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# AMMONIUM PRODUCTION BY RACES AND MATING TYPES OF *BIPOLARIS MAYDIS* AND ITS RELATIONSHIP TO MYCELIUM DRY WEIGHT, SPORULATION AND PH OF THE CULTURE MEDIUM[1]

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ABSTRACT. Isolates of *Bipolaris maydis* race T, mating types A and a, and *Bipolaris maydis* race O, mating types A and a, were compared for ammonium production at two or 10 g/l glucose on a L-asparagine-mineral salts medium in relation to mycelium dry weight, sporulation and pH of the culture medium at six, 10 and 14 d of incubation. Ammonium was detected in all media for all isolates at six days and levels were inversely related to the initial glucose levels. After 10 days, ammonium levels had increased in all media regardless of race or mating type to approximately 17.5 μmoles $NH_4^+$/ml. Thus, it appears that ammonium release from L-asparagine is a common phenomenon for *B. maydis*. At six days pH was also inversely related to glucose concentration while after 10 d the pH had increased to approximately 8.2 on all media regardless of race or mating type. The mycelium dry weight on two g/l glucose was comparable among incubation times and isolates. With 10 g/l glucose, dry weights were comparable among isolates at each incubation time while there was no change in dry weight after 10 d. Sporulation was greater with 10 g/l than with two g/l glucose for all isolates at all incubation times.

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## INTRODUCTION

There have been several studies concerning the effects of carbon and nitrogen nutrition on sporulation and growth of fungi belonging to the genera *Bipolaris* (Nisikado and Shoemaker), *Drechslera* (Ito) and *Exserohilum* (Leonard and Suggs). From these studies L-asparagine has been found satisfactory for the growth and sporulation of these fungi (Converse 1953, Tarr and Kafi 1968, Harding 1975). One interesting phenomenon was the report that the pH in cultures with L-asparagine increased from 5.2 to 8.0 after six days of incubation (Tarr and Kafi 1968).

Recently the same phenomenon was reported for *B. maydis* race T, the southern corn leaf blight pathogen. Concurrent with the increases in the pH was the production of ammonium in the culture medium. After 10 d of incubation the pH was 8.2 and ammonium levels were 17.5 μmoles/ml. In addition levels of ammonium and pH were inversely related to sporulation and dry weight (Bischoff and Garraway 1985). This study was carried out with a single isolate of *B. maydis* race T (ATCC 36180). *Bipolaris maydis* can be classified according to race (Smedegard-Petersen and Nelson 1969) or mating type (Nelson 1957). As a result four genetic variants exist in nature, *B. maydis* race T, mating type A or a and race O, mating type A or a. A preliminary study indicated that ammonium could be produced by these genetic variants (Bischoff and Garraway 1983). Therefore, this study was undertaken to determine if the changes in ammonium and pH seen with ATCC 36180 could be consistently found with other isolates of *B. maydis*.

## METHODS AND MATERIALS

Cultures of *B. maydis* were provided by Dr. E. S. Luttrell (Dept. Plant Pathology, Univ. Georgia, Athens, GA 30602). These were identified as isolates 8612-*B. maydis* race T mating type A, 8613-*B. maydis* race T mating type a, 8615-*B. maydis* race O mating type a and 8616-*B. maydis* race O mating type A. Each was maintained on a medium contain-

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ing glucose (10 g), L-asparagine (four g), $KH_2PO_4$ (1.5 g), $MgSO_4 \cdot 7H_2O$ (0.75 g), 0.1 mg of the hydrated forms of $CuSO_4$, $Fe_2(SO_4)_3$, $MnSO_4$ and $ZnSO_4$, and 20 g agar per liter of double-distilled water.

The test medium was prepared as previously described (Garraway 1973). Each ingredient was individually autoclaved for 20 min (1.2 kg/cm$^2$ pressure at 120 °C) and added to a Petri dish (100 × 15 mm). Molten agar was added to the Petri dish to bring the volume to 20 ml. Cores of mycelium, four mm in diam, were transferred from 10 day-old cultures to the test media. Cultures were incubated at 28 °C in the dark. A solid medium was used in these studies because sporulation of the fungus is inconsistent in liquid media. Comparisons of isolates for ammonium production, mycelium dry weight, sporulation and changes in the pH of the culture medium were measured on the maintenance medium with a glucose concentration of two g/l or 10 g/l at six, 10 and 14 d of incubation.

