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Effects of Intracellular Trehalose Content on *Streptomyces griseus* Spores

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The disaccharide trehalose is accumulated as a storage product by spores of *Streptomyces griseus*. Growth on media containing excess glucose yielded spores containing up to 25% of their dry weight as trehalose. Spores containing as little as 1% of their dry weight as trehalose were obtained during growth on media containing a limiting amount of glucose. Spores containing low levels of trehalose accumulated this sugar when incubated with glucose. The increase in trehalose content coincided with increases in spore refractility, heat resistance, desiccation resistance, and the time required for spore germination in complex media. Trehalose is accumulated by a wide variety of actinomycetes and related bacteria and may be partially responsible for their resistance properties.

The life cycle of members of the genus *Streptomyces* is characterized by vegetative mycelial growth followed by the formation of chains of unicellular spores (15, 19). These spores are dormant cells which are relatively resistant to desiccation, sonic vibration, enzymatic digestion, and exposure to moderately high temperatures.

Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is an important storage compound of the streptomycetes (12) and is particularly abundant in spores (4, 15). Trehalose is also a common reserve material in the dormant spores and cysts of fungi and other lower eucaryotic organisms (7, 13). The accumulation of trehalose sugar by a wide variety of desiccation-resistant eucaryotic and procaryotic organisms suggests that the sugar may play some common role associated with the resistance properties of these organisms. The level of resistance to heat or desiccation of a variety of organisms correlates with their trehalose content (5, 7, 14, 39). Trehalose protects biological membranes against the deleterious effects of dehydration (7). The resistance properties of the organisms that accumulate this sugar may be attributed in part to this protective interaction.

Streptomyces spores provide an interesting experimental system for studying trehalose accumulation and catabolism. The trehalose content of streptomycete spores varies with the sporulation conditions (20, 22). Here we describe in more detail the dependence of the trehalose content of *Streptomyces griseus* spores on culture conditions and effect of intracellular trehalose content on a variety of spore properties.

MATERIALS AND METHODS

Growth conditions. *S. griseus* NRRL B-2682 was maintained as described previously (20), except that the sporulation medium (DMC) consisted of 25 mM morpholinepropanesulfonic acid (MOPS)–10 mM potassium phosphate buffer (pH 7.0)–50 mM glucose–5.0 mM $(NH_4)_2SO_4$–0.5 mM $MgSO_4$–0.05% casein hydrolysate (Sigma Chemical Co., St. Louis, Mo.)–1.5% (wt/vol) Bacto-Agar (Difco Laboratories, Detroit, Mich.)–2.5 ml of a trace salts solution per liter. The concentrated trace salts solution consisted of 27.2 mM $CaCl_2$–7.19 mM $FeSO_4$–11.8 mM $MnSO_4$–0.174 mM $ZnSO_4$ in 0.1 N HCl. This medium was modified for particular experiments. DMC–5 mM glucose medium was identical to DMC, except that glucose was present at a final concentration of 5 mM. DM-1 medium was identical to DMC except that casein hydrolysate was omitted. For liquid cultures DMC was used without agar, and KCl was added (final concentration, 0.15 M). The addition of KCl enhanced the growth rate slightly and resulted in more dispersed mycelial growth. For liquid cultures, spores were germinated in a complex medium that consisted of 0.5% yeast extract (Difco)–0.5% casein hydrolysate in 10 mM MOPS buffer (pH 7.0). Spores were incubated in the germination medium at a final concentration of 2×10^8/ml at 33°C for 3 to 4 h. The germinated spores were harvested by centrifugation, washed 3 times with 10 mM MOPS buffer (pH 7.0), and inoculated into DMC at a final concentration of 5×10^6 spores per ml. All cultures were incubated at 30°C unless stated otherwise.

Mycelia were harvested from liquid culture by centrifugation for 10 min at 10,000 × *g* at 4°C. Spores were harvested from solid media as described previously (18) after 7 days of incubation, except that spores were suspended and washed in distilled water instead of buffer. Liquid sporulation cultures contained a mixture of spores and mycelia. Spores were harvested from liquid media by centrifugation and purified by sonic disruption of mycelia for 3 min with a sonifier (at setting 7; model 350; Branson Sonic Power Co., Danbury, Conn.), followed by centrifugation for 10 min at 6,000 × *g*. Spores were held at 0 to 4°C throughout the procedure.

