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Identification of StiR, the first regulator of secondary metabolite formation in the myxobacterium *Cystobacter fuscus* Cb f17.1

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Abstract

Myxobacteria are well established as proficient producers of natural products with numerous biological activities. Although some knowledge has been gained regarding the biosynthesis of secondary metabolites in this class of bacteria, almost nothing is known about the underlying regulatory mechanisms. In order to identify regulatory elements, we submitted the argyrin and stigmatellin producer *Cystobacter fuscus* to a random transposon mutagenesis strategy and screened 1200 mutants for the occurrence of strains showing remarkably increased or decreased production of these compounds. In addition to the identification of the stigmatellin biosynthetic gene cluster, a novel positive regulator (*stiR*) of stigmatellin production was identified after transposon recovery. In order to exclude secondary mutagenesis effects, a double cross-over mutagenesis strategy was applied to the strain resulting in the repeated generation of the transposon genotype. This strain was shown to be equally effected in natural product biosynthesis.

Keywords: Secondary metabolism; Regulation; Stigmatellin biosynthesis; Myxobacteria

1. Introduction

Myxobacteria are soil dwelling Gram-negative bacteria. They live preferentially in places that are rich in organic matter: soil, rotting plant material, the dung of various animals and on the bark of living and dead trees (Dawid, 2000; Reichenbach, 2001). Myxobacteria have two characteristic features that allow them to be distinguished readily from other bacteria; they move by gliding in swarms on solid surfaces, which make colonies spread over the culture plate, sometimes covering it completely within 6–8 days. The second characteristic of myxobacteria is unique among bacteria: under starvation conditions the cells start to aggregate within the swarm and finally form a fruiting body that contains heat and sonication resistant

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myxospores (Reichenbach, 2001). Furthermore, myxobacteria produce a large number of bioactive molecules with antifungal, antibacterial, and antitumor activities. More than 100 basic structures with nearly 500 structural variants have been identified so far, and several of these are being developed for medical applications (Reichenbach and Höfle, 1993).

Cystobacter fuscus is one of several myxobacterial species that produces a number of biologically active substances in parallel (Gerth et al., 2003; Kunze et al., 1982; Sasse et al., 2000, 2003a, 2003b). The strain *C. fuscus* Cb f17 produces stigmatellins and argyrins (Fig. 1A and B). Argyrin has antibacterial activity, while stigmatellin inhibits the electron transport in the respiratory chain of eukaryotes (Sasse et al., 2002). It shows a potent activity against the cytochorme bc_1 segment of the respiratory chain (Thierbach et al., 1984; Oettmeier et al., 1985; Matsuno-Yagi and Hatefi, 2001).

However, *C. fuscus* Cb f17 is not the only species that can produce stigmatellin. Originally, it was isolated from *Stigmatella aurantiaca* (Kunze et al., 1984) and the corresponding biosynthetic gene cluster was recently identified and characterized from this strain (Gaitatzis et al., 2002). In contrast, no sequence information related to stigmatellin or argyrin biosynthesis from *C. fuscus* is known. In fact, the authors are not aware of any molecular biological study with *Cystobacter* species.

The optimization of the production of secondary metabolites in slow growing myxobacteria using genetic means is difficult to achieve due to the poor availability, or indeed complete lack, of genetic techniques for most species. In addition, no self-replicating DNA units have been described for any myxobacterium. The alternative heterologous expression of complex biosynthetic gene clusters in other genetically related bacteria is difficult because of the high GC% content of the genome resulting in problems regarding codon usage. However, in recent efforts heterologous production has been achieved by expression of complete pathways in streptomycetes (Tang et al., 2000) and pseudomonads (Wenzel et al., 2005).

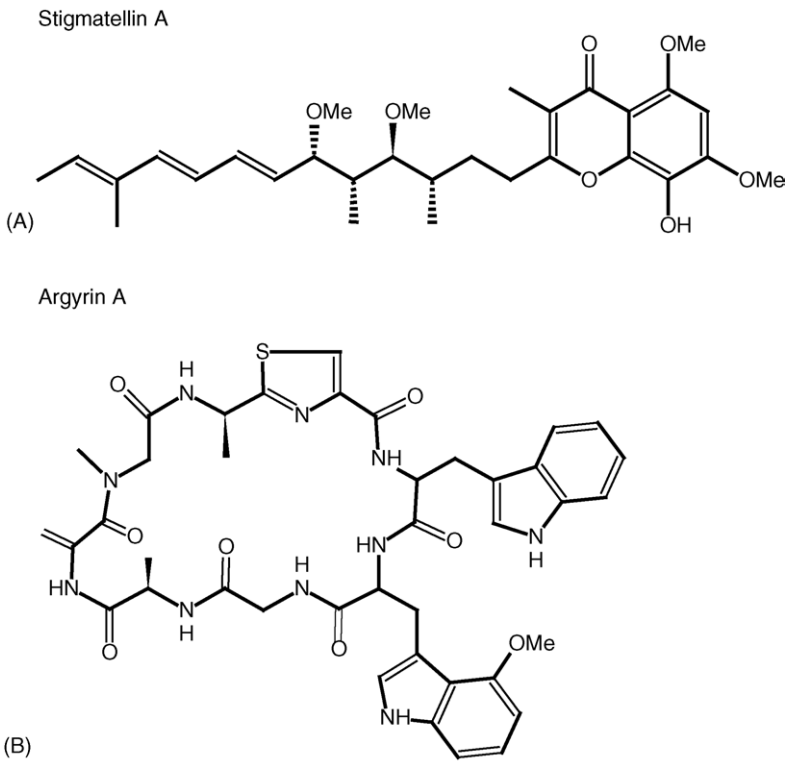

Fig. 1. The chemical structures of stigmatellin A (A) and argyrin A (B).