Influence of Carbon Sources, Amino Acids, and Water Potential on Growth and Sporulation of *Fusarium moniliforme*

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ABSTRACT

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The ability of *Fusarium moniliforme*, isolated from corn, to grow and sporulate on several carbon sources and amino acids, and on various salt solutions was tested. Macroconidial production and growth usually were greatest on soluble starch or maltose. Soluble starch was markedly superior to the other carbon sources tested. Microconidia were produced most numerously in 0.4–0.5 M KCl, concentrations too high for optimum growth or macroconidia production.

After Kurosawa (3,4) demonstrated that *Fursarium moniliforme* (Sheld.) Snyd. & Hans. stimulated plant elongation, biochemists and plant pathologists showed great interest in this fungus. Although nutrition and physiology relative to linear growth of the fungus (1,7,8) and production of gibberellins and fusaric acid (5,6) have been studied extensively, reproduction requirements have not had much attention. In a previous publication, we reported the conditions that promote formation of the perfect stage of this fungus (2). This paper reports: (i) factors that differentially promote production of macroconidia, microconidia, or mycelium; (ii) conidial germ tube length; and (iii) production of microconidia in chains.

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MATERIALS AND METHODS

We observed sporulation and growth under various nutritional and water potential regimes with several *F. moniliforme* isolates from corn (*Zea mays* L.). Most of the data presented here was obtained with an isolate (from cob rot of high lysine corn grown in Minnesota) that also was used as a tester in mating experiments.

To test the influence of carbon sources on growth and sporulation, 1% autoclaved solutions of 50 ml of each carbon source, dispensed in 150-ml Erlenmeyer flasks or as 2% agar suspensions in petri plates, were seeded with a single germinated conidium from a water agar plate. Five replicates per treatment were used. Cultures were incubated at room temperature under cool white lights for 9 days or 2 wk. After colony diameter was measured, macroconidial and microconidial numbers were

determined. Colonies were cut out and comminuted with about 150 ml of 0.1% water agar for 2 min. The ratios of macroconidia, microconidia, and mycelial fragments in this blended suspension were determined under the microscope (×400), based on 400 conidia or fragments observed. The blended suspension then was serially diluted and 1 ml of each dilution was pipetted onto Peptone-PCNB agar plates. After 4-day incubation at room temperature, the numbers of macroconidia and microconidia were estimated, based on the total numbers of colonies on the plates.

Mycelial weights produced by the fungus on various carbon sources were obtained by weighing oven-dried Millipore membranes (pore size 0.45 μm) through which flask cultures of the fungus had been filtered after 2-wk incubation of the single conidial cultures.

The influence of 21 amino acids on growth and formation of macroconida and microconidia was determined for isolate M-2a. The autoclaved media (121 C for 15 min) were made with 2% agar plus 0.03% of each amino acid; four replicate plates were used. Single germinated conidia were transferred to each test plate and incubated for 6 days, then examined as described previously for conidial numbers and growth.

Concentrations of $CaCl_2$ from 0.4 to 2.0% in 0.4% increments in 2% water agar were used to evaluate germ tube growth. Conidia were washed off the surface of a potato dextrose agar (PDA) tube with sterilized water. Then 0.5 ml of a diluted spore suspension was spread over the surface of each test plate with a glass rod, four plates per treatment. After 12-hr incubation at room temperature, 0.1% $HgCl_2$ was sprayed over the surface of each test plate to kill all germlings rapidly. Lengths of 200 germ tubes per plate were measured under the microscope.

To determine the production of macroconidia and microconidia and to make linear growth observations, KCl from 0.1–0.8 M was used in 0.1 M increments. Again, single germinated conidia from water agar plates (after 12 hr) were transferred to each of the four replicate plates of each treatment. These plates were incubated for 3 days before the measurements were made.

