Variation in Growth Rate and Colony Morphology of *Phytophthora parasitica* Induced by Metalaxyl*

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Abstract

Growing isolates P991 and P731 of *Phytophthora parasitica* on medium containing 25 µg/ml metalaxyl for 6 weeks, caused 66-74% of their single-zoospore cultures to increase the range of growth rates on metalaxyl-free medium. Metalaxyl treatment also caused each isolate to produce six colony types, including the parental type, on medium without metalaxyl. Growth rate and colony morphology of six single-zoospore cultures each representing a different colony type did not change after five successive subcultures. However, all the five colony-type variants produced single-zoospore cultures with greater variation in growth rate than did the parental type.

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Key words: variation, growth rate, colony morphology, *Phytophthora parasitica*, metalaxyl.

INTRODUCTION

Inhibitory effects of fungicides on fungal pathogens are transitory. After transfer to a fungicide-free medium, treated organisms often resume growth and produce normal colonies. Metalaxyl (N-[2, 6-dimethylphenyl]-N-[methoxyacetyl] alanine methyl ester) is a systemic fungicide widely used for controlling oomycete plant pathogens¹⁰⁾. During our recent study on the effect of metalaxyl on mating type of *Phytophthora*³⁾, we noticed that some of the single-zoospore cultures obtained from *Phytophthora parasitica* Dastur (*Phytophthora nicotianae* Breda de Haan) pre-incubated for 6 weeks on medium containing metalaxyl showed abnormal growth on metalaxyl-free medium. We, therefore, investigated the effect of metalaxyl on growth rate and colony morphology of *P. parasitica*.

MATERIALS AND METHODS

Metalaxyl treatment. Isolates P991 (A1) and P731 (A2) of *P. parasitica* obtained from Dr.G.A. Zentmyer, University of California, Riverside, were grown on V-8 agar (10% V-8 juice, 0.02% CaCO₃ and 2% agar) for four days. Four culture blocks (10×10×3 mm) from the colony margin of each isolate were placed on separate 90-mm petri dishes containing V-8 agar supplemented with 25 µm a.i./ml metalaxyl (Subdue 2E; Ciba-Geigy Corp., Greensboro, NC). Metalaxyl was added aseptically to the medium at about 60°C with a Pipetman digital microliter pipet (Rainin Instrument Co., Emeryville, CA) after autoclaving. Because the commercial formulation of metalaxyl consists mainly of metalaxyl dissolved in xylene, the medium remained sterile after addition of metalaxyl. Additional treatments consisted of V-8 agar supplemented with 75 µg/ml xylene (the concentration in the medium amended

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with metalaxyl) and V-8 agar alone. Three dishes for each treatment were sealed with Parafilm and kept in the same plastic container (40×25×15 cm) at 24°C.

Isolation of single zoospores. Both isolates P991 and P731 grew to about 20 mm in diameter after 4 weeks on V-8 agar supplemented with metalaxyl but reached the edge of the dishes containing V-8 agar alone or V-8 agar supplemented with xylene. Six mycelial blocks (10×5×3 mm) were cut from the margin of colonies on metalaxyl medium and from the edge of colonies on xylene-supplemented or control dish and placed in 10 ml sterile distilled water in separate 60-mm petri dish. After incubation at 24°C under continuous cool white fluorescent light (2,000 lux) for 2 days to induce sporangial production, zoospores were released from the sporangia by chilling the cultures at 5°C for 15 min⁶⁾. Approximately 100 zoospores from each treatment, as determined by the microliter pipet method⁷⁾ were spread on 2% water agar in separate petri dish. After incubation at 24°C for 2 days, those colonies originated from single zoospores were transferred to 5% V-8 agar (5% V-8 juice, 0.02% CaCO₃ and 2% Bacto agar). Five colonies were distributed equidistant around the edge of each dish, and developing cultures were transferred and used in the study before the colonies merged. A total of 300 single-zoospore cultures (100 each from the fungus growing on medium with metalaxyl, medium with xylene, and medium alone) was obtained from each of isolates P991 and P731.

Growth rate and colony morphology. Four mycelial discs (4 mm) were cut from the advancing margin of each single-zoospore culture and placed equidistant around the perimeter of a petri dish containing 10% V-8 agar. After incubation at 24°C for 4 days, colony morphology and linear growth were recorded. Two replicate dishes were used for each single-zoospore culture, and the experiment was repeated.

