

VARIATION IN LINEAR GROWTH AND SPORULATION OF
SINGLE SPORE ISOLATES OF *HELMINTHOSPORIUM MAYDIS*

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ABSTRACT

Single spore isolates of *Helminthosporium maydis* exhibited variation in linear growth and sporulation when cultured on corn meal agar, lactose casein hydrolysate medium, potato-dextrose agar and V-8 juice. Also, the media differed in supporting growth and inducing sporulation.

INTRODUCTION

Following the 1970 epiphytotic of southern corn leaf blight, race T of the pathogen, *Helminthosporium maydis* Nisikado and Miyake, was designated. This new race is host specific for corn lines produced with Texas (T) male-sterile cytoplasm. The studies presented in this paper were undertaken to assess the variation in sporulation and linear growth that exists in the *H. maydis* population.

Several studies have dealt with the effect of nutrition on growth and sporulation of *Helminthosporium* spp. Malca and Ullstrup (1972), working with *H. carbonum* Ullstrup and *H. turcicum* Pass. found variation in sporulation greater than variation in mycelial growth in response to several carbon (C) and nitrogen (N) sources. Maloul (1960) determined that for *H. carbonum* and *H. turcicum* the C source in the medium affected which N source was used most effectively in spore production, and the amount and rate of growth determined to a large extent the factors which favored sporulation. The work of Kafi and Tarr (1966), using five species of *Helminthosporium*, indicated that sporulation occurred over a lower range of C/N ratios than did vegetative growth. Reddy (1970) showed that lactose was poorly utilized by five species of *Helminthosporium*, but utilization was improved when half of the lactose was replaced by its hydrolytic components. Zaman and Ahmed (1971) varied the sugar content of a basic medium and found that mycelial growth of *H. oryzae* Breda de Haan increased with an increase in sugar content up to 3%. Studying *H. cynodontis* Margl., White and Johnson (1971) found that linear growth was increased by trace amounts of zinc.

MATERIALS AND METHODS

Cultural variability in linear growth and sporulation among forty-two single spore isolates of *Helminthosporium maydis* was studied using four cultural media. The six cultures from each of which seven single spore isolates were obtained were originally isolated from lesions on corn. The six original cultures are referred to as sources in this paper. The four media used were: corn meal agar (CMA) (Johnson, 1972), lactose casein

hydrolysate medium (LCHA) (Tuite, 1969), potato-dextrose agar (PDA) (Tuite, 1969), and V-8 juice agar (VJA) (Tuite, 1969). Isolates were maintained on PDA previous to the test. A 7-mm cork borer was used to cut uniform discs from the margins of cultures. These discs were transferred to the centers of five 90-mm test plates. Plates were numbered and randomized within a stack and the stacks were randomized in an incubator. Percival E-54U environmental chambers served as incubators and were maintained in complete darkness at 26 ± 2°C throughout the test. Each plate served as one replication. After four days incubation, the largest colony diameter on each of three plates was measured and the plates and stacks were rerandomized. After 48 hours, two plates, each serving as one replication, were chosen at random from each cultural source for spore counts. Spore suspensions were obtained by flooding the plates with a 5% sucrose - 0.2% "Tween 20" solution and scraping the surface of the colony. The resulting spore suspension was poured through cheesecloth into a beaker and the filtrate volume adjusted to 50 ml with the suspending solution. Spore suspensions were agitated, and a sample transferred to a hemacytometer slide. Four 1mm² grids were counted and averaged to give a spore concentration for each plate. These values were expressed as spores per milliliter and were adjusted for colony growth by dividing by the colony area to obtain the number of spores/mm².

RESULTS AND DISCUSSIONS

The range and mean values for linear growth and sporulation are presented in Table 1. The means in Table 1 are overall means for all isolates. Linear growth on LCHA, CMA, and VJA was not significantly less than on the other three media.

The degree of variability on a given medium can be used as a measure of the extent to which that medium distinguishes differences among a group of isolates. The level of probability values for the analysis of variance tests among the 42 single spore isolates for each medium are a measure of the degree of variability on each medium. These values for both linear growth and sporulation are presented in Table 2. There were no differences among the four media in distinguishing variation in linear growth. Greatest differences in sporulation among the single spore isolates were obtained on PDA and LCHA. There were no significant differences in sporulation on CMA or VJA. The linear growth data for the 42 single spore isolates by sources on each media is presented in Table 3. Ten single spore isolates from five of the sources had significantly different linear growth among the four media. The sporulation data for the 42 single spore isolates by sources on each media is presented in Table 4. Fifteen single spore isolates from the six sources had significant differences in sporulation among the four media. Comparison of Tables 3 and 4 will show that

three single spore isolates (G7, X4 and X6) had significant differences in linear growth and sporulation on the four media.

Single spore isolates from two of the sources had extremely low spore production while maintained on PDA. Tuite (1969) lists lactose casein hydrolysate medium as a good medium for inducing sporulation in *Helminthosporium* spp. The isolates from these sources

had sporulation values on LCHA significantly greater than on the other three media. These results substantiate that lactose casein hydrolysate medium is a good medium for inducing sporulation in *Helminthosporium maydis*.

All the isolates which sporulated sufficiently in culture were determined to be *H. maydis* race T in greenhouse inoculation tests of corn inbreds with the Texas (T) male-sterile and normal (N) cytoplasm.

TABLE 1:

Range and mean values for linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Media	Linear Growth (mm) ¹		Sporulation (Spores/mm ² x 1000) ²		
	Range	Mean	Range	Mean	
LCHA	78.00-31.00	59.61a ³	PDA	9.180-0.000	2.893a
CMA	74.33-40.67	58.26a	LCHA	3.965-0.000	1.585b
VJA	80.67-36.00	58.20a	VJA	3.910-0.000	1.376b
PDA	71.67-20.00	52.96b	CMA	2.565-0.000	0.271c

¹Values are means for three replications of all 42 isolates.

