JOURNAL OF BACTERIOLOGY, Nov. 1987, p. 5002–5007
0021-9193/87/115002-06$02.00/0

Vol. 169, No. 11

Metabolism of Endogenous Trehalose by *Streptomyces griseus* Spores and by Spores or Cells of Other Actinomycetes

MARK J. McBRIDE† AND J. C. ENSIGN*

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received 1 May 1987/Accepted 13 August 1987

The disaccharide trehalose is accumulated as a storage product by spores of *Streptomyces griseus*. Nongerminating spores used their trehalose reserves slowly when incubated in buffer for several months. In contrast, spores rapidly depleted their trehalose pools during the first hours of germination. Extracts of dormant spores contained a high specific activity of the enzyme trehalase. The level of trehalase remained relatively constant during germination or incubation in buffer. Nongerminating spores of *Streptomyces viridochromogenes*, *Streptomyces antibioticus*, and *Micromonospora echinospora* and nongrowing spherical cells of *Arthrobacter crystallopoietes* and *Nocardia corallina* also maintained large amounts of trehalose and active trehalase. These trehalose reserves were depleted during spore germination or outgrowth of spherical *Arthrobacter* and *Nocardia* cells into rods.

Large amounts of the disaccharide trehalose are present in the dormant spores or cysts of a variety of organisms including actinomycetes, fungi, nematodes, and brine shrimp (1, 3, 5, 6, 18). Trehalose is degraded very slowly by these organisms during periods of dormancy but is rapidly metabolized following the onset of vegetative growth (2, 11, 20, 22).

We previously reported (15, 18) the accumulation of large amounts of trehalose by spores of *Streptomyces griseus* and the effects of trehalose content on spore properties. In this report we describe the results of an investigation of the metabolism of the endogenous reserves of trehalose by dormant and germinating spores of *S. griseus*. Results of studies of the metabolism of trehalose by some other actinomycetes are also described.

MATERIALS AND METHODS

Growth conditions. *S. griseus* NRRL B-2682 spores were harvested from DMC agar as described previously (18). *Streptomyces viridochromogenes* NRRL B-1511 and *Streptomyces antibioticus* ATCC 11891 spores were harvested from DMC in the same manner. *Micromonospora echinospora* ATCC 15836 was grown in a liquid medium that was identical to DMC, except that it contained 0.1% casein hydrolysate (Sigma Chemical Co., St. Louis, Mo.) and 0.5% yeast extract (Difco Laboratories, Detroit, Mich.). Spores were harvested after incubation on a rotary shaker for 9 days at 33°C. Phase bright spherical-stage cells of *Arthrobacter crystallopoietes* ATCC 15481 and *Nocardia corallina* ATCC 4273 were obtained after 3 days of incubation with shaking at 30°C in a liquid medium composed of DM-1 (18) containing 50 mM glucose, to which was added 15 mM sodium succinate and 1 μg of thiamine per ml (final concentrations).

Mycelia of *S. griseus* were obtained from liquid DMC containing 0.15 M KCl as described previously (18).

Germination conditions for *S. griseus* spores. The complex germination medium contained 0.5% yeast extract and 0.5% casein hydrolysate in 10 mM 3-morpholinepropanesulfonic acid (MOPS) buffer (pH 7.0). The defined germination medium consisted of 10 mM potassium phosphate buffer (pH 7.0)–5 mM $(NH_4)_2SO_4$–0.5 mM $MgSO_4$–7.6 mM L-isoleucine–7.6 mM L-leucine–8.5 mM L-valine–4.9 mM L-tryptophan–0.75 mM adenosine–14 mM glucose. In some experiments glucose was replaced by 27 mM glycerol. Spores were heat activated (15 min at 45°C) in 10 mM glycine buffer (pH 9.5), rapidly cooled to 0°C, and washed 3 times with 10 mM potassium phosphate buffer (pH 7.0) by centrifugation. Activated spores were inoculated into the germination media at final concentrations of approximately 2×10^8 spores per ml. The spores were incubated in 100 ml of medium in 1-liter flasks at 33°C with shaking at 185 rpm.