The medium used to determine the number of viable CFU was identical to DMC except that it contained 0.1% casein hydrolysate and 0.5% yeast extract. Colonies were counted after incubation at 30°C for 3 and 5 days.

Trehalose accumulation. Spores (5×10^8/ml) were incubated in 10 mM potassium phosphate buffer (pH 7.0) containing 50 mM glucose at 33°C with shaking at 150 rpm to allow trehalose accumulation. The spores were harvested at various times by centrifugation, washed, and suspended in distilled water.

Analytical methods. Trehalose was measured following enzymatic hydrolysis to glucose by using a partially purified trehalase. The trehalase was obtained from extracts of an undescribed gram-negative bacterium that was isolated in

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this laboratory by enrichment culture from soil. Trehalase was purified fivefold from French pressure-disrupted cells by ammonium sulfate fractionation and hydroxyapatite column chromatography. The purified extract had no detectable activity (less than 0.1% of that observed with trehalose) with sucrose, cellobiose, lactose, gentobiose, melibiose, maltose, raffinose, starch, or glycogen as substrates. The enzyme was most active at pH 5.0.

Trehalose was extracted from spores or mycelia by boiling in water for 30 min. The suspension was centrifuged, the cell pellet was reextracted with boiling water, and the supernatant fluids were pooled. Trehalose was hydrolyzed to glucose by the partially purified trehalase (250 μg/ml in the final reaction mixture). The final reaction mixture containing trehalase and cell extract in 100 mM citrate buffer (pH 5.0) was incubated at 33°C for 45 min. The amount of trehalase in 1.0 ml of the assay mixture was sufficient to hydrolyze 300 μg of trehalose to glucose during this incubation. The reaction was stopped by immersing the tubes in a boiling water bath for 5 min. The glucose was measured enzymatically by the glucose oxidase-peroxidase method (Sigma). All values presented are averages of at least three determinations.

Total cell protein was determined by the Lowry procedure (17), except that cells were extracted with 1.0 N NaOH. Dry weight was determined by drying cells at 90°C over $CaSO_4$ to constant weight.

The content of amino acids in spore extracts was determined by high-performance liquid chromatography following derivatization with phenylisothiocyanate (6).

Chromatographic separation of radiolabeled trehalose. Spores harvested from DMC–5 mM glucose were incubated in 10 mM potassium phosphate buffer (pH 7.0) containing 50 mM [U-^{14}C]glucose (36 μCi/mmol) for 12 h at 33°C. The radiolabeled spores were washed 5 times and suspended in distilled water. Samples (0.2 ml) were transferred to scintillation cocktail (Aquasol; New England Nuclear Corp., Boston, Mass.), and the total radioactivity incorporated by the spores was measured with a scintillation spectrophotometer (Tri-Carb; Packard Instrument Co., Inc., Rockville, Md.). Trehalose was extracted from the remaining spores with boiling water as described above. After removal of spores by centrifugation, the extracts were membrane filtered (pore size, 0.2 μm), and the amounts of radioactivity in 0.2-ml samples were measured. Samples (5 to 10 μl) of the extracts were also spotted onto strips of Whatman 3 MM filter paper and chromatographed twice with *n*-propanol–ethyl acetate–water (7:1:2). In this system glucose and trehalose migrated at R_f values of 0.42 and 0.24, respectively. The strips were dried, cut into 0.5-cm-wide sections, and transferred to scintillation vials. Distilled water (0.5 ml, 70°C) was added to each vial, followed by the addition of 10 ml of Aquasol. Samples (0.1 ml) of the hot water extracts were also treated with trehalase before chromatography. The fraction of the radioactive material that migrated as trehalose and that was converted to glucose by treatment with trehalase was considered to be authentic trehalose.

The residue of the hot water extract was extracted with 5% trichloroacetic acid (TCA) at 80°C for 30 min and cooled to 0°C for 30 min. Insoluble material was removed by centrifugation at 10,000 × *g* for 5 min. The pellet was washed twice with cold 5% TCA, and the supernatant fluids were pooled. The amount of radioactivity in the hot TCA-soluble fraction (primarily nucleic acids) and TCA-insoluble fraction (primarily proteins) was measured.

