Complete genome sequence of *Pseudoalteromonas rubra* SCSIO
6842, harboring a putative conjugative plasmid pMBL6842

Baiyuan Li^{1,2}, Pengxia Wang¹, Zhenshun Zeng^{1,2}, Xingsheng Cai¹, Guanghua Wang¹,
Xiaoxue Wang^{1*}

¹Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Marine Materia Medica, RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

²University of Chinese Academy of Sciences, Beijing 100049, China

*To whom correspondence should be addressed. E-mail: xxwang@scsio.ac.cn

Keywords:

Complete genome, *Pseudoalteromonas*, cycloprodigiosin, plasmid

ABSTRACT:

Pseudoalteromonas is a genus of Gram-negative and is ubiquitously distributed in the ocean. Many *Pseudoalteromonas* species are capable of producing pigments, which can serve as an alternative source to replace synthetic pigments used in the food industry. Prodigiosins belong to a family of secondary metabolite characterized by a common pyrrolylpyrromethane skeleton, and have been successfully applied to yogurt, milk and carbonated drinks as substitutes for synthetic additives. The strain *Pseudoalteromonas rubra* SCSIO 6842 can produce cycloprodigiosin and harbors a conjugative plasmid. Here we report the complete genome of *P. rubra* SCSIO 6842 for a better understanding of the molecular basis of cycloprodigiosin production and regulation.

Pseudoalteromonas is rod-shaped, Gram-negative, non-spore forming and flagellated bacterium which belonging to class Gammaproteobacteria. *Pseudoalteromonas* is found in oceans throughout the world. Many *Pseudoalteromonas* species are capable of producing biologically active extracellular compounds including extracellular polysaccharide and pigments (Bowman, 2007). *Pseudoalteromonas* can be divided into pigmented and non-pigmented clades (Egan et al., 2002), and pigmented *Pseudoalteromonas* species seem to produce a more broad range of bioactive compounds (Holmstrom and Kjelleberg, 1999).

Natural pigments from microorganisms can serve as an alternative source to replace synthetic pigments used in the food industry (Parekh et al., 2000), and they may also have antioxidants, antifungal, and immunosuppressive activities (Darshan and Manonmani, 2015). Prodigiosins belong to a family of secondary metabolite characterized by a common pyrrolylpyrromethane skeleton, and have been successfully applied to yogurt, milk and carbonated drinks as substitutes for synthetic additives (Namazkar and Ahmad, 2013). Previous studies have shown that *Pseudoalteromonas rubra* (Gerber and Gauthier, 1979) can produce cycloprodigiosin. *Pseudoalteromonas rubra* SCSIO 6842 is a typical cycloprodigiosin-producing strain. Different from closely related *P. rubra* DSM 6842 strain (Gauthier, 1976), *P. rubra* SCSIO 6842 also harbors a conjugative plasmid. The complete genome of *P. rubra* SCSIO 6842 was sequenced in order to uncover important genomic traits related to cycloprodigiosin biosynthesis. The conjugative plasmid pMBL6842 was also sequenced in order to explore the function of horizontally transferred elements.

Pseudoalteromonas rubra SCSIO 6842 was isolated from 150 meters below surface at Bay of Bengal. The genome sequence of *P. rubra* SCSIO 6842 was determined by single molecule real-time (SMRT) sequencing platform on PacBio RS II instrument with P5-C3 chemistry (PacificBio-sciences, Menlo Park, CA) (Eid et al., 2009). To obtain the high-quality finished sequence, a strategy of Solexa paired end sequencing technology was used. The yielded raw data was 72,015 reads for 749,162,100 bases. The HGAP software (Chin et al., 2013) was used for de novo genome assembly with 63× coverage of self-corrected reads with length over 5,935,415 bp. The resulting three PacBio contigs were analyzed, and the gaps between contigs were filled through sequencing of PCR products by primer walking. The coding sequences (CDSs) were predicted using Gene Locator and Interpolated Markov ModelER (Glimmer) version 3.02 software (Delcher et al., 2007). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and tRNA/rRNA genes were identified by tRNAscan-SE (Lowe and Eddy, 1997) and RNAmmer (Lagesen et al., 2007), respectively.

