Techniques

Induction of Oospore Germination of *Phytophthora parasitica*

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ABSTRACT

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Oospores from the cross between A^1 and A^2 mating types of *Phytophthora parasitica* were exposed to light during maturation, treated with 0.25% $KMnO_4$ for 20 min, and incubated at 24 C under light on a medium consisting of glucose, lecithin, and Bacto agar (S + L medium). Under such conditions, germination of oospores from 60-day-old culture commenced within 24 hr and reached more than 90% after 10 days. Treatment with $KMnO_4$ also prevented the germination of residual mycelial fragments, chlamydospores, and sporangia present in the oospore suspension. Germination rate of oospores increased with age of culture, reaching more than 90% at the age of 56 days. Germination of oospores decreased from 95 to 44% when light was omitted during maturation of oospores, and to 15% when light was omitted during germination. Oospores did not germinate when light was not provided during maturation and germination. Oospores did not germinate in distilled water, but 28–94% did germinate on nutrient media. Among the media tested, S + L medium was the best, followed by V-8 agar and water agar. All the concentrations of $KMnO_4$ tested ranging from 0.05 to 1.0% were effective in inducing oospore germination. Treating oospores with 0.25% $KMnO_4$ for more than 20 min appeared to be harmful to oospores. Germination of oospores was completely inhibited when oospores were exposed to −15 C for 24 hr before or after treatment with $KMnO_4$.

Low and inconsistent frequency of oospore germination is one of the main obstacles in obtaining reliable data for genetic studies of *Phytophthora* (17,25). In general, oospores of self-inducing (homothallic) species of *Phytophthora* are easier to germinate than those of cross-inducing (heterothallic) species (25). High frequency of oospore germination has been reported for self-inducing species of *P. cactorum* (1), *P. heveae* (14), *P. katsurae* (13), *P. syringae* (10), *P. citricola* (15), *P. fragariae* (5), *P. vignae* (11), and *P. megasperma* (6–9). A low and inconsistent rate of germination has been reported for cross-inducing species of *P. palmivora* (4,12,18), *P. capsici* (12,16,18,19,21,24), *P. parasitica* (3,18), *P. cambivora* (18), and *P. infestans* (18,22,23).

After trying numerous methods in a preliminary test, we found an effective way of inducing oospore germination of *P. parasitica*. The detailed method is reported here. Factors affecting oospore germination of *P. parasitica* also were investigated.

MATERIALS AND METHODS

Production of oospores. A^1 (isolate 6133, ATCC 62653) and A^2 (isolate 6134, ATCC 62654) mating types of *P. parasitica* were grown on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$, and 2% Bacto agar adjusted to pH 6 with 1 N KOH) blocks (20 × 15 × 3 mm) at 24 C for 6 days in darkness. Agar blocks of A^1 and A^2 mating types were paired face to face in the center of a small petri dish (60 mm). The dish was sealed with two layers of Parafilm, kept in a moist chamber (40 × 25 × 15 cm) containing 20 ml distilled water. After incubation at 24 C for 14 days in darkness to induce oospore formation, the dish was removed from the moist chamber and further incubated at 24 C for various periods of time under cool white fluorescent light (2,000 lx) or in darkness for oospore maturation.

Germination of oospores. An oospore suspension was obtained by comminuting each pair of culture blocks with 50 ml distilled water in an Omni mixer at 4,500 rpm for 1 min. The suspension was filtered successively through 53-μm and 20-μm sieves. Oospores

retained on the 20-μm sieve were washed with 3,000 ml tap water and resuspended in 10 ml sterile distilled water.

The standard procedure for inducing oospore germination was to mix the oospore suspension with an equal volume of 0.5% $KMnO_4$ solution. After agitating the mixture 20 min on a shaker, oospores were washed on the 20-μm sieve with 2,000 ml tap water, followed by 1,000 ml sterile distilled water. About 5–20 μl of the oospore suspension containing about 50–100 viable oospores were plated on S + L medium (20). A liquid medium consisting of 1 ml basal salt solution, 10 mg lecithin (soy, refined; ICN Pharmaceutical, Cleveland, OH 44128), and 2 mg glucose in 100 ml distilled water was triturated in an Omni mixer at 6,000 rpm for 1 min and adjusted to pH 7 with 1 N KOH. The basal salt solution contained 100 mg $(NH_4)_2SO_4$, 100 mg $MgSO_4\ 7H_2O$, 30 mg $CaCl_2{\cdot}2H_2O$, 3 mg $ZnSO_4{\cdot}7H_2O$, 30 mg KH_2PO_4, and 60 mg K_2HPO_4 in 100 ml distilled water. Two grams of Bacto agar were added to the 100 ml of liquid medium, and after autoclaving, 10 mg of ampicillin, 5 mg of nystatin, and 1 mg PCNB were added to the medium to prevent growth of possible contaminants. Oospores on the medium were incubated at 24 C under light or in darkness. After 10 days, germination was assessed microscopically.