Determination of spore properties. Heat resistance was measured by injecting 0.1 ml of a spore suspension into 9.9 ml of 10 mM potassium phosphate buffer (pH 7.0) that was preheated to the test temperature. Samples of 0.5 ml were removed at various times, diluted serially with cold distilled water, and plated on the complex medium described above.

To measure desiccation resistance, 10-μl samples of a spore suspension were dried in vacuo over P_2O_5 and incubated under air for 28 days at 25°C in sealed chambers containing various salt solutions to regulate the relative humidity. An atmosphere of 0% relative humidity was maintained by using P_2O_5. Saturated solutions of NH_4NO_3, $NaNO_2$, and $CuCl_2$ were used to maintain relative humidities of 62.5, 65, and 67%, respectively (38). Viability was determined by suspending the spores in 10 mM potassium phosphate buffer (pH 7.0) containing 0.01 g of Triton X-100 per liter. Spore clumps were dispersed by 2 min of gentle sonication in a sonic water bath (Heat Systems Ultrasonics Inc.). The samples were diluted in distilled water and plated on the complex medium described above.

Germinability was determined by incubating spores (2×10^8 spores per ml) in the complex germination medium at 33°C with shaking. The percentage of spores with germ tubes was determined microscopically after 3 h of incubation.

Respiration was measured as the rate of O_2 consumption with a Clark electrode (Yellow Springs Instruments Co., Yellow Springs, Ohio). Approximately 1.2 mg (dry weight) of spores or mycelia was incubated with stirring in 3.0 ml of 10 mM MOPS buffer (pH 7.0) at 33°C.

RESULTS

Effect of culture conditions on trehalose accumulation. The amount of trehalose in spores was dependent on the growth medium. Spores grown on solid DMC medium for 7 days contained 11.8% of their dry weight as trehalose. Limiting the nitrogen or phosphorus level resulted in an increase in the trehalose content of the spores. Spores harvested from solid DMC in which $(NH_4)_2SO_4$ was omitted and casein hydrolysate was reduced from 0.05 to 0.02% contained 18.4% of their dry weight as trehalose. Spores harvested from DMC in which the phosphate concentration was reduced from 10 to 0.5 mM contained 19.2% of their dry weight as trehalose. The trehalose content of spores was dependent on the glucose concentration in the culture medium (Fig. 1). Spores grown on DMC containing 200 mM glucose contained 21% of their dry weight as trehalose, while spores grown on media containing 2 mM glucose contained 1.2% of their dry weight as trehalose. The relationship between glucose concentration in the medium and trehalose content of spores was essentially linear between 5 and 100 mM glucose. The amounts of trehalose in spores harvested from comparable liquid and solid media were similar (data not shown).

The effect of the length of incubation on agar sporulation medium on the trehalose content of spores was determined. Spores were first observed by microscopic examination at 3 to 4 days of growth, and sporulation was essentially complete by 5 to 7 days. The level of trehalose in the spores decreased progressively during extended incubation (Fig. 2). The spores remained viable, and fewer than 1% of spores had germ tubes at any time.

Trehalose is synthesized and accumulated in response to osmotic stress by some cyanobacteria (26). The effect of the external osmotic pressure on trehalose accumulation by *S. griseus* was determined by adding KCl or NaCl from 0 to 0.7 M or polyethylene glycol 6000 from 0 to 10% to liquid DMC.

The osmotic strength of the medium had no significant effect on the amount of trehalose accumulated by spores or mycelia. Spores harvested following incubation for 5 days contained 520 to 606 μg trehalose per mg of protein. Mycelia harvested after 17.5 h of incubation contained 12 to 19 μg of trehalose per mg of protein. Under some conditions, however, mycelia accumulated large amounts of trehalose. Stationary-phase mycelia of a nonsporulating spontaneous mutant of *S. griseus*, grown in DMC, and stationary-phase mycelia of the wild type, grown under conditions in which sporulation was inhibited by adding 10 mM valine to DMC, contained as much as 250 μg of trehalose per mg of protein. Trehalose is also accumulated by the mycelia of other streptomycetes which do not sporulate in liquid media (12).

