FUNCTIONAL ANALYSIS OF THE *sco2582* GENE IN *Streptomyces coelicolor*

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Introduction. *Streptomyces coelicolor* has been extensively used as a model for physiological and morphological development studies and genetic control of antibiotic production (1). Our research group has reported on the possible role of SCO2127 on carbon catabolite repression (CCR) and morphological differentiation in *Streptomyces coelicolor* (2). SCO2127 was able to bind SCO2582 in *S. coelicolor* crude extracts from cells grown in complex media. *In silico* analysis of the SCO2582 (402 amino acids) protein sequence (www.merops.sanger.ac.uk) suggests a homology with the peptidase M48 family. These proteins are classified as metallopeptidases, which require a divalent cation to show proteolytic activity. SCO2582 was detected during the stationary growth phase of *S. coelicolor*, suggesting a possible role in morphological differentiation (MD). In this microorganism, Chater, et *al*., (3) have proposed a general model of intracellular and extracellular protease cascade to explain MD. In order to identify the possible role of *sco2582* in *S. coelicolor*, the aim of this work is to evaluate the effect of a double gene copy on its ability to produce aerial mycelia and actinorhodin production.

Methods. The *sco2582* gene was amplified by PCR using the *S. coelicolor* cosmid StC123.20 as DNA template. The amplified fragment was restricted with *Bgl*II and *Nde*I and cloned into the *pIJ6902* expression vector containing apramycin (Am^R) and thiostrepton (tsr^R) resistances as well as a thioestrepton-inducible promoter p_{tipA}, used to regulate gene expression (4). The derived plasmid *pIJ825* was used to transform *S. coelicolor* M145 protoplasts. The genomic DNA from Am^R mutants was analyzed by PCR to confirm integration of *pIJ825*. *S. coelicolor* mutants were cultured on R5A agar and liquid media. Growth rate measurements and actinorhodin levels were done as reported (5).

Results. In the present work we used a *S. coelicolor* strain containing an extra copy of *sco2582* in the plasmid *pIJ825*. The wild-type M145 and a strain containing the empty vector (*pIJ6902)* were used as controls. A greater growth rate was observed in the strain with pIJ825 in submerged cultures (2.58 mg mL^{-1} dry weight), compared to the M145 and the strain containing the empty vector (1.50 and 1.90 mL^{-1} dry weight, respectively).
In regard to actinorhodin production, the strain with double copy of *sco2582* showed a slight increase in antibiotic production (53.41 μM mg^{-1} dry weight) when compared to the wild-type M145 strain (51.22 μM mg^{-1} dry weight) and the strain with the empty vector (3.29 μM mg^{-1} dry weight).
When cultured on agar plates, we found that the presence of the empty vector inhibited growth and antibiotic production (Fig. 1), A similar behavior has been reported for *S. fradiae* containing a plasmid with phiC31/*pIJ101* inserted in its genome. In contrast, *S. coelicolor* with *pIJ825* showed more blue pigment visually identified as actinorhodin, good growth and improved aerial mycelium formation.

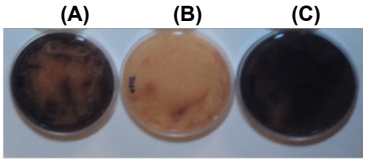

Fig. 1 Qualitative analysis of actinorhodin production by *S. coelicolor* M145 strain (A) and *S. coelicolor* strains transformed with *pIJ6902* (B) and *pIJ825* (C). The three strains were growth for 60 h in R5A agar medium.

Conclusions. Our results suggest a possible involvement of the SCO2582 metallopeptidase in the complex network development of *S. coelicolor*, typically associated with the production of secondary metabolites and aerial mycelium formation. However, with the present evidence, the specific role of *sco2582* still remains to be elucidated and will be the focus of our future work.

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