Germination was monitored microscopically and was quantitated by taking advantage of the relative resistance of dormant spores to dilute acid. The number of acid-resistant CFUs was used to measure the number of dormant spores during sporulation or germination. Samples (0.2 ml) were added to 0.8 ml of 0.125 N HCl, dispersed by gentle sonication in a sonic water bath (Heat Systems Ultrasonics Inc.) for 1 min, and incubated for an additional 4 min at 25°C. The samples were neutralized by 20-fold dilution in 50 mM potassium phosphate buffer (pH 7.1) containing 0.01 g of the nonionic detergent Triton X-100 per liter. The spores were dispersed by gentle sonication in a sonic water bath for 2 min, diluted in distilled water, and plated on the complex medium described previously (18). Colonies were counted after incubation for 3 and 5 days at 30°C. Greater than 90% of the spores of *S. griseus* survived this acid treatment, while greater than 99% of mycelial fragments were killed.

Germination of the spores of other actinomycetes and growth of the spherical-stage cells of *A. crystallopoietes* and *N. corallina* into rod-stage cells were conducted in the complex germination medium, as described above, except that heat activation was omitted and the *A. crystallopoietes* and *N. corallina* cells were incubated at 30°C instead of 33°C.

* Corresponding author.
† Present address: Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.

Incubation of spores and spherical cells in buffer or water. *Streptomyces* spores (final concentration, 3×10^8/ml) were incubated in 10 mM potassium phosphate buffer (pH 7.0) at 33°C with shaking at 185 rpm. *M. echinospora* spores were incubated in distilled water since the spores germinated in phosphate buffer. Spherical-stage cells of *A. crystallopoietes* and *N. corallina* were incubated in phosphate buffer at 30°C.

Analytical methods. Samples of spores and cells were rapidly cooled to 0 to 4°C, centrifuged at 10,000 × *g* for 10 min, and washed 3 times with cold distilled water. In some experiments cells were collected by membrane filtration (pore diameter, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.) and washed 2 times with 10 ml of cold distilled water. The results obtained from cells collected in this way were identical to those obtained from cells harvested by centrifugation.

Trehalose and protein were extracted from cells and measured as described previously (18). Trehalase activity was measured in spore and mycelial extracts. Mycelia in 10 mM potassium phosphate buffer (pH 7.0) were disrupted by sonication for 2 min in a sonifier (at power setting 7; model 350; Branson Sonic Power Co., Danbury, Conn.) while being chilled in an ice bath. Spores were broken by vortexing 0.6 ml of a spore suspension in phosphate buffer with 1.0 g of 0.1-mm-diameter glass beads for 12 5-s pulses with intermittent cooling. Spore and mycelial walls were removed by centrifugation for 5 min in a Microfuge (Beckman Instruments, Inc., Fullerton, Calif.).

Trehalase activity in cell extracts was measured in 40 mM MOPS–50 mM KCl buffer (pH 7.0). Trehalose was added to a final concentration of 100 mM to start the reaction. After incubation at 33°C for 15 min, the reaction was stopped by immersing the tubes in a boiling water bath for 3 min. The amount of glucose formed by hydrolysis of trehalose was determined by the glucose oxidase-peroxidase procedure (Sigma). One unit of activity was defined as the amount of enzyme that hydrolyzed 1.0 nmol of trehalose min^{-1} at 33°C.

Spores containing radiolabeled trehalose were obtained by incubating spores containing approximately 50 μg of trehalose per mg of protein (harvested from DMC–5 mM glucose medium) in 10 mM potassium phosphate buffer (pH 7.0) containing 50 mM [U-^{14}C]glucose (36 μCi/mmol) for 8 h at 33°C, as described previously (18). These spores were germinated in the complex germination medium. The CO_2 released was trapped in Carbosorb (United Technologies-Packard Instrument Co., Inc., Rockville, Md.), and the amount of radioactivity was measured in scintillation cocktail (Aquasol; New England Nuclear Corp., Boston, Mass.) as described previously (21). The amount of excreted spore carbon was determined by filtering samples of the germinating spore suspension through membrane filters (pore diameter, 0.2 μm) at various times throughout germination. Samples of the filtrates were mixed with Aquasol, and the amount of radioactivity was determined. The distribution of the radiolabel in spores was determined by extraction with boiling distilled water for 30 min. The suspension was centrifuged, and the cell pellet was reextracted with boiling water. The supernatant fluids were pooled and membrane filtered, and the radioactivity was determined as described above. The amount of radiolabeled trehalose in spore extracts or germination fluid was determined after paper chromatography as described previously (18).

The rate of utilization of exogenous glucose by spores was determined by radiorespirometry as described previously (21). Spores harvested from solid DMC and spores harvested from DMC–5 mM glucose medium which accumulated trehalose during 8 h of incubation with 50 mM unlabeled glucose were incubated at 33°C with 0.5 mM [U-^{14}C]glucose (5.0 mCi/mmol) in 10 mM MOPS buffer (pH 7.0), and the amount of radioactivity in CO_2 was determined.

