and streptothricin). The bacterium also owns 11 genes for nitrate and nitrite ammonification and 23 genes for ammonia assimilation but none for denitrification. Gene equipment in phosphorus metabolism (58 genes) and organic acids (43 genes) should support organic phosphate mineralization and phosphate solubilization, which are desired characteristics of PGPR (21). Additionally, 80 genes active in various degradation pathways of aromatic compounds offer growth capacities in contaminated soils.

Accession number(s). This whole-genome shotgun project was deposited at DDBJ/EMBL/GenBank under the accession number MDEN00000000. The version described in this paper is the first version, MDEN00000000.1. The 71 contigs have been deposited under the accession numbers MDEN01000001 to MDEN01000071.

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REFERENCES

1. Behrendt U, Ulrich A, Schumann P, Erler W, Burghardt J, Seyfarth W. 1999. A taxonomic study of bacteria isolated from grasses: a proposed new species *Pseudomonas graminis* sp. nov. Int J Syst Bacteriol **49:**297–308. http://dx.doi.org/10.1099/00207713-49-1-297.

- Behrendt U, Müller T, Seyfarth W. 1997. The influence of extensification in grassland management on the populations of micro-organisms in the phyllosphere of grasses. Microbiol Res 152:75–85. http://dx.doi.org/ 10.1016/S0944-5013(97)80026-2.
- Alegre I, Viñas I, Usall J, Teixidó N, Figge MJ, Abadias M. 2013. Control of foodborne pathogens on fresh-cut fruit by a novel strain of *Pseudomonas graminis*. Food Microbiol 34:390–399. http://dx.doi.org/10.1016/ j.fm.2013.01.013.
- Alegre I, Viñas I, Usall J, Anguera M, Altisent R, Abadias M. 2013. Antagonistic effect of *Pseudomonas graminis* CPA-7 against foodborne pathogens in fresh-cut apples under simulated commercial conditions. Food Microbiol 33:139–148. http://dx.doi.org/10.1016/j.fm.2012.09.007.
- Mehnaz S1, Weselowski B, Aftab F, Zahid S, Lazarovits G, Iqbal J. 2009. Isolation, characterization, and effect of fluorescent pseudomonads on micropropagated sugarcane. Can J Microbiol 55:1007–1011. http:// dx.doi.org/10.1139/w09-050.
- 6. Mikiciński A, Sobiczewski P, Puławska J, Maciorowski R. 2016. Control of fire blight (*Erwinia amylovora*) by a novel strain 49M of *Pseudomonas graminis* from the phyllosphere of apple (*Malus* spp.). Eur J Plant Pathol 145:265–276. http://dx.doi.org/10.1007/s10658-015-0837-y.
- Abadias M, Altisent R, Usall J, Torres R, Oliveira M, Viñas I. 2014. Biopreservation of fresh-cut melon using the strain *Pseudomonas graminis* CPA-7. Postharvest Biol Technol 96:69–77. http://dx.doi.org/10.1016/ j.postharvbio.2014.05.010.
- Oliveira M, Abadias M, Colás-Medà P, Usall J, Viñas I. 2015. Biopreservative methods to control the growth of foodborne pathogens on fresh-cut lettuce. Int J Food Microbiol 214:4–11. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.07.015.
- Mikiciński A, Sobiczewski P, Puławska J, Malusa E. 2016. Antagonistic potential of *Pseudomonas graminis* 49M against *Erwiniaamylovora*, the causal agent of fire blight. Arch Microbiol 198:531–539. http://dx.doi.org/ 10.1007/s00203-016-1207-7.
- Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. Nucleic Acids Res 41:D36–D42. http://dx.doi.org/10.1093/nar/gks1195.
- 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. http://dx.doi.org/10.1051/ forest:19990308.
- 12. Andrews S. 2010. FastQC: a quality control tool for high throughput

sequence data. http://www.bioinformatics.babraham.ac.uk/projects /fastqc.

- 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 singlecell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/ cmb.2012.0021.
- 14. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Ac Symp Ser 41:95–98.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. http://dx.doi.org/10.1093/bioinformatics/btt086.
- 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. http://www.ncbi.nlm.nih.gov/books /NBK174280.
- 17. 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. http://dx.doi.org/10.1186/ 1471-2164-9-75.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42: D206–D214. http://dx.doi.org/10.1093/nar/gkt1226.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. http://dx.doi.org/ 10.1128/AAC.02412-14.
- Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner P. 27 July 2016. plasmidSPAdes: assembling plasmids from whole genome sequencing data. Bioinformatics [Epub ahead of print.] http://dx.doi.org/ 10.1093/bioinformatics/btw493.
- Ahemad M, Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud U Sci 26:1–20. http://dx.doi.org/10.1016/j.jksus.2013.05.001.