Ammonium levels were determined by homogenizing three 12-mm diam cores in double-distilled water (total volume five ml) for 20 sec using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). This mixture was centrifuged at five °C for five min at 6800 *g* and a sample of the supernatant was analyzed for ammonium using Nessler's reagent and measuring absorbance at 450 nm. This technique has been tested and shown to indicate reliably the level of ammonium in the culture medium (Bischoff and Garraway 1985). Determinations made with uninoculated media indicate that autoclaving L-asparagine did not significantly contribute to ammonium levels determined after incubation with the fungus.

The pH of the culture medium was determined by cutting the agar culture into fourths and submersing them in 25 ml double-distilled water. After waiting 30 min to permit equilibration of solutes in the agar with water, the pH was determined using a pH meter.

To determine dry weight of the mycelium, five cultures from each treatment and each isolate were added to individual beakers containing 150 ml distilled water and autoclaved for 20 min at 120 °C to solubilize the agar. The mycelial mat was transferred from the beaker to an aluminum foil tare, oven dried at 100 °C, and subsequently weighed. Comparisons have been made between dry weights obtained from solid media and from liquid media which indicate no significant difference in dry weight between the two techniques (Unpubl. data).

To determine sporulation, four six-mm diam cores of mycelium with conidia were transferred to screw cap vials for each of the five replicates. A NaOH-ethanol-chlorox preservative solution was added to inactivate the fungus (Garraway 1973). The number of conidia per ml was determined on the basis of microscopic counts of 10 random fields. The number of conidia per ml and the dry weight of the mycelial cores were used to estimate the number of conidia per mg dry weight. The mycelium dry weight of the cores was determined as described above.

All data are based on two similar experiments of five replications each. Means are presented along with their 95% confidence intervals.

## RESULTS

Ammonium was detected in cultures of all isolates tested at six days of incubation. Ammonium levels were inversely related to glucose concentration at this time, and the levels were comparable among isolates. At 10 d the ammonium level in cultures with 10 g/l glucose had increased to the level observed with two g/l glucose and was comparable for all isolates. Ammonium levels failed to increase after 14 d of incubation resulting in an average ammonium level of 17.5 $\mu$moles $NH_4^+$/ml for all cultures regardless of initial glucose concentration, race or mating type (fig. 1).

The pH of the culture medium increased after six days from an initial level of 5.8. The pH while comparable among isolates, was inversely related to glucose concentration at this incubation time. At 10 d of incubation with all isolates the pH of all media had increased to 8.2. The pH failed to increase after 14 d regardless of initial glucose concentration, race or mating type (fig. 1).

When 0.0, 10.0, 15.0, 17.5 $\mu$moles $NH_4^+$/ml were added as ammonium hydroxide to an uninoculated maintenance medium, the pH of the culture medium increased respectively to 5.8, 7.1, 7.7 and 8.0. This response was similar to cultures of the fungus where at these ammonium levels a corresponding pH of 5.8, 6.8, 7.8, and 8.2 was observed (fig. 1).

Mycelium dry weight was higher with 10 g/l than two g/l glucose at six days for all isolates. Dry weight of isolates of race T was also higher than race O at 10 g/l glucose. After 10 d, dry weight with 10 g/l glucose increased to a comparable level of 95 mg for all isolates, while there were no increases in dry weight with two g/l glucose. After 14 d for either glucose treatment there was no significant increase in dry weight among isolates. Final weight

