Variable Chlorate Resistance in *Macrophomina phaseolina* from Corn, Soybean, and Soil

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ABSTRACT

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Macrophomina phaseolina isolated from soybean field soil or from corn or soybean tissue was grown on a defined medium with and without 120 mM potassium chlorate; chlorate is a nitrate analog. Growth was scored after 7 days of incubation at 30 C. Corn isolates were chlorate-resistant and grew normally, producing numerous dark microsclerotia on medium containing potassium chlorate. Isolates from soybean field soil and soybean root tissue were chlorate-sensitive. Sensitive isolates could be divided into two classes on the basis of growth on chlorate-containing medium. One class of sensitive isolates grew sparsely with a featherlike microsclerotial pattern, whereas radial growth of the other sensitive class was almost completely restricted. These observations suggest that *M. phaseolina* from corn and soybean differ in their ability to use certain nitrogenous compounds. Such differences might reflect metabolic abilities that could lead to host specialization within this genus.

Additional key words: charcoal rot, host specialization, nitrogen nutrition, *Rhizoctonia bataticola*.

Macrophomina phaseolina (Tassi) Goid. is an asexual fungus that causes charcoal rot of numerous plant species (9,23). Although the fungus has a wide host range, only one species is recognized (21). Attempts have been made to classify subspecies on the basis of microsclerotial size (18), but extreme variability within the species makes this classification scheme difficult to use. Dhingra and Sinclair (5) isolated *M. phaseolina* from five parts of a single soybean plant and observed differences in growth rate, pathogenicity, and colony development. Chromogenicity, sporulation ability, and pycnidial size are also known to vary greatly (4,6,15). Traits with less variabilty would be more useful when trying to group isolates.

Most fungi can use nitrate as a source of nitrogen (7). Nitrate uptake does not appear to occur without nitrate metabolism (14). The metabolic assimilation of nitrate is by reduction to nitrite via nitrate reductase; nitrite is then reduced to ammonia (14). Fungi unable to use nitrate, such as some Basidiomycetes, the Saprolegniaceae, and the Blastocladiales, apparently cannot sythesize nitrate reductase (22). The genetics of nitrate assimilation has been studied in *Neurospora* and *Aspergillus*. Enzyme production in these species is complex, requiring regulatory, structural, and co-factor-producing genes for the production and assembly of nitrate reductase (10). Mutations within nitrate reductase structural genes result in qualitative changes within the enzyme, whereas mutations within regulatory genes alter enzyme quantity (3).

Chlorate is an analog of nitrate and has been used to study nitrate assimilation. Reduction of chlorate to chlorite via nitrate reductase can result in chlorate toxicity in plants, algae, bacteria, and fungi (1,8,20). Other modes of action may be possible (2), but in general, chlorate-sensitive strains can reduce nitrate to nitrite and chlorate-resistant strains cannot. This paper describes a technique that differentiates isolates of *M. phaseolina* from corn and soybean, using sensitivity to potassium chlorate as the criterion.

MATERIALS AND METHODS

Cultures of *M. phaseolina* were obtained from cornstalks (*Zea mays* L.), soybean roots (*Glycine max* (L.) Merr.), and field soil. Initial isolations were made from plant tissues that had been surface-sterilized in 0.8% sodium hypochlorite for 2 min, then placed in petri dishes containing Difco lima bean agar (LBA). Soil isolates were obtained using techniques described by Mihail and Alcorn (12) or Meyer et al (11). With the exception of MPSA61 from S. M. Alcorn, University of Arizona, and MP8407 from a Kansas pasture, all soil isolates were obtained from Kansas soybean fields. Stock cultures were maintained on LBA slants at room temperature and transferred semiannually.

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Isolates to be tested were grown in the dark on LBA at 30 C. A 1-mm agar plug from a 7-day-old culture was placed in a petri dish 100 × 15 mm containing chlorate medium modified from that of Puhalla and Spieth (17). This medium contained 1.6 g of L-asparagine, 15 g of potassium chlorate, 30 g of sucrose, 2 g of $NaNO_3$, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.2 ml of trace elements solution (16), and 20 g of agar per liter. The trace elements solution contained 95 ml of distilled water, 10 g of citric acid, 10 g of $ZnSO_4 \cdot 7H_2O$, 2 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.5 g of $CuSO_4 \cdot 5H_2O$, 100 mg of $MnSO_4 \cdot H_2O$, 100 mg of H_3BO_3, 100 mg of $Na_2MoO_4 \cdot 2H_2O$, and 1 ml of chloroform. This stock solution was stored at 4 C. The pH of the medium was adjusted to 6.5 with KOH before autoclaving. Cultures were incubated in the dark at 30 C (6,15) for 7 days, then scored for growth after comparisons with cultures grown on the same medium without chlorate.

