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MOLECULAR MODELING OF THE RIBOFLAVIN GENES FROM ACIDITHIOBACILLUS FERROOXIDANS.

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Short Abstract: Acidithiobacillus ferrooxidans is a bacteria with economic relevance in metal bioleaching. Very little is known about the riboflavin genes from Acidithiobacillus ferrooxidans. Molecular modeling of enzymes involved in riboflavin biosynthesis could contribute to a better understanding of riboflavin biosynthesis when A. ferroxidans is maintained in the presence of metal sulfides.

Long Abstract:
Riboflavin is the precursor molecule for the synthesis of two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both essential in cellular metabolism. Many enzymes are involved in the synthesis of riboflavin such as GTP cyclohydrolase II (ribA gene) and 3,4-dihydroxy-2-butanone 4-phosphate (DHPB) synthase (ribB gene). Very little is known about the rib genes from Acidithiobacillus ferrooxidans, a Gram-negative bacteria with a high economic relevance due to its application in metal bioleaching. One of the metals recovered by bioleaching is copper. This metal is present in the composition of the metal sulfides chalcopyrite (CuFeS2) and bornite (Cu5FeS4) among others. RNA was isolated from A. ferrooxidans cells, maintained in the presence of bornite for 24 hs, and used in RNA arbitrarily primed PCR experiment. In this experiment, a cDNA with a higher expression in the presence of bornite was isolated. This cDNA showed similarity with the ribE gene from Geobacter sulfurreducens. The ribE gene encodes the beta subunit of riboflavin synthase (E-value 4e-55, identity 69% and similarity 83%), a key enzyme in the riboflavin biosynthesis. A search on the A. ferrooxidans ATCC23270 genome (TIGR, Unfinished Microbial Genomes - www.tigr.org) showed that the ribE gene is part of the rib operon which is comprised of the following genes: ribX > ribD > ribC > ribBA > ribE. Another riboflavin biosynthetic gene, ribB encoding for DHBP synthase, is located 1500 kb upstream from the rib operon of A. ferrooxidans. To achieve a better understanding of the proteins involved in riboflavin biosynthesis we performed homologous molecular modeling of the following enzymes : DHBP synthase, encoded by the ribB gene, beta subunit of riboflavin synthase, encoded by the ribE gene, alpha subunit of riboflavin synthase, encoded by the ribC gene and riboflavin deaminase reductase, encoded by the ribD gene. A search in the database for three dimensional protein structure (PDB – www.pdb.org) revealed 4 templates

that could be used to construct the models: DHBP synthase, PDB code 1G58/A (1.55 Å resolution; Rfree 0,231; E-value 3e-62; identity 60%); beta subunit of riboflavin synthase, PDB code 1RVV/A (2.4 Å resolution; Rfree 0,237; E-value 5e-42; identity 54%); alpha subunit of riboflavin synthase, PDB code 1I8D/A (2.0 Å resolution; Rfree 0,299; E-value 7e-27; identity 34%), and riboflavin deaminase reductase, PDB code 2B3Z/A (2.41 Å resolution; Rfree 0,277; E-value 1e-78; identity 45%). The functional and structural analysis of these enzymes could provide relevant findings that could contribute to a better understanding of riboflavin biosynthesis when A. ferroxidans is maintained in the presence of metal sulfides.