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Agarose Medium for Germination of Oospores of *Phytophthora cactorum*

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With 2 figures

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Abstract

When oospores of *Phytophthora cactorum* from 30-day-old culture were treated with 0.25% $KMnO_4$ for 20 min and incubated at 24°C under light for 10 days, 65–75% germinated on water agar and water agarose but only 1–21% germinated on V-8 agar and S+L agar. Water agarose was selected because germinated oospores formed restricted colonies on this medium that could be isolated easily. $KMnO_4$ treatment killed sporangia, chlamydospores and mycelial fragments present in oospore suspensions. Under the above conditions, approximately 44% of oospores from 10-day-old culture germinated and the optimum germination rate of about 75% was obtained when oospores reached about 20 days old.

Zusammenfassung

Agarosemedium für die Keimung von *Phytophthora cactorum*-Oosporen

Wenn Oosporen von *Phytophthora cactorum*, die von 30 Tage alten Kulturen gewonnen worden waren, mit einer 0,25%iger $KMnO_4$-Lösung für 20 Min behandelt und anschließend für 10 Tage bei 24°C mit Licht inkubiert wurden, keimten 65 bis 75% auf Wasseragar bzw. Wasseragarose aber nur 1 bis 21% auf V-8-Agar bzw. S+L-Agar. Wasseragarose wurde für die Isolation des Pilzes gewählt, weil die gekeimten Oosporen kompakte Kolonien auf diesem Nährmedium bildeten, die einfach zu isolieren waren. Die $KMnO_4$-Lösung tötete Sporangien, Chlamydosporen und Myzelfragmente, die in Oosporensuspensionen vorhanden waren, ab. Unter diesen Bedingungen keimten ca. 44% der Oosporen von 10 Tage alten Kulturen und die optimale Keimungsrate von ca. 75% wurde erzielt, wenn die Oosporen ein Alter von 20 Tagen erreicht hatten.

Introduction

Phytophthora cactorum (Lebert & Cohn) Schroeter is an important plant pathogen with a very broad host range parasitizing more than 83 genera in 44 families (Ribeiro, 1978). The fungus is also a popular organism for the study of the physiology of sexual reproduction (Elliot, 1983; Ko 1988). Oospores resulting from sexual reproduction are important survival structures and the potential source of variation in nature especially in self-inducing 'homothallic' species of *Phytophthora* (Zentmyer and Erwin, 1970). One of the major problems encountered in the study of oospores in *Phytophthora* has been their low level of germination (Blackwell, 1943; Zentmyer and Erwin, 1970). Although high germination rate of oospores of *P. cactorum* has been obtained using ingestion of water snails (Shaw 1967) or by freezing and enzyme treatments (Banihashemi and Mitchell, 1976), both methods were tedious and time consuming requiring at least 2 days to process oospores before germination on agar medium. Recently, $KMnO_4$ treatment has been found to be very effective in inducing oospore germination of *Phytophthora parasitica* Dastur (Ann and Ko, 1988) and *Phytophthora infestans* (Mont.) de Bary (Chang and Ko, 1991). The method is simple and the treatment takes only 15–30 min. We, therefore, tried it on *P. cactorum* and found that $KMnO_4$ treated oospores germinated copiously and produced colonies easy to isolate on water agarose medium. Details of the study are reported here.

Materials and Methods

Production of oospores

The isolate (121F) of *P. cactorum* used was obtained from Dr D. L. McIntosh and derived from a single zoospore. The fungus was grown on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$ and 2% agar adjusted to pH 6 with 1N KOH) blocks (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°C for formation and maturation of oospores unless otherwise stated.

Germination of oospores

An oospore suspension was obtained by triturating a culture block with 50 ml of distilled water in an Omni mixer at 4300 rpm for 30 s. The suspension was filtered successively through 53-μm and 20-μm sieves. Oospores retained on the 20-μm sieve were washed with tap water and re-suspended in 10 ml of sterile distilled water. The oospore suspension was mixed with an equal volume of freshly-prepared $KMnO_4$ solution at 0.5% (w/v). After agitating the mixture for 20 min on a shaker, oospores were washed free of $KMnO_4$ with tap water on a 20-μm sieve. About 100 oospores were spread on S+L agar (Ruben et al., 1980; Ann and Ko, 1988), 10% V-8 juice agar, 2% water agar or 0.8% water agarose (SeaKem HGT-P agarose; FMC BioProducts, Rockland, Maine, USA) (Ho and Ko, 1980). Each medium was supplemented with 100 μg/g ampicillin, 50 μg/g nystatin and 10 μg/g pentachloronitrobenzene (Ko et al., 1978) after autoclaving to prevent growth of contaminants. After incubation at 24°C under continuous cool white fluorescent light (2000 lux) for 10 days, the germination rate was assessed microscopically. Two replicates were used for each treatment and the experiments were repeated at least once.

