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Short communication

Influence of sucrose, mucin and xanthan gum on spore germination of ten different fungi

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Abstract

Germination of conidia, uredospores and basidiospores of ten diverse fungi were compared in sterile distilled water, 0.5% sucrose, 0.5% mucin and 0.5% xanthan gum. All three compounds generally increased spore germination on cellophane membranes compared to water, but mucin was the most effective. Mucin stimulated significantly more germination than sucrose for 7 of the 10 fungi, and only *Aspergillus niger* and *Colletotrichum gloeosporioides* showed greater spore germination in sucrose than in mucin. Sucrose had no effect on uredospore or basidiospore germination, but mucin stimulated germination of these spores. Mucin was more effective than xanthan gum in increasing spore germination for 9 of the 10 fungi. Animal mucin is chemically similar to the natural spore matrix of some fungi and may provide some of the benefits of the spore matrix without the drawback of possible germination inhibitors. Animal mucin may be a useful substitute for the spore matrix in inoculations of a variety of fungi.

Spores of many different fungi are produced in a water-soluble mucilaginous matrix. The conidial matrix is believed to play a role in the survival, differentiation, growth and pathogenicity of fungi. The importance of the spore matrix has been demonstrated in many fungi. The matrix protects spores from the damaging effects of freezing, UV radiation, fluctuating relative humidity and dessication (Louis and Cooke, 1985a; Louis et al., 1988; Nicholson and Moraes, 1980). The spore matrix can also promote germination and infection by sequestering toxic host phenols, adhering spores to the host surface and preventing excessive loss of ions, nutrients and other physiological molecules (Beckett et al., 1990; Braun and Howard, 1994; Moloshok et al., 1993; Nicholson et al., 1986). However, the spore matrix can also reduce spore germination because of the presence of germination inhibitors (Leite and Nicholson, 1992; Mondal and Parbery, 1992; Sparace et al., 1991).

The composition of the water soluble spore matrix has been examined for only a limited number of fungi. The spore matrix contains several proteins including enzymes, such as invertase, protease, cellulase and esterase (Louis and Cooke, 1985b; Porter, 1969). However, the major component of the matrix is composed of highly glycosylated glycoproteins (Chaubal et al., 1991; Moloshok et al., 1994; Ramadoss et al., 1985). For *Collectotrichum graminicola*, the spore matrix was analyzed and found to have an amino acid composition and carbohydrate percentage similar to that of animal mucins (Ramadoss et al., 1985).

Although the protective effect of the spore matrix is well established, conidia produced in the laboratory for infection studies are usually washed and/or diluted in water or a dilute buffer solution. This not only removes any germination inhibitors in the spore matrix but also removes the many beneficial effects provided by the matrix. This could reduce the germinability of the spores, thus requiring more spores than necessary to obtain adequate infection, particularly under conditions where spores can be exposed to a fairly non-conducive environment, such as in the field. It would therefore be desirable in many circumstances to create

an artificial spore matrix which could provide the benefits of the natural spore matrix for germination without the drawback of germination inhibitors.

The purpose of this research was to compare the relative effects of two alternative spore matrix materials on spore germination. One alternative is animal mucin, a heterologous group of highly glycosylated glycoproteins, which form the major structural component of the mucus gel (Toribara et al., 1993) and is chemically similar to fungal spore glycoprotein (Ramadoss et al., 1985). Mucin provides animals with protection and water-binding capacity (Toribara et al., 1993). Another compound which may be suitable as a spore matrix substitute is xanthan gum, an extracellular polysaccharide of the plant pathogenic bacterium, *Xanthomonas campestris* (Kennedy and Bradshaw, 1984). This compound is produced commercially and has very good gelling and antidessicant properties (Kennedy and Bradshaw, 1984; Wilson et al., 1965). It has been shown that 0.5% xanthan gum can promote the effectiveness of a mycoherbicide against its weed host (Cardina, 1986). In this study, mucin and xanthan gum were compared to water and sucrose for their effects on spore germination of fungi from very diverse groups.

