Sporulation in Bipolaris maydis: Enhancement by Xylose

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ABSTRACT

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Xylose (1.0-10.0 g/liter) stimulates sporulation, but not mycelial growth, of *Bipolaris maydis* when added as a supplement to a basal agar medium containing glucose. Sporulation on a xylose-supplemented medium was significantly higher than on a nonsupplemented control medium, whether L-asparagine or $NaNO_3$ was used as the nitrogen source or thiamine-HCl and trace elements were added. Sporulation, but not mycelial growth, was significantly higher when the nitrogen source was L-asparagine than when it was $NaNO_3$. With L-asparagine or $NaNO_3$, sporulation, but not mycelial growth, was significantly reduced by 0.1 or 1.0 mg/liter thiamine-HCl. Stimulation of sporulation in *B. maydis* by xylose and inhibition of sporulation by thiamine-HCl may provide clues to key physiological mechanisms controlling sporulation.

Additional key words: Nutrition, thiamine, Vitamin B_1, trace elements, reproduction.

The importance of asexual reproduction in the disease cycle of *Bipolaris maydis* Nisik. & Shoemaker *(Helminthosporium maydis* Nisik. & Miyake, *Cochliobolus heterostrophus* Drechs.) has provided the impetus for investigations into specific nutritional and environmental factors affecting sporulation (1, 6, 8, 14, 15, 19, 20). For several years we have been investigating nutritional factors affecting sporulation in *B. maydis* race T (6, 8), based on the assumption that precise nutritional investigations will provide clues to physiological and biochemical mechanisms controlling sporulation. Recent work with thiamine-HCl (5, 6) and with trans-cinnamic and *p*-hydroxy cinnamic acids (9) has suggested some of these mechanisms. Also, results of precise nutritional investigations of sporulation in *B. maydis* are potentially useful to those involved in critical investigations relating to physiology of pathogenesis (2, 4) and to taxonomy (12).

Recently, we evaluated the effect of several carbohydrates on sporulation in *B. maydis* race T and observed that xylose enhanced sporulation either when supplied as the main carbon source or as a supplement to a basal medium containing glucose. To verify this observation, we added xylose as a supplement to various synthetic media. This report summarizes the results of this study.

MATERIALS AND METHODS

A single-spored isolate of *B. maydis* race T, described previously (6), was used in this study. The basal medium (pH 5.5), designated as "Gx medium", used for routine culturing of the fungus and for preparation of inoculum, contained 20.0 g Difco Bacto agar, 10.0 g D(+) glucose, 2.0 g D(+)-xylose, 4.0 g L-asparagine, 1.5 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7H_2O$, and 0.1 mg each of $CuSO_4$, $MnSO_4$, $ZnSO_4$, $Fe_2(SO_4)_3$ and $NaMoO_4$ per liter of double-distilled water.

Sporulation and mycelial growth on Gx medium were compared with that on media which varied in carbohydrate regime, nitrogen source, thiamine concentration, or trace element concentration. This comparison permitted an evaluation of the stimulatory effect of xylose when included in media with various concentrations of key constituents. Carbohydrate regimes included glucose (10.0 or 20.0 g/liter); xylose (10.0 or 20.0 g/liter); glucose (10.0 g/liter) supplemented with xylose (1.0, 2.0, 5.0, or 10.0 g/liter); or xylose (10.0 g/liter) supplemented with glucose (1.0, 2.0, 5.0, or 10.0 g/liter). Nitrogen sources (1.0 g N/liter) were L-asparagine or $NaNO_3$. Trace element concentrations were 0.0, 0.1, or 1.0 mg/liter each of $CuSO_4$, $MnSO_4$, $ZnSO_4$, $Fe_2(SO_4)_3$ and $NaMoO_4$. Thiamine-HCl concentrations were 0.0, 0.1, or 1.0 mg/liter.

