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Growth Rate and Colony Morphology of Progenies of Zoospores and Oospores of *Phytophthora cactorum* causing Phytophthora rots in Apple Trees

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果樹木의 疫病을 유발하는 *Phytophthora cactorum*의 遊走子와 卵胞子의 균사생장과 균총의 형태

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ABSTRACT: Zoospore progenies of *Phytophthora cactorum* were relatively uniform and similar to their respective parent in the rate of linear extension, whereas oospore progenies were greatly various. Also, the character of colony pattern was quantitatively various in oospore progenies but not zoospore progenies. Therefore, these results suggested that multiple genes were involved in determining growth rate and colony morphology of *P. cactorum*, and support the hypothesis that species of *Phytophthora* are diploid during the vegetative phase.

KEYWORDS: *Phytophthora cactorum*, vegetative phase, multiple genes

Introduction

Some species of *Phytophthora* such as *P. cactorum, P. syringae,* and *P. megaspera* are homothalic and are capabale of completing their life cycle by single isolates. However, other species, including *P. infestans, P. palmivora,* and *P. cinnamomi* are heterothalic and require the presence of opposite mating types known as A1 and A2 for the formation of the sexual stage of the life cycle (Ko, 1988).

Boccas (1972) compared the rates of linear extension of mycelia of zoospore progeny with those of oospore progeny of self-inducing (Homothalic) *Phytophthora syringae* (Klebahn) Klebahn and found that zoospore progenies were relatively uniform, whereas oospore progenies showed great dissimilarities for growth rate. This has been looked upon as an evidence that the fungus is diploid in the vegetative state (Boccas, 1972). The variations of growth rate and colony morphology in oospore progenies from intraspecific crosses have also been found in several cross-inducing (Heterothalic) species of *Phytophthora* (Satour and Butler, 1968; Romeo and Edwin, 1969).

Recently, Ann and Ko (1990) compared the growth rate and colony morphology among progenies of zoospores and selfed oospores of this cross-inducing species of *Phytophthora parasitica,* and suggested that heterozygous polygenes were involved in determining growth rate and colony morphology of these fungi. *Phytophthora cactorum* (Lebert and Cohn) Schroeter is an important plant pathogen with a very broad host range parasitizing over 83 genera in 44 families (Ribero, 1978). Especially, *P. cactorum* is considered as a soil-borne pathogen causing Phytophthora rot of apple trees in Korea. However, there was no any report referring to growth rate and colony morphology in oospore

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and zoospore progenies of these fungi in Korea. MacIntyre and Elliot (1974) reported a variety of variations in growth rate of respective progenies of *P. cactorum*. Though MacIntyre and Elliot (1974) reported variations in growth rate of oospore progenies but not zoospore progenies of *Phytophthora cactorum*, there was no any report regarding variations of the colony morphology among progenies of zoospores and oospores of *P. cactorum* except for the rates of linear extension in these fungi. Therefore, this study was conducted to compare if multiple genes were involved in determining growth rate and colony morphology among progenies of zoospores and oospores of *P. cactorum*.

Materials and Methods

Microorganisms

The isolate of *P. cactorum* used was obtained from Dr. Ko, University of Hawaii, and derived from a single zoospore. The isolate was maintained in sterile distilled water (Ann and Ko, 1990).

Isolation of single-zoospore colonies

Three mycelial blocks (ca 10×5×2 mm) from 3 day old cultures grown on 10% V-8 agar (10% V-8 juice, 0.02% $CaCo_3$ and 2% Bacto agar adjusted to pH 6 with 1N KOH) were transferred to 10 m*l* of sterile distilled water in a petri-dish, 60 mm diam., and were incubated at 24℃ under cool white fluorescent light (2000 lux) for 2~3 days to induce the production of sporangia. Zoospores, released from sporangia by chilling at 5℃ for 15 min., were induced to encyst by agitation for 1 min. on a voltex mixer. Two drops of spore suspension containing about 100 encysted zoospores were spread on 2% water agar in a petri-dish. After incubation at 24℃ for 2~3 days, the colonies were observed microscopically and only those originating from single zoospores were transferred to V-8 agar plates (Ann and Ko, 1990).

Production of oospores

The fungus was grown on 10% V-8 agar blocks (ca 20×15×3 mm) in petri-dishes. These dishes were then sealed with two layers of parafilm and incubated in darkness for 30 days at 24℃ for formation and maturation of oospores unless otherwise stated (Chang and Ko, 1991).

