

# Draft Genome Sequence of *Serratia* sp. Strain ATCC 39006, a Model Bacterium for Analysis of the Biosynthesis and Regulation of Prodigiosin, a Carbapenem, and Gas Vesicles

Peter C. Fineran,<sup>a</sup> Marina C. Iglesias Cans,<sup>a</sup> Joshua P. Ramsay,<sup>b</sup> Nabil M. Wilf,<sup>b</sup> Desiree Cossyleon,<sup>a</sup> Matthew B. McNeil,<sup>a</sup> Neil R. Williamson,<sup>b</sup> Rita E. Monson,<sup>b</sup> S. Anette Becher,<sup>c</sup> Jo-Ann L. Stanton,<sup>d</sup> Kim Brügger,<sup>e</sup> Steven D. Brown,<sup>f</sup> George P. C. Salmond<sup>b</sup>

Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand<sup>a</sup>; Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom<sup>b</sup>; AgResearch Ltd., Invermay Agricultural Centre, Mosgiel, New Zealand<sup>c</sup>; Department of Anatomy, University of Otago, Dunedin, New Zealand<sup>d</sup>; EASIH, University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom<sup>e</sup>; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA<sup>f</sup>

***Serratia* sp. strain ATCC 39006 is a Gram-negative bacterium and a member of the *Enterobacteriaceae* that produces various bioactive secondary metabolites, including the tripyrrole red pigment prodigiosin and the  $\beta$ -lactam antibiotic 1-carbapenem-2-em-3-carboxylic acid (a carbapenem). This strain is the only member of the *Enterobacteriaceae* known to naturally produce gas vesicles, as flotation organelles. Here we present the genome sequence of this strain, which has served as a model for analysis of the biosynthesis and regulation of antibiotic production.**

Received 1 November 2013 Accepted 11 November 2013 Published 12 December 2013

**Citation** Fineran PC, Iglesias Cans MC, Ramsay JP, Wilf NM, Cossyleon D, McNeil MB, Williamson NR, Monson RE, Becher SA, Stanton J-AL, Brügger K, Brown SD, Salmond GPC. 2013. Draft genome sequence of *Serratia* sp. strain ATCC 39006, a model bacterium for analysis of the biosynthesis and regulation of prodigiosin, a carbapenem, and gas vesicles. *Genome Announc.* 1(6):e01039-13. doi:10.1128/genomeA.01039-13.

**Copyright** © 2013 Fineran et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Peter C. Fineran, peter.fineran@otago.ac.nz, or George P. C. Salmond, gpcs2@cam.ac.uk

*Serratia* sp. strain ATCC 39006 was originally isolated from *Salicornia alterniflora* and in channel water from a salt marsh in Cheesquake, NJ, in a search by the Squibb Chemical Company for bacteria producing new antibiotics (1). In addition to the  $\beta$ -lactam produced, identified as 1-carbapen-2-em-3-carboxylic acid (a carbapenem) (2), this strain synthesizes the red, linear tripyrrole pigment prodigiosin (2-methyl-3-pentyl-6-methoxyprodigiosin). Prodigiosin is a secondary metabolite with antimicrobial, anticancer, and immunosuppressant properties with derivatives in clinical trials (3, 4). *Serratia* sp. strain ATCC 39006 was used to determine the prodigiosin biosynthetic pathway, with implications for biosynthesis of the related compound, undecylprodigiosin, produced by *Streptomyces coelicolor* (4, 5). Furthermore, *Serratia* sp. strain ATCC 39006 has provided an excellent model for investigating the regulation of antibiotic biosynthesis in Gram-negative enterobacteria (4). The control of these secondary metabolites is complex and responds to quorum sensing (6–8), cyclic di-GMP signaling (9, 10), phosphate availability (7, 11), carbon source (12), Hfq (13), stationary phase (14), and drug efflux pump activity (15), among other factors. In addition, due to the ease of prodigiosin detection, this strain has been used to analyze conserved uncharacterized genes and gene products (16–18). For example, SdhE was recently investigated in this strain. SdhE is widely conserved in eukaryotes and *Alpha*-, *Beta*-, and *Gammaproteobacteria* and is essential for flavinylation and activation of succinate dehydrogenase, an enzyme central to the electron transport chain and the tricarboxylic acid cycle (17, 19, 20).

