HETEROLOGOUS EXPRESSION OF THE CEPHAMYCIN C GENE CLUSTER OF *STREPTOMYCES CLAVULIGERUS* ATCC27064 AND PRODUCTION OF CEPHAMYCIN C BY *STREPTOMYCES FLAVOGRISEUS* ATCC33331

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Introduction. *Streptomyces clavuligerus* ATCC27064 produces clavulanic acid, cephamycin C, holomycin and several additional secondary metabolites, being some of them of great interest in the pharmaceutical industry. The biosynthesis of these compounds is rather well known, but some points of the production control remain still unknown. Of special relevance is the possible requirement of upper hierarchy strain-specific regulators for antibiotic production.
In this work, the cephamycin C gene cluster has been integrated in two different *Streptomyces* hosts, but production is only achieved in the most phylogenetically close strain, *S. flavogriseus* ATCC33331.

Methods. The plasmid [Pc-*ccaR*], in which the *ccaR* gene is expressed for its own promoter, was integrated in *S.coelicolor* M1146, and *ccaR* transcription was analysed by RT-PCR. A cosmid carrying the cephamycin C cluster named [SCos-CF], was located and characterizated from a cosmid genome library of *S. clavuligerus*. This cosmid was integrated in *Streptomyces coelicolor* M1146 (Flinspach et al., 2010) and *Streptomyces flavogriseus* ATCC33331. Resistance of the exconjugants to different concentrations of cephalosporin C was analised (Table1). To test cephamycin C production, the strains were grown in 6 different media used previously either for cephamycin C production or for sporulation: TSB, TBO, ME, MEY, MS and ISP4. Cephamycin C production, both in solid and liquid cultures, was tested by bioassay using *E.coli* Ess 22-31 and confirmed by HPLC analysis of the broth.

Results. The *ccaR* gene is transcripted in *S.coelicolor* M1146::[Pc-*ccaR*] as detected by RT-PCR. Cosmid [SCos-CF] carries the cephamycin C cluster, from the *pbpA* to the *pbpR* genes. Both *S.coelicolor* [SCos-CF] and *S.flavogriseus* [SCos-CF], carrying the cephamycin C cluster integrated, were resistant to concentration of cephalosporin, between 6.5 – 7.5 mg/ml (Table1), while the parental strains were sensitive. Cephamycin C production was detected in *S.flavogriseus* [SCos-CF], but not in *S.coelicolor* [SCos-CF]. In the *S.flavogriseus* exconjugants, cephamycin C production was detected in several media, but the higher production occurred in TBO and MEY.

Table 1. Growth of the strains in presence of different cephalosporin concentrations

	6.5mg/ml	7.0mg/ml	7.5mg/ml
S.clavuligerus	++	++	+
*S.coelicolor*M1146	-	-	-
*S.coe*M1146 [SCosCF]	++	++	-
S.flavogriseus	-	-	-
S.flavo [SCosCF]	++	++	++

Conclusions. Cephamycin C production occurs in *S.flavogriseus* [SCos-CF]. but not in *S.coelicolor* [SCos-CF]. However, the *ccaR* regulator was transcripted in *S.coelicolor* M1146, showing that there is no strain-specific upper hierarchy regulators for its transcription. This suggests that the biochemical and genetics background in the *S. clavuligerus* phylogenetically close *Streptomyces flavogriseus* favours cephamycin C production.

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