Cluster of Genes of Sulfate Assimilation

Rabets, Andrii

Sulphate assimilation pathways are essential for synthesis of methionine, proteins and biosynthesis of sulfur containing antibiotics. There are many studies on sulfur metabolism in actinobacteria such as Corynebacterium and Mycobacterium, but there is no scientific evidence on the sulphur metabolism in Streptomyces that are characterised by complex secondary metabolism and produce over two-thirds of the clinically relevant natural antibiotics such as neomycin, cypemycin, grisemycin, bottromycins and chloramphenicol. Bioinformatical analysis revealed similarities in the cluster of sulfate assimilating genes in C. glutamicum and target S. Coelicolor.Two identified genes, sco6100 encoding PAPS-reductase (3'-phosphoadenosine-5'-phosphosulfatereductase) and sco6101 orphan were selected.We assumed that sulfate metabolism genes in C. glutamicum are similar in function to genes of S. Coelicolor.These genes are homologues to the all genes in order Actinomycetales including M. Tuberculosis. In order to prove this hypothesis, we implemented a cosmid based method of homologous gene deletion and recombination that enabled us to replace genes with a selective amp resistance marker.Second round of conjugation allowed deletion of antibiotic resistance genes.Obtained mutants were tested on minimal medium supplemented with different sources of sulfur compounds, corresponding to the steps of sulphur metabolic pathway.Obtained mutant growth patterns proved our hypothesis that sco6100 negative strains can’t use sulfates as a source of sulfur but only thiosulfate, sulfite, sulfide, methionine and cysthionine.However sco6101 negative strains can use only methionine.Based on the obtained results it is likely that that sco6100 and sco6101 product performs similar function as in C. Glutamicum