

Complete Genome Sequence of the Sugar Cane Endophyte *Pseudomonas aurantiaca* PB-St2, a Disease-Suppressive Bacterium with Antifungal Activity toward the Plant Pathogen *Colletotrichum falcatum*

Samina Mehnaz,^a Judith S. Bauer,^b Harald Gross^b

Department of Biological Sciences, Forman Christian College University, Lahore, Pakistan^a; Pharmaceutical Institute, Department of Pharmaceutical Biology, University of Tuebingen, Tuebingen, Germany^b

The endophytic bacterium *Pseudomonas aurantiaca* PB-St2 exhibits antifungal activity and represents a biocontrol agent to suppress red rot disease of sugar cane. Here, we report the completely sequenced 6.6-Mb genome of *P. aurantiaca* PB-St2. The sequence contains a repertoire of biosynthetic genes for secondary metabolites that putatively contribute to its antagonistic activity and its plant-microbe interactions.

Received 21 November 2013 Accepted 19 December 2013 Published 23 January 2014

Citation Mehnaz S, Bauer JS, Gross H. 2014. Complete genome sequence of the sugar cane endophyte *Pseudomonas aurantiaca* PB-St2, a disease-suppressive bacterium with antifungal activity toward the plant pathogen *Colletotrichum falcatum*. *Genome Announc.* 2(1):e01108-13. doi:10.1128/genomeA.01108-13.

Copyright © 2014 Mehnaz et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Samina Mehnaz, saminamehnaz@fccollege.edu.pk, or Harald Gross, harald.gross@uni-tuebingen.de.

Pseudomonas aurantiaca PB-St2 (syn., *Pseudomonas chlororaphis* subsp. *aurantiaca* PB-St2) (1) is a member of the group of plant-beneficial *Pseudomonas* bacteria (2). It was originally isolated from surface-sterilized stems of sugar cane grown in Pakistan (3, 4) and exhibits antagonistic activity toward the fungal phytopathogen *Colletotrichum falcatum*, the causative agent of the red rot disease of sugar cane (*Saccharum* sp. hybrids) (5). Strain PB-St2 is known to produce several secondary metabolites, such as phenazines, homoserine lactones, hydrogen cyanide, siderophores of the hydroxamate type, the lipopeptide WLIP, and lachnenoic acids A to C (3, 4, 6).

The genome of *P. aurantiaca* PB-St2 was sequenced using a combination of next-generation sequencing platforms. Genomic DNA was first subjected to 50-cycle paired-end sequencing with an Illumina GAIIX (BaseClear, Leiden, The Netherlands). The *de novo* assembly of 4,870,658 reads (45-fold median coverage) was performed using the CLC Genomics Workbench 4.0, yielding 555 contigs and a total assembled length of 6,685,786 bp. To improve the quality of the sequence, the genome was additionally sequenced using the PacBio RS technology (10-kb library, 78,395 reads, 189 Mb, 2,412 kb average length). The data collected from the PacBio RS instrument were processed and filtered (SMRT analysis software suite, CLC Genomics Workbench version 6.0.1), and the results of both sequencing platforms were subsequently used to perform a *de novo* hybrid assembly. The contigs were linked and placed into superscaffolds based on the alignment of the PacBio continuous long reads (CLR). Alignment was performed with BLASR (7). From the alignment, the orientation, order, and distance were determined using a modified version of the SSPACE Premium scaffolder 2.3 (8). The final genome is scaffolded in 23 segments and includes 6,590,922 bases with a G+C content of 63.3%. These data are comparable to those of already-reported sequenced genomes of the closely related *P. chlororaphis*

strains GP72 (9), 30-84, and O6 (10), which range from 6.6 to 6.9 Mb in size and have G+C contents of 62.8 to 63.1%.

The obtained sequence reflects a huge capacity to produce secondary metabolites on the genetic level. Gene cluster coding for the production of the cyclic lipopeptide WLIP (11), hydrogen cyanide (12), 2-hydroxyphenazine (13), and indole acetic acid (*iaaMH*) (14) was readily detected and annotated. Moreover, a candidate gene cluster coding for lachnenoic acids, biosynthetic pathways for a pyoverdine-like siderophore (15), the siderophore achromobactin (16, 17), the strong antifungal compound pyrrol-nitrin (18), an incomplete mangotoxin cluster (*mgoABCD*, syn. *pvfABCD*, but *mboABCDEF* is absent) (19, 20), an orphan tetramodular nonribosomal peptide synthetase (NRPS) gene cluster, the osmolytes *N*-acetylglutaminylglutamine amide (NAGGN) and trehalose (21, 22), homoserine lactones (*phzIR*, *cslIR*, *hdtS*) (23, 24), two bacteriocin-like proteins (25), and genes encoding exoenzymes, such as chitinases (26) and AprA (27, 28), were found in the genome.

