Techniques

A Method for Long-time Culture Storage of *Rhizoctonia solani*

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ABSTRACT

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A method is described for long-time preservation of cultures of *Rhizoctonia solani* in tubes in a dry soil containing 4% (w/w) wheat bran. The moisture level of the mixture at the time of inoculation with *R. solani* was 0.23 g water per gram (dry weight) of soil. About 1 mo at 23–27 C was required to allow the colonized mix to air dry; then it was stored at −25 C. Isolates from diverse sources survived 55 mo at −25 C. Virulence, morphology, and cultural characteristics of *R. solani* were not changed.

Additional key words: Rhizoctonia oryzae, storage of frozen agar cultures.

Rhizoctonia solani Kuehn (=*Thanatephorus cucumeris* [Frank] Donk) is a versatile fungus adapted to survive worldwide under diverse conditions. Nevertheless, its sensitivity to certain environments and the fact that it does not ordinarily produce spores, usually a requirement for lyophilization, present problems in long-time storage of cultures.

Since 1964, I have maintained a large research collection of cultures of *R. solani* at 15–25 C on potato-dextrose agar (PDA) slants in large tubes (25 × 200-mm). Sometimes the PDA was supplemented with yeast extract, malt extract, and casein hydrolysate as employed by Sherwood (as reported in [4]). Cultures were transferred every 4–5 mo. The foregoing procedure was effective but it was expensive and time-consuming.

Sinclair (4) concluded, after reviewing the literature, that the best method for long-time storage of *R. solani* was to cover the cultures with mineral oil and store below 0 C. The method was rejected because of the difficulties inherent in mineral oil storage and because I had evidence that some cultures of *R. solani* die when incubated for 2–3 mo at 5–7 C. This behavior was previously observed by Sherwood (as reported in [4]).

In spite of sensitivity to low temperatures in agar culture, the species survives winters in soil in temperate zones (3), probably as mycelium or sclerotia in or on bits of organic debris (3), suggesting that *R. solani* might survive in a dry state at low temperatures in soil supplemented with organic matter.

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This note presents data and observations on a successful method, based on the foregoing hypothesis, for storing cultures of *R. solani* for relatively long periods of time.

MATERIALS AND METHODS

Frozen agar cultures. In April 1974 a test was initiated to determine if cultures on PDA would survive below 0 C. Eighty-three isolates of *R. solani,* including each of four anastomosis groups (2), were grown on PDA for 2 wk, then placed in sealed cans at −20 C. After 16 mo, the contents were thawed by immersion of tubes in a water bath at 35 C; the entire contents of each tube were placed on a PDA plate with flat surface of the slant in contact with the agar.

Storage in soil-wheat bran mix. In March 1975 a trial was started using three of the four anastomosis-group tester isolates (2)—141, ag-3; 283, ag-4; 328, ag-2—and an isolate of *Rhizoctonia oryzae* Ryker & Gooch. The soil, a fine sandy loam, was air dried. Wheat bran was added to make a final mixture of 4% bran, w/w. Six to 8 ml of the mixture were placed in each tube (15 × 150-mm) and 3 ml of water was added. Tubes were autoclaved twice, 48 hr apart, at 121 C, then inoculated with a mycelial disk, 8 mm in diameter, cut from the margin of an actively growing colony on PDA.

Tubes, in triplicate, were incubated at 23–27 C for about 1 mo during which time the mycelium of *R. solani* permeated the medium and most of the moisture evaporated. The dried cultures then were transferred to −25 C. Also, triplicate tubes of each isolate were maintained continuously at room temperatures (23–27 C). At yearly intervals the isolates at −25 C were sampled for viability; after 55 mo all isolates were sampled.

While this limited test was in progress, two other similar tests were started. Forty-five isolates from a worldwide collection were employed in the first test. The inoculated soil-bran tubes were kept in the laboratory (23–27 C) for 24 mo then transferred to −25 C for 13 mo.

In the second test 85 isolates were stored for 20 mo at −25 C after a 1-mo drying period.

Soil moisture. The water potential of the soil-bran mixture was measured with a thermocouple psychrometer. The amount of water per gram of dry weight was determined by drying to a constant weight at 98 C.