Inoculum for seeding test media consisted of 6-mm diameter agar disks taken from fungal cultures incubated for 7 days in the dark at 28 C. The disks were removed from a 2.0-cm sampling zone which was between 1.5 cm and 3.5 cm from the center of the agar plate. Fungal cultures were incubated for 7 days in the dark at 28 C in petri dishes (100 × 15 cm) containing 20.0 ml of media. After 7 days the fungus had grown to within 1.0 cm of the edge of the medium in the petri dish. To measure the quantity of sporulation on a given medium, three 12-mm diameter disks of agar with fungus were removed from the

2.0-cm sampling zone, placed in a Clorox-ethanol-NaOH solution, and the number of conidia were estimated as described previously (6). To determine fungal dry weights, six 12-mm diameter disks were removed from the 2.0-cm sampling zone and then the mycelia were separated by filtration from the agar by melting and washing in hot distilled water. Each experiment was done at least three times with five replications per treatment.

RESULTS

Xylose stimulation of sporulation.—Sporulation of *B. maydis* was enhanced by xylose added as a supplement to a basal medium containing glucose (Table 1). There was a significant enhancement of sporulation at all concentrations of xylose supplement used. The magnitude of the increment in sporulation induced by xylose supplement was comparable at all concentrations in excess of 2.0 g/liter.

The increase in sporulation which accompanied the addition of xylose to a basal glucose medium was greater than that expected from the added carbon. For example, sporulation increased from 74,000 to 118,000 conidia/mg dry wt (Table 1) when the concentration of glucose in the basal medium was increased from 10.0 g/liter to 20.0 g/liter. In contrast, sporulation increased from 74,000 to 349,000 conidia/mg dry wt when 10.0 g/liter of xylose was added as a supplement to a basal medium containing 10.0 g/liter glucose.

The amount of sporulation on a basal medium

TABLE 1. Sporulation and mycelial growth of *Bipolaris maydis* after 7 days of incubation on glucose L-asparagine media supplemented with various concentrations of xylose, compared with that on xylose-L-asparagine media supplemented with various concentrations of glucose[a]

Carbon compound and conc (gm/liter)[a]		Sporulation: (Conidia/mg dry wt × 1,000)[b]	Mycelial growth: (mg dry wt)[c]
Glucose	Xylose		
10.0	0.0	74 ± 9	39 ± 4
20.0	0.0	118 ± 15	43 ± 7
10.0	1.0	222 ± 25	42 ± 4
10.0	2.0	286 ± 32	44 ± 6
10.0	5.0	271 ± 22	43 ± 8
10.0	10.0	349 ± 61	45 ± 4
0.0	10.0	287 ± 35	26 ± 5
0.0	20.0	291 ± 31	35 ± 4
1.0	10.0	290 ± 37	35 ± 10
2.0	10.0	282 ± 29	37 ± 6
5.0	10.0	272 ± 9	38 ± 8
10.0	10.0	312 ± 24	43 ± 5

[a]Initial pH of media, 5.5. Final pH, 5.7 ± 0.3 on media with xylose and 6.3 ± 0.3 on media lacking xylose. Inoculum was grown for 7 days on a medium containing glucose (10 gm/liter) and xylose (2 gm/liter).

[b]Each value is the mean of six replications with the mean deviation indicated.

[c]Each value represents the total mycelial dry weight from twelve 12-mm diameter disks selected randomly from locations on the agar plate containing mycelia and spores of the fungus 7 days following seeding. Mean of six replications; mean deviation is indicated.

TABLE 2. Influence of the type of carbohydrate in media for preparing inoculum source on subsequent sporulation and mycelial growth of *Bipolaris maydis* seeded to media containing either glucose, xylose, or glucose supplemented with xylose

Carbohydrate and conc (gm/liter)					
Growth medium[a]		Inoculum source medium[b]		Sporulation (Conidia/mg dry wt × 1,000)[c]	Mycelial growth (mg dry wt)[d]
Glucose	Xylose	Glucose	Xylose		
10.0	0.0	10.0	0.0	100 ± 14	53 ± 6
10.0	0.0	10.0	2.0	89 ± 23	47 ± 4
10.0	0.0	0.0	10.0	76 ± 21	51 ± 7
10.0	2.0	10.0	0.0	190 ± 25	52 ± 5
10.0	2.0	10.0	2.0	309 ± 35	55 ± 7
10.0	2.0	0.0	10.0	174 ± 22	54 ± 5
0.0	10.0	10.0	0.0	317 ± 43	37 ± 5
0.0	10.0	10.0	2.0	348 ± 50	36 ± 7
0.0	10.0	0.0	10.0	294 ± 38	42 ± 6

[a]Initial pH of growth media, 5.5. Final pH, 5.7 ± 0.3 on media with xylose and 6.3 ± 0.3 on media lacking xylose.

