Mating Type Change in *Pythium splendens* Induced by Selfing

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Abstract

When (+) and (−) isolates of *Pythium splendens* were paired on the opposite sides of a polycarbonate membrane, oospores were produced by (+) but not (−) isolate. In addition to the original mating type, the opposite mating type appeared in the progeny derived from selfed oospores of most (+) isolates tested. Occasionally self-fertile (±) and neuter (0) types were also detected. The self fertility nature of (±) was unstable and transitory, segregating into (+) and (−) types during asexual reproduction. It was postulated that selfing may have changed the mating type by altering the function of a repressor which regulated the expression of mating types with different molecular configuration.

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Key words: *Pythium splendens*, mating type change, selfing, oospores, hormone.

INTRODUCTION

When (+) and (−) isolates of *Pythium splendens* Braun were paired on opposite sides of a polycarbonate membrane, numerous oospores were produced by (+) but not by the (−) isolates⁷⁾. This suggests that sexual reproduction in this important soil-borne plant pathogen under such conditions is a result of selfing of (+) isolates in response to the hormone produced by (−) isolates. The phenomenon of hormonal heterothallism in *P. splendens* is similar to that commonly observed in *Phytophthora*¹¹⁾. Mating type change occurred frequently when cultures of both A¹ and A² mating types of the cross-inducing (heterothallic) species of *Phytophthora* were subjected to prolonged storage¹⁰⁾, exposed to certain fungicides^{2,6,10,13)} or selfing^{1,3,4,12)}. Mating type change also occurred in (+) but not (−) isolates of *P. splendens* during long-term storage⁷⁾. Our attempts to induce mating type change by exposing cultures of *P. splendens* to fungicides, as mentioned above, were not successful. However, it was found that the fungus could be induced to change mating type by selfing. Details of the study are reported here.

MATERIALS AND METHODS

Isolates. Isolate 167F of (+) mating type and isolates 169F and 117F of (−) mating type of *P. splendens* were each originated from a single sporangium. This is also referred to as a conidium due to its inability to release zoospores. Isolates 167F and 169F were

recovered from cucumber⁷⁾ and isolate 117F was obtained from Dr. M. Aragaki of University of Hawaii at Manoa¹⁵⁾. Isolates 167F-9, 167F-27, 167F-42 and 167F-53, also of (+) mating type, were each derived from a selfed oospore of isolate 167F. All isolates were stored in sterile distilled water in test tubes at 24°C⁵⁾.

Production of selfed-oospores. The polycarbonate membrane method described by Ko⁹⁾ was used to obtain selfed oospores. Three pieces of culture block (15×10×2 mm) of a 12-hr-old (+) mating type grown on 10% V-8 agar (10% V-8 juice, 0.02% CaCO₃ and 2% Bacto agar) were placed in the center of a petri plate (90 mm diam), covered with a polycarbonate membrane (0.2 μm, 90 mm diam; Nuclepore Co., Pleasanton, California 94566), and paired with the same size of V-8 agar blocks freshly inoculated with (−) mating type of isolate 117F⁷⁾. After incubation for 6 days at 24°C in darkness, the membrane and the (−) isolate were removed and the culture blocks of (+) isolate were further incubated at 24°C in darkness in a moist chamber for a total of 40 days. Neither (+) nor (−) isolate was able to grow on membrane or petri plate in the absence of agar medium to make contact with each other during the 6-day pairing.

Isolation of single oospore colonies. The methods described by Guo and Ko⁸⁾ were used to remove sporangia from oospore suspensions of *P. splendens* and to activate oospores for germination. Oospore suspensions were obtained by blending 12 culture blocks with 50 ml sterile distilled water in an Omni mixer at 3500 rpm for 1 min. The suspension was passed successively through 45-μm and 20-μm sieves. Oospores and sporan-

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gia retained on the 20- μ m sieve were washed with 1000 ml tap water and resuspended in 50 ml sterile distilled water. To eliminate sporangia, spore suspensions were incubated with an equal volume of 10% clarified V-8 juice which was prepared by centrifuging V-8 juice at $270 \times g$ for 10 min and adding 0.02% CaCO_3 to the diluted supernatant. After incubation at 24°C overnight, germinated sporangia were separated by passage through a 45- μ m screen.

The oospore suspension was mixed with an equal volume of 0.5% KMnO_4 solution and the mixture was agitated for 30 min on a shaker to activate the oospores. After activation, oospores were washed on the 20- μ m sieve with 2000 ml of tap water followed by 300 ml of sterile distilled water. A 20–40 μ l aliquot of this oospore suspension containing about 20 viable oospores was spread on S+L medium¹⁴⁾ modified by adding 2% Bacto agar, 0.15% L-sorbose, and 100 μ g/ml ampicillin to reduce the rate of germ tube extension and inhibit the growth of contaminants. The plates were then incubated at 24°C under cool white fluorescent light (2000 lux). Colonies that originated from single oospores were transferred to water agar and subcultured on 10% V-8 agar plates.

Isolation of single-sporangium colonies. A culture block (20 $\times$ 20 $\times$ 2 mm) from a 6-day-old culture grown on 10% V-8 agar was cut into small pieces and blended with 50 ml of sterile distilled water in an Omni mixer at 4500 rpm for 1 min. The sporangium suspension was passed successively through 45- μ m and 20- μ m sieves and the sporangia retained on the 20- μ m sieve were collected in 10–15 ml of sterile distilled water. A 10–20 μ l aliquot of the spore suspension containing about 30 sporangia was spread on a water agar plate. After incubation at 24°C for 2 hr, colonies that originated from a single sporangium were transferred to water agar first and then subcultured on 10% V-8 agar plate.

