TITLE: Improvement of Albocycline production by ribosomal engineering in *Streptomyces* sp. Caat 7-52

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ABSTRACT

In the last years, there was an advancement in the scientific production regarding area of microbial natural products, whose objective is producing bioactive substances from microorganisms for the development of agriculture. Into the microbiological universe, there is a highlight to the genus *Streptomyces*, which shows a versatile metabolic potential, producing compounds with insecticidal, herbicidal, bactericidal, acaricidal properties, among others, that are widely used in agriculture. Recently a natural product from macrolactones group, known as Albocycline, was discovered in *Streptomyces* sp. Caat 7-52 as a promising molecule with phytotoxic action. However, their lower titers have limited its production in great scale. Therefore, there is a necessity to improve the Albocycline production in Caat 7-52 to satisfy commercial requirements. In this work, we applied the strategy of ribosome engineering to improve Albocycline production. Firstly, the minimal inhibition concentrations (MICs) were determined into PDA plates with supplementation of different concentrations (2, 5, 10, 15, 20, and 30 mg/L) of the antibiotics Erythromycin, Streptomycin, Gentamicin and Rifampicin. Mutation induction was done by placing wild Caat 7-52 spores in five different tubes containing antibiotic solution at concentrations of 0.1 to 1.0 mg mL^{-1}, and, at intervals of three days, 50 µl of each solution was collected and inoculated into plates with PDA medium. The colonies that grew were inoculated in PDA plates with antibiotic concentration up to 10-folds MIC. To compare if any mutant had a higher production of Albocycline, the extract obtained from the mutants and wild-type strain, cultured in PD medium, was analyzed by the LC-MS, comparing the metabolic profiles of both. Up to now, 264 mutants were obtained from the four antibiotics, among them, ten mutants presented a different profile compared with the wild strain and two showed an increase of Albocycline production. The mutants, namely R111 and R138, both treated with Rifampicin, obtained 4 and 5-folds higher production of Albocycline as compared to wild-type, respectively. Our efforts have demonstrated a practical strategy for titer improvement of Albocycline and to expedite the future development of agrochemicals from microbial natural products.

KEYWORDS: Ribosome engineering, *Streptomyces*, *Actinobacteria*, Caatinga, Mass Spectrometry

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