Isolation of single-oospore colonies

Oospore suspensions were obtained by triturating each culture block (ca 15×15×3 mm) containing oospores with 50 m*l* of distilled water in an omni-mixer at 4500 rpm for 1 min. The suspension was filtered successively through a 53-μm and a 20-μm sieve. Oospores retained on the 20-μm sieve were washed with tap water and resuspended in 10 m*l* of sterile distilled water. The oospore suspension was mixed with an equal volume of fleshly prepared $KMnO_4$ solution at 0.5% (w/v). After agitating the mixture for 15 min. on a shaker, oospores were washed free of $KMnO_4$ on a 20-μm sieve with tap water. About 10~20 μ*l* of oospore suspension containing about 100~150 oospores were spread on 0.8% Seakem water agarose medium consisting of 2% Bacto agar and 0.8% Seakem water agarose (Ho and Ko, 1980). After autoclaving, the medium was supplemented with 100 μg/m*l* of ampicillin, 50 μg/m*l* of nystatin and 10 μg/m*l* of PCNB to prevent possible growths of some contaminants. Plates were incubated at 24℃ with fluorescent light. Under such conditions, oospore germination of *P. cactorum* commenced within 24 hr., and was more than 80% after 10 days. The colony originating from single oospore was transferred to V-8 agar plates.

Growth rate and colony morphology

A piece of inoculum (ca 2×2×2 mm) was placed in the center of a petri-dish containing 5% clarified V-8 agar which was prepared by centrifuging V-8 juice plus 0.2% $CaCo_3$ at 270 g for 5 min. before dilution and addition of 2% Bacto agar. After incubation at 24℃ for 3 days, linear growths were recorded. However, colony morphology was observed after incubation at 24℃ for 7 days. Four plates were used for each isolate, and this experiment was carried out two times (Ann and Ko, 1990).

Results

Growth rate

The linear extension of mycelia of 100 single-zoospore cultures isolated from *P. cactorum* was in the range of 3.3~4.0 mm/day, and similar with an average of 3.5 mm/days (Table 1). However, the linear extension of 100 single-oospore cultures was in the range of 3.4~5.4 mm/day, and varied greatly with an average of 4.8 mm/day (Table 1). The linear extensions of mycelia of 68 single zoo-

Table 1. Growth rates of zoospore and oospore progenies of *Phytophthora cactorum*

Parent	Progeny			
Isolate	Spores	No. of cultures	Linear growth (mm/day)	
			Range	Average
	Zoospores	100	3.3-4.0	3.5
		68	3.3-3.5	
		32	3.6-4.0	
P. cactorum				
	Oospores	100	3.4-5.4	4.8
		4	3.4-4.0	
		78	4.1-5.0	
		19	5.1-5.4	

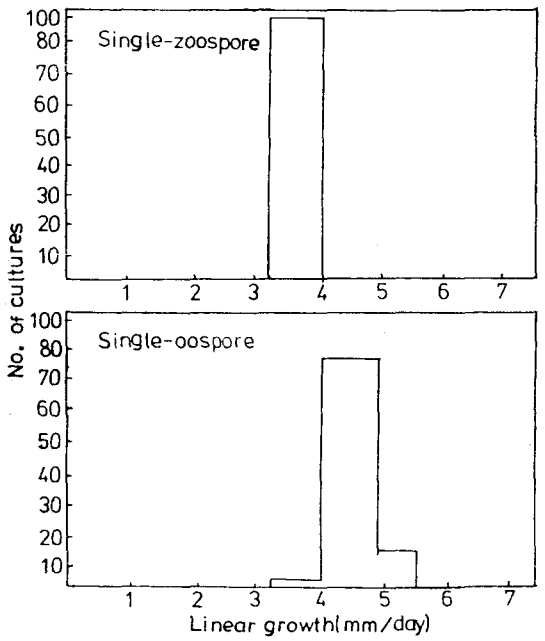

Fig. 1. Frequency distribution of growth rate among progenies of zoospores and oospores of the isolate of *Phytophthora cactorum*.

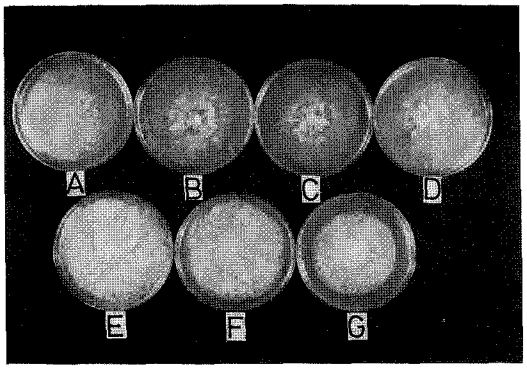

Fig. 2. Colony types displayed by *Phytophthora cactorum* on 5% V-8 agar after incubation at 24 ℃ for 7 days.

