Trehalose and Polyols as Carbon Sources for Verticillium spp.

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ABSTRACT

An isolate of *Verticillium albo-atrum* and *V. dahliae* was grown on synthetic liquid medium containing trehalose and polyols as the carbon sources. Periodic determination of the mycelial dry weight showed that trehalose and glycerol were the best sources. *Verticillium albo-atrum* grew better than *V. dahliae* on D-mannitol, D-glucitol, and ribitol. Good growth following a long lag period occurred with erythritol. Both species grew poorly on galactitol, D-xylitol, D- and L-arabitol, and *i*-inositol. *Verticillium dahliae* produced few or no microsclerotia on D-mannitol, D-glucitol, and erythritol. Phytopathology 61:339-340.

Paper chromatographic analyses of culture filtrates and mycelia of *Verticillium* spp. grown on a medium containing 15-30 g of glucose/liter (4) showed the presence of trehalose, mannitol, a pentitol, a tetritol, and several unknowns (Le Tourneau, *unpublished data*). The isolation and further characterization of these compounds is in progress. This study was made to determine if *V. dahliae* (microsclerotial form) and *V. albo-atrum* (dark mycelium form) could utilize trehalose or polyols as a sole carbon source and also form characteristic reproductive structures. The two species were obtained from Ross Watson, Department of Plant Science, University of Idaho, who in turn received them from Ivor Isaac, University College of Swansea, Wales.

Trehalose and the polyols were added to the basal medium of Malca et al. (5) equivalent to the carbon present in 10 g of D-glucose, which was used for comparison. Media were sterilized by filtration through Millipore filters (Type HA, 0.45 μ), dispensed 10 ml/50 ml Erlenmeyer flask and stoppered with cotton or plastic foam. The media were seeded with 1 ml of spore suspension prepared by growing the fungi in glucose-salts (4) medium for 7 days, then separating the spores (5). After incubation for varying periods at 25 C in the dark, 3 flasks of each treatment were harvested by centrifugation for 10 min at 12,000 *g*. The washed mycelium was then transferred to tared aluminum dishes, dried to constant wt, and weighed. Dry mycelial wt are reported as the average per flask at each harvest date.

Results are summarized in Tables 1 and 2. Dry wt are comparable to those of Malca et al. (5), even though we used 4 g of carbon/liter as opposed to their 10 g of carbon/liter. This fact may be explained by the greater surface area for growth and the shallow medium in Erlenmeyer flasks. On 10 g of carbon/liter, we obtained more growth, but the qualitative pattern was similar. Trehalose and glycerol were the best carbon sources and equivalent to D-glucose, a good carbon source for *Verticillium* spp. (2, 5). The isolate of *V. albo-atrum* consistently had a higher dry wt than the isolate of *V. dahliae*. *Verticillium albo-atrum* grew well on D-mannitol and produced considerable growth on D-glucitol and ribitol after a lag period. *Verticillium dahliae* grew poorly or not at all on these three polyols. Considerable growth with a long lag period occurred with erythritol. Both species produced wispy strands of visible growth but a negligible amount of dry wt when galactitol, D-xylitol, D-arabitol, L-arabitol, or the cyclitol, *i*-inositol, served as the carbon source. These results with two isolates indicate that *V. albo-atrum* may be able to utilize a wider variety of polyols than *V. dahliae*. Somewhat similar conclusions were made by Isaac (3), who reported that a dark mycelial type (*V. albo-atrum*) produced max growth on glycerol, while a microsclerotial type (*V. dahliae*) grew poorly. If experiments with a large number of isolates give similar results, the ability to utilize polyols might be useful in taxonomic studies of *Verticillium* spp. as with yeasts (1).

In addition to the effect on growth, the isolate of *V. dahliae* produced few or no microsclerotia on D-mannitol, D-glucitol, and erythritol media containing 4 or 10 g of carbon/liter. On the other media, black microsclerotia were visible after 7 days on both levels of carbon. Microsclerotia were produced in 7 days when *V. dahliae* was grown on a mixture of erythritol (2 g carbon/liter) plus trehalose, glucose, or glycerol (2 g carbon/liter).

TABLE 1. The effect of carbon source on the growth of *Verticillium albo-atrum*

	Days of incubation					
Carbon source	6	8	10	12	14	21
D-glucose	53[a]	97	110	90	70	
Trehalose	37	83	112	94	55	
D-mannitol	3		23	106	112	
D-glucitol	9		17	48	69	84
Ribitol				21	32	75
Erythritol	6		21	44	65	80
Glycerol	54		110	112	107	101

[a] Average dry wt (mg) per flask at each harvest.

TABLE 2. The effect of carbon source on the growth of *Verticillium dahliae*

	Days of incubation					
Carbon source	3	5	7	10	12	19
D-glucose	10[a]	55	78		60	
Trehalose	11	56	71	41	38	
D-mannitol[b]						12
D-glucitol[b]				10		35
Erythritol[b]	4		13	28	49	74
Glycerol	6	56	90	79	78	

[a] Average dry wt (mg) per flask at each harvest.
[b] No microsclerotia visible at time of final harvest.

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