



VANCOMYCIN RESISTANCE IN *STREPTOMYCES COELICOLOR* IS DEPENDENT ON THE CULTURE MEDIA AND THE GENETIC BACKGROUND

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Key words: *Streptomyces*, vancomycin, phosphate

Introduction. Vancomycin is an essential antibiotic to treat infections caused by multiple drug resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), a major killer in hospital-acquired infections. Vancomycin-resistant MRSA and enterococci have emerged as a serious health threat. Hong and coworkers (1) reported in *Streptomyces coelicolor* the first example of vancomycin resistance in a nonpathogenic and non-glycopeptide-producing bacterium. During our studies on phosphate (Pi) regulation in *S. coelicolor*, we found that vancomycin resistance in this bacterium depended on the Pi concentration of the culture medium.

Methods. For MIC determination, 10^7 spores were spread onto the plates containing a range of different vancomycin (Sigma) concentrations, and results were evaluated after 3 days of incubation at 30°C. Vancomycin disk diffusion assays were performed using commercial antibiotic disks containing 30 µg of the antibiotic (BD BBL™; Germany) and following standard procedures. The following agar-containing media were used: TSA (Tryptone Soya Broth 3 %), MMCGT (1), MMCGT-Pi (MMCGT plus 1 % potassium phosphate), MG-3.2 and MG-18.5 (2).

Results. Pi control in *S. coelicolor*, as in other bacteria, is mainly mediated by the two-component system PhoR-PhoP (3). Analyzing disruption of PhoP-regulated genes without an assigned function, we have identified a *S. coelicolor* disrupted mutant (SCO2594::Tn5062) lacking Pi regulation of vancomycin resistance. SCO2594 forms an operon with SCO2593-92 and could be implicated in exopolysaccharide synthesis. This mutant is highly resistant to vancomycin in high Pi media like, for example, TSA, at difference of the parental strain (Fig. 1). In this medium, MIC in the mutant was higher than $200 \mu\text{g ml}^{-1}$, while in parental strain was, at least, ten times lower ($20 \mu\text{g ml}^{-1}$).

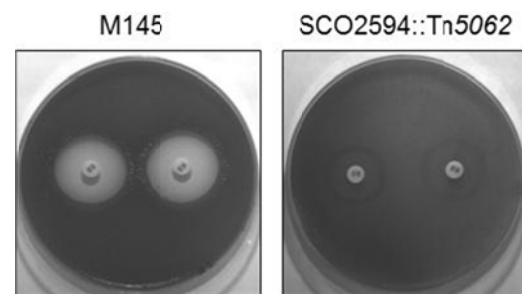


Fig.1 Vancomycin disk diffusion assays (30 µg) in TSA medium with *S. coelicolor* M145 and SCO2594::Tn5062 strains. Note the lack of inhibition halos in the mutant.

Interestingly, resistance to vancomycin in *S. coelicolor* was very influenced by the growth conditions and not only by Pi. We determined MIC values of *S. coelicolor* parental and SCO2594::Tn5062 mutant in several media and we noted that MIC values were very different depending on the medium used (Table 1).

	M145	SCO2594::Tn5062
TSA	20	>200
MMCGT	80	100
MMCGT-Pi	20	100
MG-3.2	>200	>200
MG-18.5	>200	>200

Table.1 MIC values ($\mu\text{g ml}^{-1}$) of *S. coelicolor* M145 and SCO2594::Tn5062 in low (MMCGT and MG-3.2) and high Pi media (TSA, MMCGT-Pi and MG-18.5).

Conclusions. These findings highlight the importance of the growth conditions as well as the functionality or absence of SCO2592-94 transcriptional unit in *S. coelicolor* vancomycin resistance.

Acknowledgements. This research was supported by the grant BIO2010-16094 of the Ministry of Science and Innovation (Spain).

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