Spores harvested from low-glucose media contained low levels of trehalose. These spores synthesized and accumulated trehalose during incubation in a buffered glucose solution (Fig. 3). Microscopic examination showed that less than 1% of the spores had germinated after 19 h of incubation. The viability (CFU/ml) of spores did not change during incubation for 19 h at 33°C in potassium phosphate buffer with or without glucose. When spores were incubated with radiolabeled glucose for 12 h, 9% of the glucose taken up was incorporated into macromolecules (hot water-insoluble fraction). Approximately 2.6% of the radiolabel was in the nucleic acid fraction (hot TCA soluble), while 5.4% of the radiolabel was in the protein fraction (hot TCA insoluble). Rifampin (10 μg/ml) and neomycin (20 μg/ml) blocked the incorporation of glucose into these fractions and also blocked spore germination in complex media, but had no effect on trehalose accumulation or spore viability. Protein and nucleic acid synthesis were apparently not required for trehalose accumulation.

An experiment was designed to determine how much of the glucose incorporated by spores was stored as trehalose. Spores grown on DMC–5 mM glucose medium containing a low level of trehalose (56 μg of trehalose per mg of protein) were incubated in 10 mM phosphate buffer containing 50 mM [U-^{14}C]glucose. Following 12 h of incubation, when the spore trehalose level reached 720 μg/mg of protein, the spores were harvested and the distribution of the radiolabel was determined. Trehalose accounted for 77% of the incorporated label. When spores were incubated for 12 h with glucose in the presence of rifampin and neomycin, trehalose accounted for 85% of the label incorporated. These results demonstrate that most of the glucose incorporated by spores was present as trehalose.

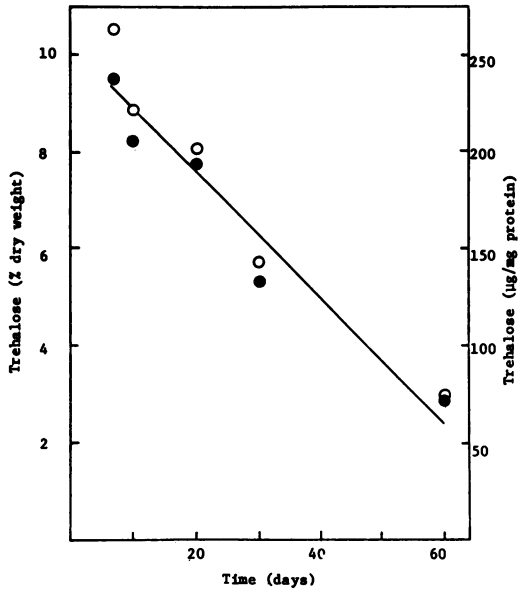

FIG. 2. Effect of time of incubation on trehalose content of spores. Spores were obtained from solid DMC containing 40 mM glucose after different times of incubation, and their trehalose content was measured. Data are expressed as percent dry weight (●) or as micrograms of trehalose per milligram of protein (○).

Effect of trehalose content on spore properties. We took advantage of the ability to alter the trehalose content of spores to determine the effect of trehalose levels on spore properties. Spores harvested from media containing 5 mM glucose were incubated in buffer containing glucose. As the trehalose content of the spores increased they became more refractile, as observed by phase-contrast microscopy (Fig. 4)

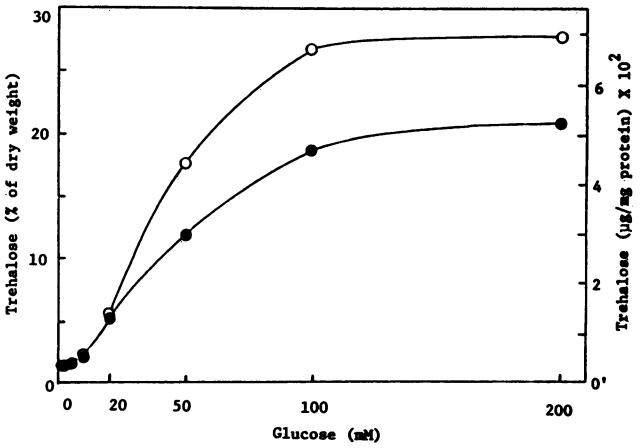

FIG. 1. Effect of glucose concentration on trehalose content of *S. griseus* spores. Spores were harvested after 7 days of incubation on solid DMC containing various concentrations of glucose and analyzed for trehalose content. Data are expressed as percent dry weight (●) or as micrograms of trehalose per milligram of protein (○).