*Serratia* sp. strain ATCC 39006 is motile by means of flagella and can swarm over surfaces aided by the production of a bio-surfactant (10). Surprisingly, this strain also produces gas vesicles, which are hollow intracellular proteinaceous organelles

that control bacterial buoyancy and allow flotation toward air-liquid interfaces (21). This is the only known enterobacterium to utilize this form of taxis naturally (21). The secretion of plant cell wall-degrading enzymes is also a feature of this bacterium, and plant pathogenicity has been confirmed in potato tuber-rotting assays (6, 9). Furthermore, this strain is virulent in a *Caenorhabditis elegans* infection model (22). The genetic analysis of *Serratia* sp. strain ATCC 39006 has been greatly facilitated by the isolation of an efficient broad-host-range generalized transducing phage (23).

Genomic DNA of *Serratia* sp. strain ATCC 39006 was sequenced using the 454 GS FLX Titanium platform (Roche) (~18 $\times$  coverage single-end data) and 36-bp Illumina single-end reads (GAIIx) (~439 $\times$  coverage). The 454 data were *de novo* assembled (Newbler v2.3), giving 53 large contigs (99.9% of sequence) from 94 total contigs. These were assembled into 5 scaffolds using PCR and Sanger sequencing (3 contigs between 200 and 1,000 bp remained). Illumina reads were mapped using BWA 0.5.8, indels were detected using GATK (24), and the sequence was polished using a custom perl script.

The *Serratia* sp. strain ATCC 39006 genome is ~4.94 Mb (G+C content of 49.2%), with 4,413 protein-encoding genes, 7 rRNA operons, and 72 tRNAs (predicted using Prodigal [25]). This sequence will now enable further analysis of the diverse and interesting biological traits that have been defined in this unusual enterobacterium.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [AWXH01000000](https://www.ncbi.nlm.nih.gov/nuccore/AWXH01000000). The version described in this paper is version [AWXH01000000](https://www.ncbi.nlm.nih.gov/nuccore/AWXH01000000).

## ACKNOWLEDGMENTS

We thank Miriam Land (ORNL) for maintaining the Microbial Annotation Genome Pipeline and Sagar Utturkar (University of Tennessee) for assistance with depositing the genome sequence into GenBank.

This work was supported by a University of Otago research grant; the BBSRC, United Kingdom; the Deans Bequest Fund, Otago School of Medical Sciences; the Marsden Fund of the Royal Society of New Zealand (RSNZ); and the BioEnergy Science Center, which is a Department of Energy (DOE) Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science. P.C.F. was supported by a Rutherford Discovery Fellowship (RSNZ), J.P.R. by a Herchel Smith Postdoctoral Fellowship from the University of Cambridge, N.M.W. by a Gates Cambridge Scholarship, and M.B.M. and J.P.R. by University of Otago Career Development Postdoctoral Fellowships. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. DOE under contract DE-AC05-00OR22725.