²Values are means for two replications of all 42 isolates.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 3: Linear growth of 42 single spore isolates of *Helminthosporium maydis* on four media—corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	Growth (mm) ¹			
	CMA	LCHA	PDA	VJA
I1	56.00a ²	62.67a	61.33a	62.33a
2	62.33a	65.67a	66.67a	65.33a
3	76.00a	78.00a	69.33a	73.33a
4	71.67a	72.33a	71.67a	74.33a
5	66.67a	63.00a	38.67b	68.33a
6	50.67a	55.00a	57.67a	49.67a
7	65.67a	61.00a	64.33a	48.00a
Z1	52.67a	31.00a	38.33a	36.00a
2	45.00a	52.33a	52.33a	49.00a
3	47.67a	52.33a	57.67a	54.00a
4	46.67c	46.67c	52.33b	58.00a
5	58.67a	67.00a	66.00a	62.33a
6	56.67a	56.67a	59.00a	58.00a
7	47.33a	59.67a	44.33a	54.33a
R1	50.67a	31.00a	38.33a	36.00a
2	56.33a	49.33a	36.00a	55.00a
3	40.67c	59.67c	48.67b	46.33bc
4	63.67a	56.33ab	28.33d	51.33bc
5	58.33ab	56.00abc	23.67d	59.67a
6	58.33a	58.33a	33.67b	64.67a
7	55.67a	55.00a	25.00a	41.33a
C1	53.33a	69.00a	67.33a	73.00a
2	65.67a	67.67a	59.33a	67.67a
4	59.00bc	62.33ab	71.00a	53.67bc
5	62.33a	61.33a	67.33a	62.33a
6	44.33a	55.67a	53.67a	60.00a
7	54.00a	60.00a	66.00a	60.00a
G1	57.67a	59.33a	50.33a	55.67a
2	55.67a	62.33a	54.67a	51.67a
3	48.67a	53.33a	48.00a	59.00a
4	47.00a	53.00a	44.67a	48.33a
5	52.67a	51.00a	34.33b	36.33b
6	52.00a	50.67a	51.33a	44.33a
7	47.00a	37.33c	31.67c	46.33ab
X1	69.33a ²	66.33a	68.00a	69.00a
2	75.00a	71.67a	41.33b	72.67a
3	66.67a	69.67a	64.67a	75.00a
4	76.33a	73.67a	65.67b	64.00b
5	62.33a	62.00a	47.33a	48.00a
6	70.00a	57.33b	41.33c	71.00a
7	61.33a	69.33a	66.00a	68.67a
8	79.33a	73.33a	67.67a	80.67a

¹Values are means of three replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 2: Level of probability values for corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA) for linear growth and sporulation

Media	Sporulation	Linear Growth
CMA	5.29	0.01**
LCHA	0.15 **	0.01**
PDA	0.01 **	0.01**
VJA	4.94	0.01**

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TABLE 4: Sporulation of 42 single spore isolates of *Helminthosporium maydis* on four media—corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	Sporulation (Spores/mm ² x 1000) ¹			
	CMA	LCHA	PDA	VJA
11	0.105b ²	3.865a	0.000b	0.000b
2	0.000a	0.000a	0.000a	0.000a
3	0.000a	0.000a	0.000a	0.000a
4	1.620a	0.000a	0.000a	0.000a
5	0.000a	0.000a	0.000a	0.000a
6	0.000b	2.320a	0.770b	0.450b
7	0.000a	0.000a	0.000a	0.000a
Z1	0.000a	3.360a	0.355a	1.000a
2	0.070b	3.905a	0.895b	0.645b
3	0.065a	3.680a	0.055a	1.055a
4	0.000a	3.680a	0.000a	0.000a
5	0.060b	1.715a	0.495b	1.515a
6	0.000a	1.215a	0.065a	0.100a
7	0.000a	0.000a	0.000a	0.000a
R1	0.185b	3.245b	8.370a	2.255b
2	0.595a	0.315a	4.340a	2.065a
3	0.285a	0.920a	0.000a	0.645a
4	0.120c	1.055bc	3.145a	1.605b
5	0.865a	2.850a	6.890a	2.860a
6	0.235a	1.155a	3.680a	1.075a
7	2.565a	3.965a	9.180a	1.185a
C1	0.715a	1.540a	2.580a	2.435a
2	0.500b	1.190ab	1.950a	1.765a
4	0.125a	0.960a	3.100a	1.955a
5	0.210c	2.195ab	2.905a	1.375bc
6	0.730a	1.305a	6.205a	2.395a
7	0.000b	1.135ab	3.145a	2.360ab
G1	0.315a	0.820a	4.160a	2.605a
2	0.225a	1.520a	3.445a	1.505a
3	0.200a	0.690a	3.405a	1.365a
4	0.340c	0.685c	3.165a	2.190b
5	0.535a	1.200a	5.345a	3.910a
6	0.260a	0.930a	2.530a	2.215a
7	0.150b	0.460b	2.075a	0.060b

TABLE 4: (continued)

Isolate	Sporulation (Spores/mm ² x 1000) ¹			
	CMA	LCHA	PDA	VJA
X1	0.335c ²	0.665bc	3.545a	1.715b
2	0.060a	1.925a	7.665a	2.145a
3	0.340c	1.845b	3.945a	1.475b
4	0.280c	1.525bc	5.010a	2.465b
5	0.100c	2.900b	7.085a	1.835bc
6	0.195b	1.280b	6.795a	1.860b
7	0.390a	1.840a	2.745a	1.620a
8	2.485a	2.230a	1.790a	0.245a

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

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