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Culture Age, Temperature, and pH Affect the Polyol and Trehalose Contents of Fungal Propagules

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The growth and conidial physiology of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus* were studied under different conditions. The effects of culture age (up to 120 days), temperature (5 to 35°C), and pH (2.9 to 11.1) were determined. Growth was optimal at pH 5 to 8 for each isolate and between 20 and 35°C, depending on the isolate. The predominant polyol in conidia was mannitol, with up to 39, 134, and 61 mg g of conidia^{-1} for *B. bassiana*, *M. anisopliae*, and *P. farinosus*, respectively. Conidia of *M. anisopliae* contained relatively small amounts of lower-molecular-weight polyols and trehalose (less than 25 mg g^{-1} in total) in all treatments. Conidia of *B. bassiana* and *P. farinosus* contained up to 30, 32, and 25 mg of glycerol, erythritol, and trehalose, respectively, g^{-1}, depending on the treatment. Conidia of *P. farinosus* contained unusually high amounts of glycerol and erythritol at pH 2.9. The apparent effect of pH on gene expression is discussed in relation to the induction of a water stress response. To our knowledge, this is the first report of polyols and trehalose in fungal propagules produced over a range of temperature or pH. Some conditions and harvesting times were associated with an apparent inhibition of synthesis or accumulation of polyols and trehalose. This shows that culture age and environmental conditions affect the physiological quality of inoculum and can thereby determine its potential for biocontrol.

Polyols and trehalose act as compatible solutes and can play a role in osmotic adjustment (6, 7). Their role in membrane and protein protection is well established (6, 11, 13–15). These compounds have been associated with resistance to environmental extremes, accelerated germination, enhanced pathogenicity, and improved storage life of fungal propagules (2, 26, 29, 32, 33). Manipulation of polyols and trehalose may therefore be important in propagules used for such purposes as biological control, soil inoculation, and cryopreservation. Of the polyols, the quantification of glycerol and erythritol is particularly important in biological control, where water availability is frequently limited (32). The low-molecular-weight polyols glycerol and erythritol are more effective in osmotic adjustment than higher-molecular-weight compounds such as mannitol (12, 28).

Two recent reports showed the effects of water availability, carbohydrate type, and carbohydrate concentration on polyols and trehalose in conidia of the entomopathogens *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus* (30, 31). Optimization of glycerol and erythritol content has increased the germination rate of these fungi, enhanced germination at low water availability, and improved pathogenicity at low relative humidity (29, 32). Optimization of trehalose content has extended the survival of conidia during long-term storage (29). Many reports show that the efficacy of entomopathogenic fungi is limited by low water availability (20, 29, 51, 53, 58). That propagules cannot normally germinate at relative humidities below 95% (equivalent to a water activity [a_w] of 0.95) has prevented the successful exploitation of these biocontrol agents (25, 32, 55).

Many studies have been carried out to optimize yield and propagule production (21, 47), to improve selection of isolates (39, 59), and to improve preparation and formulation of propagules of entomopathogens (5, 24). There has been interest in genetic manipulation of biocontrol agents to enhance osmotic tolerance (54), and many recent studies have been carried out on cuticle-degrading enzymes (36, 43, 57). The nutritional requirements for germination have also been investigated (19, 56), although the methods used in such studies may be flawed (28).

There is clearly a keen research interest in entomopathogenic fungi and their utilization for insect control. Nevertheless, there have been few attempts to improve the physiological quality of propagules by exploiting the phenotypic plasticity of fungi. As Jennings (41) states, "Phenotypic plasticity is well known to botanists. . .but rarely considered explicitly by mycologists. . . .That neglect may have been encouraged. . .by the knowledge that, when a fungus faces unfavourable environmental conditions, more resistant structures such as spores and sclerotia are formed, removing the need for the fungus to adapt vegetatively." But propagules can also adapt to environmental circumstances. The present study was carried out to determine the effect of culture age, temperature, and pH on growth and accumulation of glycerol, erythritol, arabitol, mannitol, and trehalose in conidia of *B. bassiana*, *M. anisopliae*, and *P. farinosus.*

MATERIALS AND METHODS

Organisms, growth conditions, growth measurement, and collection of conidia. Isolates of *B. bassiana* (isolate 206), *M. anisopliae* (isolate V90), and *P. farinosus* (isolate V51) were described previously (30). Storage of stock cultures (at 4°C), basic growth conditions (at 25°C), point-inoculation of media, radial growth rate measurement, collection of conidia (by wet harvesting after 30 days), and lyophilization have been detailed elsewhere (31). Media were based on Sabouraud dextrose agar (SDA) and were autoclaved for 15 min at 121°C and 1 atm (1 atm = 101.29 kPa). All media contained 21.3 g of 2-(*N*-morpholino)ethanesulfonic acid (MES) liter^{-1}, and the pH was adjusted with 2.5 M NaOH to give a final value of 5.8 ± 0.3, unless otherwise stated. The surface pH of the media was measured with a pH stick electrode (Gallenkamp) fitted with a flat probe.