The genome sequence of PB-St2 not only revealed a true capacity to produce a multitude of secondary metabolites and exoenzymes but also provides a foundation for further understanding of the lifestyle, antagonistic properties, and respective modes of action of this strain and for biosafety assessments when introduced into agricultural environments.

Nucleotide sequence accession numbers. This whole-genome sequencing project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AYUD00000000](https://www.ncbi.nlm.nih.gov/nuccore/AYUD00000000). The version described in this paper is version AYUD01000000.

ACKNOWLEDGMENTS

We gratefully acknowledge the generous contribution of the Alexander von Humboldt Foundation, which provided financial support for conducting this research (Georg Forster Fellowship awarded to S.M.).

REFERENCES

- Peix A, Valverde A, Rivas R, Igual JM, Ramírez-Bahena MH, Mateos PF, Santa-Regina I, Rodríguez-Barrueco C, Martínez-Molina E, Vélazquez E. 2007. Reclassification of *Pseudomonas aurantiaca* as a synonym of *Pseudomonas chlororaphis* and proposal of three subspecies, *P. chlororaphis* subsp. *chlororaphis* subsp. nov., *P. chlororaphis* subsp. *aureofaciens* subsp. nov., *P. chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 57(Pt 6):1286–1290. <http://dx.doi.org/10.1099/ijs.0.64621-0>.
- Mark G, Morrissey JP, Higgins P, O’Gara F. 2006. Molecular-based strategies to exploit *Pseudomonas* biocontrol strains for environmental biotechnology applications. FEMS Microbiol. Ecol. 56:167–177. <http://dx.doi.org/10.1111/j.1574-6941.2006.00056.x>.
- Mehnaz S, Baig DN, Jamil F, Weselowski B, Lazarovits G. 2009. Characterization of a phenazine and hexanoyl homoserine lactone producing *Pseudomonas aurantiaca* strain PB-St2, isolated from sugarcane stem. J. Microbiol. Biotechnol. 19:1688–1694. <http://dx.doi.org/10.4014/jmb.090.4.04022>.
- Mehnaz S, Baig DN, Lazarovits G. 2010. Genetic and phenotypic diversity of plant growth promoting rhizobacteria isolated from sugarcane plants growing in Pakistan. J. Microbiol. Biotechnol. 20:1614–1623. <http://www.jmb.or.kr/journal/viewJournal.html?year=2010&vol=20&num=12&page=1614>.
- Singh K, Singh RP. 1989. Red rot, p 169–188. In Ricaud C, Egan BT, Gillaspie AG, Jr, Hughes CG (ed), Diseases of sugarcane: major diseases. Elsevier, Amsterdam, The Netherlands.
- Mehnaz S, Saleem RSZ, Yameen B, Pianet I, Schnakenburg G, Pietraszkiewicz H, Valeriote F, Josten M, Sahl HG, Franzblau SG, Gross H. 2013. Lahorenoic acids A-C, ortho-dialkyl-substituted aromatic acids from the biocontrol strain *Pseudomonas aurantiaca* PB-St2. J. Nat. Prod. 76:135–141. <http://dx.doi.org/10.1021/np3005166>.
- Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. BMC Bioinformatics 13:238. <http://dx.doi.org/10.1186/1471-2105-13-238>.
- Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27: 578–579. <http://dx.doi.org/10.1093/bioinformatics/btq683>.
- Shen X, Chen M, Hu H, Wang W, Peng H, Xu P, Zhang X. 2012. Genome sequence of *Pseudomonas chlororaphis* GP72, a root-colonizing biocontrol strain. J. Bacteriol. 194:1269–1270. <http://dx.doi.org/10.1128/JB.06713-11>.
- Loper JE, Hassan KA, Mavrodi DV, Davis EW, Lim CK, Shaffer BT, Elbourne LD, Stockwell VO, Hartney SL, Breakwell K, Henkels MD, Tetu SG, Rangel LI, Kidarsa TA, Wilson NL, van de Mortel JE, Song C, Blumhagen R, Radune D, Hostetler JB, Brinkac LM, Durkin AS, Kluepfel DA, Wechter WP, Anderson AJ, Kim YC, Pierson LS, Pierson EA, Lindow SE, Kobayashi DY, Raaijmakers JM, Weller DM, Thomashow LS, Allen AE, Paulsen IT. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. PLOS Genet. 8:e1002784. <http://dx.doi.org/10.1371/journal.pgen.1002784>.
- Rokni-Zadeh H, Li W, Sanchez-Rodriguez A, Sinnaeve D, Rozenski J, Martins JC, De Mot R. 2012. Genetic and functional characterization of cyclic lipopeptide white-line-inducing principle (WLIP) production by rice rhizosphere isolate *Pseudomonas putida* RW10S2. Appl. Environ. Microbiol. 78:4826–4834. <http://dx.doi.org/10.1128/AEM.00335-12>.