RESULTS

Of the nine carbon sources tested, soluble starch was the most suitable for the production of macroconidia (Table 1 and 4), followed by maltose; galactose, xylose, and lactose were the least suitable. One isolate (M-29) that rarely produced macroconidia on PDA or in any of the test sugars produced them in moderate amounts on starch agar (Table 4). Furthermore, soluble starch in liquid media promoted better growth than any other C source when measured according to mycelial dry weight (Table 2).

In a comparison of different concentrations of starch and sucrose in relation to growth and sporulation of isolate M-2a, 1% concentrations were suitable for growth and macroconidia production, but microconidial numbers varied considerably (Table 3).

The amino acids tested were DL-alanine, L-arginine HCl, L-asparagine (anhydrous), L-cystine, L-cysteine HCl·H_2O, L-glutamic acid HCl, glycine, L-histidine 2HCl, DL-isoleucine, L-leucine, L-lysine·2HCl, DL-methionine, DL-phenylalanine, L-proline, DL-norleucine, DL-serine, L-threonine, L-tryptophan, L-tyrosine, and DL-valine. None favored the production of macroconidia by isolate M-2a. All amino acids except L-cysteine HCl favored microconidia production. *Fusarium roseum* 'Graminearum,' which is homothallic, was stimulated to produce mature perithecia of *Gibberella rosea* readily on the L-tryptophan and DL-valine agars.

Conidia of M-2a germinated best and produced significantly longer germ tubes (Table 5) in 1.2% $CaCl_2$ solutions (0.108 M) than in the other concentrations. In agar plates that contained various concentrations of KCl (Table 6), production of microconidia increased as the concentration increased from 0.1 M to 0.5 M; production was sharply decreased at 0.7 M and 0.8 M. However, both macroconidial production and colony diameter were largest at the lower concentrations, indicating that different water potentials probably favor different phases of the fungus. The microconidial chains increased as the concentration of KCl was increased from 0.1 M to 0.5 M, but microconidia were only in false heads in the 0.7 and 0.8 M KCl agar. Some microconidial chains were produced in plates containing 0.1 M KCl, but when these plates were incubated in a moist chamber only false heads were seen. Apparently, whether microconidia form chains or false heads depends on the water potential at the time of their production.

DISCUSSION

Although the ability of a fungus to grow on various substrates is important to infection cycles, the ability to produce the various spore states is important for distribution in nature and is of interest to plant pathologists studying the epidemiology of diseases caused by *F. moniliforme*. A previous paper (2) dealt with some of the

TABLE 1. Effect of carbon sources on growth and sporulation of *Fusarium moniliforme* on agar medium for 9 days

Carbon sources	Colony diameter (mm)	Number of macroconidia[a]	Number of microconidia[a]
D (+) xylose	41.2	4.8×10^5	5.0×10^5
D (+) glucose	40.4	5.5×10^5	4.8×10^5
D (+) mannose	40.2	4.5×10^5	3.1×10^5
D (−) fructose	42.9	6.4×10^5	2.8×10^5
Maltose	54.3	1.90×10^6	1.16×10^6
Sucrose	48.4	1.03×10^6	5.1×10^5
Lactose	52.0	6.4×10^5	6.8×10^5
Starch	51.8	3.04×10^6	4.3×10^5
Water agar	49.4	1.74×10^6	8.1×10^5

[a] Indicates number of spores per colony.

TABLE 2. Effect of different carbon sources on mycelial dry weight of *Fusarium moniliforme* grown in liquid media for 2 wk

Carbon source	Mycelial dry weight (mg)
D (+) xylose	2.0
D (+) glucose	4.0
D (+) galactose	3.5
D (−) fructose	4.5
Maltose	6.0
Sucrose	4.5
Starch	27.5[a]

[a] Significantly different from all others at 1% level of significance.

