A Selective Medium for the Quantitative Determination of Rhizoctonia solani in Soil

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ABSTRACT

An agar medium containing minerals, Dexon, chloramphenicol, streptomycin, and gallic acid and sodium nitrite as selective carbon and nitrogen sources suppresses the development of undesired microorganisms and enhances the growth of *Rhizoctonia solani* from soil. Ninety to 100% recovery of *R. solani* from soil inoculated with sclerotia was obtained with this selective-medium method. The population of *R. solani* in naturally infested soils as determined by this method ranged from 1-9 propagules/10 g dried soil. Low population of *R. solani* in soil appears to be the main reason why the soil-dilution-plate method is inadequate for determining populations of this fungus. Sensitiveness, simplicity, and quickness are the important advantages of this method. Phytopathology 61:707-710.

Additional key words: beet-seed method, soil-borne pathogens.

Various methods have been suggested for determining the prevalence of *Rhizoctonia solani* Kuehn in soil. Most involve the use of seeds (5, 7) and sections of stems (8, 9, 10) as baits for trapping the fungus, and consequently are not quantitative. Immersion-tube colonization (6) and debris-particle isolation (2) also have been suggested. However, recovery of the fungus with these methods has been unsatisfactory (7, 10). Although the dilution-plate method is widely used for determining populations of various microorganisms in soil, it has not been applied successfully to *R. solani* (1, 2). A comprehensive review of this subject was made recently by Pan (7).

Previously, no medium selective for *R. solani* has been available (11). We report here the development of a selective medium for the quantitative determination of this fungus in soil.

MATERIALS AND METHODS.—*Rhizoctonia solani* isolates R-1, R-2, and R-5 were isolated from a bean seedling, a potato tuber, and a beet seedling, respectively. Isolates R-3 and R-4 were isolated from pine seedlings. All isolates of *R. solani* were maintained on potato-dextrose agar (PDA). For soil inoculation, different isolates of *R. solani* were grown for 7 days at 26 C in sterilized soil (500 ml fine sandy loam soil plus 50 g chopped potato). Inoculated soils were prepared by mixing different ratios of inoculum and natural soil.

The basal medium used in studying the effect of gallic acid and sodium nitrite concentration on growth of *R. solani* was composed of 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 10 mg $FeSO_4 \cdot 7H_2O$, 90 mg Dexon [sodium *p*-(dimethylamino) benzenediazosulfonate, 70% wettable powder, Chemagro Co.], 50 mg chloramphenicol (Calbiochem), 50 mg streptomycin sulfate (Calbiochem), 20 g agar, and 1 liter distilled water. All the mineral salts were added to agar medium before autoclaving. Gallic acid, Dexon, chloramphenicol, and streptomycin were added to melted agar media (50 C) before pouring plates. To test the effect of different compounds on the growth of *R. solani*, agar discs (3 × 3 mm) containing mycelia of isolate R-5 were placed in the center of petri plates containing test media. After 48-hr incubation at 28 C, diam of colonies were measured. Five plates were used for each treatment, and the experiment was repeated once.

The selective-medium method is as follows: A given amount of soil was moistened with distilled water, compacted with a spatula, and evenly distributed in 10 clumps on a plate of selective medium. Fifteen plates were used for each designated amount of soil. The perimeters of the soil clumps were examined microscopically with ×10 objective after 24- and 48-hr incubation at 28 C. The number of clumps with emerging *R. solani* hyphae was then determined. *Rhizoctonia solani* was identified by the distinctive morphological characteristics of its mycelium (7).

The beet-seed colonization method was similar to that described by Pan (7). Fifty beet seeds (Detroit Dark Red) were evenly distributed over 32 g soil in a petri dish (100 × 20 mm) and covered with an additional 32 g of soil. After 48-hr incubation at 26 C, seeds were recovered and washed for 5 min with running tap water in a colander. Excess water on seeds was blotted with paper towels. Seeds were then plated on acidified PDA (0.45 ml of 50% lactic acid in 100 ml PDA, pH 4.0) using 10 seeds/plate. The number of seeds with emerging *R. solani* was determined microscopically after 24 hr.

RESULTS AND DISCUSSION.—*Development of the selective medium.*—Various inhibitors were tested for their effect on the growth of *R. solani* on PDA. Antifungal antibiotics: actidione, nystatin, and pimaricin, at 50 ppm completely inhibited the growth of *R. solani* (Table 1). Growth of *R. solani* was only slightly inhibited by Dexon at 90 ppm, chloramphenicol and streptomycin at 50 ppm, and gallic acid and sodium nitrite at 200 ppm. Dexon, chloramphenicol, streptomycin, gallic acid, and sodium nitrite were, therefore, selected for further studies.

