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Effect of chloroneb and ethazol on mating type of *Phytophthora parasitica* and *P. cinnamomi*

Pao-Jen Ann[1] and Wen-Hsiung Ko[2]

[1]*Chia-Yi Agricultural Experiment Station, Chia-Yi, Taiwan, Republic of China*

[2]*Department of Plant Pathology, Beaumont Agricultural Research Center, University of Hawaii, Hilo, HI 96720, U.S.A.*

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Abstract. When A^1 and A^2 isolates of *Phytophthora parasitica* were grown on medium containing chloroneb or ethazol, both isolates produced oospores in sectors. Single-zoospore cultures obtained from the oospore sectors consisted of both A^1 and A^2 mating types, while those obtained from non-treated cultures consisted of only the parental mating type. When A^1 and A^2 isolates of *P. cinnamomi* were grown on medium containing chloroneb or ethazol, only A^1 produced oospores in sectors. Single-chlamydospore cultures obtained from the sectors consisted of both A^1 and A^2 types. The A^2 sexual variants also gave rise to A^1 type of single-chlamydospore cultures after treatment with ethazol, indicating the reversible nature of mating type change. Both hormone production and reception were changed when A^1 mating type of *P. cinnamomi* was changed from A^1 to A^2, and vice versa.

Key words: Chloroneb; Ethazol; Mating type; *Phytophthora cinnamomi*; *Phytophthora parasitica.*

Introduction

It was reported previously that during long-term storage, A^1 and A^2 mating types of *Phytophthora parasitica* Dastur can be converted to A^2 and A^1 mating types, respectively (Ko, 1981). Conversion of A^2 to A^1 mating type also occurred when the A^2 isolate of *P. parasitica* was grown in a medium containing the fungicide, chloroneb (Ko, 1981). Subsequently another fungicide, ethazol, was found capable of converting A^1 mating type of *P. parasitica* to A^2 mating type (Ko, *et al.*, 1986).

In this paper we report the recent discovery of the ability of chloroneb and ethazol to convert both A^1 and A^2 mating types of *P. parasitica* to the opposite mating type. We also studied the effect of these two fungicides on the mating type of *P. cinnamomi* Rands. A preliminary report has been published (Ann and Ko, 1988).

Materials and Methods

Test Organisms

Isolates P991 and P731 of *P. parasitica,* and isolate P97 of *P. cinnamomi* were supplied by Dr. G. A. Zentmyer. Isolate 6127 of *P. cinnamomi* was isolated from a root of ohia [*Metrosideros collina* (Forst.) Gray subsp. *polymorpha* (Gaud.) Rock]. Each isolate used originated from a single zoospore and was maintained on V-8 agar (10% V-8 juice, 0.02% $CaCO_3$ and 2% Bacto agar).

Induction of Mating Type Change

Four pieces of agar cultures (ca. $4 \times 4 \times 3$ mm) of A^1 or A^2 mating type of *P. parasitica* were transferred to a plate of V-8 agar supplemented with 20 μg/ml chloroneb (Terraneb SP, 65% active, Kincaid Enterprises, Nitro, West Virginia 25143) or 20 μg/ml ethazol (Truban WP, 30% active, Mallinckrodt, St. Louis, Missouri). Formation of oospore sectors on the medium

during incubation at 24°C for one month was used as an indication of the appearance of the opposite mating type (Ko, 1981).

Isolation of Single Spores

Single-zoospore cultures of *P. parasitica* were obtained using the method described by Ko (1981). Pieces of cultures (ca. 10×5×3 mm) cut from oospore sectors were placed in 10 ml of sterile distilled water in a small Petri dish (60 mm diam.) and incubated at 24°C under fluorescent light for 2 days to induce production of sporangia by mycelia. Zoospores were released from sporangia by chilling the culture at 5°C for 15 min. About 100 zoospores were spread on 2% Bacto water agar in a Petri plate. After incubation at 24°C for 2 days, the colonies originating from single zoospores were transferred to V-8 agar plates. Five colonies were evenly distributed around the edge of each plate.