RESULTS

On the metalaxyl medium isolates P991 and P731 of *P. parasitica* were strongly inhibited. Growth first was visible after 7 days, reaching approximately 20 mm in diameter after 4 weeks. Between week 4 and 6, growth of either isolate was not detectable. Medium containing xylene was not inhibitory to

Fig. 1. Growth-rate distributions of single-zoospore cultures obtained from isolate P991 of *Phytophthora parasitica* grown for 6 weeks on V-8 agar alone (A), V-8 agar containing xylene (B) and V-8 agar containing metalaxyl at 25 µg/ml (C). CV: coefficient of variation.

Fig. 2. Growth-rate distributions of single-zoospore cultures obtained from isolate P731 of *Phytophthora parasitica* grown for 6 weeks on V-8 agar alone (A), V-8 agar containing xylene (B) and V-8 agar containing metalaxyl at 25 μ g/ml (C). CV: coefficient of variation.

Table 1. Colony types in single-zoospore cultures of *Phytophthora parasitica* obtained from isolates grown at 24°C for 6 weeks on V-8 agar alone, V-8 agar supplemented with 75 μ g/ml xylene and V-8 agar supplemented with 25 μ g/ml metalaxyl

Isolate and treatment	Colony type ^{a)} and no. of cultures						Total
	A	B	C	D	E	F	
P991							
Metalaxyl	43	17	2	35	2	1	100
Xylene	100	0	0	0	0	0	100
None	100	0	0	0	0	0	100
P731							
Metalaxyl	54	32	2	7	4	1	100
Xylene	100	0	0	0	0	0	100
None	100	0	0	0	0	0	100

a) Single-zoospore cultures grown on V-8 agar at 24°C for 5 days. Symbols: A, typical, irregular zonal pattern, abundant aerial growth; B, uniform texture, abundant aerial growth; C, uniform fine texture, moderate aerial growth, usually slow or intermediate in growth; D, zonal pattern, little aerial growth, radiate; E, irregular mosaic pattern, moderate aerial growth, non-radiate, usually intermediate in growth; F, irregular pattern, little aerial growth, radiate, usually slow or intermediate in growth.

either isolate. Colonies of both isolates on medium containing xylene and unsupplemented reached the edge of culture dishes within 4 days.

Growth rates of single-zoospore cultures obtained from isolates P991 and P731 over 6-week period on xylene-supplemented medium and unsupplemented were very uniform. The range of growth rates of single-zoospore cultures from the fungus growing on xylene-supplemented medium was 6.3–7.0 mm/day for isolate P991 and 6.3–7.9 mm/day for isolate P731, while that from the fungus growing on medium alone was 6.1–6.8 mm/day for isolate P991 and 6.1–7.7 mm/day isolate P731 (Figs. 1 and 2). However, exposure of these isolates to metalaxyl for 6 weeks greatly increased the ranges of growth rates among their single-zoospore cultures on medium without metalaxyl; 2.3–7.3 mm/day for P991 (Fig. 1) and 2.6–7.1 mm/day for P731 (Fig. 2). Metalaxyl treatment induced change in growth rate in 66 and 74% of single-zoospore cultures of isolates P991 and P731, respectively.

Fig. 3. Colony patterns displayed by single-zoospore cultures obtained from *Phytophthora parasitica* treated with metalaxyl. Cultures were grown on V-8 agar at 24°C for 5 days. Upper left, type A (parental type); upper center, type B; upper right, type C; lower left, type D; lower center, type E; lower right, type F. Isolates P991 and P731 displayed the same types.

Table 2. Variation in growth rates among single-zoospore cultures from isolates each representing one of the six colony types from isolate P991 of *Phytophthora parasitica* grown for 6 weeks on V-8 agar containing 25 µg/ml metalaxyl

Isolate	Colony type	Original growth rate (mm/day)	Single-zoospore cultures		
			Range of growth rate (mm/day)	CV (%) ^{b)}	Total no.
P991-1 ^{a)}	A	6.1	5.1-6.4	4.8	64
P991-2M	A	5.6	5.3-6.4	4.7	60
P991-14M	B	6.3	3.0-6.6	18.1	75
P991-1M	C	2.5	0.4-3.8	40.0	60
P991-23M	D	5.0	1.3-5.9	53.3	72
P991-12M	E	5.6	4.0-6.8	7.5	60
P991-19M	F	4.9	1.0-6.1	43.6	60

a) Single-zoospore culture from non-treated control.

b) CV, coefficient of variation.