Effect of gallic acid concentration on the growth of

TABLE 1. Effect of inhibitors on the growth of *Rhizoctonia solani* on potato-dextrose agar

Compound	Concn	Diam of colonies[a]
	ppm	*mm*
None		25.0
Dexon[b]	90	20.4
Chloramphenicol	50	20.8
Streptomycin sulfate	50	22.8
Actidione	50	0
Nystatin	50	0
Pimaricin	50	0
Gallic acid	200	22.0
Sodium nitrite	200	19.0

[a] Based on two experiments and five replicates/treatment. Measured after 2 days' incubation at 28 C.
[b] Sodium *p*-(dimethylamino)benzenediazosulfonate, 70% wettable powder.

R. solani was studied on the basal medium supplemented with 200 ppm $NaNO_2$ and different concentrations of gallic acid. When gallic acid was added as a carbon source, it stimulated the growth of *R. solani* at concentrations below 800 ppm (Fig. 1). It was inhibitory at 1,600 ppm. The optimum concentration of gallic acid for the growth of *R. solani* was 200-400 ppm. The higher rate was preferred for the selective medium because gallic acid was reported to inhibit other fungi (4). Effect of sodium nitrite concentration on the growth of *R. solani* was also studied, using the basal medium supplemented with 400 ppm gallic acid and different concentrations of sodium nitrite. Sodium nitrite was stimulatory to the growth of *R. solani* at all concentrations tested, when added as a nitrogen source. The optimum concentration of sodium nitrite for the growth of *R. solani* was 200 ppm. Fifty ppm chloramphenicol and streptomycin was used because this concentration has been commonly used for inhibiting soil bacteria and actinomycetes, while 90 ppm Dexon was selected because this amount was reported to be active against phythiaceous fungi, but not *R. solani* (7).

From the above results, the following medium, selective for *R. solani*, was formulated: 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 10 mg $FeSO_4 \cdot 7H_2O$, 0.2 g $NaNO_2$, 0.4 g gallic acid, 90 mg Dexon, 50 mg chloramphenicol, 50 mg streptomycin, and 20 g agar in 1 liter distilled water.

The selective medium was tested for its effect on the growth of soil microorganisms and isolates R-1, R-2, R-3, and R-4 of *R. solani*, which differ in growth rate and pathogenicity. Agar discs containing *R. solani* were inserted into natural soil, and each was placed on a plate of selective medium. After 24-hr incubation at 28 C, all isolates of *R. solani* grew away from the soil clumps on the medium. Other fungi did not grow on the medium until after 48 hr. Growth of *R. solani* at that time was about 10-32 mm away from the soil clumps. Neither bacteria nor actinomycetes developed on the selective medium. The medium is, therefore, considered selective for growth of *R. solani* from soil.

Plates containing selective medium were kept in darkness in an incubator at 24 C to study the effect of storage on the selective medium. After 2, 4, 8, 16, and 32 days' incubation, isolate R-1 of *R. solani* was coated with natural soil and placed on the center of the plate. Growth of *R. solani* and other soil microorganisms was determined after 48-hr incubation at 28 C. Five plates were used each time, and the experiment was repeated once. After 32 days' storage, the selective medium was still effective in suppressing undesired soil microorganisms and supporting rapid growth of *R. solani*. The selective medium must be stored in darkness because Dexon is sensitive to light.

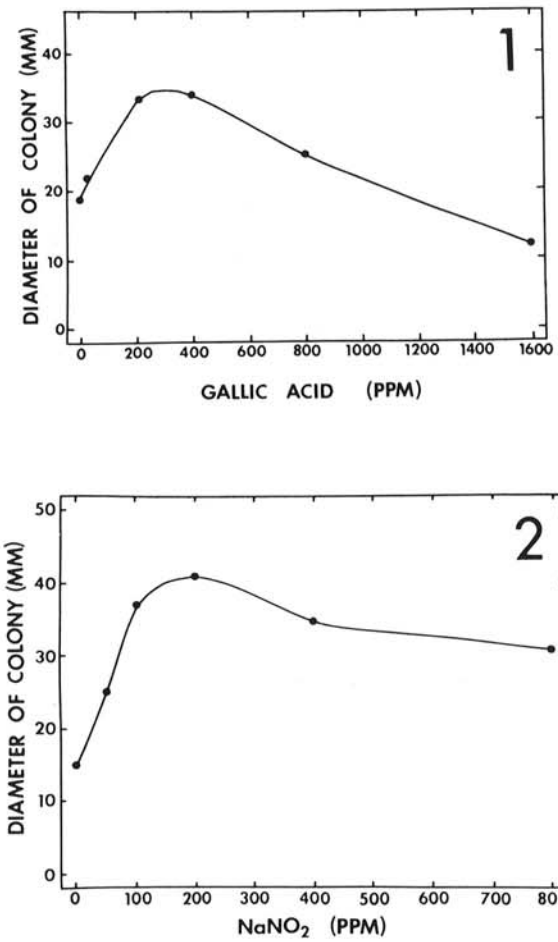

Fig. 1-2. **1)** Effect of gallic acid concentration on growth of *Rhizoctonia solani* on a basal medium supplemented with 200 ppm sodium nitrite. **2)** Effect of sodium nitrite concentration on growth of *R. solani* on a basal medium supplemented with 400 ppm gallic acid. The basal medium contained 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 10 mg $FeSO_4 \cdot 7H_2O$, 90 mg Dexon, 50 mg chloramphenicol, 50 mg streptomycin, 20 g agar, and 1 liter distilled water.

