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Redirection of metabolism during nutrient feeding in fed-batch cultures of *Bacillus thuringiensis*

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Abstract During sporulation, *Bacillus thuringiensis* produces insecticidal crystal inclusions (Cry proteins) encoded by *cry* genes. In fed-batch cultures (FBCs), spores and Cry protein yields are usually low, so we therefore studied the pattern of metabolic changes occurring in batch cultures and FBCs of a *B. thuringiensis* strain having a *cry1Aa* promoter-*lacZ* fusion, and their effect on sporulation and *cry1A* gene expression. In FBCs, there was a redirection of bacterial metabolism and a reduction in the specific growth rate during feeding, even when the nutrient concentration was higher than at the beginning of batch culture. These physiological changes suggest that the transition state is set up during feeding and this set-up seems to have a negative effect on both sporulation and *cry1Aa* expression. When the filtrate of a culture in the transition state was added to a batch culture early in the first exponential growth phase, it delayed sporulation and *cry1Aa* expression, thus suggesting that a soluble cellular factor that blocked sporulation might be excreted during the transition state. Citrate production usually started during the transition state but, when a medium rich in free amino acids was fed, citrate was produced from the first growth phase and sporulation was nearly blocked.

Introduction

Bacillus thuringiensis is a spore-forming bacterial host for a variety of Cry proteins, which are produced during sporulation. To form a dormant spore, the bacteria undergo a cellular differentiation process that includes a transition state. *B. thuringiensis* is closely related to *B. subtilis*, in which complex regulatory circuits govern the changes in gene expression that occur upon entry into the stationary phase. During the previous phase, the transition state, the utilization of nutrients is maximized and the cell expresses functions that allow it to scavenge any alternative nutrients. Therefore, the transition state is a crossroad in the life cycle of the cell, when information is gathered and processed and when functions are expressed that will allow entry into whichever metabolic state or developmental path is ultimately chosen, but before any final commitment is made (Phillips and Strauch 2002).

Fed-batch culture (FBC) of *B. thuringiensis* is an alternative to increase the production of spores and Cry proteins although, while high cell densities are reached, yields of spores and Cry proteins are low (Arcas et al. 1987; Kang et al. 1992; Liu et al. 1994; Avignone-Rossa and Mignone 1993). Speculation to explain this phenomenon calls for a requirement of some internal energy source (Kang et al. 1992; Liu et al. 1994). However, in most cases, only the time-courses of cell growth and residual glucose and the production of heat-resistant spores or free spores, were followed, but no attention was given to metabolic changes and the cellular differentiation process.

In this report, we describe a study of the pattern of metabolic changes occurring in batch cultures (BCs) and FBCs of *B. thuringiensis* and their effect on sporulation efficiency and *cry1Aa* expression. Our results indicate that there is a redirection of cellular metabolism concomitant with a decrease in the specific cell growth rate during feeding, even when nutrients are plentiful. These findings suggest that the transition state is set up during feeding and has a negative effect on sporulation and *cry1Aa* expression.

Materials and methods

Bacterial strains

The *B. thuringiensis* strain BtpHTcry1A2, containing a *cry1Aa-lacZ* fusion, used here was derived from strain Cry (−)B (Stahly et al. 1978) by transformation with the plasmid

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