Genome Sequence of the Oocydin A-Producing Rhizobacterium Serratia plymuthica 4Rx5

Miguel A. Matilla, a,b Zulema Udaondo, c George P. C. Salmond a

aDepartment of Biochemistry, University of Cambridge, Cambridge, United Kingdom
bDepartment of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain
cDepartment of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

ABSTRACT Serratia plymuthica 4Rx5 was isolated from the rhizosphere of oilseed rape due to its antagonistic properties against plant-pathogenic fungi. The strain 4Rx5 produces the antifungal and antioomycete heterumalide, oocydin A. Analysis of its genome revealed the presence of various gene clusters putatively involved in the biosynthesis of additional secondary metabolites.

Serratia plymuthica strains are frequently isolated from the rhizosphere of agriculturally relevant crops and are considered efficient biocontrol agents (1, 2). Their biocontrol properties have been associated with the production of antibiotics (1–6), hydrolytic enzymes (1, 2), and their ability to trigger systemic resistance (2, 7).

Serratia plymuthica 4Rx5 was originally isolated by Berg and coworkers (1) from the rhizosphere of oilseed rape after screening for bacteria that showed hydrolytic activities and antagonism against phytopathogenic fungi. More recently, strain 4Rx5 has been used as a model bacterium for the investigation of biosynthesis and regulation of the antifungal oocycin A (8). In contrast to other oocydin A-producing strains, the biosynthesis of this polyketide in 4Rx5 was shown to be regulated by an N-acyl-L-homoserine lactone-based quorum-sensing system (8). Additionally, 4Rx5 was shown to produce chitinases and proteases (1) and siderophores (M. A. Matilla and G. P. C. Salmond, unpublished data).

The genomic DNA of 4Rx5 was extracted from stationary-phase cells grown in lysogeny broth (9) using a Qiagen DNeasy kit. A single-end shotgun library for 454 pyrosequencing was prepared using a Roche GS FLX Titanium rapid library preparation kit and was run on a picotiter plate for a Roche Applied Science Genome Sequencer FLX instrument according to the manufacturer’s specifications. Read quality was monitored with the inclusion of control reads and using the 454 sequencing system software package v2.6 (Roche) using default settings. The resulting 319,495 reads were de novo assembled using Newbler v2.6 with default parameters. An approximately 25× coverage of the estimated genome size was obtained, and the assembly resulted in 20 contigs larger than 1,000 bp. The largest contig was 1,704,970 bp, and the N50 contig size was 511,280 bp. The genome was automatically annotated using NCBI Prokaryotic Genome Annotation Pipeline v4.2 (10).

The draft genome sequence of 4Rx5 comprises 5,367,478 bp with an overall G+C content of 54.7%. Automated genome annotation predicted 4,870 protein-coding sequences, 75 pseudogenes, 1 CRISPR array, 6 rRNAs, 71 tRNA genes, and 9 noncoding RNAs. In addition to the polyketide synthase biosynthetic cluster responsible for the production of oocycin A (5), bioinformatic analyses using antiSMASH (11) predicted 4 additional uncharacterized gene clusters putatively involved in the production of polyketides and nonribosomal peptides. Scrutiny of the genome also revealed the presence of a biosynthetic cluster responsible for production of the antifungal.
metabolite pyrrolnitrin (12). Genome comparison analyses showed that the genome of 4Rx5 is highly similar to that of Serratia plymuthica 4Rx13 (13). The strain 4Rx13 produces a broad range of volatile organic compounds (VOCs) (13–15), some of them possessing antifungal properties (14). The bicyclic terpene sodorifen was the major VOC emitted by 4Rx13 (15), and the sodorifen gene cluster was identified in the genome of 4Rx5. Altogether, our results highlight the potential of 4Rx5 for the biocontrol of phytopathogenic fungi and oomycetes. Further research will elucidate the spectrum of secondary metabolites produced by this bacterium.

Data availability. The sequences obtained by this whole-genome shotgun project have been deposited in DDBJ/EMBL/GenBank under the accession number PESE00000000.

ACKNOWLEDGMENTS

We thank Kornelia Smalla for the very generous donation of the bacterial strain. We also thank Shilo Dickens for technical support. The sequencing of this strain was undertaken at the Department of Biochemistry at the University of Cambridge (United Kingdom).

