COMPLEX REGULATION OF AURICIN BIOSYNTHESIS IN *STREPTOMYCES AUREOFACIENS* CCM3239

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Introduction. Bacteria of the genus *Streptomyces* belong to the main producers of bioactive natural products including many antibiotics. We previously identified a gene cluster, *aur1*, in *Streptomyces aureofaciens* CCM3239, which was responsible for the production of angucycline polyketide antibiotic auricin (1). Intriguingly, auricin was produced in very narrow growth phase interval after entry into stationary phase. This specific production of auricin was due to a strict transcriptional regulation of the auricin biosynthetic gene cluster. Expression of the auricin biosynthetic genes is under control of the pathway-specific positive regulator Aur1P that belongs to the family of response regulators of bacterial two-component signal transduction systems (2). In addition, the SARP-family positive regulator Aur1PR3 has been found to be involved in auricin regulation (3). Expression of both *aur1P* and *aur1PR3* genes is negatively regulated by the TetR family Aur1R repressor (4).
In the present study we investigated an additional SARP-family regulator, Aur1PR4, involved in auricin regulation.

Methods. Growth of *S. aureofaciens* CCM3239 and analysis of auricin production by TLC and HPLC were done as described in (3). Disruption of the *aur1PR4* gene in *S. aureofaciens* CCM3239 was done by the same method as described in (3). Transcription of the *aur1PR4p* promoter was determined by S1-nuclease mapping using RNA isolated from different growth phases of *S. aureofaciens* strains grown in liquid Bennet medium, essentially as described in (4). Electrophoretic mobility shift assay (EMSA) of the *aur1PR4p* promoter was done using a purified Aur1P (2) and a ^{32}P-labelled 500 bp DNA fragment comprising the *aur1PR4p* promoter region (nucleotide positions -306 to +195 with respect to the TSP)

Results. Previously we found that disruption of the *bpsA* gene in *S. aureofaciens* CCM3239 resulted in increased auricin production. A possible explanation of this increase may be a polar effect of the resistance marker gene on the downstream *sa9* gene (5). This gene encodes a protein with similarity to SARP family of streptomycetes antibiotic regulators. Actually, disruption of the gene (renamed to *aur1PR4*) in *S. aureofaciens* CCM3239 dramatically affected auricin production. Transcriptional analysis of the *aur1PR4* gene revealed a single promoter, *aur1PR4p*, induced just before auricin production. The *aur1PR4p* promoter was not affected in the *aur1R* mutant, but it was absent in the *aur1P* mutant. The effect of *aur1P* upon *aur1PR4* expression is likely direct, as Aur1P protein bound the *aur1PR4p*.

Conclusions.
1, Disruption of the *aur1PR4* gene encoding SARP-family transcriptional regulator dramatically affected auricin production.
2, The single *aur1PR4p* promoter was absent in the *S. aureofaciens aur1P* mutant.
3, Aur1P protein directly binds the *auri1PR4p* promoter
4, The results indicate a complex regulation of auricin biosynthesis by the two-component response regulator Aur1P directing expression of primary polyketide biosynthetic genes and two SARP-family regulators, Aur1PR3 and Aur1PR4, likely directing expression of tailoring biosynthetic genes. These two SARP-encoding genes are under differential control of the auricin-specific regulators Aur1P and Aur1R.

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