Isolation and Identification of Pyrenochaeta terrestris from Soil on Dilution Plates

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ABSTRACT

An agar medium containing sorbose, chloramphenicol, and TBZ was developed on which propagules of *Pyrenochaeta terrestris* present in soil can be isolated and easily recognized as pink-pigmented, restricted colonies.

In naturally infested soil, the fungus was associated with plant debris particles and was also present as free resting bodies.

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Pyrenochaeta terrestris (Hansen) Gorenz, Walker & Larsen, which causes the pink root disease of onions, has recently become established in Israel. A rapid method for identification of this pathogen from infected roots was suggested by Watson, who used a low nutrient medium and sterile chopped wheat straw; the latter turns pink only on contact with *P. terrestris* (5). However, no method has been reported for direct isolation and identification of this pathogen from soil. In the present work, both a medium and a technique were developed for the isolation and identification of *P. terrestris* on soil dilution plates.

Watson's medium (5) without wheat straw was used as the basal medium. Sorbose proved to be a suitable carbon source to increase selectivity of the medium because of its known paramorphogenic effect (1, 4). Colonies of *P. terrestris* originating from mycelial fragments remained restricted and unpigmented on medium containing 0.5% sorbose. Pink pigmentation of the restricted colonies was induced by the addition of 2-(4′-thiazolyl)benzimidazole (TBZ). However, when added before incubation, TBZ inhibited fungal growth. These two findings were exploited in devising a technique for the identification of *P. terrestris* isolated from soil on dilution plates.

Sandy loam soil (pH 6.8) was inoculated with different amounts of homogenized mycelium of *P. terrestris* which had been grown on potato-dextrose broth for 7 days at 25 C. One-ml aliquots of 10^{-2} soil dilution in 0.1% water agar were pipetted onto each of 60-mm diam plates (10 plates/treatment). Four ml of medium containing 0.3% $NaNO_3$, 0.1% $MgSO_4 \cdot 7 H_2O$, 0.5% sorbose, 250 μg/ml chloramphenicol and 2% Bacto agar were pipetted into each of the plates and rotated. TBZ (20% active ingredient in 7% hypophosphorous acid) was diluted in distilled water and 0.3-ml aliquots added to the surface of the medium after 4 days of incubation at 25 C to give a final concn of 1 μg/ml, active ingredient. The TBZ solution was evenly spread with a Drigalski glass rod. The pink, restricted colonies of *P. terrestris* could be easily counted after an additional 48 hr of incubation.

TABLE 1. Effect of inoculum density[a] of *Pyrenochaeta terrestris* in soil on disease incidence of onion seedlings[b] (cv 'Egyptian') and numbers of colonies/g soil, appearing on soil dilution plates

Inoculum (g mycelium/kg soil)	Infection (%)	No. colonies/g soil[c]
0	0	0
0.1	53	30
0.5	80	125
1.0	93	230
2.0	99	500

[a] Inoculum was grown on potato-dextrose broth for 7 days at 25 C, washed with distilled water, weighed, and homogenized in a glass homogenizer.

[b] One hundred onion seedlings were sown in 17 × 17 × 7 cm plastic boxes containing 1 kg soil and kept for 23 days in Wisconsin tanks at 27 C (duplicates).

[c] Counted on sorbose-TBZ medium.

The identification of *P. terrestris* colonies on dilution plates made from a naturally infested soil was verified as follows: Pink restricted colonies and those unpigmented (30 colonies each) were tested by Watson's method (5). Only the pink colonies induced straw pigmentation.

Evaluation of the method for quantitative assay of artificially infested soil showed a correlation between increasing inoculum concns in soil and the numbers of pink colonies/g soil on the dilution plates. Increasing inoculum concn also increased the percentage onion seedlings infected (Table 1).

Siemer and Vaughan (3) found propagules of *P. terrestris* mainly in 0.5-1.0 mm soil particles and stated that the pathogen is associated with undecomposed organic debris. In order to determine the number and kind of *P. terrestris* propagules in soil and their association with roots and other organic debris, 100-g samples of naturally infested soil were taken from a heavily infested field. The organic particles were separated from the soil by the method of Boosalis and Scharen (2). They were divided into four groups according to their size, and plated on Watson's medium (5). In addition, soil dilution plates

TABLE 2. Assay for the presence of *Pyrenochaeta terrestris*[a] in plant debris particles separated from naturally infested soil

Particle	Size (mm)	Particles/100 g soil: Colonized by *P. terrestris*	Particles/100 g soil: Total tested
roots	> 8	74	84
roots	4-8	43	93
roots	< 4	59	96
unidentified	< 4	12	103

[a] On Watson's medium (5).

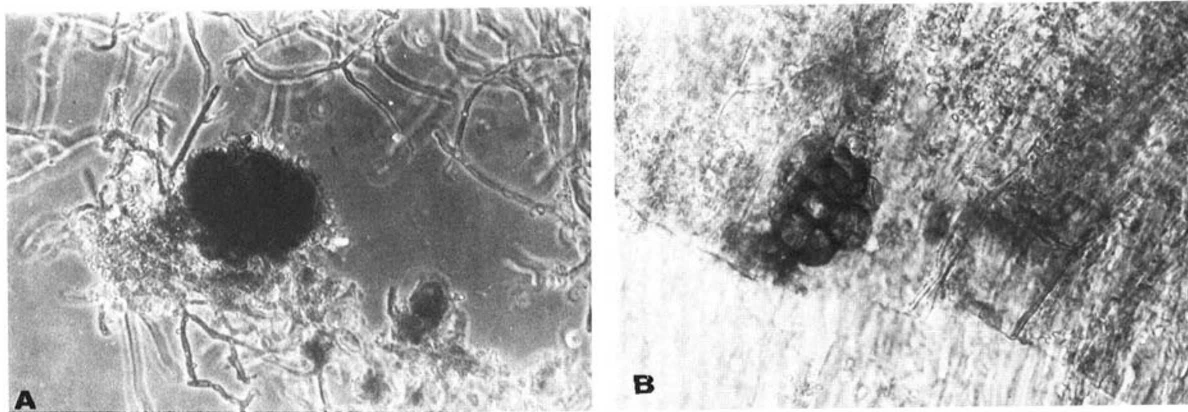

Fig. 1-A, B. A) Germinated resting body of *Pyrenochaeta terrestris* taken from a dilution plate made from naturally infested soil (× 78). **B)** A resting body of *P. terrestris* on an infected root of onion (× 156).

were made to obtain more information on free propagules in soil. Of 376 organic debris particles separated from the soil, 188 were colonized with *P. terrestris* (Table 2). By soil dilution plate-count, there were 20,000 colonies/100 g soil. Colonies originating from single resting bodies free of organic matter were observed on the dilution plates (Fig. 1-A). Resting bodies were also found on colonized roots (Fig. 1-B).

LITERATURE CITED

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