Draft Genome Sequences of Endophytic *Pseudomonas* spp. Isolated from Grapevine Tissue and Antagonistic to Grapevine Trunk Disease Pathogens

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ABSTRACT Endophytic strains of *Pseudomonas* were isolated from grapevine tissues and exhibited antagonistic activity against several grapevine trunk disease pathogens. The draft genome sequences of the four strains revealed the presence of putative gene clusters that may impart biocontrol activity against plant pathogens.

Species within *Pseudomonas* may be beneficial or detrimental to plant production systems. Efficacy has been established for *Pseudomonas* spp. as biocontrol agents against late blight and scab of potato (1, 2), *Rhizoctonia* root rot on bean (3), damping-off and root rot in tomato (4), black root rot of tobacco, and take-all disease of wheat (5, 6). In grapevines, *Pseudomonas* spp. are found in the phyllosphere (7–9) and inner tissues (10–12) and are known to suppress *Botrytis cinerea* (13, 14) and *Rhizobium vitis* (15).

Pseudomonas isolates BCA13, BCA14, and BCA17 were obtained from grapevine canes exhibiting *Botryosphaeria* dieback (BD) in Wagga Wagga, New South Wales (NSW), Australia. The canes were stripped of bark, surface sterilized, and placed on nutrient agar. Emerging bacteria were streaked onto King's B medium to obtain single colonies. All isolates inhibited BD and *Eutypa* dieback (ED) pathogens in culture and reduced BD infection *in planta* (our unpublished data). A fourth isolate, JMN1, was obtained from an asymptomatic vine in Harden (NSW, Australia) by suspending internal trunk wood shavings in Ringer's solution. Single colonies were selected by streaking on King's B medium at 25°C. JMN1 was not antagonistic to BD and ED pathogens. The four isolates were identified by amplification and sequencing of the 16S rRNA and *rpoD* genes. Gene sequences were subjected to BLASTn searches of the NCBI database, and reference sequences were selected for phylogenetic analyses. Sequence alignment was completed with Clustal W, and a neighbor-joining tree was constructed within MEGA 7 (16). The four isolates were found to be closely related to *Pseudomonas poae*.

Each isolate was grown in nutrient broth for 24 h at 25°C and then harvested for DNA extraction using the Gentra Puregene bacterial DNA extraction kit (Qiagen), following the manufacturer's specifications. Shotgun library preparation and Illumina sequencing (HiSeq 2500 platform) were conducted by the Australian Genome Research Facility, resulting in 12,569,718 reads (150-bp paired ends; Table 1). Data were generated with the Illumina bcl2fastq pipeline version 2.20.0.422. Draft genomes were assembled using the Unicycler assembler, implementing an optimizer for SPAdes 3.13.0 (17). *k*-mer lengths between 0.2 and 0.95 of total read length were examined, and contigs of <200 bases were removed. Annotation was completed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) 4.7 (18) and the Rapid Annotations

TABLE 1 Genome information and accession numbers of four *Pseudomonas* strains isolated from grapevine tissue

Strain	SRA accession no.	No. of reads	Assembly size (bp)	No. of contigs	N_{50} value (bp)	G+C content (%)	No. of coding genes	No. of RAST subsystems represented
BCA13	SRX5463364	3,189,991	6,318,228	34	1,278,996	60.18	5,171	370
BCA14	SRX5463365	2,798,878	6,322,821	34	562,240	60.18	5,666	403
BCA17	SRX5463366	3,028,714	6,318,257	28	760,227	60.18	5,559	403
JMN1	SRX5463367	3,552,135	6,322,966	29	691,035	60.18	5,564	401

using Subsystems Technology server, implementing RAST*tk* (19). Default parameters for all software programs were used, unless otherwise specified.

Gene clusters which may play a role in the control of plant pathogens were identified. Queries of the Plant-bacteria Interaction Factors Resource (PIFAR) (20) found that each strain of *Pseudomonas* contains a remarkable number of putative biocontrol gene clusters, including those responsible for lipopeptide antibiotics, siderophores, proteases, detoxification, lipopolysaccharides, multidrug resistance, microbe-associated molecular proteins (MAMPs), and biofilms. antiSMASH 4.0 (21) was implemented to detect gene clusters responsible for the biosynthesis of secondary metabolites, resulting in the identification of clusters containing nonribosomal peptide synthetases with known activity as antimicrobial agents, including poaeamide, rhizomide, and rhizoxins.

Data availability. The genome sequences for BCA13, BCA14, BCA17, and JMN1 are available under NCBI BioProject number PRJNA522029, with annotated assemblies available under accession numbers SGWK00000000, SGWJ00000000, SGWI00000000, and SGWH00000000, respectively. The Sequence Read Archive (SRA) accession numbers are listed in Table 1. Sequence reads were deposited in the NCBI SRA under the accession numbers SRR8667294 to SRR8667297.