RESULTS

Trehalose content and trehalase activity during the growth cycle of *S. griseus*. The trehalose and trehalase contents of the cells were measured during spore germination, growth, and sporulation of the culture. For this experiment the spores were germinated synchronously by heat shock, followed by incubation for 6 h in the defined germination medium. The germinated spores were washed and inoculated into DMC, in which mycelial growth and sporulation occurred. The trehalose content of the cells decreased rapidly during the first 15 h of germination and growth (Fig. 1). The specific activity of trehalase decreased during this time, from 63 to 16 units per mg of protein, and remained essentially constant thereafter. The growth rate began to decrease and the trehalose content of the cells began to increase slowly at 20 to 24 h. Spores were first microscopically visible at approximately 26 h. At this time the acid resistance counts began to increase. Microscopic examination of the cultures between 100 and 170 h revealed masses of branched mycelia and chains of spores. After 170 h of incubation, the trehalose content and trehalase activity were determined in both the mycelia-spore mixture and in a partially purified spore preparation obtained by sonic disintegration of mycelia followed by differential centrifugation. The specific contents (amounts per milligram of protein) of trehalose and trehalase, respectively, increased from 150 μg and 29 units in the spore-mycelium mixture to 235 μg and 46 units in the purified spore fraction.

Trehalose metabolism in nongerminating spores. Spores utilized their trehalose reserves slowly when incubated aerobically at 33°C in phosphate buffer (Fig. 2). Trehalose was metabolized at an essentially linear rate. After incubation for 75 days 28% of the initial content of trehalose

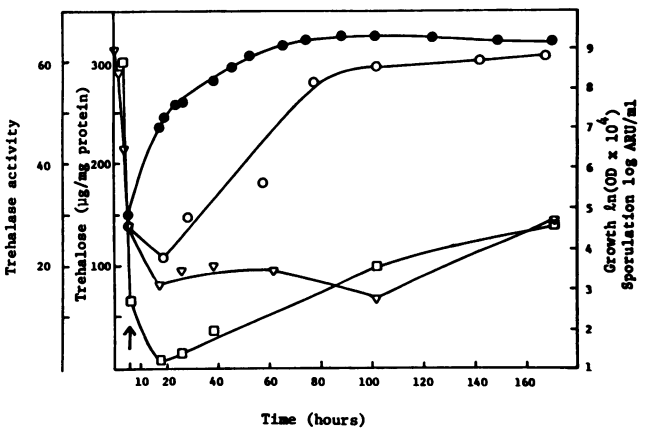

FIG. 1. Trehalose content and trehalase activity during growth of *S. griseus*. Spores were germinated for 6 h in the defined germination medium, washed 3 times with distilled water, and inoculated into DMC at a concentration of 5×10^6 spores per ml. The arrow at 6 h denotes the time of inoculation of the germinated spores into DMC. Symbols: ●, growth (ln [$OD_{600} \times 10^4$], where OD_{600} is the optical density at 600 nm); ○, sporulation (log acid-resistant units/ml); □, trehalose (micrograms per milligram of protein); spores contained 472 μg of trehalose per mg of protein at 0 h; ▽, units trehalase activity (1 unit is equal to 1.0 nmol of trehalose hydrolyzed per mg of protein per min).

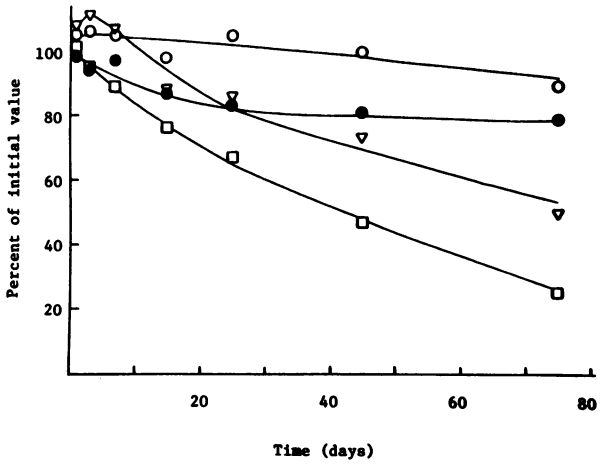

FIG. 2. Trehalose metabolism in nongerminating *S. griseus* spores. Spores were incubated in 10 mM phosphate buffer (pH 7.0) at 33°C. The data are expressed as a percentage of the initial values per milliliter. Symbols: □, trehalose (initial value, 467 μg/mg of protein); ▽, total trehalase activity (initial value, 58 nmol of trehalose hydrolyzed per mg of protein per min); ○, total protein; ●, viability.