Pathogenicity. At the end of the test periods, nine isolates were selected to determine if the storage procedures had any noticeable effects on pathogenicity. Five isolates were selected from the 45 stored at 23–27 C for 24 mo and four from the 85 placed at −25 C immediately after the soil-bran mixture had dried. Radish seed (*Raphanus sativus* L. 'Cherry Belle') were surface disinfested by immersion in a 0.5% solution of sodium hypochlorite for 5 min, rinsed with distilled water and distributed on water agar, 10 seeds per plate. After the hypocotyl was about 1 cm in length a mycelial plug, 8 mm in diameter, of each isolate of *R. solani* taken from the margin of an actively growing colony on Weinhold's Medium A (5) was placed in the center of each plate, five plates for each isolate. Control plates were not inoculated. All plates were incubated at 23–27 C under fluorescent lighting for 10 days.

RESULTS

Frozen agar cultures. Only 44 of the 83 isolates survived; there was no apparent relationship between survival and source of culture or anastomosis group. Mycelium of some of the surviving isolates emerged only from the thin part of the slant.

Storage in soil-wheat bran mix. At all sampling times *R. solani* and *R. oryzae* from storage at −25 C grew vigorously and produced typical cultures when small amounts of the soil-bran mixture were transferred to PDA. The same isolates of *R. solani* stored at 23–27 C were viable, but colonies formed on PDA were not vigorous and only a few centers of growth emerged from soil or bran particles. *R. oryzae* did not survive after 55 mo of storage at 23–27 C.

In the test with 45 isolates, 42 grew vigorously and produced normal colonies; three isolates failed to grow when the soil-bran mixture was transferred to PDA. Two of them were heavily contaminated with bacteria, and one with *Aspergillus* sp. In the second test, 83 of the 85 isolates produced normal colonies and two isolates failed to grow. In one of these the soil-bran mix was contaminated with bacteria; in the other the tube contents were moist. Apparently the tube was incubated at −25 C before drying was complete.

Soil moisture. At the time of inoculation with *R. solani* the soil-bran mixture contained 0.21–0.24 g of water per gram dry weight. The total average water potential was −5 bars; about −4.5 bars was due to the effect of substances dissolved from the bran on the osmotic potential of the soil solution. Water potential at the termination of the storage periods ranged from −620 to −1,100 bars.

Pathogenicity. Seven of the nine isolates killed and colonized all of the radish seedlings; two isolates belonging to ag-3 and isolated from sclerotia on potato tubers were essentially avirulent. Although I did not test pathogenicity of the potato isolates prior to storage at −25 C, the results were not surprising. In 1945 Houston (1) stated that isolates from potato ".....must be classified as very slightly pathogenic on all hosts other than potato."

Morphology of *R. solani* in the soil-bran mix. After the 1-mo prestorage period, examinations were made of tube contents of 10 isolates to observe the growth forms of *R. solani.* Five isolates had produced masses of monilioid cells, runner hyphae, and irregular swollen hyphae. One isolate formed all of these structures plus discrete small sclerotia and in four isolates only irregular hyphae were observed. The mycelium, monilioid cells, and sclerotia were associated mostly with particles of wheat bran. Runner hyphae were present in pore spaces.

DISCUSSION

Isolates of *R. solani* stored in the dry soil-wheat bran system can be expected to survive for at least 4 yr at −25 C and for 2 yr at 23–27 C and retain the wild-type features of morphology, vegetative vigor, and pathogenicity. I did not extensively explore the possibilities of different soil types or different sources of organic matter other than wheat bran. However, in preliminary studies, I used a cornmeal-soil mixture and soil alone without added organic matter and obtained poor results.

These results suggest the importance of having dry, contaminant-free cultures for storage; only moist or contaminated cultures failed to survive.

The amount of water needed per tube to obtain the proper initial moisture level would probably vary with different soils. I chose a sandy loam because its structure permitted ease of handling and rapid growth of the mycelium of *R. solani.* The role of wheat bran was significant; it provided nutrients and appeared to increase pore space in the system by swelling during autoclaving.

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