Single-zoospore cultures obtained from isolates P991 and P731 grown on medium with xylene and unsupplemented for 6 weeks were uniform and similar in colony appearance to their respective parents (Table 1). However, after exposure to metalaxyl for 6 weeks, each isolate produced zoospores that gave rise to six colony types, including the parental type A, on medium without metalaxyl (Table 1, Fig. 3). Metalaxyl treatment induced changes in colony appearance in 57% of single-zoospore cultures of isolate P991. About 65% of the variants belonged to colony type D, which produced zonal, radiate growth and little aerial growth. The same metalaxyl treatment induced changes in colony appearance in 46% of single-zoospore cultures of isolate P731. About 70% of these variants belonged to colony type B, having a uniform texture and abundant aerial growth.

When isolates of zoospore cultures each representing one of the six colony types from P991 were successively subcultured five times, their growth rates were not significantly different from the original rates and none of them changed colony appearance. However, all the five colony-type variants produced single-zoospore cultures with greater variation in growth rate than did the parental type (Table 2). The range of growth rate among single-zoospore cultures from isolate P991-2M was similar to that from the control isolate. All the single-zoospore cultures were similar to their parents in colony

appearance.

When single-zoospore cultures were obtained from isolates P991 and P731 grown on medium containing 25 $\mu\text{g/ml}$ metalaxyl for 4 weeks, variants were detected only in one of three tests (data not shown). In that positive test, 28% of the single-zoospore cultures from P991 were morphological variants and 22% were growth-rate variants. Single-zoospore cultures from isolate P731 produced 12% morphological variants and 14% growth-rate variants.

DISCUSSION

Exposure of *P. parasitica* to metalaxyl for 6 weeks resulted in considerable variation for growth rate and colony appearance among the zoospore progeny. Such effect of metalaxyl is similar to segregation for growth rate and colony morphology resulting from selfing of this fungus²⁾. The mechanism for induction of variation by metalaxyl in *P. parasitica* remains to be investigated. Holt and Paxton⁵⁾ also observed variation in virulence after successive transfers of *Phytophthora megasperma* f. sp. *glycinea* on medium amended with metalaxyl.

Consistent changes of colony morphology and growth rate in *P. parasitica* occurred only after exposure to metalaxyl for 6 weeks but not 4 weeks. Whether long-term exposure to other fungicides also can cause similar changes of fungi deserves further investigations.

Fungi usually are inhibited by fungicides only in the presence of these chemicals, and revert to normal growth and appearance after being transferred to a medium free from these chemicals. Although development of resistance to fungicides by fungi after their exposure to those chemicals is relatively common, reports on changes in other characteristics induced by fungicides are very rare. Durieu-Trautmann and Tavlitzki⁴⁾ reported that chloramphenicol and ethidium-bromide induced stable change of *Ustilago cynodontis* from hyphal cells to yeast-like colonies. Shaw and Elliot⁹⁾ also obtained some stable morphological variants of *P. cactorum* when a wild type was treated with streptomycin. Recently several fungicides including metalaxyl have been found to be capable of inducing mating-type change and subsequently change in hormone production and reception in species of *Phytophthora*^{1,3,6,8)}.

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和 文 摘 要

T.T. CHANG and M.H. KO: メタラキシル剤によって誘導される疫病菌 *Phytophthora parasitica* の菌糸伸長と菌そう形態の変異

疫病菌 *Phytophthora parasitica* (P991 および P731 菌株) をメタラキシル剤 (25 $\mu\text{g/ml}$) を含む培地で6週間培養したところ, その単遊走子分離菌株の66~74% がメタラキシルを含まない培養に比べて生長速度の変動幅が増大した。このメタラキシル処理菌株を普通培地に移植すると, 菌そうの形態, 性状は6種(親株型を含む)に類別された。これら6形態株に由来する単遊走子分離菌株は, 引き続き5回の移植培養によっても菌糸伸長速度および菌そう形態に変化は認められなかった。ただし, 菌そう形態変異株の方が親株に比べれば伸長速度の変異幅が大きく現れた。