- Laville J, Blumer C, von Schroetter C, Gaia V, Défago G, Keel C, Haas D. 1998. Characterization of the *hcnABC* gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0. J. Bacteriol. 180: 3187–3196.
- Huang L, Chen MM, Wang W, Hu HB, Peng HS, Xu YQ, Zhang XH. 2011. Enhanced production of 2-hydroxyphenazine in *Pseudomonas chlororaphis* GP72. Appl. Microbiol. Biotechnol. 89:169–177. <http://dx.doi.org/10.1007/s00253-010-2863-1>.
- Spaepen S, Vanderleyden J, Remans R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31: 425–448. <http://dx.doi.org/10.1111/j.1574-6976.2007.00072.x>.
- Ravel J, Cornelis P. 2003. Genomics of pyoverdine-mediated iron uptake in pseudomonads. Trends Microbiol. 11:195–200. [http://dx.doi.org/10.1016/S0966-842X\(03\)00076-3](http://dx.doi.org/10.1016/S0966-842X(03)00076-3).
- Challis GL. 2005. A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. Chembiochem 6:601–611. <http://dx.doi.org/10.1002/cbic.200400283>.
- Berti AD, Thomas MG. 2009. Analysis of achromobactin biosynthesis by *Pseudomonas syringae* pv. *syringae* B728a. J. Bacteriol. 191:4594–4604. <http://dx.doi.org/10.1128/JB.00457-09>.
- Hammer PE, Hill DS, Lam ST, van Pee KH, Ligon JM. 1997. Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. Appl. Environ. Microbiol. 63:2147–2154.
- Vallet-Gely I, Opota O, Boniface A, Novikov A, Lemaitre B. 2010. A secondary metabolite acting as a signalling molecule controls *Pseudomonas entomophila* virulence. Cell. Microbiol. 12:1666–1679. <http://dx.doi.org/10.1111/j.1462-5822.2010.01501.x>.
- Carrión VJ, Arrebola E, Cazorla FM, Murillo J, de Vicente A. 2013. The *mbo* operon is specific and essential for biosynthesis of mangotoxin in *Pseudomonas syringae*. PLOS One 7:e36709. <http://dx.doi.org/10.1371/journal.pone.0036709>.
- Kurz M, Burch AY, Seip B, Lindow SE, Gross H. 2010. Genome-driven investigation of compatible solute biosynthesis pathways of *Pseudomonas syringae* pv. *syringae* and their contribution to water stress tolerance. Appl. Environ. Microbiol. 76:5452–5462. <http://dx.doi.org/10.1128/AEM.00686-10>.
- Sagot B, Gaysinski M, Mehiri M, Guigonis JM, Le Rudulier D, Alloing G. 2010. Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. Proc. Natl. Acad. Sci. USA 107:12652–12657. <http://dx.doi.org/10.1073/pnas.1003063107>.
- Laue BE, Jiang Y, Chhabra SR, Jacob S, Stewart GS, Hardman A, Downie JA, O’Gara F, Williams P. 2000. The biocontrol strain *Pseudomonas fluorescens* F113 produces the *Rhizobium* small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl)homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. Microbiology 146(Pt 10): 2469–2480.
- Maddula VS, Zhang Z, Pierson EA, Pierson LS III. 2006. Quorum sensing and phenazines are involved in biofilm formation by *Pseudomonas chlororaphis* (*aureofaciens*) strain 30-84. Microb. Ecol. 52:289–301. <http://dx.doi.org/10.1007/s00248-006-9064-6>.
- Parret AH, de Mot R. 2002. Bacteria killing their own kind: novel bacteriocins of *Pseudomonas* and other γ-proteobacteria. Trends Microbiol. 10:107–112. [http://dx.doi.org/10.1016/S0966-842X\(02\)02307-7](http://dx.doi.org/10.1016/S0966-842X(02)02307-7).
- Folders J, Algra J, Roelofs MS, van Loon LC, Tommassen J, Bitter W. 2001. Characterization of *Pseudomonas aeruginosa* chitinase, a gradually secreted protein. J. Bacteriol. 183:7044–7052. <http://dx.doi.org/10.1128/JB.183.24.7044-7052.2001>.
- Anderson LM, Stockwell VO, Loper JE. 2004. An extracellular protease of *Pseudomonas fluorescens* inactivates antibiotics of *Pantoea agglomerans*. Phytopathology 94:1228–1234. <http://dx.doi.org/10.1094/PHYTO.2004.94.11.1228>.
- Siddiqui IA, Haas D, Heeb S. 2005. Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor with activity against the root-knot nematode *Meloidogyne incognita*. Appl. Environ. Microbiol. 71: 5646–5649. <http://dx.doi.org/10.1128/AEM.71.9.5646-5649.2005>.