

Draft Genome Sequence of *Pseudomonas fluorescens* LMG 5329, a White Line-Inducing Principle-Producing Bioindicator for the Mushroom Pathogen *Pseudomonas tolaasii*

Maarten G. K. Ghequire, Hassan Rokni-Zadeh,* Peyman Zarrineh,* René De Mot

Centre of Microbial and Plant Genetics, University of Leuven, Heverlee-Leuven, Belgium

* Present address: Peyman Zarrineh, School of Computer Science, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran; Hassan Rokni-Zadeh, Department of Biotechnology and Molecular Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.

M.G.K.G. and H.R.-Z. contributed equally to this article.

***Pseudomonas tolaasii*, the causative agent of *Agaricus bisporus* brown blotch disease, can be identified by the white line reaction, occurring upon confrontation of the tolaasin-producing mushroom pathogen with “*Pseudomonas reactans*,” producing the lipopeptide white line-inducing principle (WLIP). The draft genome sequence of the WLIP-producing indicator *Pseudomonas fluorescens* strain LMG 5329 is reported here.**

Received 6 May 2013 Accepted 24 June 2013 Published 25 July 2013

Citation Ghequire MGK, Rokni-Zadeh H, Zarrineh P, De Mot R. 2013. Draft genome sequence of *Pseudomonas fluorescens* LMG 5329, a white line-inducing principle-producing bioindicator for the mushroom pathogen *Pseudomonas tolaasii*. *Genome Announc.* 1(4):e00383-13. doi:10.1128/genomeA.00383-13.

Copyright © 2013 Ghequire et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Maarten G. K. Ghequire, maarten.ghequire@biw.kuleuven.be, or René De Mot, rene.demot@biw.kuleuven.be.

The white line test (1) enables the detection of the mushroom pathogen *Pseudomonas tolaasii*, relying on formation of a visible precipitate in the agar medium between colonies of this pathogen and an indicator strain, referred to as “*Pseudomonas reactans*.” This diagnostic phenotype stems from the interaction of the *P. tolaasii* virulence factor, tolaasin, with another diffusible cyclic lipopeptide, the white line-inducing principle (WLIP) from *P. reactans* (2). The WLIP biosynthetic system of the white line-indicator organism *Pseudomonas fluorescens* LMG 5329 (NCPPB 3149) was characterized (3). Here, we report the draft genome sequence of this mushroom isolate that is closely related to *P. fluorescens* SBW25 (4) and other *P. fluorescens* subclade 3 strains (5).

Genomic DNA was subjected to 100-cycle paired-end sequencing with an Illumina HiSeq 2000 (Genomics Core Facility, KU Leuven). Following FastQC quality assessment of raw data (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>), reads were trimmed to 50 bp for *de novo* assembly by Velvet (6). Assembly of 16,486,578 reads (56-fold median coverage) yielded 293 contigs with an N₅₀ value of 55,400 bp. The total assembled length is 6,875,357 bp, with a G+C content of 60.5%, and the largest contig is 164,530 bp. Automated annotation using RAST (7) assigned a total of 6,324 protein-coding genes, along with 63 tRNAs.

In addition to WLIP nonribosomal peptide synthetase (NRPS) genes, the LMG 5329 genome carries a *pvfC* homologue, which is involved in the production of a putative signaling molecule (8), and four pyoverdine NRPS genes, suggesting the production of a decapeptidic siderophore identical to *P. fluorescens* strain 18.1 pyoverdine (9) but different from the heptaepitidic *P. fluorescens* SBW25 pyoverdine (3, 10). In addition, systems for the acquisition of Fe²⁺ (11) and heme (12) are present. The importance of iron metabolism for the organism is further inferred from multi-

ple extracytoplasmic function (ECF) sigma factors assigned to the regulation of iron metabolism. The ECF complement includes a component of the cell surface-signaling system PUMA3 (13). Several genes can be linked to surface-associated determinants of a sessile lifestyle: genes encoding poly-β-1,6-N-acetylglucosamine (14), alginic, Pel and Psl polysaccharides (15), Fap amyloid protein (16), Flp pili (17), and phosphate-binding protein PstS (18). The proteome includes no homologues of the cell surface proteins LapA or LapF (18). In addition to the Tat secretory genes, clusters for type I, type II, type III (two systems), type V, and type VI secretion and for fimbrial chaperone-usher systems are predicted. Interbacterial antagonistic proteins include a nuclelease-type soluble (S) pyocin, Rhs proteins, and a contact-dependent inhibitory factor (19, 20). Also notable is the apparent acquisition of a mobile element encoding mercury reductase-based resistance as found in *Pseudomonas putida* (21) and a β-proteobacterial-type methylamine utilization operon (22).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. ASGY00000000. The version described in this paper is version ASGY00000000.1.

ACKNOWLEDGMENTS

This work was financially supported by grant G.0393.09N from FWO-Vlaanderen (to R.D.M.). P.Z. was supported by a grant from IPM (CS1391-4-20).

We acknowledge A. Sanchez-Rodriguez for assistance with sequence assembly.

REFERENCES

1. Wong WC, Preece TF. 1979. Identification of *Pseudomonas tolaasii*: the white line in agar and mushroom tissue block rapid pitting tests. *J. Appl. Bacteriol.* 47:401–407.

