THE GLUCOSE KINASES FROM *Streptomyces peucetius* var. *caesius*.

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Introduction. Glucose phosphorylating enzymes mainly are hexokinases or glucokinases (Gk). The Gks group is able to utilize phosphoryl donors such as ATP, inorganic phosphate (polyphosphate) or ADP. Gks which use polyphosphate (pp) have been found in some Gram-positive bacteria as *Microlunatus phosphovorus*, *Arthrobacter* sp., and the pathogenic actinobacteria *Mycobacterium tuberculosis* (1). Only in *Streptomyces aureofaciens* it was observed a pp-Gk activity (2) and recently, several peptides correspondent to a pp-Gk enzyme were found in the proteome from *S. coelicolor* cultured in glucose (3). Because it has been assigned a regulatory role to the ATP-Gk from *S. coelicolor* in the carbon catabolite repression (CCR) mechanism (4) it was of our interest to search for other Gk activities in *Streptomyces peucetius* var. *caesius* a strain which overproduces an antitumoral compound.

Methods. *S. peucetius* and *S. peucetius* var. *caesius* were grown under shaking at 180 rpm in medium NDYE (10 mM $NaNO_3$, 1 mM K_2HPO_4, 1.5 mM $MgSO_4$, 2 g/L yeast extract) supplemented with 100 mM glucose or mannose or 20 mM glutamate. After incubation, cells were harvested by centrifugation at 6000X*g*. pH and residual carbon source were determined in supernatant. Pelleted cells were sonicated, and from the extract obtained, it was estimated the protein concentration (Bradford method) and Gk activity as described by Imriskova *et al.* (5).

Results. In NDYE medium, *S. peucetius* var. *caesius* showed its logarithmic growth phase from 0 until 12 h of incubation. After that time the bacteria reaches the stationary phase and starts anthracyclines production. In the presence of glucose, Gk activity was higher compared to that obtained in mannose and glutamate. In order to verify the number of enzymes present in the cellular extracts, we carried out electrophoresis under native conditions which were revealed by activity (zymograms). In all zymograms we found an activity protein band at 124 kDa. The protein band was analyzed by spectrometry mass and we detected two possible Gk proteins, one ATP-dependent (previously reported by Imriskova *et al.* 2001) and other pp-dependent, similar to the reported enzyme from *Streptomyces avermitilis*. In order to confirm the presence of both enzymes in *S. peucetius* var. *caesius* we carried out a Glk time course experiment. In this way, the existence of both activities was corroborated. The ATP-dependent Gk appeared during the logarithmic growth phase and the pp-dependent Gk showed maximum expression during the middle of the stationary phase. Both Gks where present when the culture medium had glucose as sole carbon source, but not with glutamate. As a control we decided to use the parental strain *S. peucetius* to carry out a time course and to compare the results to that obtained with *S. peucetius* var. *caesius.* This strain is a doxorubicin producer bacteria obtained by chemical treatment of the wild type *S. peucetius* strain. The wild type strain showed a growth curve similar to that of the doxorubicin producer however, the pp-Glk production of the wild type bacteria was significantly lower than the production observed in the overproducing mutant (Fig.1). The differences detected between both strains could be an effect of the antitumoral overproduction which requires larger metabolic flux from glucose which could be provided by an extra Gk.

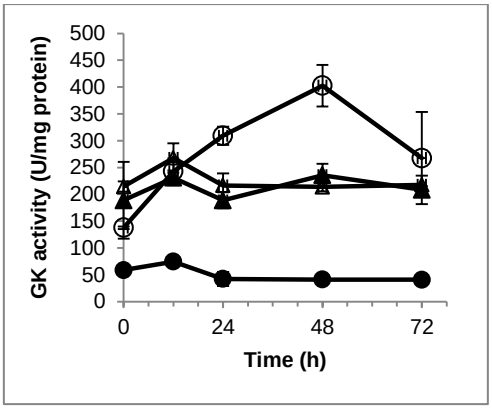

Fig 1. Time course of ATP-Gk (▲, Δ) or pp-Gk (○,●) production in *S. peucetius* (closed symbols) and *S. peucetius* var. *caesius* (open symbols).

Conclusions. *S. peucetius* and *S. peucetius* var. *caesius* have two Gk activities, in contrast to the model *S. coelicolor* which has only one activity. This fact points to a different CCR mechanism which should take into account the metabolic sum of these two activities.

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