CHARACTERIZATION OF TRANSITION STATE REGULATOR AbrB IN BATCH CULTURES OF *Bacillus thuringiensis* USING POLYCLONAL ANTIBODY ANTI-AbrB

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Introduction. AbrB is one of the most important regulators of the transition state. It is involved in the regulation of various cellular functions, also has the ability to activate, suppress or prevent the inappropriate expression of various genes(1). From a Bioprocess point of view, AbrB might be influence the synthesis of many industrial products synthesized by the *Bacillus* genera. However, most of studies on AbrB are made in flask cultures. The characterization AbrB in *Bacillus thuringiensis* (*Bt*) has not been reported. The aim of this study was characterized the transition state AbrB in batch cultures of *Bt*. We used a recombinant AbrB expressed in *E. coli* as antigen for the production of polyclonal antibodies anti-AbrB obtained from rabbits.

Methods. *abrB* gene of *Bt kurstaki* HD73 was cloned with vector pJET and AbrB protein was expressed in the vector pQE30Xa in *E. coli*. The polyclonal antibody was purified by affinity column with protein A *S. aureus* and tested for reactivity of the immunoglobulin (IgG's) obtained (dilution of 1:200 to 10). Serial dilutions of the recombinant protein AbrB (0.01 10 µg/ml) was tested. *Bt* pHT1kAc was grown in clean medium (CL) based on glucose and soy peptone and MHS medium based on glucose and soybean meal. Medium. Batch cultures were performed in 7-l reactor with 4-l of medium culture at 600 rpm, 30°C, 1 vvm. Cellular counts, spores and *cry1Ac* expression were quantified. AbrB protein synthesis was followed by ELISA and Western blot using the recombinant AbrB as positive control.

Results. The purified AbrB and In order to verify the immunoglobulin isotype once purified, was analyzed (Fig. 1).

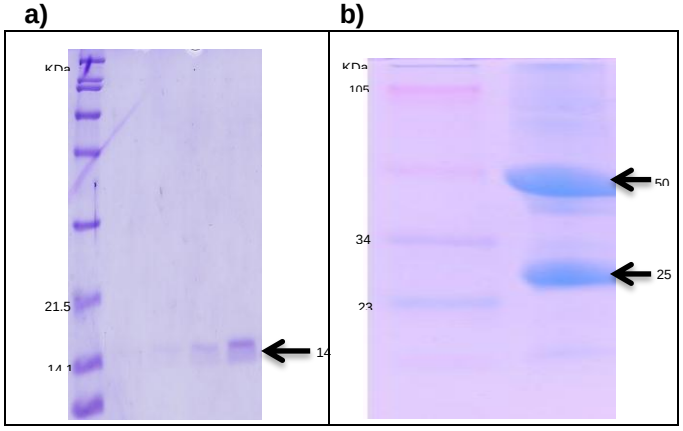

Fig.1 Displays denaturing polyacrylamide electrophoresis (15%), a)Purified protein AbrB, b)Purified immunoglobulins.

According to the presence of bands with molecular weights of 25 and 50 kDa, we verified the isotype γ (gamma) of the obtained IgG's.

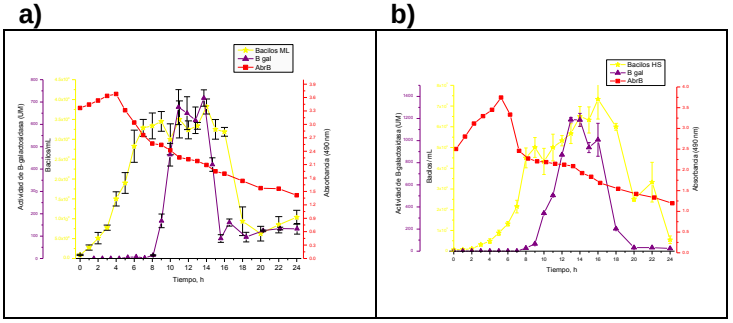

Figure 2. Kinetics of growth, *cry1Ac* expression and AbrB synthesis in bacth cultures of *Bt* pHT1kaAc. AbrB synthesis was evaluated by ELISA. The purified antibody was used at a concentration of 25 µg/ml. a) CL, b) MHS.

AbrB synthesis in batch cultures were detected from the beginning of fermentation became maximal at 4h and 5 h for ML and MHS respectively. The changes in expression profiles may be attributable to the medium composition and availability of nutrients. Maximum AbrB synthesis coincided with the middle of the exponential growth (2). Other reports mentioned that AbrB expression became maximal from the late exponential phase until de end of transition state (3).

Conclusions. The AbrB protein expression occurred from the beginning and middle of exponential growth. Hence, AbrB is a regulator of the transition phase from latency to exponential phase, but not a transition regulator from the exponential phase to stationary phase as reported. Now, we are conducted the homologous recombination of *Bt* to evaluate the effect of AbrB overexpression on metabolism, *cry* expression and sporulation.

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