remained. Trehalase activity also declined steadily, but 50% of the activity remained at 75 days. The protein content of the spores remained fairly constant throughout the incubation period. Spore viability decreased slowly during the first 20 days, but 80% of the spores survived incubation for 75 days. Microscopic examination at various times showed that fewer than 1% of the spores had germ tubes. It appears, therefore, that most of the trehalose was utilized to support the endogenous metabolism of the spores. Nearly identical results were obtained when spores were incubated in 10 mM MOPS buffer (pH 7.0) or distilled water (data not shown). Spores incubated in DM-1 growth medium lacking only the carbon and energy source (DM-salts) also utilized their trehalose reserves slowly, retaining 48% of their trehalose and 79% of their trehalase activity after incubation for 25 days. If the amount of trehalase activity found in spore extracts (58 units per mg of protein) was fully active within the dormant spores, the trehalose pools would be nearly completely hydrolyzed during incubation at 33°C for 30 min. Some mechanism of control of trehalase activity must be operative in dormant spores.

Trehalose metabolism during spore germination. *S. griseus* spores germinated rapidly and synchronously when incubated in the complex germination medium. This is illustrated in Fig. 3 by the decrease in acid-resistant counts and by germ tube formation. The trehalose content of germinating spores decreased rapidly. Only 12% of the initial content of trehalose remained in spores after 3 h of germination. The addition of 15 mM glucose or trehalose to the germination medium had no effect on the rate of utilization of endogenous trehalose (data not shown). Total trehalase activity remained fairly constant throughout germination. The pattern of trehalose mobilization and trehalase activity was essentially the same during germination in the defined germination medium (data not shown).

The spores of both *S. viridochromogenes* and *M. echinospora* release a large fraction of their total spore carbon into the medium during germination (7). Depletion of the trehalose pools of *S. griseus* spores during germination might thus involve leakage of trehalose into the surrounding medium. To test this hypothesis, spores were rapidly cooled to 0°C at various times during germination in the complex germination medium and incubated at this temperature for 1 h. There was no significant decrease in the trehalose content of the spores during the period of arrested germination. This suggests that passive leakage through the cell membrane is not involved in the loss of trehalose from germinating spores. Furthermore, trehalose was not detected in the culture fluid after germination of spores for 4 h in the defined germination medium containing glycerol as carbon and energy sources.

The fate of trehalose during germination was determined by using spores with radiolabeled trehalose pools. Approximately 84% of the accumulated radiolabel was accounted for as trehalose, while 7% was in the hot water-insoluble (macromolecule) fraction of the spores. During germination, 29% of the radioactivity was recovered as $^{14}CO_2$ (Fig. 4A), and 33% of the radioactivity was recovered in the culture fluid (Fig. 4B). Results of paper chromatographic analysis showed that less than 10% of the radioactive material released into the germination fluid was trehalose. Approximately 15% of the excreted material migrated with glucose