Fungi were grown over a pH range of 2.9 to 11.1. Media were adjusted with nonmetabolizable zwitterionic buffers as far as possible [MES-NaOH, *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES)–NaOH, 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES)–NaOH, and 3-(cyclo-hexylamino)-1-propanesulfonic acid (CAPS)–NaOH] (34), but glycine-HCl and citric acid-

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TABLE 1. Mean pH values of buffers used in growth media and final pH values of media at 25°C[a]

Buffer	pH of buffer	pH of medium
Glycine-HCl	2.70	2.88
Citric acid-Na_2HPO_4	3.95, 5.25	4.04, 4.91
MES-NaOH	5.90	5.50
HEPES-NaOH	6.50, 8.00	6.75, 7.73
CHES-NaOH	8.50	8.64
CAPS-NaOH	9.40, 11.20	9.35, 11.10

[a] The pH values listed are accurate to ±0.10 for the pH of buffers and to ±0.20 for the final pH of media.

Na_2HPO_4 were used to obtain pH values of less than 5. Buffers were made up as described by Dawson et al. (17), and the pH was adjusted prior to sterilization by the addition of HCl, Na_2HPO_4, or NaOH. However, the components of the CAPS-NaOH buffer were filter sterilized and then added to the media after autoclaving. The water availability of pH-adjusted media was 0.984 ± 0.003 a_w. The modification of the pH of the media is summarized in Table 1. Media were semiliquid at pH 2.9, so the surface was covered with sterile nylon mesh to support the mycelium.

Extraction and detection of polyols and trehalose. Extractions were carried out with 5-mg samples of conidia that were sonicated in AnalaR water (Merck) and then immersed in a boiling water bath as described previously (31). Samples (in microcentrifuge tubes) were sonicated to optimize polyol extraction and placed into a boiling water bath to maximize trehalose extraction (28). Extracts were filtered through a 0.2-μm-pore-size particle filter and then injected onto a Dionex series 4500 high-performance liquid chromatograph (HPLC). The HPLC was fitted with a CarboPac PA1 column (4 by 250 mm), a CarboPac PA1 guard column, and a Dionex pulsed electrochemical detector (with a gold electrode) and connected to a Hewlett-Packard 3390A integrator. The mobile phase was 100 mM NaOH (pH 14), and the flow rate was 1 ml min^{-1}. The limits of detection were 1.6 μg ml^{-1} for polyols and 2.8 μg ml^{-1} for trehalose. The HPLC protocol has been detailed elsewhere (28, 31).

The conidia were freeze-dried for polyol and trehalose determinations. Even without freeze-drying, aerial conidia are desiccated structures, so expression of concentrations in molar terms is inappropriate. Furthermore, the use of molar values would deny differences of intracellular concentration and may imply that the polyols and trehalose are located exclusively in the cytosol (and not in the cell wall, for example). However, such compounds may accumulate outside the cell membrane (22, 58). For these reasons, quantities are expressed as milligrams of carbohydrate per gram of conidia, and these units are consistent with those used in earlier reports (29–32).

Replication and statistical treatment of results. Measurements of pH and water availability were made on three different samples of medium. Mycelial growth rate measurements were made on three separate cultures per treatment and in two perpendicular directions across each colony. Analyses of standard solutions of polyols and trehalose were carried out three times. The polyol and trehalose contents of conidia were determined separately for three samples taken from three replicate plates. The C-Stat program (Cherwell Scientific Publishing) was used to derive means and standard deviations and to carry out one-way analyses of variance. The error mean square was obtained from the one-way analyses of variance and used to calculate the least significant difference ($P < 0.05$) between treatments.

RESULTS

Growth in relation to temperature and pH. Under the conditions of this study, *B. bassiana* grew optimally at about 25°C, *M. anisopliae* grew optimally at 30 to 35°C, and *P. farinosus* grew optimally at about 20°C (Fig. 1a). The growth rates for each isolate were near optimal (i.e., 80 to 100% of the maximum value) at 25°C. The growth rate was determined on SDA from pH 2.9 to 11.1, at 25°C (Fig. 1b). The growth of each isolate was optimal from pH 5 to 8 and declined sharply outside this pH range.

