DETERMINATION OF COSMOMYCIN BIOSYNTHETIC GENE CLUSTER ENDS IN *Streptomyces olindensis* DAUFPE 5622

The study of the gene cluster and biosynthetic pathway of anthracycline cosmomycin D, CosD, in *Streptomyces olindensis* has been made by different approaches including RT-PCR and genome walking. Sequencing of *S. olindensis* genome was carried out to identify a contig with the CosD biosynthetic cluster. This cluster includes about 40 genes and can be considered one of the largest among anthracyclines. *In silico* analysis of the products encoded by each ORF was performed by grouping genes according to their functionality.

In this project, we attempt to elucidate the extremes of the cosmomycin cluster. For this, we analyze the relative expression of the candidate genes by RT-PCR, during the cosmomycin production, using the *Hrd*B gene as housekeeping. *Hrd*B is the main sigma factor in *Streptomyces* and represents the main housekeeping regulator. RNA extraction was performed using TRIzol from Invitrogen and Illustrative RNAspin kits from GE Healthcare and PureLink RNA from Ambion. RT-PCR was performed using the Invitrogen SuperScript III First-Strand kit, and Applied Biosystems SYBR Green PCR Master Mix on an Applied Biosystems StepOnePlus Real Time PCR System.

The results obtained allow us to find the extremes of the cosmomycin cluster. We detected the overexpression of the ORFs 17, 18, 19, 20, 52 and 54, while studying ORFs 15-20, 52, 54-56. ORF 17, 54 and 55 where statistically significate with a p-value of 0,039 (ORF17), 0,0069 (ORF54) and 0,021 (ORF55); while ORF 55 was under-expressed.

On the right side of the cluster is located the extreme of ORFs 17-20, which contains the antitumoral self-resistance genes. The expression levels of the 17-20 ORFs obtained by RT-PCR agree with the studies carried out previously in our laboratory, it was proposed the mechanism of self-resistance of *S. olindensis* to CosD; conforming to ORF 17 (cosP) daunorubicin resistance UvrA-like protein, ORF 18 (cosU) glutathione peroxidase, ORF 19 (cosJ) multidrug ABC transporter permease, and ORF 20 (cosI), ABC transporter ATP binding protein. On the other side, the ORFs located are ORF 52, SARP family transcriptional regulator, ORF 53, acyl carrier protein, and ORF 54, glucose-1-phosphate thymidyltransferase; proteins involved in biosynthesis regulation, aglycone modification and sugar biosynthesis of the anthracycline cosD.

By means of these results we can conclude that the cosmomycin D cluster in *Streptomyces olindensis*, comprised of 37 ORFs.

Área de conhecimento: Genética de Microrganismos e Bioinformática

Sub-área: Genética Microbiana

Palavras Chaves: PCR Real Time, *Streptomyces*, BGC, cosmomycin

Agências Financiadoras: CAPES

Instituições dos autores: Laboratório de Bioprodutos, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo

Autores: Ortiz-Castro, R.E., Cunha, L.L., Garrido, L.M., Padilla, G.