Zoospores of *P. cinnamomi* were difficult to obtain from oospore sectors using the methods described above. The following method was subsequently developed to solve the problem. Three pieces of culture blocks (ca. 3 × 3 × 3 mm) cut from oospore sectors were transferred to a sterilized disc of cellophane (90 mm in diam.) laid on V-8 agar. After incubation at 24°C for 7 days, mycelial mats containing oospores and chlamydospores were scraped off the cellophane with a spatula, and were triturated with 50 ml of sterile distilled water in an Omni mixer chamber at 4,500 rpm for 1 min. The suspension was passed through a 53 μm sieve. Spores retained on the sieve were spread on a selective medium consisting of V-8 agar supplemented with 100 μg/ml ampicillin, 50 μg/ml nystatin and 10 μg/ml pentachloronitrobenzene (Ko *et al.*, 1978). Nine plates each containing about 100 chlamydospores were used. After incubation at 24°C for 24h, colonies originating from single chlamydospores were transferred to V-8 agar plate.

Determination of Mating Type

Mating type of each single-spore culture was determined by pairing a small piece of culture block (ca. 3 × 3 × 3 mm) with the same size of A^1 tester (P991) or A^2 tester (P731) of *P. parasitica* on a piece of V-8 agar block (ca. 10 × 10 × 3 mm) in a Petri plate. Ten isolates were tested in each plate. After incubation at 24°C in darkness for 6 days, agar blocks were examined microscopically. Those isolates forming oospores when paired with A^2 tester were A^1. Similarly those forming oospores with A^1 tester were A^2 and those forming oospores with both A^1 and A^2 tester were A^1A^2 which also formed oospores in the absence of the testers.

Results

Phytophthora parasitica

When A^1 and A^2 isolates of *P. parasitica* were grown on medium containing chloroneb, both isolates produced oospores in sectors indicating the appearance of the opposite mating type. All the single-zoospore cultures obtained from the sectors were self sterile. Among these cultures 64 were A^1 and 4 were A^2 from P991, and 2 were A^1 and 50 were A^2 from P731 (Table 1). Without treatment all the single-zoospore cultures showed the same mating type as their respective parent. When the same isolates were grown on medium containing ethazol, both isolates also produced oospores in sectors. The mating type ratios of single-zoospore cultures were 138 A^1 : 2 A^2 from P991 and 6 A^1 : 44 A^2 from P731 (Table 1).

Phytophthora cinnamomi

When A^1 and A^2 wild types of *P. cinnamomi* were grown on medium containing chloroneb or ethazol, only A^1 (P97) produced oospores in sectors in the presence of ethazol. Single-chlamydospore cultures obtained from the sectors consisted of 110 A^1, 35 A^2 and 2 A^1A^2 (Table 2). A^2 wild type (6127) did not produce oospore sectors in medium containing chloroneb or ethazol. The A^1 isolate (P97-1) derived from a single chlamydospore of isolate P97 produced oospores in sectors when grown on medium containing chloroneb. Single-chlamydospore cultures obtained from the sectors consisted of 70 A^1, 1 A^2 and 1 A^1A^2. The A^2 sexual variant P97-2 also produced oospores in sectors when grown on medium containing ethazol. The mating type ratio of single-chlamydospore cultures was 12 A^1, 64 A^2 and 6 A^1A^2 (Table 2).

A^1 wild type (P97), A^2 sexual variant (97-2) and A^1 revertant (P97-2-1) of *P. cinnamomi* were each paired with A^1 (P991) and A^2 (P731) testers of *P. parasitica* using the polycarbonate membrane technique (Ko, 1978) to determine their hormone production and reception. A^1 isolate was capable of inducing sexual reproduction of A^2 tester (production of hormone α^1) and

Table 1. *Mating type distribution in single-zoospore cultures from A^1 and A^2 isolates of* ***Phytophthora parasitica*** *grown on medium containing chloroneb or ethazol*

Isolate	Mating type	Treatment	No. of single-zoospore cultures	
			A^1	A^2
P991	A^1	Chloroneb	64	4
P731	A^2	Chloroneb	2	50
P991	A^1	Ethazol	138	2
P731	A^2	Ethazol	6	44
P991	A^1	None	136	0
P731	A^2	None	0	70

