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SEQUENCE ANALYSIS OF THE RIBOFLAVIN BIOSYNTHESIS GENES FROM ACIDITHIOBACILLUS FERROOXIDANS.

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Short Abstract: Acidithiobacillus ferrooxidans is a bacteria with economic relevance in metal bioleaching. We describe here the sequence analysis of the riboflavin operon in this bacteria. Expression analysis of the rib genes was performed by Real-Time PCR. The results could contribute to a better understanding of the bioleaching process by Acidithiobacillus ferrooxidans.

Long Abstract:
Acidithiobacillus ferrooxidans is a Gram-negative bacterium with a high economic relevance due to its application in metal bioleaching. One of the metals recovered by this process is copper. This metal is present in the composition of the metal sulfides chalcopyrite (CuFeS2) and bornite (Cu5FeS4) among others. A. ferrooxidans cells were grown in the presence of iron and then they were maintained in the presence of chalcopyrite and bornite for 24 hours. The cells grown in the presence of iron were used as control. RNA isolated from these cells were used in RNA arbitrarily primed PCR experiments that resulted in the identification of a 255 bp cDNA with a higher expression in the presence of bornite. The complete sequence of this gene was obtained from the A. ferrooxidans ATCC23270 genome (TIGR, Unfinished Microbial Genomes - www.tigr.org). The obtained sequence was compared with GenBank sequences using the Blast algorithm and a similarity with ribE from Geobacter sulfurreducens was observed. This gene encodes the beta subunit of riboflavin synthase (E-value 4e-55, identity 69% and similarity 83%). The riboflavin synthase is a key enzyme for the bacterium since it catalyses the riboflavin biosynthesis. Riboflavin is the precursor molecule for the synthesis of two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both essential in cellular metabolism and redox reactions. Sequence analysis using the Expasy - ProtParam tool (http://www.expasy.org/tools/protparam.html) revealed a predicted isoeletric point of 6,29 and a molecular weight of 16,64 kDa of the protein. The predicted cellular localization, using the PSORTb v.2.0.4 (http://www.psort.org/psortb/) revealed that this protein is located in the cytoplasm. Using the complete ribE gene sequence we were able to identify a larger contig in the TIGR database. Computer analysis of this contig showed that the ribE gene was located in a rib operon. The order of the genes in this operon was as following : ribX > ribD > ribC > ribBA > ribE. Further analysis of the

genome resulted in the identification of another riboflavin biosynthetic gene, ribB, that encodes for DHBP synthase and is located 1500 kb upstream from the rib operon. The relative expression value of the riboflavin biosynthetic genes from A. ferrooxidans were determined by Real-Time PCR. Further characterizations of these genes expression were performed with RNA obtained from A. ferrooxidans cells grown in different conditions such as phosphate starvation and pH and temperature different from the optimum. Preliminary results show that the relative expression of these genes change according to the growth condition. In addition, the characterization of the rib genes expression in A. ferrooxidans grown in the presence of the metal sulfides chalcopyrite and bornite could contribute to a better understanding of the bioleaching process by Acidithiobacillus ferrooxidans.