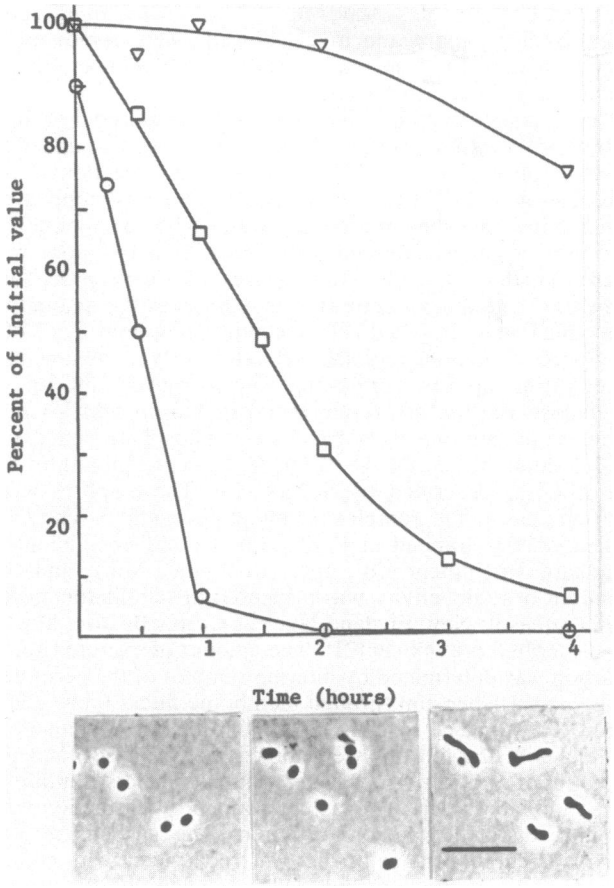

FIG. 3. Trehalose metabolism in germinating *S. griseus* spores. Spores were germinated in the complex germination medium. Data are expressed as a percentage of the initial values per milliliter. Symbols: □, trehalose (initial value, 524 μg/mg of protein); ▽, trehalase (initial value, 60 nmol of trehalose hydrolyzed per mg of protein per min); ○, germination index (acid-resistant units per milliliter). The phase-contrast photomicrographs at the bottom of the figure illustrate the progress of germination at 0, 2, and 4 h, from left to right, respectively. Bar, 10 μm.

on the paper chromatograms. Germinated spores retained 38% of the initial radioactivity of dormant spores. The distribution of this radiolabel within germinated spores was quite different than that in dormant spores. Approximately 78% of the radiolabel in germinated spores was in the hot water-insoluble fraction. The spores apparently utilized their endogenous trehalose as an energy source and for the synthesis of cell components during germination.

It is possible that trehalose was released from the germinating spores and hydrolyzed by exocellular trehalase. The glucose produced might then have been metabolized by the spores. This idea was tested by incubating the radioactively labeled spores in the complex germination medium containing 10 mM unlabeled trehalose or 100 mM Tris hydrochloride buffer (pH 7.0). Tris is a competitive inhibitor of trehalase (10, 19). We verified this for *S. griseus* trehalase (data not shown). The presence of unlabeled trehalose in the germination medium (Fig. 4) or the addition of Tris to inhibit exocellular trehalase (data not shown) had no effect on the course of germination or the distribution of radioactivity. This indicates that trehalose mobilization during germination is due to increased hydrolysis within the spores.

Spores that were incubated under nongerminating conditions did not metabolize or excrete large amounts of their trehalose reserves. Dormant spores incubated in buffer for 4 h at 33°C converted less than 1.5% of the radioactive pools to $^{14}CO_2$ and released less than 1.0% of the initial radioactivity into the buffer. The distribution of the radioactivity between spore pool and macromolecule fractions was not altered by incubation in buffer.

Extracts of dormant spores contain a high specific activity of trehalase, and yet the spores metabolize their endogenous reserves of trehalose slowly. The possibility that turnover of trehalose is involved in the stability of the trehalose pool of spores was tested. Spores were incubated with [U-^{14}C]glucose, and the amount of glucose converted to CO_2 was measured by radiorespirometry. Dormant spores metabolized glucose to CO_2 at the constant rate of 1.3 ± 0.3 nmol (mg protein)$^{-1}$ min^{-1} over a 2-h incubation period. Following incubation in the complex germination medium for 4 h, metabolically active germinated spores metabolized 5.6 ± 0.4 nmol of glucose (mg protein)$^{-1}$ min^{-1}. Since dormant spores metabolized glucose to CO_2 at approximately 23% of the rate of germinated spores, it is reasonable to assume that some of any glucose resulting from intracellular trehalose hydrolysis in nongerminating spores would be converted to CO_2. However, spores with radiolabeled trehalose pools generated little $^{14}CO_2$ during incubation in buffer (Fig. 4A), indicating that turnover of trehalose within nongerminating spores is unlikely to be significant.

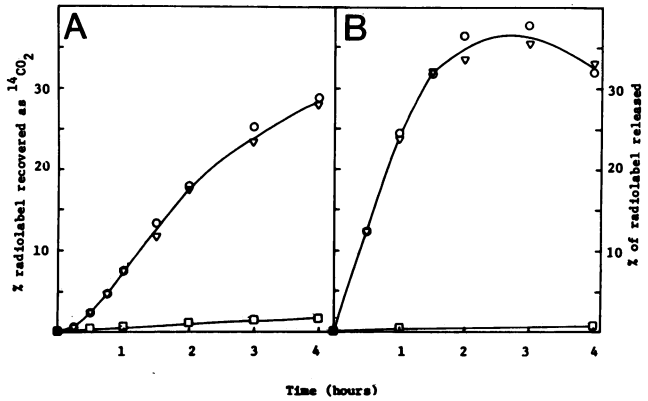

FIG. 4. Metabolism of labeled trehalose pools by dormant and germinating *S. griseus* spores. Spores with labeled trehalose pools were incubated in 10 mM MOPS buffer (pH 7.0; □), or germinated in the complex germination medium (○) or the germination medium supplemented with 10 mM trehalose (▽). (A) Percentage of label respired to $^{14}CO_2$. (B) Percentage of label released into the medium.

