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Trehalose Metabolism in Germinating Spores of *Streptomyces hygroscopicus*

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Evidence is presented which indicates that utilization of trehalose is an early event in spore germination in *Streptomyces hygroscopicus*. Early in spore germination and before any increase in cell mass, the activity of trehalase increased more than 15-fold, while the intracellular content of trehalose fell to very low levels. On the other hand, increased activity of the trehalose phosphate synthetase or disappearance of glycogen did not occur until later in germination, during which time cell mass was also increasing.

The activity of the enzyme trehalase (EC 3.2.1.28) increases dramatically during germination of spores of *Neurospora* (7) and of *Dictyostelium discoideum* (1). Along with this increase in trehalase activity, there was a rapid decline in the levels of trehalose, suggesting that this sugar may supply the energy necessary for spore germination. Since trehalose is also stored in streptomycetes (2), it was of interest to examine the role of this sugar during germination of spores of *Streptomyces hygroscopicus*. Therefore, the activity of various enzymes involved in the synthesis and degradation of trehalose, as well as the levels of intermediates, were determined during the initial stages of spore germination.

For sporulation, *S. hygroscopicus* was grown in the medium of Hickey and Tresner (6). Spores were removed aseptically and stored at −20 C until used. Germination was in nutrient broth and was followed by examination with a microscope and by changes in wet weight. During the first 20 h of germination, the spores did not show any significant microscopic changes, and no outgrowth was observed. Spores were harvested at various stages of germination and were disrupted by sonic treatment. Cell debris was removed by centrifugation, and the supernatant liquid was used to assay for various enzymatic activities. No significant enzymatic activity for the enzymes was found in the pellet from this centrifugation. Trehalose phosphate synthetase (3), trehalose phosphate phosphatase (8), and trehalase (5) were assayed as described previously. Samples of spores were also extracted in boiling ethanol glucose. These compounds were separated and determined as described previously (4). Glycogen was extracted with 30% KOH and determined colorimetrically (4).

Figure 1 shows the changes in the activities of several enzymes involved in trehalose metabo-

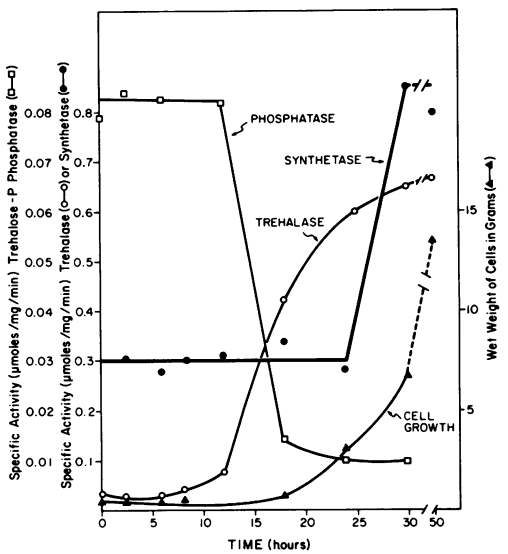

FIG. 1. *Levels of trehalose phosphate synthetase* (●), *phosphatase* (□), *and trehalase* (○) *activities during the germination of spores of S. hygroscopicus* (▲). *Results are expressed as specific activities (micromoles of product per milligram of protein per minute) of the enzymes at various times during germination. At the times indicated, a sample of the cells was removed and isolated by centrifugation, and the enzymatic activities were measured as described previously (3, 5, 7).*

lism during the early stages of germination. Trehalase activity began to increase at 12 h, well before any change in cell mass was seen, and by 20 h when the first increases in mass were observed trehalase activity had increased more than 15-fold. On the other hand, the trehalose phosphate synthetase did not show any change in activity during the first 20 to 25 h but then increased along with cell mass. The phosphatase was highest in spores during the first 12 h but then decreased to very low levels as germination ensued.

The levels of various intermediates was also examined during the early stages of germination (Fig. 2). Trehalose was fairly high in ungerminated spores but rapidly decreased as germination proceeded until by 25 h it had reached very low levels. A small amount of trehalose phosphate was found in spores, and this appeared to be converted to trehalose in the first few hours. No free glucose was detected at any time, indicating that trehalose was rapidly metabolized once it was cleaved by the trehalase.

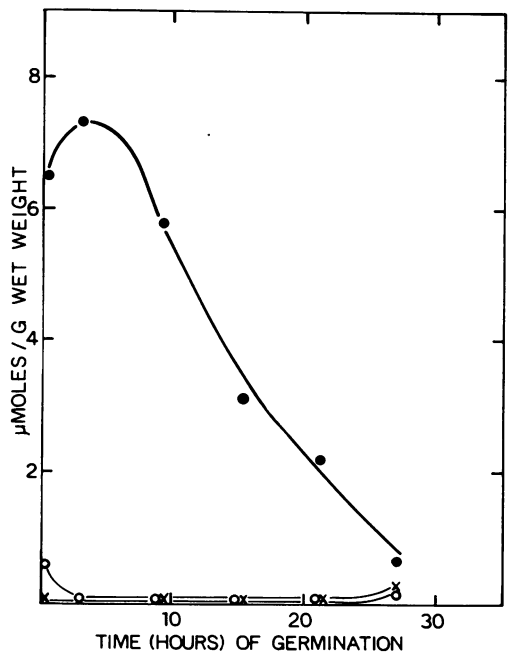

FIG. 2. *Levels of trehalose phosphate* (○), *trehalose* (●), *and glucose* (×) *in germinating S. hygroscopicus spores. Samples of cells were removed at the indicated times and extracted as described previously* (*4*).

The levels of glycogen were also examined in germinating spores. The amount of glycogen remained fairly constant at about 40 to 45 mg/g of dry cells during the first 13 h of germination, and then began to fall rather slowly. Thus, at 18 h the level had fallen to 34 mg/g and at 24 h to about 20 mg/g. These data suggest that catabolism of glycogen occurs somewhat later in germination than catabolism of trehalose, and support the notion that utilization of trehalose is important for early events in spore germination.

These data also suggest that the degradation and metabolism of trehalose is an early event in spore germination and that trehalose may supply the energy necessary for the initial stages of germination. In this regard, spore germination in streptomycetes appears to resemble that found in fungi.

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