Determination of mating type. The mating type of each isolate was determined by pairing a small piece of culture with another piece of (+) tester (isolate 167F) or (–) tester (isolate 169F) on a block (15 $\times$ 10 $\times$ 2 mm) of V-8 agar in a petri plate. Five blocks were placed at equal distance along the edge of the plate. After incubation at 24°C in darkness for 6 days, those

isolates forming oospores with the (+) tester were designated (–). Similarly those forming oospores with the (–) tester were designated (+). Those isolates forming oospores with both (+) and (–) tester and in single culture were designated ($\pm$). The (0) type was used to designate the isolates that were not able to form oospores with either (+) or (–) tester.

RESULTS

Oospore progeny of (+) isolates

When paired on the opposite sides of a polycarbonate membrane, oospores were produced by (+) isolate 167F but not (–) isolate 117F of *P. splendens*. Among the 117 selfed-oospore progeny of (+) isolate obtained, 62 cultures were of the parental (+) type, while 35 cultures become (–) type (Table 1). The oospore progeny also included 19 self-fertile cultures belonging to ($\pm$) type and one neuter culture belonging to (0) type. Mating type change did not occur in the asexual progeny. All the 110 single-sporangium cultures of isolate 167F tested belonged to the parental (+) type.

Selfed-oospore cultures were obtained from four (+) isolates selected from oospore progeny of isolate 167F. Some members of progeny produced by three of these isolates were of the (–) type (Table 1). Isolates 167F-9 and 167F-42 produced oospore progeny consisting of (+), (–), ($\pm$) and (0) types. Oospore progeny produced by isolate 167F-53 contained (+), (–) and (0) types. Among nine oospore cultures obtained from isolate 167F-27, eight were of the (+) type and the other was of the ($\pm$) type. About the same number of sporangium cultures obtained from each of these four isolates were all of the parental (+) type.

Sporangium progeny of ($\pm$) isolates

Single-sporangium cultures were obtained from six ($\pm$) isolates selected from oospore progeny of isolate 167F. The asexual progeny from all the six ($\pm$) isolates contained both (+) and (–) types (Table 2). Three of them also included one ($\pm$) type in their sporangium progeny.

Table 1. Mating type distribution in selfed-oospore progeny of (+) isolates of *Pythium splendens*

Parental isolate	No. of single-oospore cultures ^{a)}				
	Total scored	Mating type			
		(+)	(–)	($\pm$)	(0)
167F	117	62	35	19	1
167F-9	37	23	7	5	2
167F-27	9	8	0	1	0
167F-42	17	2	4	5	6
167F-53	18	13	4	0	1

a) Oospores were obtained by hormonal stimulation using the polycarbonate membrane method⁹⁾.

Table 2. Mating type distribution in sporangium progeny of ($\pm$) selfed-oospore cultures of isolate 167F of *Pythium splendens*

Parental isolate	No. of single-sporangium cultures			
	Total scored	Mating type		
		(+)	(–)	($\pm$)
1	17	10	6	1
2	21	2	19	0
3	24	19	4	1
4	17	2	15	0
5	18	2	15	1
6	18	5	13	0

DISCUSSION

Results from this study show that in addition to aging⁷⁾, mating type change from (+) to (−) in *P. splendens* can also occur as a result of selfing. The mating type did not change from (−) to (+) even during long-term storage⁷⁾. It is, therefore, considered possible that in *P. splendens* sexual reproduction may have started only after the conversion of certain ancestral members from the original (+) to (−) type during the evolutionary process. Therefore, the possible evolutionary origin of sex in this species differs from that of the closely related species of *Phytophthora* in which both A¹ and A² types can be changed to the opposite type during long-term storage¹⁰⁾ or as a result of exposure to various fungicides^{2,6,10,13)}.

It is conceivable that similar to the mechanism postulated by Ko¹⁰⁾ for *Phytophthora*, the expression of mating type in heterothallic species of *Pythium* may be regulated by a repressor which represses the expression of (+) mating type with one molecular configuration, and (−) mating type with another configuration. Selfing may have changed the mating type by reversing the function of the repressor.

The self-fertile character of (±) type that resulted from selfing of (+) isolates of *P. splendens* is unstable and appears to be a transitional state in the process of mating type change from (+) to (−) because almost all their asexual progeny were either (+) or (−) type. Unstable self-fertile (A¹A²) had also been generated by an A¹ isolate of *Phytophthora parasitica* Dast. through aging¹⁰⁾ and *Phytophthora infestans* (Mont.) de Bary through exposure to metalaxyl⁶⁾ and by an A² isolate of *P. parasitica* through exposure to chloroneb¹⁰⁾. Both A¹ and A² isolates of *Phytophthora cinnamomi* Rands also generated A¹A² type after being exposed to chloroneb or ethazol²⁾.

Conversion of (+) type to neuter (0) type in *P. splendens* as a result of selfing is unique. Neuter type (A⁰) which has been found in various species of *Phytophthora* has never been generated in the process of mating type change in this genus¹¹⁾.

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和 文 摘 要

Li-Yun GUO・Wen-Hsiung KO: 自殖によって誘導された *Pythium splendens* の交配型の変化

Pythium splendens の(+)型と(−)型の分離株をポリカーボン膜の表裏に接触させて培養すると、(+)株側では自殖により卵胞子が形成されたが、(−)側では形成されなかった。この方法により、(+)株側では形成された卵胞子の後代の交配型を検定した結果、ほとんどの株でもとの(+)型に加え、正反対の(−)型も出現することが明らかになった。さらに自家和合性の(±)型や無性の(0)型も検出された。(±)株の自家和合性の性質は不安定で一時的であり、無性生殖により(+)型と(−)型に分離した。本実験より、自殖は、異なった分子配列により交配型の表現を制御しているレプレッサーの機能を変えることによって、交配型を変化させたのではないかと想定される。