Table 2. *Mating type distribution in single-chlamydospore cultures from A^1 and A^2 isolates of* ***Phytophthora cinnamomi*** *grown on medium containing ethazol*

Isolate	Origin	Mating type	Treatment	No. of single-chlamydospore cultures		
				A^1	A^2	A^1A^2
P97		A^1	Ethazol	110	35	2
P97-1	P97	A^1	Chloroneb	70	1	1
P97-2	P97	A^2	Ethazol	12	64	6

Table 3. *Hormone production and reception in wild type and sexual varaint of* ***Phytophthora cinnamomi***

Isolate	Origin	Mating type	Hormone production[1]		Hormone reception[2]	
			α^1	α^2	α^1	α^2
P97		A^1	+	−	−	+
P97-2		A^2	−	+	+	−
P97-2-1		A^1	+	−	−	+

[1] +, productive; −, non-productive.
[2] +, responsive; −, non-responsive.

forming oospores after being stimulated by A^2 tester (presence of hormone α^2 receptor). A^2 sexual variant was capable of inducing sexual reproduction of A^1 tester (production of hormone α^2) and forming oospores after being stimulated by A^1 tester (presence pf hormone α^1 recepter) indicating that both hormone production and reception were changed in the sexual variant (Table 3). Hormone production and reception of the A^1 revertant were the same as A^1 wild type.

Discussion

Results from this study show that chloroneb and ethazol can cause the mating type change of both A^1 and A^2 of *P. parasitica* and *P. cinnamomi*. The fact that A^2 isolate P97-2 that were derived from A^1 isolate P97 through ethazol treatment gave rise to A^1 chlamydospores after treatment with ethazol, clearly shows the reversibility of mating type change in *P. cinnamomi*. Similar phenomenon was observed in *P. parasitica* (Ko. 1981, Ko *et al.*, 1986), Like *P. parasitica* (Ko, 1988), the finding of reversible change of mating type from A^1 to A^2 in *P. cinnamomi* does not support the hypothesis that A^1 is homozygous recessive aa, and that A^2 is heterozygous Aa (Sansome, 1980) because the homozygous recessive character should not segregate.

Results concerning hormone production and reception of wild type, sexual variant and revertant of *P. cinnamomi* are compatible with postulation of the presence of a repressor in *Phytophthora* which represses the expression of A^1 mating type with one molecular configuration, and A^2 type with another configuration (Ko, 1981). Chloroneb and ethazol may have changed the mating type by reversing the function of the repressor.

The nature of the A^1A^2 type of *P. cinnamomi* obtained after chemical treatment has not been studied. Similar phenomenon was observed when isolate P991-SZ18 of *P. parasitica* was treated with chloroneb (Ko, 1981). The A^1A^2 type was found to be a transitional state in the process of mating type change. All the single-hyphal tip cultures derived from it were self-sterile and behaved as the parental type.

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Chloroneb 和 ethazol 對 *Phytophthora parasitica* 和 *P. cinnamomi* 配對型的影響

安寶貞[1] 柯文雄[2]

[1]台灣省農業試驗所嘉義分所植保系

[2]美國夏威夷大學植物病理系

將 *P. parasitica* 的 A^1和 A^2菌株培養於含有 chloroneb 或 ethazol 的培養基，兩配對型的菌株均在培養基產生局部卵孢子區。自這些卵孢子區分離的單游走子菌株含有 A^1和 A^2配對型，然而分離自無處理菌落的單游走子菌株只含有與母菌株相同的配對型。將 *P. cinnamomi* 的 A^1和 A^2菌株培養於含有 chloroneb 或 ethazol 的培養基，只有 A^1菌株形成局部卵孢子區。自這些卵孢子區分離的單厚膜孢子菌株含有 A^1和 A^2配對型。當 A^2性變異株經 ethazol 處理後，自其分離之單厚膜孢子菌株中也出現 A^2配對型。此結果顯示兩配對型可以互相轉變。當 *P. cinnamomi* 的配對型自 A^1轉變成 A^2時，賀爾蒙的形成與接受也同時改變，反之亦然。