Pseudomonas koreensis A9 genome insights in heterotrophic sulfide oxidation

A. Novo[1], I. S. Moreira[1], I. Vasconcelos[1] and **P. M. L. Castro[1]**

[1] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, apartado 2511, 4202-401 Porto, Portugal

Biological oxidation of hydrogen sulfide to sulfate is one of the major reactions of the global sulfur cycle. In the presence of a suitable electron acceptor, bacteria can oxidize hydrogen sulfide, sulfur, sulfite, thiosulfate, and various polythionates under alkaline, neutral, or acidic conditions. The first step of hydrogen sulfide oxidation is its conversion to sulfur or polysulfide in many phototrophic and chemotrophic bacteria by flavocytochrome c, or by sulfide: quinone reductase, which are located in the periplasm and the periplasmic surface of the cytoplasmic membrane, respectively [1,2]. In the last decade, our understanding on the acidophilic bacteria has greatly advanced, however knowledge on the physiology and genetics of the heterotrophic sulfur-oxidizing is more scarce, and compared to autotrophic sulfur-oxidizing bacteria these heterotrophs are more versatile [3]. This work aims at the identification of the genes that encode enzyme(s) responsible for H_2S oxidation in the sequenced genome of *Pseudomonas koreensis* A9. This bacterial strain was isolated from an odour biofilter installed in a WWTP, that displayed high sulfide tolerance and growth on with sulfide on concentrations up to 16 mM. Activity tests were conducted using crude cell extract as catalyst and measuring the formation of sulfate as the reaction product. Maximum sulfate concentration achieved was 71,6 $mg.L^{-1}$ and a specific activity 2,9 $U.mg^{-1}$ of protein, but when this extract was purified by acetone precipitation and ultrafiltration, results of specific activity reached 67,3 $U.mg^{-1}$ what is very promising compared to the pure sulfide oxidase from *Arthrobacter* sp. (65,1 $U.mg^{-1}$) [4]. Total genomic DNA from strain A9 was sequenced and assembled by a 454-pyrosequencing system Genome Sequencer FLX with GS FLX Titanium. The genome draft was annotated using the Rapid Annotation System Technology (RAST) [5]. The sequencing produced 269 031 reads with an average length of 651 bases. DNA sequencing of *P. koreensis* A9 and short read de novo assembly generated the 6 376 154 bp draft genome with 60,1 % G+C content. The de novo read assembly produced 73 contigs with pegs, the longest had 747 025 bases. This genome encodes 5 738 predicted coding genes distributed by 542 sub-systems from SEED [5]. Out of 5 738 protein coding genes sequences, 50% were annotated with functional specifications, while the remaining encoded hypothetical proteins. The A9 genome was screened for sulfide oxidation related genes by browsing the RAST subsystems associated to S metabolism. Functional screening analysis reveals that A9 possesses the genes required for sulfide oxidation to sulfate, with sulfite as the intermediate. The orthologous sequences of these enzymes were found on peg's 3615, peg.2931 and peg. 4936. In addition, rhodanese-like, glutathione: sulfur transferase, thiol peroxidase, among others were identified in the genome which demonstrate the ability and versatility of A9 to sulfide bioremediation applications.

Key words: Hydrogen sulfide; Heterotrophic sulfur oxidation; *Pseudomonas koreensis* A9; Genome analysis

References

[1] Mohapatra BR, Gould WD, Dinardo O, Koren DW (2008) An Overview of the Biochemical and Molecular Aspects of Microbial Oxidation of Inorganic Sulfur Compounds. Clean. 36: 823 – 829

[2] Yin H, Zhang X, Li X, He Z, Liang Y, Guo X, Hu Q, Xiao Y, Cong J, Ma L, Niu J, Liu X (2014) Whole-genome sequencing reveals novel insights into sulfur oxidation in the extremophile Acidithiobacillus thiooxidans. BMC Microbiology. 14:179

[3] Liu Y, Yang H, Zhang X, Xiao Y, Guo X, Liu X (2016) Genomic Analysis Unravels Reduced Inorganic Sulfur Compound Oxidation of Heterotrophic Acidophilic Acidicaldus sp. Strain DX-1. BioMed Research International. Article ID 8137012. 8 pages

[4] Mohapatra BR, Gould WD, Dinardo O, Papavinasam S, Revie RW (2006) Optimization of culture conditions and properties of immobilized sulfide oxidase from Arthrobacter species. Journal of Biotechnology. 124:523–531

[5] Overbeek et al. (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Research. 42:D206–D214