[b]Cultures of *B. maydis* were grown for two successive 7-day cycles at 28 C in the dark on a medium containing glucose (10 gm/liter) and xylose (2 gm/liter), followed by one 7-day cycle on the inoculum source medium, prior to use for seeding the various growth media.

[c]Each value is the mean of six replications with mean deviation indicated.

[d]Each value represents the total mycelial dry wt from 12-mm diameter disks selected randomly from locations on the agar plate containing mycelia and spores of the fungus 7 days following seeding. Mean of six replications; mean deviation is indicated.

containing xylose alone was comparable to that on a glucose medium supplemented with 2.0 g/liter or more of xylose. The amount of sporulation was not significantly altered when a basal medium containing xylose was supplemented with various concentrations of glucose ranging from 1.0 to 10.0 g/liter (Table 1).

Mycelial growth on a basal medium containing glucose was comparable to that on one supplemented with xylose (Table 1). Also, mycelial growth on media containing 20.0 g/liter xylose or on xylose media containing 1.0, 2.0, 5.0, or 10.0 g/liter glucose was comparable to that on a glucose medium. But mycelial growth on a medium containing 10.0 g/liter xylose only was significantly less than on a medium containing 10.0 g/liter glucose (Table 1).

Source of inoculum in relation to xylose stimulation of sporulation.—Since the carbohydrate regime had a profound effect on the sporulation of *B. maydis*, the possibility existed that the carbohydrate regime used in preparing the source of inoculum for seeding experimental media might have an effect as well. To test this hypothesis, the fungus was cultured for two consecutive 7-day cycles on a glucose (10.0 g/liter) medium, then transferred to a glucose medium, a Gx medium, or a xylose (10.0 g/liter) medium. After 7 days of incubation at 28 C in the dark, these media were used as a source of inoculum for seeding experimental glucose, Gx, or xylose media (Table 2).

The magnitude of sporulation on a glucose medium supplemented with xylose was significantly higher when the inoculum source medium contained glucose supplemented with xylose than when it contained either glucose alone or xylose alone (Table 2). Sporulation on xylose and glucose media was not significantly affected by the type of carbohydrate in the inoculum source medium. Also, mycelial growth was insensitive to the type of carbohydrate used in the inoculum source medium.

Nitrogen source, and thiamine and trace element concentration in relation to xylose stimulation of sporulation.—For this study, $NaNO_3$ was compared with L-asparagine because the changes in pH of the medium which accompany the utilization of $NaNO_3$ by *B. maydis* are comparable to those which accompany the utilization of L-asparagine. Consequently, this permitted a comparison of the effect of an organic and an inorganic nitrogen source under similar pH conditions (Table 3). The thiamine-HCl concentrations (0.1 and 1.0 mg/liter) used previously were found to inhibit growth and sporulation and to alter metabolism of the fungus (5, 6). For tests with trace elements, an attempt was made to determine whether the stimulatory effect of xylose would be expressed in the absence of trace elements or in the presence of a trace element concentration (1.0 mg/liter) which was 10 times greater than that used in the Gx medium.

Sporulation, but not mycelial growth, was enhanced by a xylose supplement when $NaNO_3$ was used as a nitrogen source instead of L-asparagine (Table 3). On either nitrogen source, thiamine-HCl (0.1 and 1.0 mg/liter) caused a significant reduction in sporulation. In the presence or absence of thiamine-HCl, the amount of sporulation was less on $NaNO_3$ media than on comparable L-asparagine media. Thiamine had no significant effect on mycelial growth.

The stimulatory effect of a xylose supplement was also obtained on a basal glucose medium lacking trace elements. Sporulation was 3,000, 32,000, and 39,000 conidia/mg dry wt, respectively, on a basal glucose medium with 0.0, 0.1, and 1.0 mg/liter of a trace element mixture. The magnitude of sporulation on comparable media supplemented with xylose was 8,000, 72,000, and 79,000 conidia/mg dry wt, respectively.