TABLE 3. Effect of different concentrations of sucrose and starch on growth and sporulation of *Fusarium moniliforme* 6 days after inoculation on agar media

Concentration (%)	Sucrose			Starch		
	Colony diameter (mm)	Macroconidia (%)	Microconidia (%)	Colony diameter (mm)	Macroconidia (%)	Microconidia (%)
1	45.4	47.8	33.2	51.8	50.4	33.6
3	49.6	38.7	40.3	51.2	51.8	22.2
5	55.8	24.7	60.4	51.2	49.0	40.1
10	54.2	44.8	38.2	46.0	22.1	70.0

TABLE 4. Effect of carbon source on macroconidia production by different isolates of *Fusarium moniliforme* on agar media

Carbon source	Macroconidia production[a] with isolates of *F. moniliforme*			
	M-13	M-15g	M-29	M-30
D (+) xylose	+	+++	−	+
D (+) galactose	+++	++	−	++
D (+) glucose	+++++	++++	−	++++
D (−) fructose	++++	++++	−	++
Maltose	+++++	+++++	−	+++++
Sucrose	++++	+	−	+++++
Lactose	++	+	−	++
Soluble starch	++++	+++++	++	++++

[a] +++++ = macroconidia produced very abundantly.
++++ = macroconidia produced abundantly.
+++ = moderate numbers of macroconidia produced.
++ = several macroconidia observed (in each microscope field).
+ = very few macroconidia produced.
− = no macroconidia observed.

TABLE 5. Growth of germ tubes of conidia from *Fusarium moniliforme* (M-2a) on water agar containing different concentrations of $CaCl_2$

Treatment	Average length of germ tube (μm)
2% water agar only	12.53 ± 1.47
$CaCl_2$ 0.4% (0.036M)	13.02 ± 1.40
$CaCl_2$ 0.8% (0.072M)	13.58 ± 0.42
$CaCl_2$ 1.2% (0.108M)	16.94[a] ± 2.38
$CaCl_2$ 1.6% (0.144M)	13.86 ± 0.84
$CaCl_2$ 2.0% (0.180M)	11.20 ± 0.70

[a] Significantly different from all other means at the 1% level.

conditions necessary for the production of the perfect stage of this heterothallic plant pathogen. Both spore states are important in fungus distribution; plant pathologists use the asexual spores for identification of the pathogen in culture. An isolate from nature, such as M-29, which so rarely produces macroconidia on common substrates used in laboratory culture, could be mistaken for another genus (such as *Cephalosporium*). When placed on soluble starch agar, however, it produced moderate numbers of macroconidia.

Production of microconidia in chains is also a taxonomic feature of this species. We observed previously (2) that often *F. moniliforme* isolates formed microconidia in chains only during the first few days after isolation. Later and in subcultures, only false heads were observed. In this work we found that whether microconidia formed in chains or in false heads depended on water potential. These observations have a bearing on whether the isolates fall into the cultivar Subglutinans. In general, available water favored the production of macroconidia, and microconidia were formed in false heads, but when water was less available,

TABLE 6. Effect of different KCl concentrations on growth and production of *Fusarium moniliforme* (M-2a)[a]

Treatment	Colony diameter (mm)	Relative abundance of	
		Microconidia[b]	Macroconidia[b]
0.1 M KCl	3.2	+++	+++++
0.2 M KCl	3.3	+++	+++++
0.3 M KCl	2.7	++++	+++
0.4 M KCl	2.3	+++++	+++
0.5 M KCl	2.2	+++++	++
0.6 M KCl	2.2	+++	++
0.7 M KCl	1.7	++	−
0.8 M KCl	1.7	++	−

[a] Microconidia only formed in false heads at concentrations of KCl at 0.7 and 0.8 M and formed abundantly in chains at 0.4 and 0.5 M.
[b] +++++ = conidia produced abundantly.
++++ = many conidia produced.
+++ = conidia produced in moderate numbers.
++ = several conidia observed.
+ = few conidia produced.
− = no conidia observed.

microconidia were borne in chains on aerial conidiophores. Macroconidia, on the other hand, were formed close to the substrate. This phenomenon could be important for the natural dispersal of the fungus. Microconidia in chains probably are more easily airborne than those in false heads, in which the microconidia usually adhere in a moisture film. Macroconidia usually are distributed in water.

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