2. Mortishire-Smith RJ, Nutkins JC, Packman LC, Brodey CL, Rainey PB, Johnstone K, Williams DH. 1991. Determination of the structure of an extracellular peptide produced by the mushroom saprotroph *Pseudomonas reactans*. *Tetrahedron* 47:3645–3654.
3. Rokni-Zadeh H, Li W, Yilmaz E, Sanchez-Rodriguez A, De Mot R. 2013. Distinct lipopeptide production systems for WLIP (white line-inducing principle) in *Pseudomonas fluorescens* and *Pseudomonas putida*. *Environ. Microbiol. Rep.* 5:160–169.
4. Silby MW, Cerdeño-Tárraga AM, Vernikos GS, Giddens SR, Jackson RW, Preston GM, Zhang XX, Moon CD, Gehrig SM, Godfrey SA, Knight CG, Malone JG, Robinson Z, Spiers AJ, Harris S, Challis GL, Yaxley AM, Harris D, Seeger K, Murphy L, Rutter S, Squares R, Quail MA, Saunders E, Mavromatis K, Brettin TS, Bentley SD, Hothersall J, Stephens E, Thomas CM, Parkhill J, Levy SB, Rainey PB, Thomson NR. 2009. Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. *Genome Biol.* 10:R51.
5. Loper JE, Hassan KA, Mavrodi DV, Davis EW, Jr, 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, III, 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. doi:10.1371/journal.pgen.1002784.
6. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829.
7. 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. doi:10.1186/1471-2164-9-75.
8. 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.
9. Kilz S, Lenz C, Fuchs R, Budzikiewicz H. 1999. A fast screening method for the identification of siderophores from fluorescent *Pseudomonas* spp. by liquid chromatography/electrospray mass spectrometry. *J. Mass Spectrom.* 34:281–290.
10. Moon CD, Zhang XX, Matthijs S, Schäfer M, Budzikiewicz H, Rainey PB. 2008. Genomic, genetic and structural analysis of pyoverdine-mediated iron acquisition in the plant growth-promoting bacterium *Pseudomonas fluorescens* SBW25. *BMC Microbiol.* 8:7. doi:10.1186/1471-2164-8-7.
11. Cao J, Woodhall MR, Alvarez J, Cartron ML, Andrews SC. 2007. EfeUOB (YcdNOB) is a tripartite, acid-induced and CpxAR-regulated, low-pH Fe²⁺ transporter that is cryptic in *Escherichia coli* K-12 but functional in *E. coli* O157:H7. *Mol. Microbiol.* 65:857–875.
12. Alontaga AY, Rodriguez JC, Schönbrunn E, Becker A, Funke T, Yukl ET, Hayashi T, Stobaugh J, Moënne-Locoz P, Rivera M. 2009. Structural characterization of the hemophore HasAp from *Pseudomonas aeruginosa*: NMR spectroscopy reveals protein-protein interactions between holo-HasAp and hemoglobin. *Biochemistry* 48:96–109.
13. Llamas MA, van der Sar A, Chu BCH, Sparrius M, Vogel HJ, Bitter W. 2009. A novel extracytoplasmic function (ECF) sigma factor regulates virulence in *Pseudomonas aeruginosa*. *PLoS Pathog.* 5:e1000572. doi:10.1371/journal.ppat.1000572.
14. Itoh Y, Rice JD, Goller C, Pannuri A, Taylor J, Meisner J, Beveridge TJ, Preston JF, III, Romeo T. 2008. Roles of *pgaABCD* genes in synthesis, modification, and export of the *Escherichia coli* biofilm adhesin poly-β-1,6-N-acetyl-D-glucosamine. *J. Bacteriol.* 190:3670–3680.
15. Mann EE, Wozniak DJ. 2012. *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiol. Rev.* 36:893–916.
16. Dueholm MS, Søndergaard MT, Nilsson M, Christiansen G, Stensballe A, Overgaard MT, Givskov M, Tolker-Nielsen T, Otzen DE, Nielsen PH. 2013. Expression of Fap amyloids in *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* results in aggregation and increased biofilm formation. *Microbiologyopen* 2:365–382. doi:10.1002/mbo3.81.
17. Bernard CS, Bordi C, Termine E, Filloux A, de Bentzmann S. 2009. Organization and PprB-dependent control of the *Pseudomonas aeruginosa* *tad* locus, involved in Flp pilus biology. *J. Bacteriol.* 191:1961–1973.
18. Duque E, de la Torre J, Bernal P, Molina-Henares MA, Alaminos M, Espinosa-Urgel M, Roca A, Fernández M, de Bentzmann S, Ramos JL. 2013. Identification of reciprocal adhesion genes in pathogenic and non-pathogenic *Pseudomonas*. *Environ. Microbiol.* 15:36–48.
19. Poole SJ, Diner EJ, Aoki SK, Braaten BA, t'Kint de Roodenbeke C, Low DA, Hayes CS. 2011. Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. *PLoS Genet.* 7:e1002217. doi:10.1371/journal.pgen.1002217.
20. Ruhe ZC, Low DA, Hayes CS. 2013. Bacterial contact-dependent growth inhibition. *Trends Microbiol.* 21:230–237.
21. Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, van der Lelie D. 2011. Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. *FEMS Microbiol. Rev.* 35:299–323.
22. Gak ER, Tsygankov YD, Chistoserdov AY. 1997. Organization of methylamine utilization genes (*mau*) in 'Methylobacter flagellatum' KT and analysis of *mau* mutants. *Microbiology* 143:1827–1835.