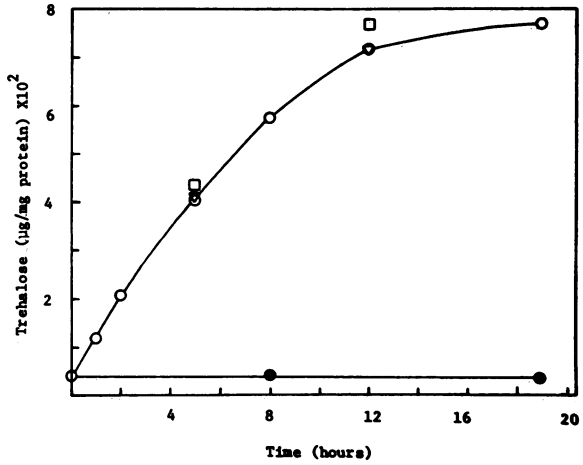

FIG. 3. Accumulation of trehalose by spores. Spores containing low levels of trehalose obtained from solid DMC–5 mM glucose medium were incubated in 10 mM phosphate buffer (pH 7.0; ●); 10 mM phosphate buffer–50 mM glucose (○); 10 mM MOPS buffer (pH 7.0)–50 mM glucose (□); or 10 mM phosphate buffer containing 50 mM glucose, rifampin (10 μg/ml), and neomycin (20 μg/ml) (▽).

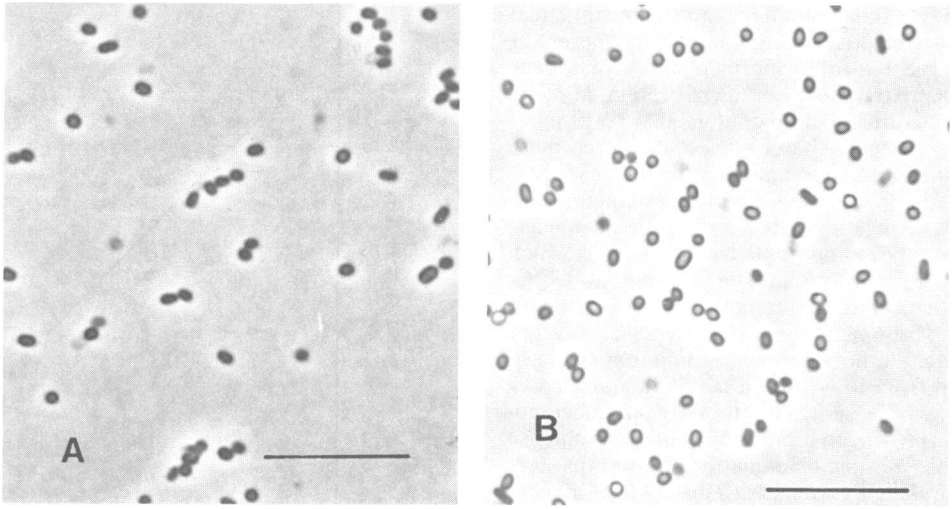

FIG. 4. Phase-contrast photomicrographs of *S. griseus* spores containing different amounts of trehalose. (A) Spores containing 41 μg of trehalose per mg of protein were harvested from solid DMC–5 mM glucose. (B) Spores harvested from DMC–5 mM glucose medium were incubated in 10 mM phosphate buffer (pH 7.0) containing 50 mM glucose for 12 h at 33°C. These spores contained 721 μg of trehalose per mg of protein. Bar, 10 μm.

and as measured by increased optical density (Fig. 5). The refractility of spores containing low levels of trehalose did not change during incubation in buffer alone. Spores that formed on solid media under conditions that allowed the accumulation of large amounts of trehalose (greater that 200 μg of trehalose per mg of protein) were also consistently more phase bright than spores containing small amounts of trehalose (less than 75 μg of trehalose per mg of protein) (data not shown).