Effect of culture age on polyols and trehalose. The mannitol content of *B. bassiana*, *M. anisopliae*, and *P. farinosus* conidia was greatest at 7 to 25, 10 to 120, and 7 to 25 days, respectively (Fig. 2). Conidia contained less than 12 mg of glycerol or arabitol g^{-1} at all sample times, regardless of isolate. The trehalose content was greatest in conidia of *B. bassiana* and *P. farinosus* between 25 and 40 days, but conidia of *M. anisopliae* contained only small amounts of trehalose (less than 8 mg g^{-1}), regardless of incubation period.

Polyols and trehalose in relation to temperature. Conidia of *B. bassiana* and *P. farinosus* contained less than 6 mg of polyols g^{-1} at 5°C (Fig. 3). Conidia contained less than 2.5 mg of trehalose g^{-1} at 5°C, regardless of isolate. The mannitol and erythritol contents of *B. bassiana* and *P. farinosus* conidia were greater at high temperatures (up to 25°C). These two species did not produce sufficient conidia at 35°C for HPLC analyses to be carried out. Of the compounds studied, *M. anisopliae* conidia contained predominantly mannitol, regardless of temperature, but the concentration was highest between 15 and 35°C (from 100 to 150 mg of mannitol g^{-1}). The trehalose content of *B. bassiana* and *P. farinosus* conidia was maximal at 20 to 30°C.

Polyols and trehalose over a range of pH. Conidia contained the highest level of mannitol at pH 4.9 to 9.4 (Fig. 4). However, conidia of *M. anisopliae* contained significantly ($P < 0.05$) more mannitol than the other isolates, i.e., up to 103 mg g^{-1}. On media with a pH of less than 4, the mannitol concentration in conidia was less than 3 mg g of $conidia^{-1}$, regardless of isolate. Conidia of *B. bassiana* contained more arabitol (7.7 mg

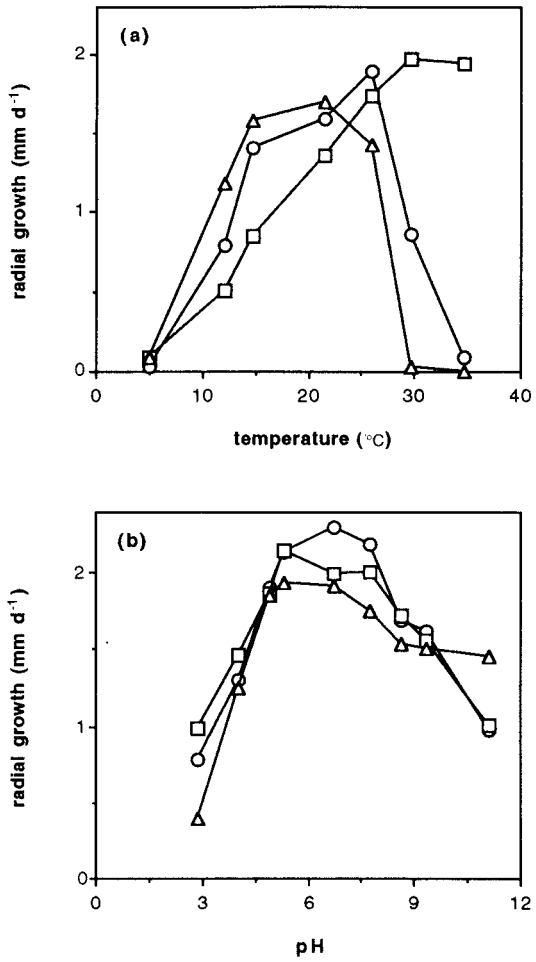

FIG. 1. Mean radial growth rates (in millimeters per day) of *B. bassiana* (○), *M. anisopliae* (□), and *P. farinosus* (△) on SDA in relation to temperature (a) and on SDA modified by the addition of different buffers over a range of pH values, at 25°C (b). The least significant differences ($P < 0.05$) for growth rates of *B. bassiana*, *M. anisopliae*, and *P. farinosus* were as follows: (a) 0.06, 0.08, and 0.07, respectively; (b) 0.18, 0.26, and 0.08, respectively. d^{-1}, per day.

