Trehalase: Induction in *Bacillus cereus*[1,2]

The disaccharide trehalose is a storage sugar in resting stages[3], and when hydrolyzed by trehalase can serve as an energy source for germination in a number of microbial systems[4-6]. Its utilization has been recorded in *Bacillus anthracis*, *B. polymyxa*, *B. mycoides*, and *B. macerans*[7], and various Gram negative species can ferment it[8]. We observed that *B. cereus* can also ferment trehalose, as shown by acid production in a defined medium with trehalose as the sole carbon source. This utilization was prevented in our system by 2% glucosamine hydrochloride (w/v), an inhibitor of trehalase activity[9]. These observations have led us to experiments which directly demonstrate the existence of an inducible trehalase in *B. cereus*.

Experimental procedure. *Bacillus cereus* was grown at 30 °C in a nutrient broth with or without trehalose. Whole cells were not used for enzyme determinations because our assay for trehalase depends upon the production of glucose from the trehalose substrate, and this glucose was utilized by intact organisms. Therefore, cells were collected by centrifugation, washed twice with distilled water, and disrupted by a modification of the method of LAMANNA and MALLETTE[10]. To each 1 ml of the washed pellet we added 1 ml of water and 3 g of 0.2 mm glass homogenizing beads which had been washed to neutrality. The mixture was ground for 3 min at full speed in a micro Sorvall blender fitted with a paraffin plug to prevent foaming. The beads and blender were washed several times with water, the washings being pooled and centrifuged at $12{,}000 \times g$ for 30 min to remove cell debris and intact cells. The decanted supernatant was saturated with ammonium sulfate. Material for assay was obtained by centrifuging this latter solution at $44{,}000 \times g$ for 20 min, discarding the supernatant and resuspending the pellet in water. Protein was determined by the method of LOWRY et al.[11]. The trehalase assay was as described by GUSSIN and WYATT[9], except that the pre-incubation of cell extract and substrate was carried out for 120 min. Enzyme activity was a linear function of time and enzyme concentration.

Results and discussion. Trehalase activity could be demonstrated only in medium supplemented with trehalose, indicating the inducible nature of this enzyme in *B. cereus*. When trehalose, at a final concentration of 1%, was added to 6 h broth cultures, low levels of trehalase (Specific activity = 2.2 nmoles glucose/min per mg protein) appeared within 30 min and then gradually decreased over a period of 6.5 h to a specific activity of 0.99. The induction was then repeated under different conditions. Replicate 250 ml flasks containing 50 ml of nutrient broth were inoculated with 0.5 ml of a 12 h broth culture of *B. cereus*. After 6 h of incubation cells were collected by aseptic filtration and transferred to fresh broth containing 2% trehalose (w/v). In this instance there was a dramatic increase in both the amount of enzyme produced and the period of time over which it was produced (Table). Control cells transferred to fresh broth without inducer showed no trehalase activity. The decline in activity in cells induced with 1% trehalose was probably due to depletion of the inducer and/or some essential factor from the medium in which cells had grown for 6 h.

In order to determine if induction required the synthesis of protein, cells were grown in nutrient broth and inducer or in broth, inducer and 200 µg/ml puromycin[12]. In the former experiment induction occurred as usual (S.A. = 20.592), while in the latter induction was inhibited by puromycin (S.A. = 0.326). There was no significant cell death due to exposure to the antibiotic; the viable counts in incubations without puromycin were 1.36×10^8 cells/ml and in incubations with the inhibitor were 1.25×10^8 cells/ml. Thus, the presence of trehalase in induced cells required the synthesis of new protein.

Conclusion. In *Bacillus cereus* the disaccharide trehalose can induce the formation of its hydrolytic enzyme trehalase. The induction can be inhibited by puromycin, showing that the synthesis of new protein is required.

Induction of trehalase activity in *Bacillus cereus*

Time (h)	Specific activity (nmoles glucose/min per mg protein) Without trehalose	With 2% trehalose
6	0.016	–
6.5	–	2.532
7	–	17.724
8	–	23.724
9	–	12.495
10	2.414	17.159
11	0.183	–

Zusammenfassung. Es wird gezeigt, dass *Bacillus cereus* die Disaccharid Trehalose fermentieren kann. Die für diese Fermentwirkung benötigte Trehalase wurde durch das Substrat ermöglicht, während Puromycin den Vorgang verhinderte. Dies beweist die Notwendigkeit einer neuen Proteinsynthese.

LINDA H. CRABBE[13], A. E. S. GUSSIN[14] and ELIZABETH A. TYRRELL

Department of the Biological Sciences, Smith College, Northampton (Massachusetts 01060, USA), 10 March 1969.

[1] Publication No. 1 from the Department of the Biological Sciences of Smith College.

[2] This work was supported in part by National Science Foundation Grants to A.E.S.G. and by an Alfred P. Sloan Foundation Summer Participant Grant to L.H.C. We thank Miss JUDITH G. ARNOLD for technical assistance.

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[13] Present address: Department of Bacteriology, University of California, Berkeley (California 94720, USA).

[14] Address reprint requests to this author at Smith College in Northampton.