The work in the Salmond laboratory and the sequencing in this report were funded by the Biotechnology and Biological Sciences Research Council (BBSRC, United Kingdom). Miguel A. Matilla was supported by the EU Marie-Curie Intra-European Fellowship for Career Development (FP7-PEOPLE-2011-IEF) grant 298003 and the Spanish Ministry of Economy and Competitiveness Postdoctoral Research Program, Juan de la Cierva (JCI-2012-11815). The funders had no role in study design, data collection and interpretation, or the decision to work for publication.

REFERENCES

1. Berg G, Roskot N, Steidle AE, Eberl L, Zock A, Smalla K. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobia isolated from different Verticillium host plants. Appl Environ Microbiol 68:3328–3338. https://doi.org/10.1128/AEM.68.7.3328-3338.2002.
2. De Vleesschauwer D, Høfte M. 2007. Using Serratia plymuthica to control fungal pathogens of plants. CAB Rev 2:1–12.
3. Matilla MA, Drew A, Udaondo Z, Krell T, Salmond GP. 2016. Genome sequence of Serratia plymuthica A153, a model rhizobacterium for the investigation of the synthesis and regulation of haterumalides, zeamine, and andrimid. Genome Announc 4:e00373-16. https://doi.org/10.1128/genomeA.00373-16.
4. Matilla MA, Nogellova V, Morel B, Krell T, Salmond GP. 2016. Biosynthesis of the acetyl-CoA carboxylase-inhibiting antibiotic, andrimid in Serratia is regulated by Hfq and the LysR-type transcriptional regulator, AdmX. Environ Microbiol 18:3635–3650. https://doi.org/10.1111/1462-2920.12341.
5. Matilla MA, Stockmann H, Leeper FJ, Salmond GP. 2012. Bacterial biosynthetic gene clusters encoding the anti-cancer haterumalide class of molecules: biogenesis of the broad spectrum antifungal and anti-oomycete compound, oocydin A. J Biol Chem 287:39125–39138. https://doi.org/10.1074/jbc.M112.401026.
6. Hellberg JEEU, Matilla MA, Salmond GPC. 2015. The broad-spectrum antibiotic, zeamine, kills the nematode worm Caenorhabditis elegans. Front Microbiol 6:137. https://doi.org/10.3389/fmicb.2015.00137.
7. Ryu CM, Choi HK, Lee CH, Murphy JF, Lee JK, Kloepper JW. 2013. Modulation of quorum sensing in acylhomoserine lactone-producing or -degrading tobacco plants leads to alteration of induced systemic resistance elicited by the rhizobacterium Serratia marcescens 90-166. Plant Pathol J 29:182–192. https://doi.org/10.5423/PPJ.SI.11.2012.0173.
8. Matilla MA, Leeper FJ, Salmond GPC. 2015. Biosynthesis of the antifungal haterumalide, oocydin A, in Serratia, and its regulation by quorum sensing, RpoS and Hfq. Environ Microbiol 17:2993–3008. https://doi.org/10.1111/1462-2920.12839.
9. Bertani G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli. J Bacteriol 62:293–300.
10. Tatouova 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.
11. Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucolieri R, Lee SY, Fischbach MA, Muller R, Wohleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. https://doi.org/10.1093/nar/gkw537.
12. van Pee K-H, Ligon JM. 2000. Biosynthesis of pyrrolnitrin and other phenylpyrrole derivatives by bacteria. Nat Prod Rep 17:157–164. https://doi.org/10.1039/a902138b.
13. Weise T, Thurmer A, Brady S, Kai M, Daniel R, Gottschalk G, Piechulla B. 2014. VOC emission of various Serratia species and isolates and genome analysis of Serratia plymuthica 4Rx13. FEMS Microbiol Lett 352:45–53. https://doi.org/10.1093/femsle/ntu021.
14. Kai M, Effmert U, Berg G, Piechulla B. 2007. Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen Rhizoctonia solani. Arch Microbiol 187:351–360. https://doi.org/10.1007/s00203-006-0199-0.
15. Kai M, Crespo E, Cristescu SM, Harren FJM, Francke W, Piechulla B. 2010. Serratia odorifera: analysis of volatile emission and biological impact of volatile compounds on Arabidopsis thaliana. Appl Microbiol Biotechnol 88:965–976. https://doi.org/10.1007/s00253-010-2810-1.