The linear growth rates of selected isolates were examined in race tubes (19) containing medium with and without chlorate. Race tubes were constructed by bending sections 16 mm × 50 cm of Pyrex glass tubing to a 45-degree angle 4 cm from each end. Tubes were filled with 20 ml of molten medium and the ends plugged with cotton. After being autoclaved 20 min at 121 C, tubes were cooled on a level surface. The length of each tube covered by medium was about 40 cm. Each tube was inoculated with a 1-mm agar plug of microsclerotia, about 1 cm from one end of the agar, and incubated in the dark at 30 C. Growth was measured each day by marking the advancing edge of the colony.

RESULTS

Table 1 lists the growth response of *M. phaseolina* isolated from corn, soybean, and soil when grown in petri dishes on a defined medium containing 120 mM potassium chlorate. The rating scale used was a comparison with growth on medium lacking chlorate. Three growth patterns (Fig. 1) were observed: restricted growth, feathery spreading growth, and dense growth like that obtained when grown on medium without chlorate. In general, isolates from corn grew more rapidly on the defined medium containing chlorate than did isolates from soybean or soil. Incubation on chlorate medium for 1 wk at 30 C gave definitive results. By contrast, the three colony types had dense growth and could not be distinguished from one another on LBA + 120 mM potassium chlorate. All

TABLE 1. Growth response of *Macrophomina phaseolina* isolates from corn, soybean, or soil on medium containing 120 mM potassium chlorate

Isolate	Source	Growth on chlorate medium[a]
MP8206	Corn	Dense
MP8217	Corn	Dense
MP8207	Corn	Feathery
MP8308	Corn	Dense
MP8309	Corn	Dense
MP8311	Corn	Dense
MP8346	Corn	Dense
MP8312	Corn	Dense
MPSA61	Soil	Feathery
MP8301	Soil	Feathery
MP8108	Soil	Restricted
MP8401	Soil	Restricted
MP8404	Soil	Feathery
MP8407	Soil	Feathery
MP8212	Soybean	Feathery
MP8215	Soybean	Restricted
MP8304	Soybean	Restricted
MP8320	Soybean	Feathery
MP8305	Soybean	Feathery
MP8321	Soybean	Feathery
MP8326	Soybean	Feathery
MP8330	Soybean	Feathery
MP8336	Soybean	Feathery

[a] Dense growth is like that on medium without chlorate. Feathery (sparse, spreading growth) and restricted (inhibited growth) indicate chlorate sensitivity.

