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Studies towards the production of candicidin by Streptomyces sp. CBMAI 2043

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Abstract
The aim of this study was to explore two gene clusters from Streptomyces sp. CBMAI 2043. One of them was the candicidin gene cluster, a polyketide annotated form the strain whole genome sequencing. By deleting important genes it is possible to have clues about the biosynthetic pathways and released intermediates. The second gene is a unknown non ribosomal. Using the same approach we expected to ,correlate the gene cluster to the chemical entity. These studies will help to have a better understanding of the whole matabolite profile of the wild type strain.

Key words:
Streptomyces, biosynthesis, PKS, NRPS.

Introduction

Streptomyces is a genus composed by Gram-positive bacteria mainly found in soil. The large diversity of secondary metabolites expressed by this genus, like antibiotics, antiparasitic agents, herbicides, enzymes and others, is the reason why it is so studied over the world[1]. Candicidin is a polyene macrolide produced by a type I modular PKS[2]. The name was chosen due to the strong activity of this metabolite against some species of *Candida* and the first description of candicidin is dated from 1953 by Lechevalier *et al.* in their studies with *Streptomyces griseus*[3].

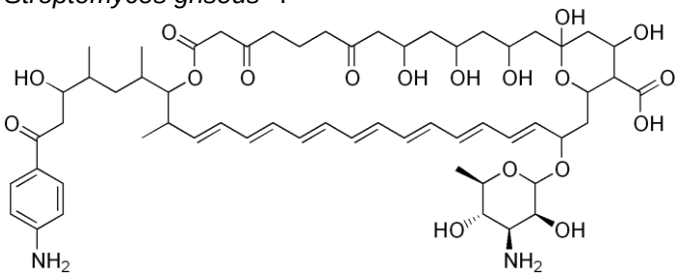

Image 1. Candicidin structure

Results and Discussion

The current research project is based in two gene clusters annotated from the whole genome sequence of *Streptomyces* sp. CBMAI 2043 (previous *Streptomyces wadaymensis* A23). The production of candicidin is regulated by three genes as shown in Figure 2 and is observed in culture extracts of CBMAI 2043. We are also investigating a cryptic gene cluster responsible for the biosynthesis of a unknown non-ribosomal peptide (Figure 3). Our first approach corresponds to a deletion of a region of each cluster to associate it to the chemical entity. Alternatively the whole gene cluster will be transferred to a heterologous host as a way to improve the production of candicidin and identify the unknownmetabolite.

Image 2. Candicidin gene cluster

Image 3. Unknown metabolite gene cluster.

Our first aim was to select a region of each cluster to promote the gene deletion. The deletion plasmids were constructed using Gibson Assembly and are based on pYH7, a suicide vector. The construction contain flanking arms with homology to the corresponding region in the genome to be deleted. The correct sequence of the deletion plasmid was verified by restriction enzymes (Figure 4). These constructions will be transferred to the *Streptomyces* strain by conjugation using the methylase deficient strain *E. coli* ET12567.

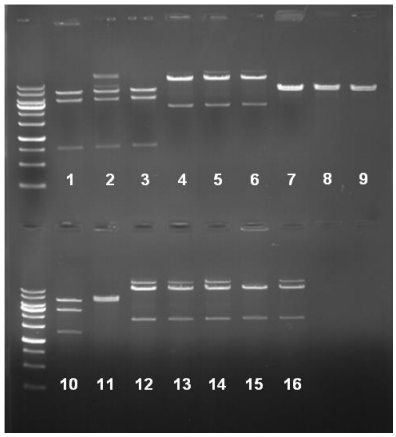

Image 4. Digestion rection of the deletion plasmids to confirm the correct contruction

Conclusions

Making a deletion in a gene cluster region should promote the biosynthesis interruption and can shed light to new information about biosynthetic intermediates. The next steps involves comparison of metabolite profiles after conjugation and double crossing over.

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