REFERENCES

1. Caulier S, Gillis A, Colau G, Licciardi F, Liépin M, Desoignies N, Modrie P, Legrève A, Mahillon J, Bragard C. 2018. Versatile antagonistic activities of soil-borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and other potato pathogens. Front Microbiol 9:143. https://doi.org/10.3389/fmicb.2018.00143.

2. Arseneault T, Goyer C, Filion M. 2013. Phenazine production by *Pseudomonas* sp. LBUM223 contributes to the biological control of potato common scab. Phytopathology 103:995–1000. https://doi.org/10.1094/PHYTO-01-13-0022-R.

3. D'Aes J, Hua GK, De Maeyer K, Pannecoucque J, Forrez I, Ongena M, Dietrich LE, Thomashow LS, Mavrodi DV, Hofte M. 2011. Biological control of Rhizoctonia root rot on bean by phenazine- and cyclic lipopeptide-producing *Pseudomonas* CMR12a. Phytopathology 101:996–1004. https://doi.org/10.1094/PHYTO-11-10-0315.

4. Solanki MK, Singh RK, Srivastava S, Kumar S, Kashyap PL, Srivastava AK, Arora DK. 2014. Isolation and characterization of siderophore producing antagonistic rhizobacteria against *Rhizoctonia solani*. J Basic Microbiol 54:585–597. https://doi.org/10.1002/jobm.201201564.

5. Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Defagao G. 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. Mol Plant Microbe Interact 5:4–13. https://doi.org/10.1094/MPMI-5-004.

6. Cook RJ, Weller DM. 1987. Management of take-all in consecutive crops of wheat or barley, p 41–76. *In* Chet I (ed), Innovative approaches to plant disease control. John Wiley & Sons, Inc., New York, NY.

7. Portillo M, Franques J, Araque I, Reguant C, Bordons A. 2016. Bacterial diversity of Grenache and Carignan grape surface from different vineyards at Priorat wine region (Catalonia, Spain). Int J Food Microbiol 219:56–63. https://doi.org/10.1016/j.ijfoodmicro.2015.12.002.

8. Perazzolli M, Antonielli L, Storari M, Puopolo G, Pancher M, Giovannini O, Pindo M, Pertot I. 2014. Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. Appl Environ Microbiol 80:3585–3596. https://doi.org/10.1128/AEM.00415-14.

9. Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC. 2014. Unravelling the diversity of grapevine microbiome. PLoS One 9:e85622. https://doi.org/10.1371/journal.pone.0085622.

10. Deyett E, Roper MC, Ruegger P, Yang J, Borneman J, Rolshausen PE. 2017. Microbial landscape of the grapevine endosphere in the context of Pierce's disease. Phytobiomes 1:138–149. https://doi.org/10.1094/PBIOMES-08-17-0033-R.

11. West ER, Cother EJ, Steel CC, Ash GJ. 2010. The characterization and diversity of bacterial endophytes of grapevine. Can J Microbiol 56:209–216. https://doi.org/10.1139/W10-004.

12. Bell CR, Dickie GA, Harvey WLG, Chan J. 1995. Endophytic bacteria in grapevine. Can J Microbiol 41:46–53. https://doi.org/10.1139/m95-006.

13. Ait Barka E, Gognies S, Nowak J, Audran J, Belarbi A. 2002. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote grapevine growth. Biol Control 24:135–142. https://doi.org/10.1016/S1049-9644(02)00034-8.

14. Trotel-Aziz P, Couderchet M, Biagianti S, Aziz A. 2008. Characterization of new biocontrol agents *Acinetobacter, Bacillus, Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea*. Environ Exp Bot 64:21–32. https://doi.org/10.1016/j.envexpbot.2007.12.009.

15. Khmel IA, Sorokina TA, Lemanova NB, Lipasova VA, Metlitski OZ, Burdeinaya TV, Chernin LS. 1998. Biological control of crown gall in grapevine and raspberry by two *Pseudomonas* spp. with a wide spectrum of antagonistic activity. Biocontrol Sci Technol 8:45–57. https://doi.org/10.1080/09583159830423.

16. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054.

17. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e105595. https://doi.org/10.1371/journal.pcbi.1005595.

18. 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.

19. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RAST*tk*: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.

20. Martínez-García PM, López-Solanilla E, Ramos C, Rodríguez-Palenzuela P. 2016. Prediction of bacterial associations with plants using a supervised machine-learning approach. Environ Microbiol 27:4847–4861. https://doi.org/10.1111/1462-2920.13389.

21. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos ELC, Kim H, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 45:36–41. https://doi.org/10.1093/nar/gkx319.