The genome features of *P. rubra* SCSIO 6842 are summarized in Table.1. The genome size is 913,780 bp. The complete genome of *P. rubra* SCSIO 6842 consists of two circular chromosomes of 1,311,023bp (Chromosome I, 47% G+C), 4,532,889 bp (Chromosome II, 47.9% G+C) and one circular plasmid, named as pMBL6842 (69,868 bp, 53% G+C). It includes 5,193 protein coding genes (CDSs), 116 tRNAs and 31 rRNAs. A putative cycloprodigiosin biosynthetic gene cluster (12 open reading frames) of *P. rubra* SCSIO 6842 was revealed by BLASTP search against the prodigiosin biosynthesis gene cluster in *Hahella chejuensis* KCTC 2396. Additionally, a total of 66 open reading frames was identified in plasmid pMBL6842 including one encoding the histone-like nucleoid-structuring protein H-NS. The availability of complete genome of *P. rubra* SCSIO

6842 will help to improve our understanding of the microbial behavior of pigment production in *Pseudoalteromonas* species.

Table 1

Genome features of *Pseudoalteromonas rubra* SCSIO 6842.

Attributes	Chromosome I	Chromosome II	Plasmid pMBL6842
Length (bp)	1,311,023	4,532,889	69,868
G + C content(%)	47	47.9	53
Protein-coding genes (CDSs)	1080	4047	66
rRNA operons	0	31	0
tRNA genes	1	115	0

Nucleotide sequence accession number

The complete genome sequence of *P. rubra* SCSIO 6842 has been deposited in GenBank under the accession numbers CP013611 (chromosome I), CP013612 (chromosome II), CP013613 (plasmid pMBL6842). The strain is available from China General Microbiological Culture Collection Center (CGMCC) in China under the accession number no CGMCC 1.15540.

Acknowledgements

This work was supported by the National Basic Research Program of China (2013CB955701), the National Science Foundation of China (31290233 and 41406189) and the Chinese Academy of Sciences (XDA11030402). XW is the 1000-Youth Elite Program recipient in China.

Reference

- Bowman, J.P., (2007) Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. *Marine Drugs* 5, 220-241.
- Darshan, N., Manonmani, H.K., (2015) Prodigiosin and its potential applications. *Journal of Food Science and Technology-Mysore* 52, 5393-5407.
- Delcher, A.L., Bratke, K.A., Powers, E.C., Salzberg, S.L., (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23, 673-679.
- Egan, S., James, S., Holmstrom, C., Kjelleberg, S., (2002) Correlation between pigmentation and antifouling compounds produced by *Pseudoalteromonas tunicata*. *Environmental Microbiology* 4, 433-442.
- Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., deWinter, A., Dixon, J., Foquet, M., Gaertner, A., Hardenbol, P., Heiner, C., Hester, K., Holden, D., Kearns, G., Kong, X., Kuse, R., Lacroix, Y., Lin, S., Lundquist, P., Ma, C., Marks, P., Maxham, M., Murphy, D., Park, I., Pham, T., Phillips, M., Roy, J., Sebra, R., Shen, G., Sorenson, J., Tomaney, A., Travers, K., Trulson, M., Vieceli, J., Wegener, J., Wu, D., Yang, A., Zaccarin, D., Zhao, P., Zhong, F., Korlach, J., Turner, S., (2009) Real-Time DNA Sequencing from Single Polymerase Molecules. *Science* 323, 133-138.
- Gauthier, M.J., (1976) *Alteromonas rubra* sp-nov, a new marine antibiotic-producing bacterium. *International Journal of Systematic Bacteriology* 26, 459-466.

- Gerber, N.N., Gauthier, M.J., (1979) New prodigiosin-like pigment from *Alteromonas rubra*. *Applied and Environmental Microbiology* 37, 1176-1179.
- Holmstrom, C., Kjelleberg, S., (1999) Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *Fems Microbiology Ecology* 30, 285-293.
- Lagesen, K., Hallin, P., Rodland, E.A., Staerfeldt, H.-H., Rognes, T., Ussery, D.W., (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research* 35, 3100-3108.
- Lowe, T.M., Eddy, S.R., (1997) tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* 25, 955-964.
- Namazkar, S., Ahmad, W., (2013) Spray-dried prodigiosin from *Serratia marcescens* as a colorant. *Biosciences Biotechnology Research Asia* 10, 69-76.
- Parekh, S., Vinci, V.A., Strobel, R.J., (2000) Improvement of microbial strains and fermentation processes. *Applied Microbiology and Biotechnology* 54, 287-301.