Fungal isolates used in this study are listed in Table 1. Except for *P. pelargonii-zonalis* and *H. annosum*, the fungi were grown on potato dextrose media with 3% agar (PDA) for 4–5 days. Plates were flooded with 10 mL of sterile distilled water and gently scraped with a sterile glass rod to dislodge the spores. The cultures were filtered through sterile cheesecloth to remove mycelial fragments. The filtered spores were centrifuged at 600 rpm for 20 min, the supernatant decanted and the spore sediment was washed twice with sterile distilled water. The washed spores were suspended in 1 mL sterile distilled water and the concentrations determined using a hemocytometer. Uredospores of *P. pelargonii-zonalis* were scraped from infected geranium leaves (*Pelargonium* x *hortorum*), and then washed and counted as described above. Basidiospores of *H. annosum* were collected from fruiting bodies produced in cultures grown *in vitro* on pine stem sections according to Chase and Ullrich (1985) and then washed and counted as described above.

Spores were suspended in either sterile distilled water, 0.5% mucin type III partially purified from porcine stomach (Sigma, St. Louis, MO), 0.5% xanthan gum Keltrol T (Merck and Co., Chicago, IL) or 0.5% sucrose to a final concentration of 10^6 conidia mL^{-1}. A 10 μL droplet of spore suspension was spotted onto a sterile cellophane membrane placed on a glass slide coated with 1.5% water agar. Four droplets were placed equidistant on a glass slide on four separate pieces of cellophane membrane, and the slides were incubated at 25°C in a humid chamber for the times listed in Table 1. Following incubation, the spores were stained in a solution of cotton blue in lactophenol which prevented further germ-tube growth (Dhingra and Sinclair, 1995). The cellophane membrane was mounted onto a slide and the number of conidia in four randomly chosen microscope fields ($\times$400 power) were counted on each of the four cellophane pieces for each fungus. The percentage of germinated conidia was determined. A conidium was defined as having germinated if the germ-tube was at least as long as the width of the conidium. Four microscope fields comprised one replication, and there were three replications per experiment. Each experiment was repeated once. Because no significant differences were found between the replications of each experiment (Barltett's test, $P = 0.05$), the data were combined for analysis of variance (ANOVA) using the ANOVA procedure of the Statistical Analysis System (SAS Institute, Cary, NC).

Table 1. Species of fungi and host origin of isolates used in the spore germination study

Species	Host	Incubation time (h)[a]
A. niger	Onion	22
B. cinerea	Strawberry	6
C. gloeosporioides	Mallow	8
F. oxysporum	Ginseng	9
G. roseum	Strawberry	22
H. annosum	Pine	22
L. maculans	Canola	22
T. roseum	Rose	8
V. dahliae	Potato	13
P. pelargonii-zonalis	Geranium	6

[a] Time required for at least 40% of germ tubes to grow a distance at least as great as the width of the spore at 25°C.

Preliminary experiments indicated that 0.5% (w/v) mucin significantly increased conidial germination of *C. graminicola* (data not shown), and this amount was adopted for all of the fungi tested in this study. For comparison, sucrose and xanthan gum were used at the same concentration. Experiments were also conducted to determine the time required for each fungus to have 40% or more of the spores produce a germ-tube in water that was approximately as long as the width of the spore (Table 1).

Table 2. Relative effectiveness ranking of water, sucrose, xanthan and mucin on germination of ten fungi

Species	Percent germination			
	Water	Sucrose	Mucin	Xanthan
A. niger	43.4 ± 1.04[a]	90.2 ± 0.75	69.9 ± 1.23	47.0 ± 1.17
B. cinerea	70.0 ± 0.85	94.1 ± 0.68	95.2 ± 0.65	92.3 ± 0.71
C. gloeosporioides	63.1 ± 0.89	95.0 ± 0.64	83.8 ± 1.02	62.9 ± 1.05
F. oxysporum	60.1 ± 0.97	66.2 ± 0.95	81.9 ± 1.01	75.1 ± 0.86
G. roseum	68.6 ± 0.37	81.7 ± 0.58	95.6 ± 0.43	80.3 ± 0.50
H. annosum	59.2 ± 0.78	59.6 ± 0.67	74.1 ± 2.67	72.4 ± 1.68
L. maculans	67.4 ± 0.68	67.4 ± 0.95	97.2 ± 0.33	81.2 ± 0.79
T. roseum	62.0 ± 1.26	92.7 ± 0.81	97.6 ± 0.65	96.1 ± 0.71
V. dahlii	63.6 ± 0.78	62.0 ± 0.47	92.7 ± 0.34	74.1 ± 0.42
P. polygoni-zonalis	65.8 ± 0.95	65.6 ± 1.14	95.9 ± 0.39	80.6 ± 0.72