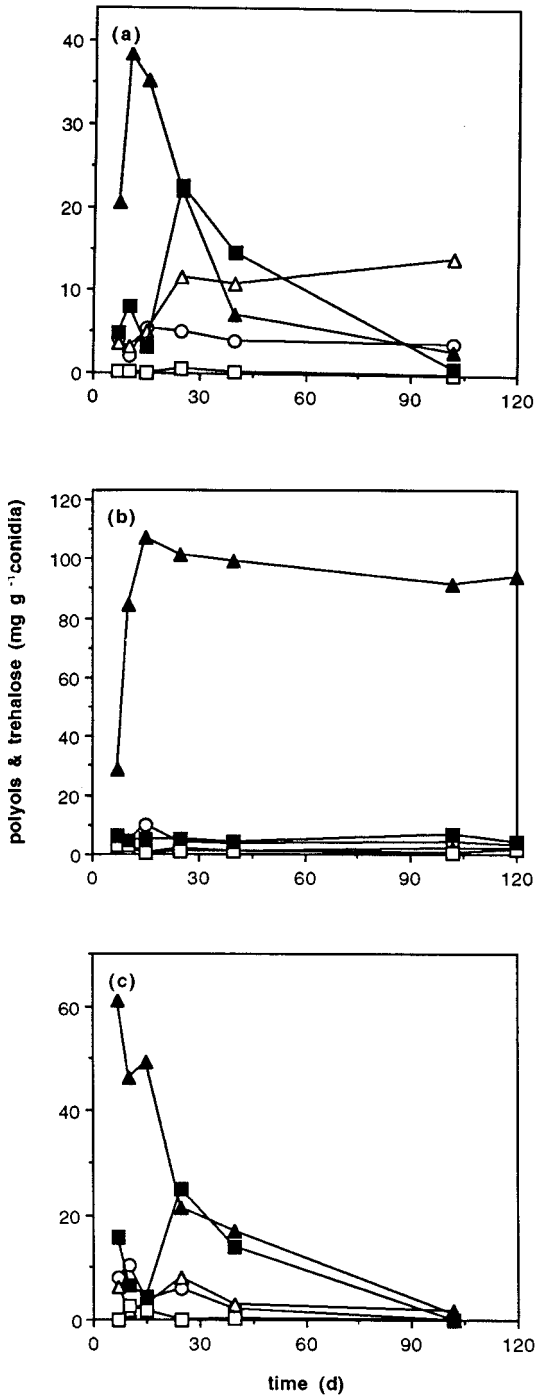

FIG. 2. Mean polyol and trehalose contents (in milligrams per gram of conidia) of *B. bassiana* (a), *M. anisopliae* (b), and *P. farinosus* (c) conidia that were harvested at different times over a period of 120 days. Cultures were grown on SDA at 25°C. The least significant differences ($P < 0.05$) for glycerol (○), erythritol (△), arabitol (□), mannitol (▲), and trehalose (■) were as follows: (a) 1.83, 3.88, 0.42, 6.92, and 14.16, respectively; (b) 2.02, 1.17, 1.65, 7.47, and 4.39, respectively; (c) 2.24, 2.42, 1.08, 9.78, and 3.86, respectively. d, days.

g^{-1}) at pH 2.9 than at higher pH values. In contrast, there were large increases in the lower-molecular-weight polyols glycerol and erythritol in conidia of *P. farinosus* (up to 59.7 mg g^{-1} in total) at pH 2.9. There was significantly less ($P < 0.05$) trehalose in conidia from cultures grown at pH 2.9 than in those from cultures, grown at higher pH, values, regardless of isolate. Insufficient conidia were produced at pH 11.1 for HPLC analyses to be carried out. The optimal conditions for accumulation of specific polyols and trehalose are shown in Table 2. For comparison, the results of two other studies of these fungal isolates are also summarized in Table 2.

DISCUSSION

Growth in relation to temperature and pH. Each isolate grew optimally at different temperatures, but the growth of each was either optimal or near optimal at 25°C, the standard temperature for the rest of the study. It is important to understand the effects of temperature on growth because increases of more than 10°C can occur during solid-substrate culture of entomopathogens (see below). The range of pH over which optimal growth occurred (pH 5 to 8) is the same as that reported for other species of entomopathogenic fungi, including *Metarhizium flavoviride* (40). In general, growth of entomopathogens is optimal over a broad range of pH. Some other fungal species grow over a more narrow pH range, and optimum growth appears to correspond to a specific pH value (8). This implies that entomopathogenic fungi may regulate cytosolic pH more effectively than many other species. The ability of entomopathogens to grow below pH 7 is desirable during industrial production of inocula. This enables the pH of the substrate to be reduced to inhibit the growth of contaminants such as bacteria (4), without affecting yield.

Growth of the isolates under study has been characterized previously over a range of a_w values, carbohydrate types, and carbohydrate concentrations (30, 31) (Table 2). Water availability, carbohydrate type and concentration, culture age, temperature, and pH are not independent and separate variables. Variation in carbohydrate type or concentration results in changes in water availability (31). Water availability affects the growth response of fungal cells to variation in pH (48). The optimum pH for fungal growth varies with temperature (8), and this implies that the optimum temperature for growth is dependent on pH. Temperature and water availability are interdependent in their effects on microbial growth (9). One multifactorial study (49) demonstrated interactions between temperature, water availability, and pH in their effects on fungal growth. As Bull and Bushnell (10) pointed out, "there is a tendency to treat each factor separately but this approach is doomed because it implies acceptance of independently acting factors. . .an appreciation of the interaction of such factors is essential for the full understanding of fungal. . .growth." There is, therefore, a limit to the value of studies that allow for only one variable at a time. For this reason, care must be taken when interpreting the information given in Table 2 (for instance). The effects of increased carbohydrate concentration, for example, may be partly attributable to the associated decrease in a_w.