DISCUSSION

Enhancement of sporulation in *B. maydis* race T by xylose added as a supplement to a glucose-containing medium appeared to be independent of the type of nitrogen source, the trace element concentration, or the thiamine-HCl concentration. Also, xylose was a more effective carbon source for growth and sporulation than was glucose. Pathogenicity and virulence of *B. maydis* race T were not altered detectably following repeated transfers to media containing xylose (M. O. Garraway, *unpublished*). Therefore, media used in the foregoing study should be useful to others investigating the biology and physiology of this pathogen.

Since xylans are a major constituent of corn cell walls (2, 16) and *B. maydis* race T produces significant amounts of xylanase (2), it can be assumed that xylose is released and available to the fungus from walls of infected corn tissues. Recently, Carter (3) found a significantly greater

TABLE 3. Enhancement of sporulation in *Bipolaris maydis* by xylose added as a supplement to media with either L-asparagine or $NaNO_3$ and with various concentrations of thiamine

Nitrogen source[a]	Thiamine conc (mg/liter)	Sporulation (conidia/mg dry wt × 1,000)[b]		Mycelial growth (mg dry wt)[bc]	
		Without xylose	With xylose	Without xylose	With xylose
L-asparagine	0.0	68 ± 12	157 ± 20	37 ± 5	40 ± 6
	0.1	34 ± 10	87 ± 14	38 ± 4	42 ± 5
	1.0	38 ± 6	90 ± 16	36 ± 6	41 ± 5
$NaNO_3$	0.0	20 ± 7	32 ± 4	28 ± 3	35 ± 4
	0.1	7 ± 3	18 ± 4	31 ± 5	35 ± 5
	1.0	6 ± 2	19 ± 5	34 ± 3	32 ± 5

[a]Initial pH of medium, 5.5. Final pH on L-asparagine-containing media was 5.7 ± 0.3 with xylose and 6.3 ± 0.3 without. Final pH on $NaNO_3$-containing media was 6.7 ± 0.2 with or without xylose.

[b]Each value is the mean of five replications with mean deviation indicated.

[c]Each value represents the total mycelial dry wt from twelve 12-mm diameter disks selected randomly from locations on the agar plate containing mycelia and spores of the fungus 7 days following seeding. Mean of five replications; mean deviation is indicated.

activity of polygalacturonase in extracts of infected leaves with Texas male-sterile cytoplasm, 1, 2, and 3 days following inoculation, than in extracts of infected leaves with normal cytoplasm. Also, sporulation on infected leaves of Texas male-sterile cytoplasm corn cultivars is significantly greater 3 days following inoculation than on infected leaves of normal-cytoplasm corn cultivars (17, 18, 21). In view of our demonstration of sporulation enhancement by xylose, Carter's observations together with those of Bateman et al. (2) could mean that cell-wall-degrading enzymes contribute indirectly to the relatively high level of sporulation seen on the Texas male-sterile cytoplasm cultivars. Obviously, differences in leakage of electrolytes (7) and nutrients (8, 11) from infected leaves of either cultivar contribute to differences in the amount of sporulation.

It is inferred from this study that xylose may have a regulatory effect on some process(es) in *B. maydis* race T that determines the amount of sporulation. Since xylose does not stimulate mycelial growth, the xylose effect may be relatively selective. Clues to the mechanism(s) by which xylose promotes sporulation may come from recent studies with thiamine (5) and with cinnamic acid derivatives (9). It has been suggested that thiamine might inhibit growth and sporulation of *B. maydis* race T by causing an abnormal buildup of acetaldehyde in the fungus (5). Because the metabolism of xylose is known to be associated with the generation of NADH (13), the presence of xylose in the growth medium for *B. maydis* could provide an enhanced level of the reduced cofactor needed for conversion of toxic compounds, such as aldehydes, to less toxic products. Alternatively, xylose or its metabolic products could suppress or inactivate enzymes, such as peroxidase (9, 10), the activities of which appear to relate to the level of sporulation.

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