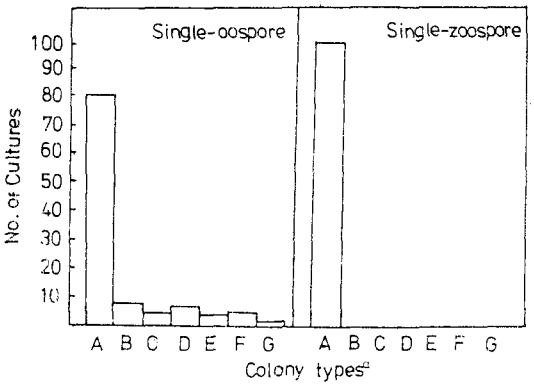

Fig. 3. Colony distribution of oospore and zoospore progenies of *Phytophthora cactorum*.
[a] Symbols: A, Uniform texture, Aerial growth with white star-like mark in the center of colony; B, Uniform texture, Abundant aerial growth; C, Somewhat irregular texture, Abundant aerial growth, Intermediate in growth; D, Uniform texture, Abundant aerial growth with aerial white area in the center of colony; E, Uniform texture, Highly abundant aerial growth, High in growth; F, Uniform texture, Highly abundant aerial growth with entirely dense white colors, High in growth; G, Uniform texture, Highly abundant aerial growth with dense white color in the center of colony, Slow in growth.

spore cultures were in the range of 3.3~3.5 mm/day, whereas 78 single oospore cultures were within the scope of 4.1~5.0 mm/day (Table 1). Comparing the linear extension of mycelia of 100 single-zoospore cultures with 100 single-oospore cultures, it was obvious that single-oospore cultures were far greater variation for growth rate than single-zoospore cultures (Fig. 1).

Colony morphology

Oospore progenies of isolates of *P. cactorum* appeared seven types which designated A, B, C, D, E, F and G, respectively (Fig. 2, 3). The colony type of the isolate belonged to A type with uniform texture, aerial growth, and star-like mark in the center of the colony. All the zoospore progenies of the isolates were within the scope of A type, and similar to their respective parent in colony appearance, whereas colony morphology among oospore progenies varied greatly (Fig. 3).

Dicussion

Caten and Jinks (1968) found that zoospore progenies of *P. infestans* were greatly various in growth rate and colony morphology. Therefore, they suggested that these characters were under cytoplasmic control. However, we found that zoospore progenies of *P. cactorum* were very uniform and similar to their respective parent in the rate of linear extension and colony appearance.

Consequently, it may be concluded that zoospore progenies of *P. cactorum* didn't release quantitative variations in the rate of linear extension and colony appearance. Since these characters differ from the results with *P. infestans* (Caten and Jinks, 1968), it can be suggested that the characters are probably controlled by nuclear genes in zoospore progenies of *P. cactorum* (Ann and Ko, 1990). Since on the contrary, oospore progenies of *P. cactorum* displayed a continuous quantitative variation in growth rate and colony appearance, it can be suggested that these characters are under cytoplasmic control.

Boccas (1972) observed a continuous growth rate distribution for oospore progenies of *P. syringae*, homothalic fungi, and also suggested that multiple genes probably were involved in determining the rate of linear extension in these fungi. Also, he mentioned that variation distribution for oospore progenies of *P. syringae* was greater in range than the variation released upon zoospore progenies. The quantitative variation in growth rate of oospore progenies but not zoospore progenies of *P. cactorum* was consistent with the hyphothesis that species of *Phytophthora* were diploid in the vegetative state (Samson, 1966).

Ann and Ko (1990) also found that a continuous quantitative variation in the amount of amylase produced among progenies of selfed oospores but not zoospores of *P. parasitica*, and suggested that amylase production in *P. parasitica* was controlled by heterozygous multiple genes. However, we didn't demonstrated whether amylase production in *P. cactorum*, homothalic fungi, was controlled by multiple genes. From the viewpoint of our results, the inheritance of growth rate of *P. cactorum* is similar to that of *P. syringae* (Boccas, 1972). The character of colony type in the isolate of *P. cactorum* was greatly various in oospore progenies but not zoospore progenies. Therefore, it is considered possible that multiple genes also are involved in determining the appearance of colonies in these fungi.

摘 要

*Phytophthora cactorum*으로부터 분리된 遊走子는 균사 생장이 상대적으로 균일하고 균사 생장분포가 母體 菌株와 유사하였으나, 분리된 卵胞子는 균사 생장분포가 매우 다양하였다. 또한, 균총의 특징은 난포자에서 크게 다양한 반면, 유주자에서는 균일하였다. 이 결과는 *P. cactorum*의 균사생장과 균총의 모양을 결정하는데 있어 다양한 유전자가 관여하고 있으며, 몇몇 역병균이 생장하는 동안에 이배체성 생식을 나타낼 수 있다는 점을 시사한다.

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