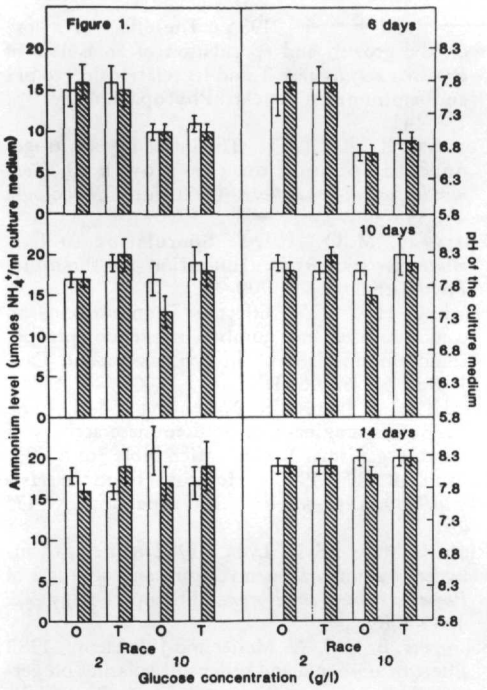


FIGURE 1. Ammonium and pH levels in cultures of *B. maydis* at six, 10 or 14 d of incubation with glucose concentrations of two g/l or 10 g/l. The race of the fungus is indicated on the horizontal axis. Solid bars represent mating type a while slashed bars represent mating type A. Values represent means of five replications, and the 95% confidence interval is indicated.

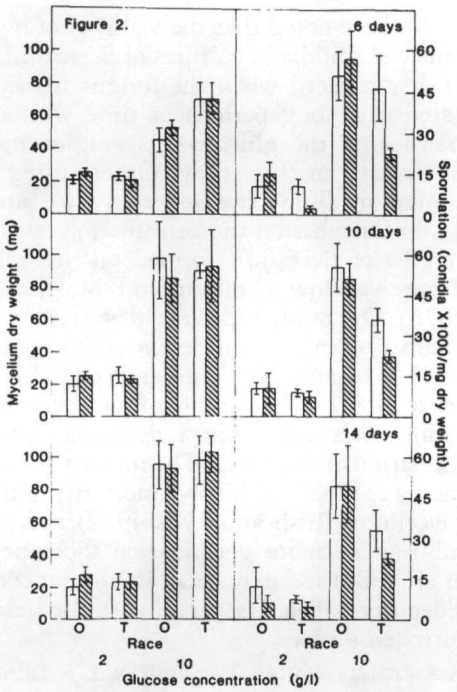


FIGURE 2. Mycelium dry weight and sporulation of *B. maydis* at six, 10 or 14 d of incubation with glucose concentrations of two g/l or 10 g/l. The race of the fungus is indicated on the horizontal axis. Solid bars represent mating type a while slashed bars represent mating type A. Values represent means of five replications, and the 95% confidence interval is indicated.

was approximately 50% of the glucose initially present, 95 mg with 10 g/l (200 mg/20 ml medium) glucose and 22 mg with two g/l (40 mg/20 ml medium) glucose (fig. 2).

Sporulation was also higher with 10 g/l than with two g/l glucose at six days. Levels of sporulation observed at six days were similar to those observed at 10 or 14 d for all isolates. Sporulation of the isolate *B. maydis* race T mating type A was consistently lower than the other isolates tested (fig. 2).

## DISCUSSION

Based on our studies, it appears that ammonium release from L-asparagine is a general phenomenon for both races and mating types of *B. maydis*. It also appears that high initial glucose levels delay the release of ammonium from L-asparagine during the first six days of incubation while this effect is no longer present after 10 d.

Concurrent with the production of ammonium was an increase in the pH of the culture medium. A similar response in pH was observed when ammonium hydroxide was added to the culture medium. Two hypotheses might explain this relationship. The pH increase may be due to the extrusion of ammonium hydroxide from the fungus into the culture medium. Alternatively, L-asparagine might react extracellularly with an asparaginase resulting in the formation of L-aspartate and ammonium. This enzyme is well documented in fungi (Imada et al. 1973). The preferential uptake of L-aspartate would cause ammonium to accumulate and the pH to increase.

We have noted that the viability of mycelia and conidia in cultures of *B. maydis* is greatly reduced when the fungus is incubated over long periods of time without transfer on the glucose-L-asparagine medium used in this study. Others using a similar medium have reported that after 14 d of incubation the germination rate of conidia of *B. maydis* formed on this medium was low (Trainor and Martinson 1978). Our study indicated that at 10 d of incubation ammonium levels had increased to 17.5 $\mu$moles $NH_4^+$/ml and the pH had risen to 8.2. These high pH and ammonium levels may interact resulting in the formation of ammonia. The toxicity of ammonia to fungi can involve mortality of the mycelium (Rush and Lyda 1982) and inhibition of spore germination (Schippers et al. 1982) and may be an important consideration when L-asparagine is used as a nitrogen source.

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## EDITOR'S NOTE

New manuscripts usually will be published within 7 months of acceptance in *The Ohio Journal of Science.*