TABLE 1. Trehalose mobilization by spores and spherical cells of actinomycetes

Organism	% Trehalose remaining following incubation in[a]:				
	Phospate buffer			Germination medium	
	3 h	3 days	10 days	1 h	3 h
S. griseus	100	95	84	67	12
S. viridochromogenes	140	113	110	33	15
S. antibioticus	ND[b]	118	ND	ND	25
M. echinospora	98	95	ND	40 (1.5 h)[c]	16 (4 h)
N. corallina	ND	58	50	42 (1.5 h)	16 (4 h)
A. crystallopoietes	ND	65	44	74 (2 h)	34 (6 h)

[a] Spores and cells were incubated in phosphate buffer or germination medium as described in the text. Initial trehalose contents, in micrograms per milligram of protein, were as follow: *S. griseus*, 519; *S. viridochromogenes*, 436; *S. antibioticus*, 195; *M. echinospora*, 493; *N. corallina*, 305; and *A. crystallopoietes*, 544.

[b] ND, Not determined.

[c] Numbers in parentheses indicate incubation times that were different from those indicated.

The accumulation of trehalose is a trait common to many actinomycetes and related organisms (18). *A. crystallopoietes* and *N. corallina* grow slowly as spherical cells in defined media containing glucose as the sole carbon and energy sources (8, 9). We found that the spherical cells of both organisms contained large amounts of trehalose. The spherical cells grow into rods following transfer to media that support rapid growth (8, 9).

Spores or spherical cells of a variety of actinomycetes utilized their trehalose reserves slowly during incubation under conditions that did not allow germination or growth (Table 1). The spores germinated and the *A. crystallopoietes* and *N. corallina* spherical-phase cells grew into rods after they were transferred to the complex germination medium. Trehalose was rapidly mobilized by each organism during this period. Extracts of spores or spherical cells contained trehalase activity. The specific activity of trehalase was not

TABLE 2. Trehalase activity in actinomycete cell extracts[a]

Organism	Trehalase activity[b] of cells incubated in:				
	Phospate buffer			Germination medium	
	0 h	3 days	10 days	1 h	3 h
S. griseus	64	60	52	51	29
S. viridochromogenes	36	32	33	34	30
S. antibioticus	18	21	ND[c]	ND	17
M. echinospora	4	2	ND	4 (1.5 h)[d]	4 (4 h)
N. corallina	19	25	26	15 (1.5 h)	12 (4 h)
A. crystallopoietes	18	24	19	21 (2 h)	17 (6 h)

[a] Spores and cells were incubated as described in footnote *a* to Table 1. Extracts were prepared by disruption of spores or cells by agitation with glass beads.

[b] Nanomoles of trehalose hydrolyzed (milligrams of protein)$^{-1}$ minute^{-1}, at 33°C.

[c] ND, Not determined.

[d] Numbers in parentheses indicate incubation times that were different from those indicated.

greatly altered by incubation of the cells in buffer or in the complex germination medium (Table 2). The pattern of trehalose metabolism was remarkably similar among these organisms.

DISCUSSION

Trehalose is present in large quantities in *S. griseus* and is most abundant in spores in which it may enhance spore resistance (1, 4, 16–18).

A high specific activity of trehalase was found in *S. griseus* at all stages of the life cycle. Trehalase activity was highest in extracts of spores. The level of total spore trehalase activity did not change drastically during incubation in buffer or germination media. The specific activity of *S. griseus* trehalase decreased during germination (Table 2), probably as a result of the increase in total protein.

Spores metabolized their trehalose reserves extremely slowly during incubation in buffer. We have observed previously (18) that spores incubated for extended periods of time on solid media also slowly deplete their trehalose pools.

The stability of the trehalose pool in nongerminating spores was probably not due to hydrolysis and resynthesis of trehalose. Dormant spores metabolized exogenously added glucose to CO_2. If the endogenous trehalose were hydrolyzed to glucose within the spores, a large part of this glucose would be metabolized to CO_2. Spores with radioactive trehalose pools did not generate significant amounts of radiolabeled CO_2 when incubated in buffer. These observations indicate that trehalose is not rapidly hydrolyzed to glucose within dormant spores.

