THE CLAVULANIC ACID GENE CLUSTERS IN TWO ACTINOMYCETES: HETEROLOGOUS EXPRESSION OF *S. clavuligerus* ATCC 27064 CLAVULANIC ACID CLUSTER IN *S. flavogriseus* ATCC 33331

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Introduction. In a survey of genomes we found the complete cluster for clavulanic acid (CA) biosynthesis in the actinomycete *S. flavogriseus*. This CA cluster contain blocks of genes conserved in the same order as those of *S. clavuligerus* CA cluster but ensambled in a different organization. The *Streptomyces*-activator regulatory protein (SARP) CcaR, that activates clavulanic acid genes expression is present as a separate unit in *S. flavogriseus*. However, whereas *ccaR* in *S. clavuligerus* is located inside the cephamycin C cluster, in *S. flavogriseus* is in the middle of the clavulanic acid cluster

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Methods. Clavulanic acid production was quantified by bioassay using *Klebsiella pneumoniae* ATCC 29665 (Romero *et al*. 1984). In addition, samples derivatized with imidazole were quantified by HPLC as described by Foulstone and Reading (1982). RNA was obtained using the RNeasy kit (Quiagen) and RT-PCR was tested as indicated by Santamarta *et al.* (2011).

Results. *S. flavogriseus* ATCC3331 was grown in different complex and defined media but clavulanic acid production was undetectable by bioassays or HPLC analysis. RT-PCR analysis showed that *ccaR* was expressed, but the expression of five genes, *cas2, car, orf12, orf14* and *orf16* is very low or absent in all the media tested. It was of interest to know whether the *S. flavogriseus* CcaR protein was functional. Therefore we introduced in *S. flavogriseus* the clavulanic acid gene cluster from *S. clavuligerus* located in cosmid SCos-CA. The new strain, *S. flavogriseus SCos-CA,* produced CA in TBO and MEY media but not in other media tested (SA, TSB, MG, R5. ISP4). Confirmation of the structure of the CA produced by *S. flavogriseus SCos-CA* was obtained by LC-MS.

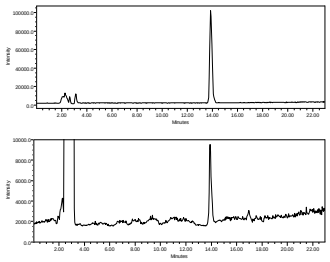

Fig.1 Detecting mass chromatograms at m/z 198. (λ = 220 nm). Up: CA standard. Down: broth of *S.flavogriseus SCos-AC* in TBO medium.

Conclusions. *S. flavogriseus* contains a silent CA cluster and does not produce clavulanic. *S. clavuligerus* CA cluster, present in cosmid SCos-CA is expressed heterologously in *S. flavogriseus* and the genetically modified strain is able to produce clavulanic acid.

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