Effect of culture age on polyols and trehalose. The only polyols detected were glycerol, erythritol, arabitol, and mannitol, regardless of species and experimental conditions. Conidia did not accumulate other polyols that could be detected within the range of sensitivity of the pulsed electrochemical detector (28). Pulsed electrochemical detection (with a gold electrode) represents the most sensitive method that is widely available to quantify carbohydrates by HPLC or gas chromatography (42). The polyol and trehalose contents of *B. bassiana* and *M. anisopliae* conidia from SDA were low compared with those from other treatments, regardless of culture age. This would be expected because the cultures grown on SDA, under the standard conditions of this study, were relatively stress-free. After

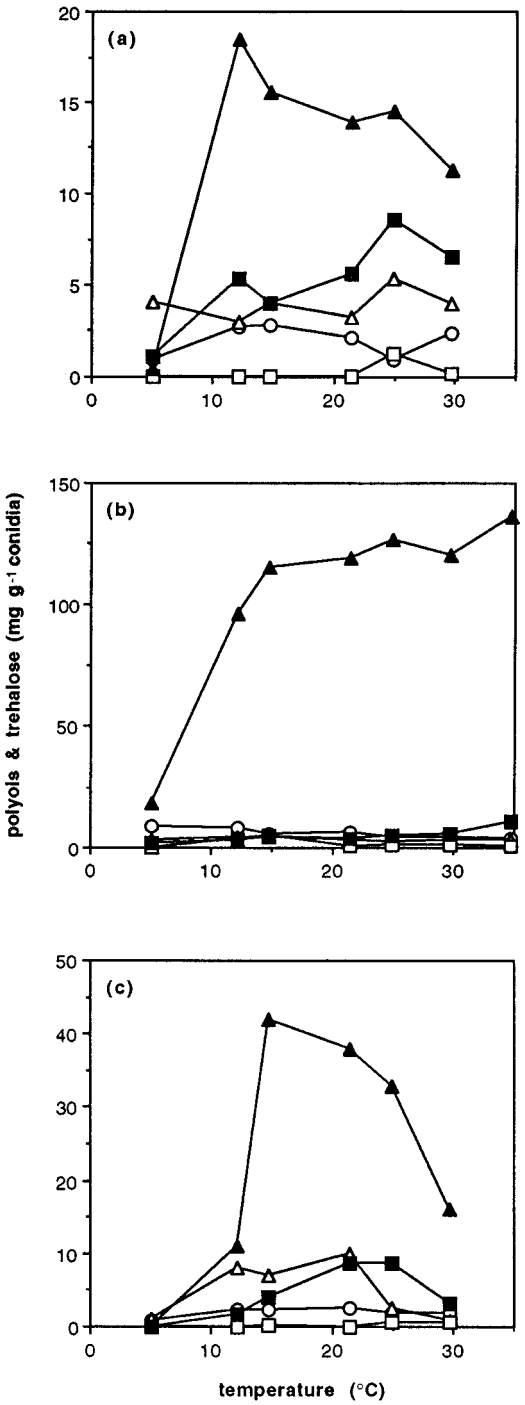

FIG. 3. Mean polyol and trehalose contents (in milligrams per gram of conidia) of *B. bassiana* (a), *M. anisopliae* (b), and *P. farinosus* (c) conidia that were obtained from cultures grown at different temperatures, on SDA. The least significant differences ($P < 0.05$) for glycerol (○), erythritol (△), arabitol (□), mannitol (▲), and trehalose (■) were as follows: (a) 0.74, 1.16, 0.56, 3.70, and 1.76, respectively; (b) 2.05, 1.26, 0.70, 14.83, and 2.21, respectively; (c) 0.92, 2.49, 0.62, 3.78, and 1.06, respectively.

30 days, there was a progressive decrease in the polyol and trehalose contents of *B. bassiana* and *P. farinosus* conidia. The rest of the study was carried out with a 30-day incubation period because the main compounds of interest (glycerol, erythritol, and trehalose) were, in general, at their highest concentrations at this time.

There have been few studies of temporal changes of polyols in fungal propagules. One study, of *Geotrichum candidum* arthrospores, showed that the total polyol content gradually increased over a 10-day incubation period (16). However, studies of conidial polyols have been limited to the disappearance of carbohydrates during germination (e.g., see reference 1). Hall et al. (27) found that entomopathogenic conidia from 3-day cultures germinated more rapidly than those from older cultures, even when spore production was still in an exponential phase. These authors did not carry out a physiological investigation, but polyol content can determine germination rate (2, 32). That culture age affects the polyol content of conidia and that this correlates with changes in germination rate suggest that incubation period may be a critical determinant of inoculum quality. It is, therefore, important to understand the effect of culture age on polyol content of entomopathogenic conidia. The ideal period of incubation may depend on species and isolate and factors such as the type of substrate used (32).