## REFERENCES

- Parker WL, Rathnum ML, Wells JS, Jr, Trejo WH, Principe PA, Sykes RB. 1982. SQ 27,860, a simple carbapenem produced by species of *Serratia* and *Erwinia*. *J. Antibiot.* 35:653–660.
- Coulthurst SJ, Barnard AM, Salmond GP. 2005. Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nat. Rev. Microbiol.* 3:295–306.
- Williamson NR, Fineran PC, Gristwood T, Chawrai SR, Leeper FJ, Salmond GP. 2007. Anticancer and immunosuppressive properties of bacterial prodiginines. *Future Microbiol.* 2:605–618.
- Williamson NR, Fineran PC, Leeper FJ, Salmond GP. 2006. The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol.* 4:887–899.
- Williamson NR, Simonsen HT, Ahmed RA, Goldet G, Slater H, Woodley L, Leeper FJ, Salmond GP. 2005. Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amylypyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*. *Mol. Microbiol.* 56:971–989.
- Fineran PC, Slater H, Everson L, Hughes K, Salmond GP. 2005. Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol. Microbiol.* 56:1495–1517.
- Slater H, Crow M, Everson L, Salmond GP. 2003. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol. Microbiol.* 47:303–320.
- Thomson NR, Crow MA, McGowan SJ, Cox A, Salmond GP. 2000. Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol. Microbiol.* 36:539–556.
- Fineran PC, Williamson NR, Lilley KS, Salmond GP. 2007. Virulence and prodigiosin antibiotic biosynthesis in *Serratia* are regulated pleiotropically by the GGDEF/EAL domain protein, PigX. *J. Bacteriol.* 189:7653–7662.
- Williamson NR, Fineran PC, Ogawa W, Woodley LR, Salmond GP. 2008. Integrated regulation involving quorum sensing, a two-component system, a GGDEF/EAL domain protein and a post-transcriptional regulator controls swarming and RhlA-dependent surfactant biosynthesis in *Serratia*. *Environ. Microbiol.* 10:1202–1217.
- Gristwood T, Fineran PC, Everson L, Williamson NR, Salmond GP. 2009. The PhoBR two-component system regulates antibiotic biosynthesis in *Serratia* in response to phosphate. *BMC Microbiol.* 9:112.
- Fineran PC, Everson L, Slater H, Salmond GP. 2005. A GntR family transcriptional regulator (PigT) controls gluconate-mediated repression and defines a new, independent pathway for regulation of the tripyrrole antibiotic, prodigiosin, in *Serratia*. *Microbiology* 151:3833–3845.
- Wilf NM, Williamson NR, Ramsay JP, Poulter S, Bandyra KJ, Salmond GP. 2011. The RNA chaperone, Hfq, controls two luxR-type regulators and plays a key role in pathogenesis and production of antibiotics in *Serratia* sp. ATCC 39006. *Environ. Microbiol.* 13:2649–2666.
- Wilf NM, Salmond GP. 2012. The stationary phase sigma factor, RpoS, regulates the production of a carbapenem antibiotic, a bioactive prodigiosin and virulence in the enterobacterial pathogen *Serratia* sp. ATCC 39006. *Microbiology* 158:648–658.
- Gristwood T, Fineran PC, Everson L, Salmond GP. 2008. PigZ, a TetR/AcrR family repressor, modulates secondary metabolism via the expression of a putative four-component resistance-nodulation-cell-division efflux pump, ZrpADBC, in *Serratia* sp. ATCC 39006. *Mol. Microbiol.* 69:418–435.
- Gristwood T, McNeil MB, Clulow JS, Salmond GP, Fineran PC. 2011. PigS and PigP regulate prodigiosin biosynthesis in *Serratia* via differential control of divergent operons, which include predicted transporters of sulfur-containing molecules. *J. Bacteriol.* 193:1076–1085.
- McNeil MB, Clulow JS, Wilf NM, Salmond GP, Fineran PC. 2012. SdhE is a conserved protein required for flavinylation of succinate dehydrogenase in bacteria. *J. Biol. Chem.* 287:18418–18428.
- McNeil MB, Iglesias-Cans MC, Clulow JS, Fineran PC. 2013. YgfX (CptA) is a multimeric membrane protein that interacts with the succinate dehydrogenase assembly factor SdhE (YgfY). *Microbiology* 159:1352–1365.
- McNeil MB, Fineran PC. 2013. Prokaryotic assembly factors for the attachment of flavin to complex II. *Biochim. Biophys. Acta* 1827:637–647.
- McNeil MB, Fineran PC. 2013. The conserved RGxxE motif of the bacterial FAD Assembly factor SdhE is required for succinate dehydrogenase flavinylation and activity. *Biochemistry* 52:7628–7640.
- Ramsay JP, Williamson NR, Spring DR, Salmond GP. 2011. A quorum-sensing molecule acts as a morphogen controlling gas vesicle organelle biogenesis and adaptive flotation in an enterobacterium. *Proc. Natl. Acad. Sci. U. S. A.* 108:14932–14937.
- Coulthurst SJ, Kurz CL, Salmond GP. 2004. *luxS* mutants of *Serratia* defective in autoinducer-2-dependent “quorum sensing” show strain-dependent impacts on virulence and production of carbapenem and prodigiosin. *Microbiology* 150:1901–1910.
- Evans TJ, Crow MA, Williamson NR, Orme W, Thomson NR, Komi-topoulou E, Salmond GP. 2010. Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, *Serratia* and *Pantoea*. *Microbiology* 156:240–247.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytisky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20:1297–1303.
- Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.