Spores transferred to conditions which promoted germination rapidly metabolized their trehalose reserves. Endogenous trehalose was catabolized to CO_2 and intermediary metabolites which were either incorporated into macromolecules or excreted.

The metabolism of endogenous trehalose in spores and cells of other actinomycetes followed a pattern similar to that observed for *S. griseus*. Trehalose was metabolized slowly by nongrowing cells but was rapidly mobilized following a shift to conditions that allowed growth. The specific activity of trehalase remained fairly constant during incubation in buffer or in the germination medium. The levels of trehalase activity in extracts of *M. echinospora* were much lower than those in extracts of the other bacteria tested. This was not due to any difference in pH or ionic strength optima of the enzyme (data not shown). The level of trehalase activity in the *M. echinospora* spores may be insufficient to account for the rapid mobilization of trehalose that was observed during germination. Spores of *S. viridochromogenes* and *M. echinospora* excrete a large fraction of their spore carbon, some of which is trehalose, during germination (7). This probably accounts for some of the mobilization of trehalose during germination of these spores.

Hey-Ferguson et al. (11) reported trehalose utilization accompanied by an increase in trehalase activity during germination of *S. hygroscopicus* spores. In their experiments germination was slow, with no apparent changes in spore morphology and no growth during the first 20 h of incubation in the germination medium. Trehalase activity was measured in spore extracts that were prepared by sonication. We were unable to achieve significant breakage of spores of the same strain of *S. hygroscopicus* during 10 min of sonication at the highest power setting. The initial lower level of trehalase in spore extracts might have resulted from inactivation of the enzyme during sonic disruption. These data, indicating increased trehalase activity during *S. hygroscopicus* spore germination do not agree with our results, which show that there is no such increase during the germination of spores of several other *Streptomyces* species. Trehalase activity was detected in extracts of dormant *S. hygroscopicus* spores (11). From Fig. 1 of that study (11) we calculated that dormant spore extracts hydrolyzed approximately 30 nmol of trehalose (mg of protein)$^{-1}$ min^{-1}. This value is approximately one-half of the level of trehalase activity that we measured in extracts of dormant *S. griseus* spores.

Trehalose is present in fungal spores and is rapidly metabolized during germination (20). Extracts of the dormant spores of many fungi also contain a high specific activity of trehalase (20). The mechanism by which trehalose mobilization is regulated in these fungal spores is unclear, but it may involve localization of trehalose and trehalase in separate compartments of the dormant spore and the subsequent removal during germination of the barrier separating them.

Trehalase activity increases slowly during germination of the spores of some fungi, including *Neurospora crassa*, *Aspergillus oryzae*, and *Dictyostelium discoideum* (12, 13, 20). This may be due to de novo synthesis of trehalase. In these organisms the increase in trehalase activity occurs after the onset of trehalose hydrolysis. Extracts of dormant spores of these fungi do contain trehalase (12, 13, 14, 20). Apparently, this basal level of trehalase in the spores is sufficient to allow rapid trehalose mobilization early in germination.

Trehalase activity increases rapidly during germination of spores of *Phycomyces blakesleeanus*, *Pichia pastoris*, *Candida utilis*, and *Mucor rouxii* and following the transfer of stationary-phase *Saccharomyces cerevisiae* cells to favorable growth conditions (20). These organisms appear to activate trehalase by a cyclic AMP-dependent phosphorylation reaction.

The coexistence of trehalose and trehalase in *S. griseus* spores during prolonged incubation in buffer and the rapid utilization of trehalose during germination requires some mechanism to regulate trehalose catabolism in the spores. Trehalose and trehalase may be maintained in separate compartments within the spore. Alternatively, the physicochemical conditions of the dormant spore might render trehalase inactive. Whatever the mechanism, rapid trehalose metabolism commences at an early stage of germination. Continuing studies in this laboratory are directed toward defining the basis for the apparent inactivity of trehalase in dormant spores and the rapid increase in trehalose utilization that occurs during germination.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI-13948 from the National Institutes of Health and by the College of Agriculture and Life Sciences, University of Wisconsin. M.J.M. was supported by Cellular and Molecular Biology Training Grant 5-T32-GM07215.

