

## Genome Sequence of the Pigment-Producing Bacterium *Pseudogulbenkiania ferrooxidans*, Isolated from Loktak Lake

Sampada Puranik, Reshma Talkal, Asifa Qureshi, Anshuman Khardenavis, Atya Kapley, Hemant J. Purohit

Environmental Genomics Division, National Environmental Engineering Research Institute (CSIR-NEERI), Nehru Marg, Nagpur, India

The whole genome of a pigment-producing isolate from a lake in northern India, *Pseudogulbenkiania ferrooxidans* strain EGD-HP2, has been sequenced to study the spectrum of biosynthesis of secondary metabolites. The genome annotation data revealed an operon for violacein, which showed homology with the reported operon of a *Chromobacterium* sp., and also a quinone cofactor.

Received 22 November 2013 Accepted 26 November 2013 Published 26 December 2013

Citation Puranik S, Talkal R, Qureshi A, Khardenavis A, Kapley A, Purohit HJ. 2013. Genome sequence of the pigment-producing bacterium *Pseudogulbenkiania ferrooxidans*, isolated from Loktak Lake. Genome Announc. 1(6):e01115-13. doi:10.1128/genomeA.01115-13.

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

Address correspondence to Hemant J. Purohit, hj\_purohit@neeri.res.in.

pigment-producing strain was isolated from Loktak Lake in northern India. To understand the possible nature of the pigment, whole-genome sequencing of the isolate was done. The draft genome data were analyzed for biosynthesis of pigment and secondary metabolites. According to the annotated data, the genome was confirmed to be that of a Pseudogulbenkiania ferrooxidans strain. The functional categories of genes were identified through the SEED Viewer. Of the various secondary metabolic pathways, we identified the complete operon for violacein biosynthesis. The operon consisted of all the genes which are involved in biosynthesis of violacein, including vioA (tryptophan-2-monoxygenase), vioB (violacein biosynthesis protein), vioC (monoxygenase), vioD (tryptophan hydroxylase), and vioE [proto(deoxy)violaceinic acid synthase], along with important regulatory elements (1, 2). The violacein pathway of this strain shows homology with the reported pathway from Chromobacterium violaceum. An additional interesting feature of this strain, which we mapped using RAST (Rapid Annotations using Subsystems Technology) (3, 4), is the presence of a dehydrogenase flavoprotein, a quinone cofactor, encoded by the *lodB* gene.

The pathway analysis shows the presence of various important subcategories, like secondary metabolism, stress response (mainly to oxidative stress), iron acquisition and metabolism, and aromatic compound metabolism. These are the subcategories involved in distant cause-effect relationships, with the violacein production being under the influence of a quorum-sensing mechanism. Identification of such genes and investigations of the details of promoter regulation through sequence analysis using B-PROM could reveal strategic interrelations of networking, which might elucidate the molecular aspects of dye production (5, 6). These data might open new avenues into industrial production of secondary metabolites. Natural pigments, like violacein, also display advantageous biological activities, such as antiprotozoal, antileishmanial, antioxidant, antibacterial, and anticancer effects (7–10). With sequencing technology becoming more affordable, the draft-genome approach could be used for rapid screening of pigment-producing strains from unique habitats with potential secondary metabolites of commercial value.

The high-quality reads of 387,863 bp were assembled into 301 contigs by use of GS Assembler/CLC Genomics Workbench with optimized parameters (v2.3). The draft genome sequence has an average GC content of 64.1%. The genome was annotated using RAST v4.0 and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes /static/Pipeline.html). NCBI PGAAP annotated 301 high-quality contigs into genes, 4,392 coding sequences (CDS), 80 tRNA genes, 4 copies of 5S rRNA genes, 1 copy of a 16S rRNA gene, and 8 pseudogenes.

**Nucleotide sequence accession number.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. AVPH000000000. The version described in this paper is the first version.

## ACKNOWLEDGMENTS

This work is supported by CSIR-NEERI. We thank the director of the CSIR-National Environmental Engineering Research Institute (NEERI) for providing the necessary facilities. Sampada Puranik thanks CSIR for providing a Senior Research Fellowship.

## REFERENCES

- Hoshino T. 2011. Violacein and related tryptophan metabolites produced by *Chromobacterium violaceum*: biosynthetic mechanism and pathway for construction of violacein core. Appl. Microbiol. Biotechnol. 91: 1463–1475.
- Zhang X, Enomoto K. 2011. Characterization of a gene cluster and its putative promoter region for violacein biosynthesis in *Pseudoalteromonas* sp. 520P1. Appl. Microbiol. Biotechnol. 90:1963–1971.
- 3. 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.
- 4. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, Cohoon M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuyl D,

Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rückert C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res. 33:5691–5702.

- Sobha Rani T, Bapi RS. 2009. Analysis of *n*-gram based promoter recognition methods and application to whole genome promoter prediction. In Silico Biol. 9:S1–16.
- Leon LL, Miranda CC, De Souza AO, Duràn N. 2001. Antileishmanial activity of the violacein extracted from *Chromobacterium violaceum*. J. Antimicrob. Chemother. 48:449–450.
- 7. Konzen M, de Marco D, Cordova CA, Vieira TO, Antônio RV,

Creczynski-Pasa TB. 2006. Antioxidant properties of violacein: possible relation on its biological function. Bioorg. Med. Chem. 14:8307–8313.

- 8. Martins D, Frungillo L, Anazzetti MC, Melo PS, Durán N. 2010. Antitumoral activity of L-ascorbic acid-poly-D,L-(lactide-co-glycolide) nanoparticles containing violacein. Int. J. Nanomedicine 5:77–85.
- Hakvåg S, Fjærvik E, Klinkenberg G, Borgos SE, Josefsen KD, Ellingsen TE, Zotchev SB. 2009. Violacein-producing *Collimonas* sp. from the sea surface microlayer of coastal waters in Trøndelag, Norway. Mar. Drugs 7:576–588.
- Kodach LL, Bos CL, Durán N, Peppelenbosch MP, Ferreira CV, Hardwick JC. 2006. Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. Carcinogenesis 27:508–516.