A Modified Soil-Over-Culture Method for Inducing Basidia in Thanatephorus cucumeris

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ABSTRACT

In the induction of basidia by *Rhizoctonia solani* with the soil-over-culture method, dextrose and yeast extract were the major components of the primary medium. Tests were performed to determine the optimum concentrations of these components for basidial production. Experiments with soil pH, soil moisture, soil compaction, and mycelium age at the time of overlay were designed to study the effects of those variables on sporulation. The following soil-over-culture method is proposed: (i) inoculate the fungus to 20 ml of yeast extract-dextrose agar in 9 cm petri dishes, (ii) incubate at room temperature until the fungus colony covers the agar surface or up to 2 days later, (iii) cover the agar surface with 90 g of oven dry soil (silty clay) to make a soil layer approximately 1 cm deep, (iv) adjust the soil reaction to pH 8-9, (v) maintain the soil moisture at 30% (by weight), and (vi) incubate at room conditions.

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Many methods are reported for inducing the perfect state of *Rhizoctonia solani* Kuehn (= *Thanatephorus cucumeris*) (Frank) Donk and related fungi in vitro (2, 7). These methods can be grouped into three principal categories: sporulation on agar, on soil, and on plant tissues. However, none of these methods can be satisfactorily applied to induce sporulation of all isolates and species of *Rhizoctonia*. Among these methods, the soil-over-culture method (7) is most widely accepted because of its simplicity, but details of the optimum conditions for this method are not known. We report results of experiments designed to determine optimum substrate, inoculum, and physical conditions for vegetative growth and sporulation of *R. solani*.

An isolate of *R. solani*, K-1, was used as a test fungus throughout these experiments and a total of seven isolates were used in confirmatory tests. Potato-dextrose agar (PDA, Difco) plus 0.5% yeast extract served as primary substrate for fungal growth. A silty clay from Georgia was used as secondary substrate for hymenium formation. All experiments had four replications of treatments and repeated twice.

Potato-yeast extract-dextrose agar (PYDA) was prepared by boiling 250 g of sliced fresh potato for 30 minutes, and adding to the decanted boil-water 5 g of yeast extract, 20 g of dextrose, 15 g of agar, and distilled water to make the mixture up to 1,000 ml. Different concentrations of yeast extract in PDA were also prepared by adding 0.1, 0.25, 0.5, 0.75, and 1.0% yeast extract to PDA.

Mycelial disks 10 cm in diameter were cut from the periphery of 3-day-old colonies grown on PDA, transferred to PYDA in petri dishes, and incubated at 28 C in the dark. After a variable growth period, the cultures were removed from the incubator and the colonies on the agar surface were covered with soil.

The effects of soil moisture on hymenium formation was evaluated. Replicate groups of petri dish cultures with colonies of *R. solani* on PYDA received 100 g of oven-dried soil and their moisture levels were adjusted to 5, 15, 25, 35, and 45% by weight. Readjustment of soil moisture was made twice a day to compensate for the loss of water by evaporation. For testing the effects of different soil compaction, 30, 60, 90, and 120 g of silty clay were added to the 9-cm diameter petri dishes which contained fully grown colonies of *R. solani* on PYDA. The added soil was packed to a depth of not more than 1 cm. For comparing the effects of different soil reactions, petri dishes with colonies of *R. solani* received 100 g of soil, which was then adjusted to different pH levels. Desired soil pH was obtained by adding suitable amounts of 1N HCl and 1N KOH. Soil moisture was maintained at 35% water (by weight) by daily addition of distilled water adjusted to the respective pH levels.

No hymenium formation was found on water agar and dextrose agar. Very scant hymenia were found on potato-dextrose agar, yeast extract agar, and potato-yeast extract agar. Dextrose-yeast extract agar and PYDA were the best media for both hymenial and basidial development. From this analysis, it was clear that dextrose and yeast extract were the two major components of the primary substrate. However, the effect of potato extract seemed rather minor, and effects of yeast extract and dextrose were negligible except when combined together. Yeast extract at 0.75% concentration had the best effect on hymenial development. Yeast extract at 0.25 and .05% slightly reduced hymenium production. A sharp decrease of hymenia was found when PDA contained 0.1 or 1.0% yeast extract.

Mycelial maturity was positively related to initiation and development of hymenia. Both very young (1-2 days) and very old (8 or more days) colonies failed to sporulate. When the colony had just covered the agar surface (3-4 days), hymenial initiation took only 3 days after covering with soil, and further hymenial development was also good. Although a slightly older colony (5 days) delayed hymenium initiation for one day, further hymenial development was excellent and it almost completely covered the soil surface. This indicated that hymenial initials were formed from hyphae of a limited age (3-7 days old).

A suitable amount of silty clay (90 g) in a limited space (9 cm diameter by 1 cm deep) induced the best hymenial growth. The effect of soil compaction on hymenial initiation may have resulted from its effects on the moisture condition of soil. The best soil moisture for hymenial development in our tests was 30% by weight, which agrees with earlier reports (5). Petri dishes which contained less than 90 g of soil dried rapidly in open air, and hence basidia were not formed readily.

In previous studies (1, 5), good vegetative growth of *R. solani* has been reported at a soil pH of 4.1 to 8.1. Hymenial development, however, occurred when soil pH varied from 6 to 10. Another report (4) showed that

hymenial development was best at a higher soil pH (8.0), while saprobic growth was best at pH 7. Stretton et al. (7) found that under the influence of light, hymenia appeared after 8-24 hours of constant or fluctuating light intensities of 4 to 1,450 ft-c., and Flentje (2) had similar results under 200 to 2,000 ft-c. We obtained excellent sporulation under laboratory conditions. A number of workers had shown that hymenia developed when air temperature varied from 20 to 30 C (2, 3, 6, 8), and when the relative humidity ranged from 40-60% (7) or as high as 100% (3, 6).

From the above results, a modification of the soil-over-culture method was devised. This modification consisted of: (i) inoculate the fungus to 20 ml of yeast extract-dextrose agar (yeast extract, 7.5 g; dextrose, 20 g; agar, 15 g; and distilled water to make up 1,000 ml) in a 9-cm diameter petri dish, (ii) incubate at room temperature until the fungal colony covers the agar surface or up to 2 days later, (iii) cover the agar surface with 90 g of oven-dry soil (silty clay) to make a soil layer approximately 1 cm deep, (iv) adjust the soil pH to 8-9, (v) maintain the soil moisture at 30% water (by weight), and (vi) place the petri dish on a laboratory bench exposed to direct sunlight.

The efficiency of the modified soil-over-culture method was compared with that of Stretton et al. (7) for its ability to induce basidia in seven isolates of *R. solani*. The modified method induced all isolates to sporulate, while the latter was successful in only four. The most practical value of the modified method will probably be its broader application.

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