Spores containing large amounts of trehalose germinated more slowly and with less synchrony than did spores containing low levels of trehalose (Fig. 5). Germ tubes were formed by 88% of the spores that contained a low level of trehalose during a 3-h incubation in complex germination medium, while only 12% of spores which had accumulated trehalose during 12 h of incubation with glucose germinated under the same conditions. The latter spores were viable since during extended incubation more than 95% of the spores germinated. The relationship between trehalose content of spores and germination was not linear. The reason for the slower germination of spores containing higher levels of trehalose is not known. The germinability of spores containing low levels of trehalose did not change following incubation for 19 h in phosphate buffer at 33°C.

Spores became more resistant to exposure to 60°C as they accumulated trehalose during incubation in phosphate buffer containing glucose (Fig. 5). The same relationship between resistance to exposure to 60°C and trehalose content was found with spores containing 41, 160, and 670 μg of trehalose per mg of protein that were harvested from solid DMC containing different concentrations of glucose (data not shown).

The effect of trehalose content on heat resistance was dependent on the temperature of the heat treatment (Table 1). Spores with small trehalose pools (46 μg of trehalose per mg of protein) were less resistant to exposure to a temperature of 65°C than were spores containing high levels of trehalose (675 μg of trehalose per mg of protein). The differences in decimal reduction times (which is the time for a 10-fold reduction in numbers of survivors) at 65°C were significant and indicated survival differences of approximately 2 orders of magnitude following heating for 2 min. Spores which accumulated trehalose during incubation with glucose in the presence of rifampin and neomycin were equally resistant to exposure to 65°C as spores incubated in buffered glucose alone. The inhibitors did not affect the heat resistance of spores that were incubated in buffer. Spores containing low or high levels of trehalose were equally resistant to heating at 55°C. The heat resistance properties of spores were the same following accumulation of trehalose

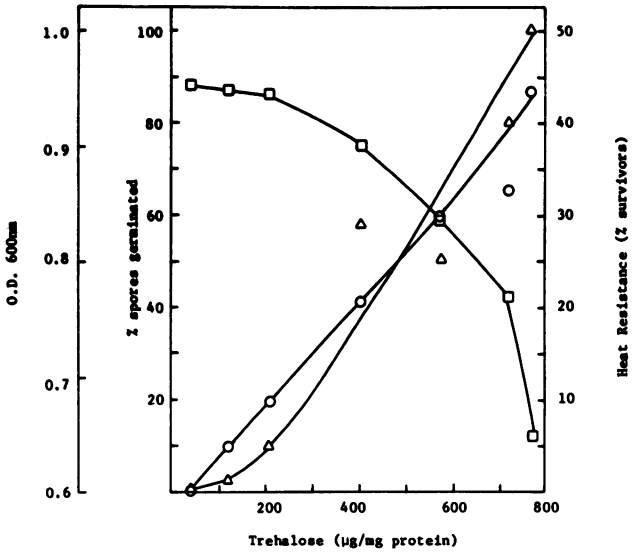

FIG. 5. Effect of trehalose content of spores on various spore properties. Spores obtained from solid DMC–5 mM glucose medium were incubated in 10 mM phosphate buffer containing 50 mM glucose. Following incubation for various times to allow formation of different amounts of trehalose in the spores, the optical density (○; OD_{600}, optical density at 600 nm), heat resistance (△; percentage of spores surviving incubation at 60°C for 10 min), and germinability (□; percentage of spores with germ tubes after 3 h of incubation in complex medium) were determined.

during incubation with glucose in phosphate or MOPS buffers.

Spores with low levels of trehalose were much more heat resistant than vegetatively growing cells. Spores that were incubated for 4 h in the complex germination medium at 33°C to initiate vegetative growth contained 41 μg of trehalose per mg of protein and had a decimal reduction time at 55°C of approximately 1.2 min. The decimal reduction time of spores at this temperature was approximately 32 min (Table 1).