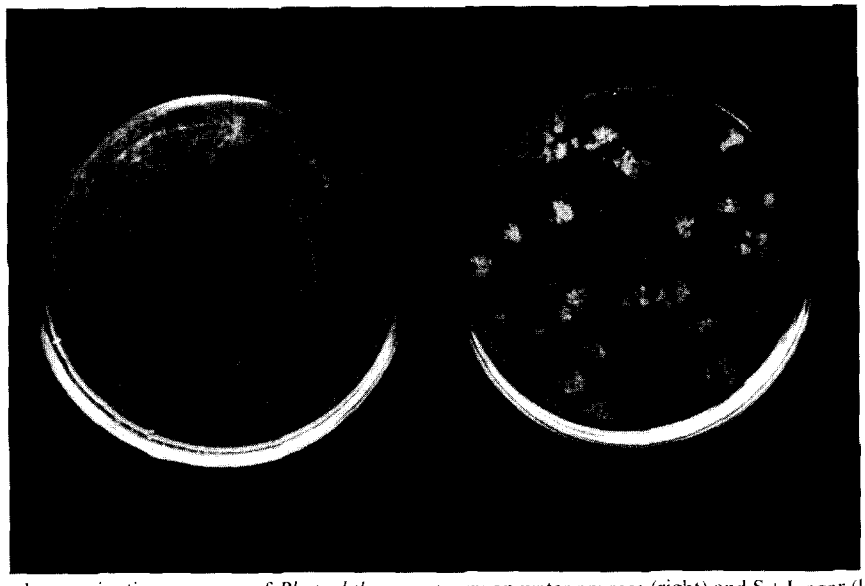

Fig. 1 Colony formation by germinating oospores of *Phytophthora cactorum* on water agarose (right) and S+L agar (left). All colonies on each plate originated from oospores

Table 1
Effects of media on germination of oospores of *Phytophthora cactorum*

Germination medium	Germination (%)[b]
S+L agar[a]	21 B[c]
V-8 agar	1 B
Water agar	65 A
Water agarose	75 A

[a] The medium consisted of basal salts, glucose and lecithin (Ruben et al., 1980).
[b] Oospores (30-day-old) were treated with 0.25% of $KMnO_4$ for 20 min followed by incubation at 24 °C 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.

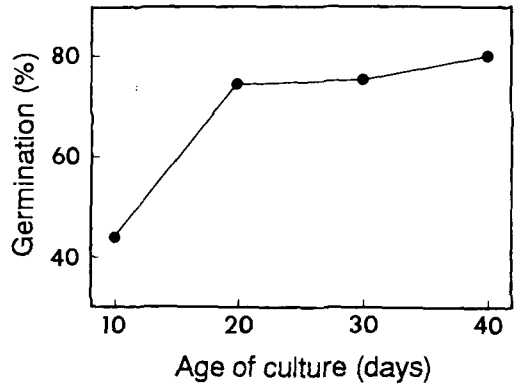

Fig. 2 Effect of culture age on germination of oospores of *Phytophthora cactorum*. Oospores were treated with 0.25% $KMnO_4$ for 20 min and incubated on water agarose at 24 °C under light for 10 days

Results

When oospores of *P. cactorum* from 30-day-old culture were treated with 0.25% $KMnO_4$ for 20 min before incubation on different agar media at 24 °C under light, 65–75% germinated on water agar and water agarose but only 1–21% germinated on V-8 agar and S+L agar (Table 1, Fig. 1). Although germination of *P. cactorum* oospores on water agar was not significantly different from that on water agarose, the latter was selected for subsequent experiments because germinated oospores formed restricted colonies that could be isolated easily on water agarose (Fig. 1) but not on water agar.

To study the effect of age of culture on oospore gemination, oospores were harvested at different time intervals, treated with 0.25% $KMnO_4$ for 20 min, and plated on water agarose for 10 days at 24 °C under light. Approximately 44% of oospores from 10-day-old culture germinated, and the optimum germination of about 75% was obtained when oospores reached about 20 days old (Fig. 2).

Discussion

Since Seakem agarose is practically free of nutrients (Ho and Ko, 1980), the present result suggests that oospores of *P. cactorum* did not require nutrients for germination. This is consistent with the previous finding that *P. cactorum* oospores germinated in sterile distilled water (Banihashemi and Mitchell, 1976). Different species of *Phytophthora* appear to have different nutritional requirements for oospore germination. Without nutrients, *P. parasitica* oospores did not germinate even after activation with $KMnO_4$ (Ann and Ko, 1988). Our study also found that although S+L medium stimulated germination of oospores of *P. parasitica* (Ann and Ko, 1988) and *P. infestans* (Chang and Ko, 1991), it was inhibitory to that of *P. cactorum*.

Oospore suspensions of *P. cactorum* obtained in this study also contained some sporangia, chlamydospores and viable mycelial fragments. $KMnO_4$ treatment appears to kill all these propagules as none of them were able to germinate or grow on the media tested. Destruction of asexual structures by 20-min $KMnO_4$ treatment is more convenient than by 48-h passage through water snails (Shaw, 1967) or 24-h freezing treatment (Banihashemi and Mitchell, 1976).

Our results showed that oospores of *P. cactorum* required a relatively short period of time for maturation. Shaw (1967) also obtained high germination rates with oospores from 21-day-old culture. Different species of *Phytophthora* apparently require different periods of time for maturation. Optimum germination rates of oospores were obtained when culture age reached about

10 days for *P. infestans* (Chang and Ko, 1991), 30 days for *Phytophthora katsurae* Ko et Chang (Ko and Arakawa, 1980) and 50 days for *P. parasitica* (Ann and Ko, 1988).

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