PHOU AND PHOSPHATE CONTROL IN *STREPTOMYCES COELICOLOR*

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Introduction. *Streptomyces coelicolor* is a Gram-positive soil bacterium and it must be able to adapt to changes of the environment like nutrient starvation. Inorganic phosphate (Pi) is one of the main nutrients limited in soil. Pi controls the biosynthesis of many classes of secondary metabolites; most of them are regulated negatively by high Pi concentration in the media (1). Bacterial two-component systems allow microorganisms to overcome rapid environmental changes (1). As in other bacteria, the two-component PhoR–PhoP system responds to Pi limitation in *Streptomyces* (1). PhoP is directly responsible for activation or repression of the Pho regulon genes, including *phoU*, which is unknown whether takes part or not in the signal transduction cascade, as in other bacteria (1). PhoU was first defined in *Escherichia coli* and it seems to be essential for Pho regulon repression at high Pi conditions (2). In *Streptomyces*, the function of PhoU has not been determined.
Therefore, the main goal of this study is to determine the effect of PhoU in activation or repression of the *S. coelicolor* Pho system.

Methods. Vector pHZphoU (this study) carrying the kanamycin resistance cassette was used to interrupt *phoU*. Plasmid pLUX-*glpQ1* (3), carrying the *luxAB* genes from *Vibrio harveyi* fused to *S. coelicolor glpQ1* promoter, was introduced by conjugation in *S. coelicolor* M145 and *phoU* disrupted mutant strains. The respective exconjugants were cultured in MG medium with high (MG-18.5) and low (MG-3.2) Pi concentrations (4). Culture samples were analyzed at the level of growth, Pi consumption and *glpQ1* promoter activity. Transcription of *glpQ1* is totally dependent on PhoP activation (3), and therefore is used in this study as PhoP induction reporter.

Results. The growth of *phoU* disrupted mutant was not affected neither on Pi-limited (MG-3.2) nor Pi-replete (MG-18.5) conditions (data not shown). Moreover, *phoU* disrupted mutant behaved similarly to parental strain in terms of Pho regulon induction under Pi-replete conditions (Fig. 1A).
As shown in Fig 1B, *glpQ1* promoter activity in both strains was induced under Pi-limitation (*i.e.* in MG-3.2 Pi was starved before 42 hours of culture) although *glpQ1* promoter activity was significantly higher in the mutant. Actually, luciferase values were more than double at 42 and 68 h of culture.

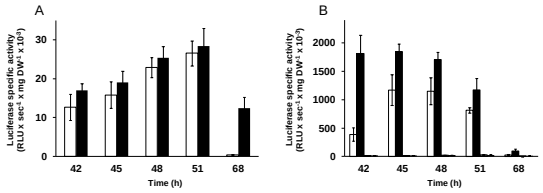

Fig.1 *glpQ1* promoter activity of *S. coelicolor* M145 (white bars) and *phoU* disrupted mutant (black bars) in MG-18.5 (A) and MG-3.2 (B). Error bars correspond to the mean of 4 cultures replicates.

Conclusions. In *S. coelicolor*, similar to *E. coli*, PhoU seems to be a negative modulator of Pho activation (2). However, contrary to *E. coli*, disruption of *phoU* does not produce a constitutive activation of the *S. coelicolor* Pho system. The strong PhoU effect under Pi-limitation conditions matches with the PhoP-dependent expression profile of *phoU* in *S. coelicolor* (1). In summary, PhoU is activated by PhoP in Pi-limited conditions and PhoU, in turn, seems to repress PhoP activation; thus preventing this system to be out of control.

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References.

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