Spores with large amounts of trehalose were more resistant to desiccation than were spores containing low levels of trehalose (Table 2). Spores were especially susceptible to long-term incubation at relative humidities near 65% saturation. The spores of many *Streptomyces* spp. are most sensitive to desiccation at this relative humidity (23).

The results presented above suggest that trehalose accumulation is important to the resistance properties of *S. griseus* spores. However, the resistance of spores to ultraviolet irradiation, sonication, or repeated cycles of rapid freezing and slow thawing were unaffected by changes in trehalose content (data not shown). The endogenous respiration rate of spores was also unaffected by trehalose content. Spores with either high or low levels of trehalose consumed 0.5 to 0.7 μM O_2 (mg of protein)$^{-1}$ min^{-1} at 33°C. The endogenous metabolic rate of vegetative mycelia 18 h old was much higher [4.2 μM O_2 (mg of protein)$^{-1}$ min^{-1}].

Spores of *Streptomyces* contain large pools of amino acids (16). Pools of amino acids, especially proline, have been correlated with increased resistance of bacterial cells (28). We analyzed hot water extracts of *S. griseus* spores by high-performance liquid chromatography for their amino acid contents. Spores containing low levels of trehalose contained large amounts of glutamate (140 μg/mg of spore protein) and much smaller amounts of other amino acids. There were no significant differences in the levels of any amino acids in spores that accumulated trehalose during incubation with glucose (data not shown). It is therefore unlikely that the accumulation of amino acids is involved in the increased resistances of spores following incubation in buffered glucose.

Actinomycetes are noted for their desiccation resistance (36). We analyzed a variety of actinomycetes and related bacteria for their trehalose content. All of the actinomycetes that we tested, including five *Streptomyces* species (*S. griseus* NRRL B-2682, *S. antibioticus* ATCC 11891, *S. hygroscopicus* ATCC 10976, *S. rimosus* ATCC 10970, and *S. viridochromogenes* NRRL B-1511); four *Frankia* strains ($ACN1^{AG}$, CpI1, $EAN1_{pec}$, and $EuI1_c$); *Micromonospora echinospora* ATCC 15836; *Actinoplanes utahensis* ATCC 14539; *Dactylosporangium aurantiacum* NRRL 8111; *Nocardia corallina* ATCC 4273; *Arthrobacter crystallopoietes* ATCC 15481; and *Corynebacterium fascians* ATCC 12974, accumulated trehalose (greater than 100 μg/mg of protein) (data not shown).

TABLE 1. Trehalose accumulation and heat resistance of *S. griseus* spores[a]

Medium	Trehalose (μg/mg of protein)	DRT[b] (min) at:	
		55°C	65°C
Water (control)	50 ± 9[c]	35 ± 8	0.8 ± 0.05
Buffer	46 ± 10	32 ± 5	0.75 ± 0.1
Buffer-rifampin-neomycin[d]	65 ± 10	ND[e]	0.75 ± 0.05
Buffer-glucose	675 ± 75	30 ± 5	4.2 ± 0.5
Buffer-glucose-rifampin-neomycin	718 ± 50	ND	3.8 ± 0.4

[a] Trehalose-deficient spores obtained from DMC-5 mM glucose medium were incubated either in water at 0°C (control) or in 10 mM potassium phosphate buffer (pH 7.0) in the presence or absence of 50 mM glucose for 12 h at 33°C.
[b] DRT, Decimal reduction time.
[c] Standard deviations were calculated from at least three determinations.
[d] Concentrations of 10 μg of rifampin and 20 μg of neomycin per ml were used.
[e] ND, Not determined.

TABLE 2. Effect of trehalose content of spores on desiccation resistance at different relative humidity values

Relative humidity	Percent survival for[a]:	
	Spores containing 53 μg of trehalose/mg of protein[b]	Spores containing 774 μg of trehalose/mg of protein[c]
0	4.7 ± 1.3[d]	23 ± 1.0
62.5	2.8 ± 2.0	58 ± 11.0
65	0.03 ± 0.015	2 ± 1.2
67	0.18 ± 0.15	19 ± 6.6
100	29.0 ± 3.3	65 ± 11.0

[a] Spores were dried and incubated at the indicated relative humidities for 28 days at 25°C.
[b] Spores harvested from solid DMC-5 mM glucose medium.
[c] Spores harvested from solid DMC-5 mM glucose medium and incubated with glucose for 12 h.
[d] Standard deviations were calculated from at least three determinations.