[a] Each value represents the mean of two experiments with three replicates per experiment followed by the standard error.

Ten different fungi were tested for spore germination in water, sucrose, mucin and xanthan gum (Table 2). The spores examined were conidia from eight Ascomycetes/Deuteromycetes, and uredospores or basidiospores from two Basidiomycetes. Germination of the ten fungi ranged from 43 to 68% in water, 62 to 95% in sucrose, 70 to 97% in mucin and 47 to 96% in xanthan gum (Table 2). In seven species, germination was best in mucin, in two species, *A. niger* and *C. gloeosporioides*, germination was best in sucrose, and in *B. cinerea*, germination was best in mucin but this was not significantly different from that in sucrose. Germination in mucin was always better than that in water. Xanthan gum gave the second best result in six species, *F. oxysporum*, *H. annosum*, *L. maculans*, *T. roseum*, *V. dahliae* and *P. polygoni-zonalis*, but was better than water for all ten fungi except *C. gloeosporioides*. In two species, *B. cinerea* and *T. roseum*, germination was similar in xanthan and mucin. Germination in sucrose was equal to or slightly less than that in water for *H. annosum*, *L. maculans*, *V. dahliae* and *P. pelargonii-zonalis* but was better than that in water for the other six species. The pH of water was not affected by the addition of either xanthan gum or sucrose. However, mucin did lower the pH of water by 0.37 pH unit, which could have affected spore germination.

The germination of a fungal spore is a complex process involving spore swelling and then emergence and growth of the germ-tube. Associated with this are many changes including an increase in respiration and protein and nucleic acid biosynthesis, and it is possible that many factors could alter these processes and thus affect spore germination (Griffin, 1994). The three substances tested in this study generally increased spore germination for the fungi tested compared to distilled water. Of the three materials, spore germination was generally best in mucin. If the effect of the materials was to provide only a carbon source to the developing germ-tube, then one would expect that sucrose would have a similar effect on spore germination as the two polymers. For some fungi, sucrose did have an equal or greater effect on spore germination, but mucin was more effective for 7 of the 10 fungi examined. For the two Basidiomycetes, *P. pelargonii-zonalis* and *H. annosum*, sucrose had no significant effect on spore germination, whereas mucin greatly stimulated both fungi. Their spores may lack sufficient invertase activity to utilize the added sucrose for germination. Unlike conidia, uredospores and basidiospores are considered to be self-sufficient in nutritional requirements for germination (Bartnicki-Garcia, 1984). If the increase in spore germination due to mucin was related solely to the polymer nature of the compounds, then one would expect that the effect of mucin and xanthan gum on spore germination would be similar. However, spore germination was greater in mucin than in xanthan gum for all the fungi tested except for *H. annosum*, where there was no significant difference between the two adjuvants. It appears that mucin can provide certain spores with unknown factors or an environment that can enhance their germination. Mucin could affect one or many different processes involved in spore germination, for example, reducing the loss of nutrients and ions from germinating spores. Mucin has also been used in

growth room studies for plant inoculations with conidia of *L. maculans*, and it has been found to be very beneficial in improving the adhesion of spores to the plant surface and increasing the chances for infection (Mahuku, unpublished).

Further research is needed to understand how an animal glycoprotein enhances the germination of plant pathogenic and saprophytic fungi; however, the similar chemical composition of mucin and the natural mucilage of fungal spores provides a starting point for such an investigation.

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