Two replicates were used for each treatment, and all the experiments were repeated at least twice.

RESULTS

Oospore germination. The method found to be effective in inducing oospore germination was to expose oospores of *P. parasitica* to light during maturation after their formation and treat them with 0.25% $KMnO_4$ for 20 min before incubation on S + L medium at 24 C under light. Under such conditions, germination of oospores commenced within 24 hr and reached more than 90% after 10 days. Oospores germinated by producing germ mycelia. The germ tube of the germinating oospore usually penetrated the oogonial wall. Occasionally it also emerged through the oogonial stalk and antheridium.

Effect of different treatments on germination of other propagules. Oospore suspension of *P. parasitica* obtained in this study also contained some mycelial fragments, chlamydospores, and sporangia that may germinate on the medium and interfere with counting germination rate and isolating germinating oospores. Without treatment, about 76% of these propagules germinated on S + L agar. Treatment with 0.25% $KMnO_4$ for 20 min, however, prevented germination of these propagules. After the oospore suspension of *P. parasitica* was exposed to −15 C for 24 hr, about 25% of other propagules were still capable of germinating on S + L agar.

Factors affecting oospore germination. For studying the effect of light and age of culture on oospore germination, oospores were harvested at different time intervals. Exposure to light during maturation of oospores after their formation and during germination is essential for obtaining high germination rates in relatively short periods of time. Under such conditions, germination rate of *P. parasitica* oospores increased with increasing age of culture, reaching more than 90% at the age of 56 days (Fig. 1). When light was provided during the maturation process, some oospores started to germinate directly in culture in 35 days, and the number of germinating oospores increased with increasing time of exposure to light. Germination of oospores from 56-day-old culture decreased from 95 to 44% when light was omitted during maturation of oospores and to 15% when light was omitted during germination (Fig. 1). Oospores did not germinate if light was not provided during maturation and germination.

To determine the effect of nutrients on germination of oospores, oospores from 60-day-old culture incubated under light were treated with 0.25% $KMnO_4$ for 20 min and incubated at 24 C under light for 10 days on S + L agar, 5% V-8 agar, potato-dextrose agar, and water agar. Oospores of *P. parasitica* apparently required nutrients for germination. Oospores did not germinate in distilled water, but 28 to 94% did germinate on nutrient media (Table 1). Among the media tested, S + L medium was the best, followed by V-8 agar and water agar. Potato-dextrose agar was not effective in supporting oospore germination.

Oospores from 60-day-old culture incubated under light were also used for studying the effect of $KMnO_4$ concentration and time of exposure to $KMnO_4$ on oospore germination. After $KMnO_4$ treatment, oospores were incubated on S + L agar at 24 C under light for 10 days. With a 20-min treatment, all the concentrations of $KMnO_4$ tested, ranging from 0.05 to 1.0%, were effective in inducing oospore germination (Fig. 2). Germination of oospores was greatly increased by treatment with 0.25% $KMnO_4$ solution for 5, 10, and 20 min, but not for 40 or 60 min (Fig. 3). None of the oospores germinated after 60-min treatment with 0.25% $KMnO_4$ solution. Germination of oospores also was completely inhibited when oospores were exposed to −15 C for 24 hr before or after treatment with 0.25% $KMnO_4$ for 20 min. Without $KMnO_4$ treatment, oospore germination was 17% after exposure to −15 C for 24 hr.

DISCUSSION

Only 3 to 12% germination of oospores of *P. parasitica* were obtained in the previous reports (3,18). In this study, more than 90% of the oospores of *P. parasitica* germinated within 10 days when oospores were exposed to light during maturation and treated with 0.25% $KMnO_4$ for 20 min before incubation on S + L medium under light. Treatment with $KMnO_4$, exposure to light during maturation and germination, and the use of proper

TABLE 1. Effect of nutrients on germination of oospores from the cross between A^1 and A^2 mating types of *Phytophthora parasitica*

Germination medium	Germination (%)[b]
S + L medium[a]	94 A[c]
V-8 agar	46 B
Potato dextrose agar	28 C
Water agar	43 B
Distilled water	0 D

[a] The medium consisted of basal salts, glucose, and lecithin (20).
[b] Oospores (60 days old) were treated with 0.25% $KMnO_4$ for 20 min followed by incubation at 24 C under light for 10 days.
[c] Data followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