The fate of polyols as cultures age is uncertain. They may be slowly metabolized and ultimately used in respiration or converted to higher-molecular-weight compounds such as glycogen. They may move out of conidia by transport into the mycelium or by diffusion into the conidiophore wall. That polyols can move more or less freely through pores in the cell membrane has been shown previously (46). It is also possible that some conidia are produced late in the incubation period and that they contain a smaller amount of polyols than those produced earlier. It is certain, however, that the mean polyol content of conidia declines with increasing culture age, regardless of species. This must contribute to the decline of viability that is observed as cultures age.

Polyols and trehalose in relation to temperature. The accumulation of polyols and trehalose in conidia was dependent on temperature and decreased above 25°C (*B. bassiana* and *P. farinosus*) and below 14°C (all three isolates). Although the mannitol content of *B. bassiana* and *P. farinosus* conidia was greatest at about 15°C, the amounts of trehalose and other polyols in conidia were highest at about 20 or 25°C under the standard conditions of this study. For this reason, and to standardize experimental conditions, all three species were grown at 25°C for the rest of the study.

Polyols have been associated with thermotolerance and protein stability at high temperature (23, 45). However, accumulation of low-molecular-weight polyols was not as pronounced as it was in other studies of entomopathogenic conidia (30–32). That there was no marked accumulation of polyols at high temperature suggests that the maximum temperature used in the present study (35°C) was not high enough to destabilize hydrated cell components such as enzymes. An increased intracellular trehalose concentration has been often associated with tolerance of heat shock (e.g., 3). However, the trehalose content of conidia did not increase at high temperatures. Trehalose appears to interact with hydrated cell components slightly differently from polyols such that it is most effective in situations that cause shock or absolute water stress (e.g., heat shock or desiccation) (15). Trehalose would not therefore normally be expected to accumulate as a compatible solute under steady-state conditions of high temperature, even if temperatures were sufficiently high to destabilize enzymes and membranes.

There appears to have been no information available on the effect of temperature on conidial polyols and trehalose. Bartlett and Jaronski (4) said "it is surprising that [the effect of temperature on growth and sporulation] has been stressed so