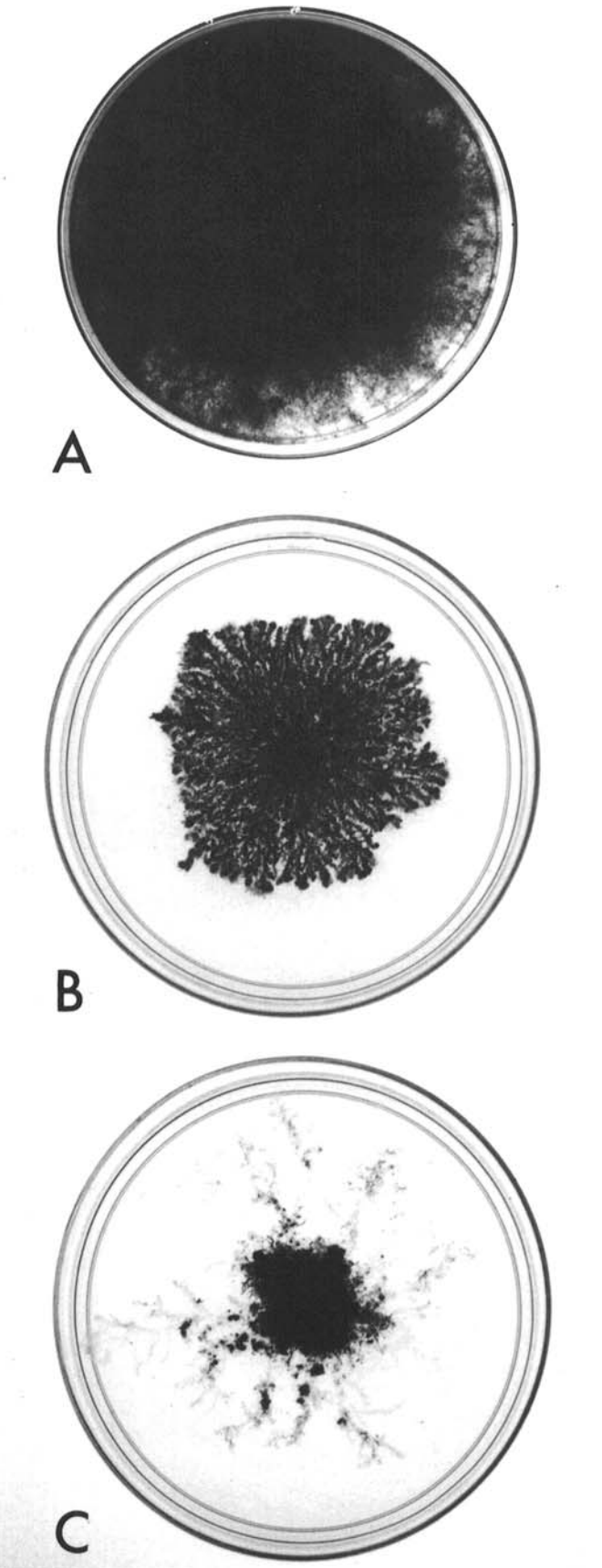

Fig. 1. Growth patterns of *Macrophomina* isolated from corn and soybean tissue and field soil when grown on a medium containing 120 mM potassium chlorate in petri dishes 100 × 15 mm. **A,** Dense growth pattern, **B,** feathery growth pattern, and **C,** restricted growth pattern.

isolates had dense growth when grown on the defined medium lacking chlorate and could not be differentiated. Altering the chlorate concentration in the defined medium to 60 or 240 mM did not change the resistance response found in corn isolates. Soybean isolates showing a feathery growth on 120 mM chlorate were difficult to score at the lower concentration because dense and feathery growth sometimes appeared similar. However, increasing chlorate concentrations from 120 to 240 mM did not alter the growth response of soybean isolates. The inoculum pieces used were small to reduce carryover from LBA to the chlorate test medium. If large agar plugs were used, sensitive isolates sometimes grew in a dense pattern for a short time. Although tests using mycelium or microsclerotia as inoculum yielded similar results, microsclerotial inoculum was routinely used throughout this study.

Linear growth rates of MP8305 (from soybean) and MP8346 (from corn) were determined by growing the cultures in race tubes. When grown on defined medium without chlorate, the isolates grew at similar rates (Fig. 2). When grown on the chlorate medium, MP8305 grew at a slower rate whereas chlorate-resistant MP8346 was not affected. The average linear growth rate of five corn isolates (MP8206, MP8207, MP8311, MP8317, and MP8346) was significantly faster than that of the five soybean isolates (MP8212, MP8304, MP8305, MP8320, and MP8336) when they were grown on the defined chlorate medium (Fig. 3). This difference in growth

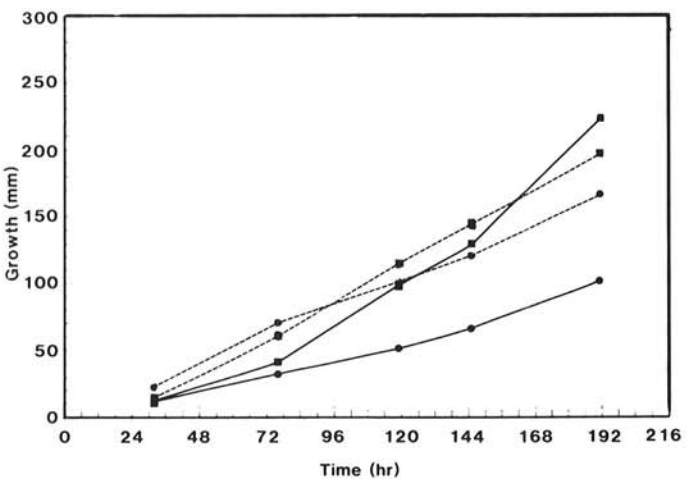

Fig. 2. Growth rates of MP8305 from soybean (•) and MP8346 from corn (■) on a defined minimal medium with (——) and without (- - - -) 120 mM potassium chlorate.