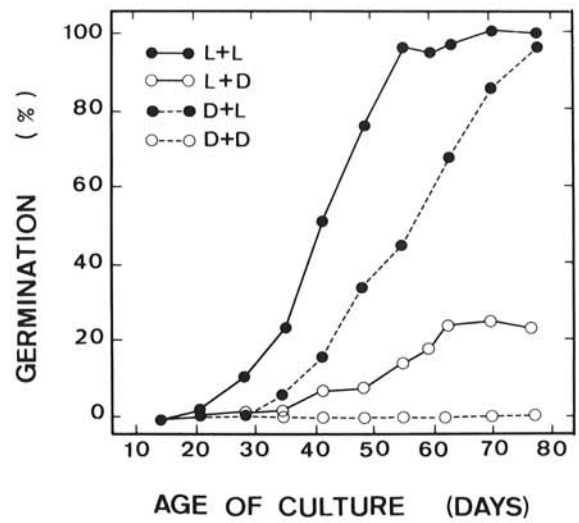

Fig. 1. Effect of age of culture and light on germination of oospores from the cross between A^1 and A^2 mating types of *Phytophthora parasitica*. Oospores were treated with 0.25% $KMnO_4$ for 20 min and incubated on S + L medium at 24 C for 10 days. L + L, oospores exposed to light during maturation and germination; D + L, oospores kept in darkness during maturation but exposed to light during germination; L + D, oospores exposed to light during maturation but kept in darkness during germination; D + D, oospores kept in darkness during maturation and germination.

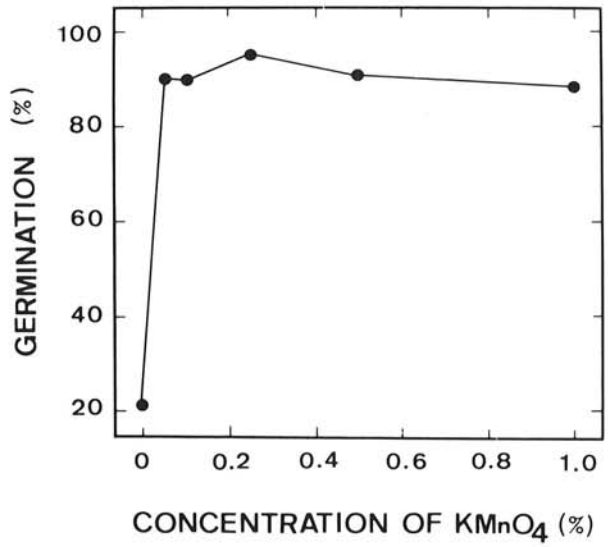

Fig. 2. Germination of oospores from 60-day-old culture under light, from the cross between A^1 and A^2 mating types of *Phytophthora parasitica* after treatment for 20 min in various concentrations of $KMnO_4$ solution. Germination was recorded after incubation on S + L agar at 24 C under light for 10 days.

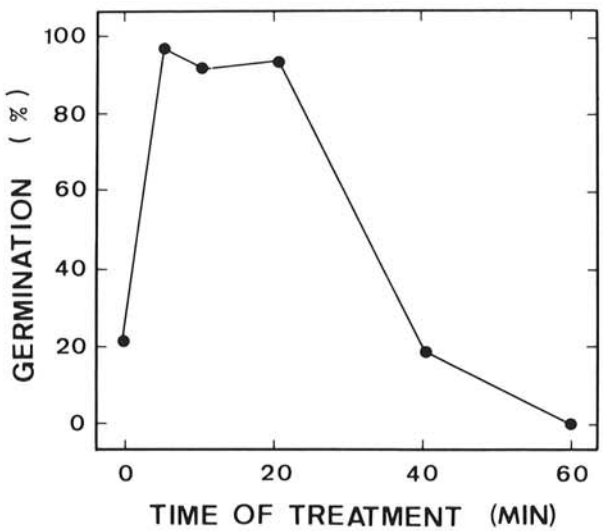

Fig. 3. Germination of oospores from 60-day-old culture under light, from the cross between A^1 and A^2 mating types of *Phytophthora parasitica*. Oospores were pretreated in 0.25% $KMnO_4$ solution for 0, 5, 10, 20, 40, or 60 min. Germination was recorded after incubation on S + L medium at 24 C under light for 10 days.

germination medium appear to be the most important factors for obtaining consistent high frequency of oospore germination of *P. parasitica*. In addition to being very effective in inducing oospore germination, the method also has the advantage of being free from interference by residual mycelial fragments, chlamydospores, and sporangia present in the oospore suspension.

It is not known if the same method is also effective in inducing germination of oospores of other cross-inducing *Phytophthora*. Ruben et al (20) reported that $KMnO_4$ treatment in combination with desiccation of culture and heat treatment stimulated synchronous germination of oospores of *Pythium aphanidermatum*. Recently El-Hamalawi and Erwin (6) also reported that treatment with $KMnO_4$ and incubation in alfalfa root exudate under light stimulated oospore germination of self-inducing *P. megasperma* f. sp. *medicaginis*. Beake et al (2) showed that pretreatment of oospores of *P. m.* f. sp. *medicaginis* had no detectable effect on the oospore cytoplasm or outer layers of the oospore wall, but that it did reveal the microfibrillar substructure of the thick inner layer of the oospore wall.

The mechanism by which freezing treatment renders oospores of *P. parasitica* vulnerable to $KMnO_4$ treatment and vice versa is still not known. Oospores may have been weakened by low temperature or $KMnO_4$ due to damage or alteration of cell-wall structure.

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