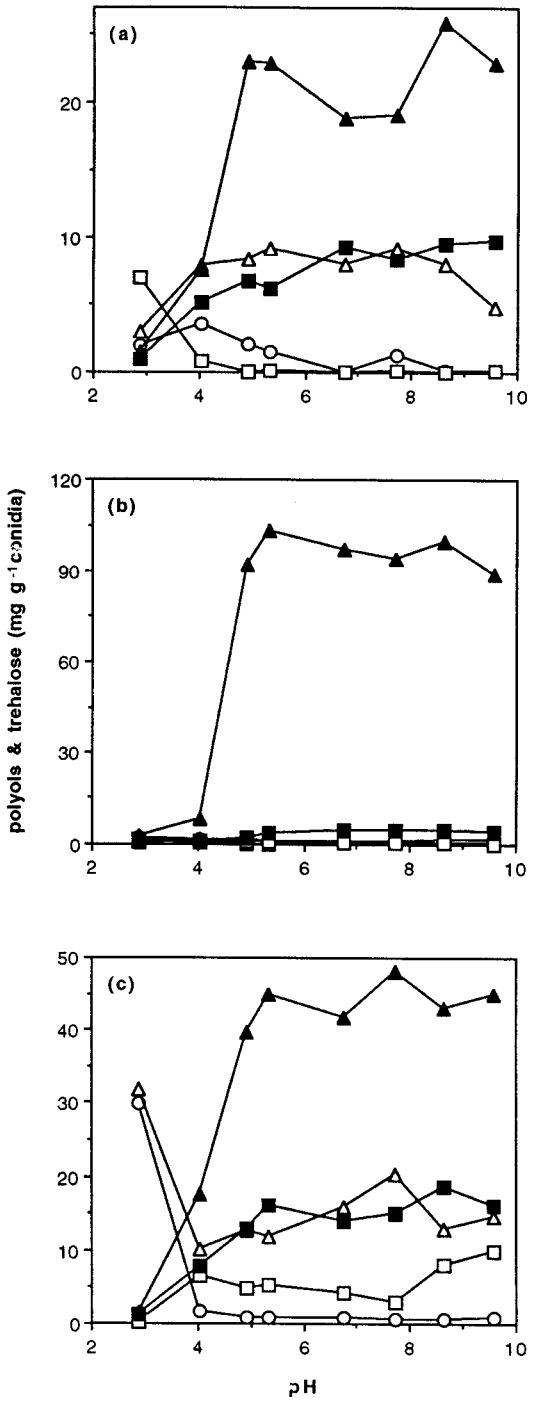

FIG. 4. Mean polyol and trehalose contents (in milligrams per gram of conidia) of *B. bassiana* (a), *M. anisopliae* (b), and *P. farinosus* (c) conidia that were obtained from cultures grown over a range of pH values on SDA modified by the addition of different buffers. The least significant differences ($P < 0.05$) for glycerol (○), erythritol (△), arabitol (□), mannitol (▲), and trehalose (■) were as follows: (a) 0.98, 3.86, 2.30, 3.81, and 5.38, respectively; (b) 0.67, 0.56, 0.64, 14.06, and 2.74, respectively; (c) 4.87, 4.50, 2.26, 5.69, and 6.71, respectively.

little in the production of mycoinsecticides with solid substrates." The present study substantiates their concern by showing that temperature has a significant effect on polyol and trehalose accumulation. The temperature of large-scale solid-substrate cultures can rise by as much as 10 to 15°C, reaching 40°C (4). Fermentations that generate excessive heat must be cooled. However, marked temperature reductions may result in a reduction in the polyol and trehalose contents of conidia. As mentioned, the polyol and trehalose contents affect the quality of biocontrol inocula. This should be considered when regulating temperature during large-scale fermentation for production of conidia.

Polyols and trehalose over a range of pH. There was little effect of pH on polyol and trehalose accumulation in conidia between pH 5 and 10. Lactic acid and HCl are sometimes used to decrease the pH of industrial substrates to between 3.5 and 4.5, thereby inhibiting the growth of other microorganisms (4). In the present study, the polyol content of conidia produced below pH 4.5 was, on the whole, reduced. However, at pH values as low as 2.9, there were accumulations of glycerol and erythritol in conidia of *P. farinosus* but not *B. bassiana* or *M. anisopliae*. Acidification of the medium may therefore be desirable to enhance the content of low-molecular-weight polyols for some isolates or species but not for others. Only trace amounts of trehalose were present at pH values below 4.5, regardless of species, and this may reduce the shelf life of conidia. The main part of this study was carried out at a moderate pH value (5.8) that did not adversely affect the polyol and trehalose contents of conidia under the standard conditions of the study.

Cells respond directly to water stress by producing low-molecular-weight polyols (28, 30, 31). The mechanism by which polyol synthesis is controlled operates at a genetic level. So-called "osmotic" control of gene expression involves a disruption of DNA-associated water leading to the relaxation of supercoiled DNA and may involve a conformational change of regulatory proteins (37, 38). These structural changes can have a direct effect on replication and transcription (see reference 50). Extreme pH, osmolytes, ethanol, and heat are all sources of water stress that can affect the structural state of DNA (38, 44, 50). Different sources of stress elicit the same metabolic response (compatible solute accumulation) because they all cause the same basic problem, namely, water stress. Extreme acidic or alkaline conditions will disrupt hydrogen bonding in cell components such as enzymes and lipid bilayers, thereby having an adverse effect on cell integrity and metabolism. Karem and Foster (44) carried out a study of bacterial DNA and showed that pH has a direct effect on supercoiling and gene expression. This may explain how low pH leads to the accumulation of glycerol and erythritol in conidia of *P. farinosus*. What remains uncertain, however, is why such extreme conditions did not elicit the same response in *B. bassiana* and *M. anisopliae*. Maybe the synthesis and accumulation of low-molecular-weight polyols is enhanced in the mycelium of each isolate. Furthermore, the isolates studied may not typify their respective species. These possibilities were not investigated in the present study.

In some studies of intracellular polyol accumulation, emphasis has been placed on total polyol concentrations. When considering the function of polyols in osmotic adjustment, there is little point in totalling values because some polyols are ineffective in this capacity (12, 28). Furthermore, polyols of different molecular weights may be differentially effective as compatible solutes (7, 12). Higher-molecular-weight polyols (e.g., mannitol) cause a slight inhibition of enzyme activity compared with low-molecular-weight polyols (e.g., glycerol) at equivalent concentrations (7). In consequence, to place emphasis on total polyol concentrations can be misleading in studies of cell metabolism.