Another desiccation-resistant bacterium commonly found in soil, *Myxococcus xanthus*, forms resistant myxospores. Bacon et al. (2) observed that glycerol-induced myxospores of *Myxococcus xanthus* (ATCC 25232) accumulated large amounts of carbohydrate. We suspect that this carbohydrate is trehalose. Analysis of myxospores harvested 14 h after induction with glycerol revealed that they contained 240 μg of trehalose per mg of protein.

DISCUSSION

Trehalose is accumulated in a wide variety of eucaryotic organisms as a preparation for dormancy (7). Trehalose is also found in a variety of actinomycetes and related bacteria including species of the genera *Streptomyces*, *Nocardia*, *Mycobacterium*, and *Corynebacterium* (13); *Frankia* (25); *Micromonospora* (11); *Arthrobacter* (33); *Cellulomonas* (29); *Micrococcus* (1); *Microbacterium* (37); *Bacterionema* (30); *Propionibacterium* (31); and *Rhodococcus* (35). Trehalose accumulation appears to be characteristic of these bacteria.

Trehalose is accumulated in response to osmotic stress by some cyanobacteria (26). The trehalose content of *S. griseus* cells was independent of the concentrations of KCl, NaCl, and polyethylene glycol 6000 in the growth medium. Mycelia of *S. griseus* and *S. californicus* accumulate large amounts of proline, glutamine, and alanine to maintain turgor when grown in media of high osmotic strength (21).

S. griseus spores containing large amounts of trehalose were more resistant to exposure to high temperatures (60 to 65°C) than were spores with low levels of trehalose. Trehalose accumulation cannot be entirely responsible for the heat resistance of dormant spores since spores of *S. griseus* containing low or high levels of trehalose were much more resistant to heat than were vegetatively growing cells. Resistance to more moderate temperatures (55°C) was not enhanced by increased trehalose content. We do not know the reason for these results. Loss of viability may be due to the fact that different spore components were damaged at the different temperatures. A similar phenomenon was observed with *Bacillus* endospores (3).

Spores of *S. viridochromogenes* containing 1.3 and 9.7% of their dry weight as trehalose were approximately equally resistant to exposure to 60°C (22). Heat resistance was tested only at this temperature. It is possible that a correlation between heat resistance and trehalose content would have been observed at higher temperatures.

Spores of *S. griseus* with large pools of trehalose were more resistant to desiccation than were spores with low levels of trehalose. Cruz-Martin et al. (10) reported recently that a decrease in trehalose content of *S. antibioticus* spores following depletion of trehalose reserves by incubation in buffer or germination medium was accompanied by a parallel decrease in resistance to freeze-drying (10). These data suggest that increased desiccation resistance may be a characteristic property of spores containing elevated levels of trehalose and do not exclude the possibility that other events associated with spore starvation or germination caused the observed decreased level of resistance. Our comparison of desiccation resistance of dormant spores that differ primarily in their trehalose contents confirms that there is a correlation between trehalose content and desiccation resistance of *Streptomyces* spores.

The accumulation of trehalose may alter spore resistances in several ways. Increased survival to stress may be due to the use of trehalose for cell repair processes. Trehalose may be an ideal reserve material because it is a stable, nonreducing sugar. Reducing sugars react with proteins and could be detrimental if stored in large quantities in the cytoplasm. Trehalose may perform a more direct role in conferring resistance through its physical interactions with cell macromolecules or by its effect on the osmolarity of the cytoplasm. The protective interactions of trehalose with biological membranes have been demonstrated (7, 27). Several lines of evidence indicate that trehalose interacts with the polar head groups of membrane phospholipids (7–9, 24). This could maintain the membranes in their hydrated configuration in the absence of water.

Actinomycetes are primarily terrestial organisms, and so are constantly subjected to the stresses of desiccation and temperature fluctuation. Trehalose has been considered to be directly involved in the desiccation resistance of a variety of organisms (7). The results of this study, involving comparisons of the resistance properties of *S. griseus* spores containing high and low levels of trehalose, provide further support for this hypothesis.

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