



## Identification of *Streptomyces clavuligerus* genes involved in a resistance system against cephamycin and clavulanic acid

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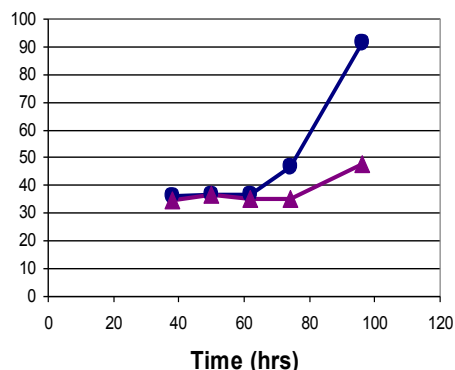
**Introduction.** It has been seen that both a microorganism's sensitivity to its own antibiotic and the ability to excrete it to the medium can reduce or even stop it<sup>(a)</sup>. For this reason, these microorganisms have developed different resistance mechanisms, such as direct modification on the antibiotic and modifications on the action sites and excretion systems by membrane integral proteins<sup>(b)</sup>. In that sense, the *cmcT*, *pbp74*, *bla* and *orf13* genes located within biosynthetic clusters of cephamycin and clavulanic acid in *Streptomyces clavuligerus*<sup>(c)</sup> could be related to these resistance systems. Therefore, our objective in the present work is to analyze both the relation between these genes and the effect of each of them on the biosynthesis of cephamycin and clavulanic acid (CA).

**Methods.** In order to analyze the effect of the genes of interest on the production of CA and cephamycin, deleted mutants were obtained through REDIRECT technology. In order to observe the relation between these genes during the biosynthesis of CA and cephamycin, studies of expression were done during trophophase and idiophase of the cultures with the different mutant and parental strains.

### Results.

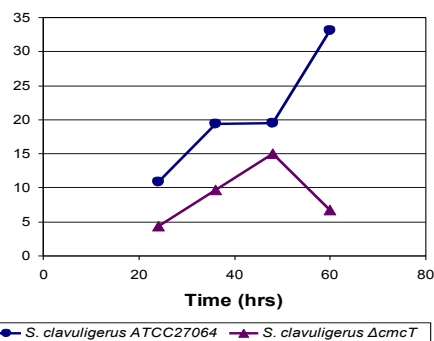
The analyses of resistance to cephalosporin and Pen G, showed that when eliminating the *cmcT* gene, the *S. clavuligerus* resistance increases. The other strains are being analyzed.

**Specific Production of Cefamycin C by *S. clavuligerus*  $\Delta cmcT::apr$  and *S. clavuligerus* ATCC27064 in TSB medium**



—●— *S. clavuligerus* ATCC27064 —▲— *S. clavuligerus*  $\Delta cmcT$

**Specific Production of Clavulanic Acid by *S. clavuligerus*  $\Delta cmcT::apr$  and *S. clavuligerus* ATCC27064 in SA medium**



—●— *S. clavuligerus* ATCC27064 —▲— *S. clavuligerus*  $\Delta cmcT$

	Cephalosporin mg/ ml			Penicillin G µg/ ml				
	5.5	7.5	10	85	95	100	150	250
<b>Wt</b>	+++	+++	-	+/-	-	-	-	-
<b><math>\Delta cmcT::apr</math></b>	+++	+++	++	+++	+++	+++	+/-	-

Studies of expression of the *cmcT*, *pbp74*, *bla* and *orf13* genes were realized in the different strains.

**Conclusions.** When the *cmcT* gene is deleted from the *Streptomyces* genome, the production of both clavulanic acid and cephamycin C reduces in relation to the wild type strain. The  $\Delta cmcT::apr$  mutant is more resistant to cephalosporin and penicillin G than *S. clavuligerus* ATCC27064. The studies of expression show that the *cmcT*, *pbp74*, *bla* and *orf13* genes are implicated in the CA and cephalosporin production. Furthermore, a relation is observed between said genes during the biosynthesis of the antibiotics.

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### References.

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