The analyses of conidial polyols and trehalose in the present study will be most pertinent to conidia produced on solid

TABLE 2. Optimal conditions for the production and accumulation of specific polyols and trehalose in conidia of *B. bassiana*, *M. anisopliae*, and *P. farinosus*

Isolate	Compatible solute produced (mg g of conidia^{-1})	Optimal conditions (range of values)[a]					
		Culture age (days) (7–102 or –120)	Temp (°C) (5–30 or –35)	pH (2.9–9.4)	Carbohydrate type[b]	Carbohydrate concn (g of added carbon liter^{-1}) (3– or 16–55, –95, or –143)[c]	a_w[d] (0.938–0.998)
B. bassiana 206	Glycerol				Glycerol	55	
	Erythritol	25–102		4–8.6	Glycerol	55	0.938–0.998
	Arabitol			2.9			
	Mannitol	7–25	12–30	5.5–9.4	Any	32–44	0.938–0.998
	Trehalose	25–40	12–30	6.8–9.4	Trehalose	95–143	0.97–0.998
M. anisopliae V90	Glycerol	15	5–12		Glycerol	44–55	0.938
	Erythritol				Glycerol	44–55	0.938–0.973
	Arabitol				Glucose, trehalose	55–95, 95–143[e]	0.97–0.988
	Mannitol	10–120	12–35	4.9–9.4	Any		0.938–0.998
	Trehalose		35		Trehalose + KCl[f]	143	0.998
P. farinosus V51	Glycerol	7–10		2.9	Glycerol	55–95	0.938–0.961
	Erythritol	7–25	12–22	2.9–9.4	Glycerol	32–95	0.938–0.961
	Arabitol			8.6–9.4	Glucose	95–143	0.938–0.97
	Mannitol	7–25	15–25	4.9–5.3	Any		0.938–0.998
	Trehalose	7–40	22–25	4.9–9.4	Trehalose	143	0.982–0.998

[a] The conditions that led to outstanding accumulations of specific compatible solutes are underlined. Optimal carbohydrate concentrations are only applicable for the corresponding carbohydrate types listed.
[b] See reference 31. Carbohydrates included glycerol, glucose, trehalose, and starch.
[c] See reference 31.
[d] Modified with polyethylene glycol 600 or KCl (28, 30).
[e] These ranges of values correspond to media containing glucose and trehalose, respectively.
[f] See reference 32.

media in vitro, on solid substrates in industrial fermentations, or on the insect surface. Mycelia, blastospores, and submerged conidia that are produced in liquid media in vitro, in submerged fermentations in industry, and in the insect hemolymph may differ physiologically from aerial conidia. This said, the response of fungal cells to environmental stresses (especially those that, directly or otherwise, cause water stress) is highly conserved. Any physiological differences between aerial conidia and submerged cells are therefore likely to relate to the state of desiccation of the former and the enhanced metabolic activity of the latter.

Conclusions. Despite the ongoing research effort to improve the efficacy of biocontrol fungi, there has been surprisingly little information available about compatible solute accumulation. Heale (35) reviewed the potential impact of molecular biology on insect control by entomopathogenic fungi. Although Heale stated that biocontrol is "strictly limited by adverse low humidity," no indication was given about how fungal germination and growth might be improved at reduced water availability. The lack of understanding of the polyol physiology of propagules has prevented the application of molecular techniques to the improvement of compatible solute production and osmotic adjustment.

A recent review of the impact of biotechnology on entomopathogens was mainly concerned with the biochemistry and molecular biology of cuticle-degrading enzymes (36). Such enzymes have been associated with host specificity but do not normally limit infection rate. By contrast, manipulation of intracellular carbohydrates can enhance infection rate. Compatible solute accumulation can be enhanced by varying incubation period, temperature, and pH (present study) as well as a_w, carbohydrate type, and carbohydrate concentration (30–32) (Table 2). In addition, O_2 supply was shown to have a significant impact on carbohydrate flux through the pentose phosphate pathway (leading to erythritol production, for example) and glycolysis (leading to glycerol production) in *Aspergillus nidulans* (18). A recent report by Prenerová (52) showed that blastospores of *P. farinosus* germinated more quickly and were more pathogenic if pretreated by aeration while suspended in nutrient solution. Blastospores may synthesize polyols under these conditions, but the carbohydrate content of these propagules was not investigated. Culture oxygenation is an area that may therefore warrant further investigation.

The marked accumulation of glycerol and erythritol in *P. farinosus* conidia at low pH may be a response to pH-induced water stress. At low pH, DNA and regulatory proteins may undergo conformational changes that determine gene expression. None of the treatments in this study led to outstanding accumulations of polyols or trehalose compared with those of earlier reports (31, 32) (Table 2). For example, the maximum glycerol and trehalose concentrations were only 35 and 5%, respectively, of some of those obtained elsewhere (32). However, some culture conditions led to an apparent inhibition of polyol and trehalose synthesis or accumulation. That culture age and environmental conditions affect the physiological quality of conidia has important implications for the production of propagules for purposes such as biocontrol, soil inoculation, and cryopreservation.

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