Sensitivity of the selective medium.—Among methods previously reported, use of beet seeds as bait for trapping *R. solani* is the most sensitive (7). Sensitivity of the selective-medium method for recovering *R. solani* was compared with the beet-seed method, using the same four *R. solani* isolates in inoculated soil.

TABLE 2. Comparison of the sensitivity of the beet-seed and selective-medium methods for recovery of *Rhizoctonia solani* from inoculated soil[a]

Isolate	*R. solani* recovered (no./g dried soil)	
	Beet seed	Selective medium
R-1	3.5	16
R-2	12	16
R-3	0.25	44
R-4	9.5	260

[a] Soils were mixed with different amounts of inoculum grown in sterilized soil supplemented with 10% potato.

Twenty and 0.5% inoculum were used for isolate R-3, and for R-1, R-2, and R-4, respectively, because they gave the highest recovery among concentrations tested with the beet-seed method. The amount of inoculated soil plated on selective medium was 0.1-2.5 g/plate. Population of *R. solani* isolates R-1, R-2, R-3, and R-4 as determined by the selective-medium method was, respectively, about 4, 1.3, 176, and 27 times higher than that determined by the beet-seed method (Table 2).

The possibility that one *R. solani* propagule in soil may colonize more than one beet seed was studied by placing two sclerotia of *R. solani* isolate R-1 about 4 cm apart in a petri plate containing 50 beet seeds in 64 g soil. After 48 hr, the number of beet seeds colonized by two sclerotia in fine sandy loam, sandy loam, and silty clay loam were 10, 6, and 5, respectively. No beet seeds were colonized by *R. solani* in controls which did not have added sclerotia. The number of beet seeds colonized by *R. solani* was greater than that of *R. solani* propagules present. Moreover, the type of soil used considerably affected the number of beet seeds colonized. These results further suggest that baiting methods are not quantitative.

Sensitivity of the selective medium was also studied by mixing 5, 10, or 15 sclerotia of *R. solani* isolate R-1 obtained from a potato-dextrose agar culture with 15 g natural soil. Each inoculated soil was plated on 15 plates, using 1 g distributed in 10 clumps/plate. Ninety to 100% recovery of *R. solani* from soil was obtained. Therefore, this method is suitable for quantitative determination of *R. solani* in soil. In addition to sensitiveness, simplicity and quickness are the two other advantages of this method.

Determination of R. solani population in naturally infested soils with the selective medium.—Soil collected from a naturally infested field was used to study the relation between the amount of soil plated and the number of *R. solani* propagules recovered. Fifteen plates were used for each designated amount of soil. The population of *R. solani* appeared to be very low. *Rhizoctonia solani* was not recovered when 0.01 and 0.1 g soil/plate was used. Even when 1 and 2 g soil/plate were used, respectively, only 6 and 16 clumps out of 150 clumps on 15 plates contained *R. solani*. When naturally infested soil was used, the average growth of *R. solani* after 48 hr was about 12 mm away from soil, whereas the average growth of the other fungi was about 5 mm.

The population of *R. solani* in eight other soil samples collected from infested potato and bean fields on two islands of Hawaii was determined using 15 plates for each sample and 1 g of soil/plate. The population of *R. solani* in these soil samples ranged from 1-9 propagules/10 g dried soil. In comparison with the population of other soil-borne pathogens, this number is extremely low.

We, therefore, studied the disease-inducing potential of soil containing this amount of inoculum. Twenty beet seeds were planted in a 250-ml beaker containing 24 g moist soil inoculated with *R. solani* isolate R-1 at a concentration of 0, 1, or 9 sclerotia/10 g dried soil. Three beakers were used for each treatment. After 6 days, the per cent pre-emergence damping-off was 0, 50, and 90 in soil containing 0, 1, and 9 sclerotia/10 g dried soil, respectively. When naturally infested soils were used, pre-emergence damping-off of beet also occurred. Apparently, the population of 1-9 propagules of *R. solani*/10 g dried soil is capable of causing disease. Therefore, low populations of *R. solani* in soil may account for the unsuccessful application of the dilution-plate method to this fungus.

The combination of selective inhibitors against undesired microorganisms and the carbon and nitrogen sources selective for *R. solani* constituted the selective medium. The antibiotics, chloramphenicol and streptomycin, were used to inhibit both bacteria and actinomycetes, while the fungicide, Dexon, which is not inhibitory to *R. solani* (7), was used to prevent the growth of pythiaceous fungi. Gallic acid (4) and sodium nitrite (3) were selected as the carbon and nitrogen sources for *R. solani* because they are inhibitory to or unmetabolizable by many other fungi. Both compounds are stimulatory to the growth of this fungus.

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