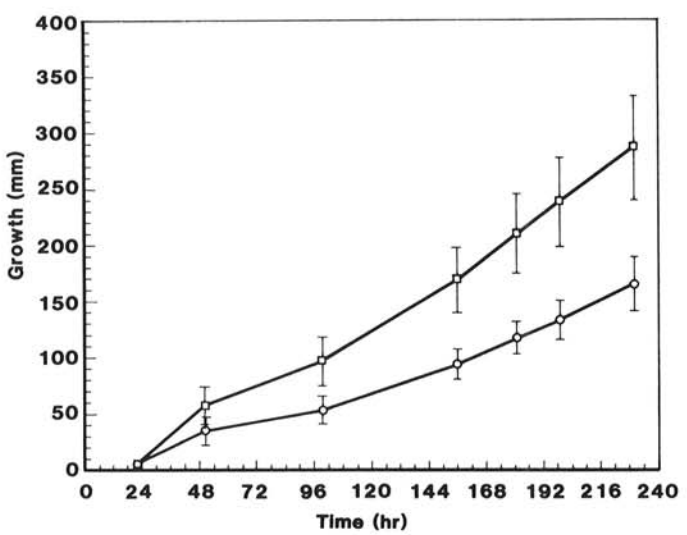

Fig. 3. Average growth rate of five corn (□) and five soybean (o) isolates on medium containing 120 mM potassium chlorate. Variation is indicated as standard deviation about the mean.

rate suggests that the difference between the isolates may be related to the regulation of nitrogen source utilization rather than the simple ability to block the toxic effects of chlorate.

DISCUSSION

M. phaseolina isolated from corn tissue can be distinguished from isolates obtained from soybean field soil or soybean root tissue by using a defined medium containing 120 mM potassium chlorate. Corn isolates, in general, are chlorate-resistant and grow densely on chlorate medium. Soybean isolates, in general, are chlorate-sensitive and show either a restricted or feathery growth pattern.

Although *M. phaseolina* can use nitrate as a nitrogen source (6), corn isolates do not grow as well as soybean isolates in shake flask culture on medium containing KNO_3 as a sole nitrogen source (C. A. S. Pearson, J. F. Leslie, and F. W. Schwenk, *unpublished*). This growth difference suggests that differences in chlorate sensitivity among these isolates may be in a regulatory control locus rather than in a locus coding for a portion of the nitrate reductase molecule. For example, the sensitive isolates could be constitutive for nitrate reductase, whereas resistant strains might have an inducible or repressible control mechanism for this enzyme (10).

Our results indicate that corn and soybean isolates of *M. phaseolina* differ from one another in chlorate sensitivity. In other fungi, such sensitivity is related to nitrate assimilation and the ability to produce nitrate reductase (3,10). This correlation has not yet been demonstrated for *M. phaseolina*. Because isolates unable to use nitrate must use other nitrogen sources, strains resistant and sensitive to chlorate are being examined for their ability to use other nitrogenous compounds. Soil isolates obtained from soybean fields responded like those isolates obtained from soybean tissue; both are chlorate-sensitive. Studies, using this chlorate resistance trait, of isolates from other hosts and soils from a variety of locations and under various cropping sequences are in progress (C. A. S. Pearson, J. F. Leslie, and F. W. Schwenk, *unpublished*).

Using chlorate resistance to group fungal isolates may be useful when studying phytopathogenic species, such as *M. phaseolina*, with extreme variability and/or no perfect stage. Pate (13) examined several plant species for nitrogenous compounds within the xylem and found nitrogen composition to be a function of species. Thus, two hosts differing in nitrogen composition could place selection pressure on the parasite and lead to host specialization by different strains. *M. phaseolina* from corn and soybean, which are differentially sensitive to chlorate, may be showing signs of such specialization.

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