LITERATURE CITED

1. **Brana, A. F., C. Mendez, L. A. Diaz, M. B. Manzanal, and C. Hardisson.** 1986. Glycogen and trehalose accumulation during colony development in *Streptomyces antibioticus*. J. Gen. Microbiol. **132**:1319–1326.
2. **Clegg, J. S.** 1978. Hydration-dependent metabolic transition and the state of cellular water in *Artemia* cysts, p. 117–153. *In* J. H. Crowe and J. S. Clegg (ed.), Dry biological systems. Academic Press, Inc., New York.
3. **Crowe, J. H., L. M. Crowe, and D. Chapman.** 1984. Preserva-

tion of membranes in anhydrobiotic organisms: the role of trehalose. Science **223**:701–703.
4. **Cruz-Martin, M., L. A. Diaz, M. B. Manzanal, and C. Hardisson.** 1986. Role of trehalose in the spores of *Streptomyces*. FEMS Microbiol. Lett. **35**:49–54.
5. **Elbein, A. D.** 1974. The metabolism of γ,γ-trehalose. Adv. Carbohydr. Chem. **30**:227–256.
6. **Ensign, J. C.** 1978. Formation, properties and germination of actinomycete spores. Annu. Rev. Microbiol. **32**:185–219.
7. **Ensign, J. C., M. J. McBride, L. J. Stoxen, A. Bertinuson, M. Pomplun, and A. Ho.** 1986. The life cycle of *Streptomyces*: germination and properties of spores and regulation of sporulation, p. 777–790. *In* G. Szabo, S. Biro, and M. Goodfellow (ed.), Biological biochemical and biomedical aspects of actinomycetes. Akademiai Kiado, Budapest.
8. **Ensign, J. C., and R. S. Wolfe.** 1964. Nutritional control of morphogenesis in *Arthrobacter crystallopoietes*. J. Bacteriol. **87**:924–932.
9. **Heinzen, R. J., and J. C. Ensign.** 1974. Effect of growth substrates on morphology of *Nocardia corallina*. Arch. Microbiol. **103**:209–217.
10. **Hey, A. E., and A. D. Elbein.** 1968. Partial purification and properties of a trehalase from *Streptomyces hygroscopicus*. J. Bacteriol. **96**:105–110.
11. **Hey-Ferguson, A., M. Mitchell, and A. D. Elbein.** 1973. Trehalose metabolism in germinating spores of *Streptomyces hygroscopicus*. J. Bacteriol. **116**:1084–1085.
12. **Hill, E. P., and A. S. Sussman.** 1964. Development of trehalase and invertase activity in *Neurospora*. J. Bacteriol. **88**:1556–1566.
13. **Horikoshi, K., and Y. Ikeda.** 1966. Trehalase in conidia of *Aspergillus oryzae*. J. Bacteriol. **91**:1883–1887.
14. **Jackson, D. P., A. H. Chan, and D. A. Cotter.** 1982. Utilization of trehalose during *Dictyostelium discoideum* spore germination. Dev. Biol. **90**:369–374.
15. **Kendrick, K. E., and J. C. Ensign.** 1983. Sporulation of *Streptomyces griseus* in submerged culture. J. Bacteriol. **155**:357–366.
16. **Koepsel, R., and J. C. Ensign.** 1984. Microcycle sporulation of *Streptomyces viridochromogenes*. Arch. Microbiol. **140**:9–14.
17. **McBride, M. J.** 1986. The effect of trehalose content on *Streptomyces griseus* spores, p. 847–849. *In* G. Szabo, S. Biro, and M. Goodfellow (ed.), Biological, biochemical and biomedical aspects of actinomycetes. Akademiai Kiado, Budapest.
18. **McBride, M. J., and J. C. Ensign.** 1987. Effects of intracellular trehalose content on *Streptomyces griseus* spores. J. Bacteriol. **169**:4995–5001.
19. **Nakano, M., and B. Sacktor.** 1984. Renal trehalase: two subsites at the substrate binding site. Biochim. Biophys. Acta **791**:45–49.
20. **Thevelein, J. M.** 1984. Regulation of trehalose mobilization in fungi. Microbiol. Rev. **48**:42–59.
21. **Tisa, L., M. McBride, and J. C. Ensign.** 1982. Studies of growth and morphology of *Frankia* strains $EAN1_{pec}$, $EuI1_c$, CpI1, and $ACN1^{AG}$. Can. J. Bot. **61**:2768–2773.
22. **Womersley, C., and L. Smith.** 1981. Anhydrobiosis in nematodes. I. The role of glycerol, myo-inositol and trehalose during desiccation. Comp. Biochem. Physiol. **70B**:579–586.