Differential Medium for Identification of *Rhizoctonia solani* AG-3

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ABSTRACT

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Anastomosis groups (AG) of *Rhizoctonia solani* may coinhabit soil and differ in pathogenicity and epidemiology characteristics. Therefore, methods for rapid identification and separation of AG-types would be desirable. Separation of *Rhizoctonia solani* AG-3 from other *Rhizoctonia* AG-types was studied on Stewart's medium and on potato-dextrose agar (PDA). One hundred twenty-six isolates of *R. solani*, representing AG-1, AG-2, AG-3, and AG-4 and four isolates of *R. solani*-like fungi were grown on these media. After incubation on Stewart's medium, all AG-3 isolates were consistently and uniformly brown, whereas all other isolates were white. On PDA or other common media, the colony appearance of AG-3 isolates was less characteristic. Stewart's medium might be a useful and rapid tool for differentiating isolates of *R. solani* AG-3.

Rhizoctonia solani Kühn, a fungus species composed of seven known anastomosis groups (AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, and AG-BI) (4), is an important soilborne pathogen. *R. solani* AG-3 is frequently associated with root rot and black scurf of potatoes (3,8).

The distinction of *R. solani* isolates according to AG has been emphasized (5–7,10). Although the groups can be identified serologically (1), hyphal anastomosis (7) is more reliable and widely used. Both methods, however, are impractical for large-scale epidemiology studies. Serology studies depend on the availability of antiserum with suitable titer and nonspecificity toward other AG-types. Tests for hyphal anastomosis between paired isolates are both laborious and time-consuming.

Attempts to separate *R. solani* isolates into AG-types based on colony morphology after growing on potato-dextrose agar (PDA) yeast and oatmeal have failed (9). Sherwood (10) found that AG-2 and AG-3 isolates grown on PDA were similar in colony appearance and usually darker than other AG-types. AG-3 and AG-4, commonly isolated from potato fields in Idaho (3) and California (12), are tentatively separated on the basis of colony appearance on PDA after 2 wk of incubation at 24–28 C (A. R. Weinhold, *personal communication*).

Preliminary attempts to separate AG-3 from AG-4 on the basis of differential growth at low temperatures or on reaction in culture media amended with pH-indicator (Czapek's bromophenol blue, pH 3–4.6) or scopoletin (5–10 ppm in PDA) were unsuccessful. Additional experiments indicated that isolates of these two AG-types could be distinguished by colony color within 1 wk on a medium developed by Stewart (11). On this medium, originally developed for isolating pectinolitic bacteria, colonies of *R. solani* AG-3 consistently appeared brown, whereas those of AG-4 were white.

The purpose of this study was to investigate the use of Stewart's medium for rapid separation of *R. solani* AG-3 from other AG-types. A preliminary report has been published (2).

MATERIALS AND METHODS

One hundred twenty-six isolates of *R. solani* and four isolates of *R. solani*-like

Table 1. Isolates of *R. solani* (AG-1, AG-2, AG-3, and AG-4) and *R. solani*-like species tested for colony color on Stewart's medium

Isolates: group[a] and number	Host	Accession or geographic origin
R. solani AG-1 (white)[b]		
1	*Brassica caulifora*	ATCC 13248[c]
1	*Ponderosa pine*	California
2	...[d]	California
1	*Picea glauca*	Canada
1	*Phaseolus* sp. (leaves)	Costa Rica
1	*Macroptilium* sp.	Florida
2	*Oryza sativa*	Louisiana
1	*Raphanus sativus*	Japan
1	*Brassica* sp.	Wisconsin
R. solani AG-2 (white)[b]		
1	...	Australia
1	...	California
1	*Raphanus sativus*	California
2	*Beta vulgaris*	Colorado
1	*Beta vulgaris*	Michigan
1	*Daucus carota*	Minnesota
2	...	Minnesota
2	*Beta vulgaris*	Japan
1	*Scirpus* sp.	Japan
2	...	Japan
R. solani AG-3 (brown)[b]		
1	*Phaseolus* sp.	ATCC 14006
1	...	Australia
8	*Solanum tuberosum* (tuber)	California
10	*S. tuberosum* (tuber)	Canada
1	*S. tuberosum*	Ecuador
5	*S. tuberosum* (root)	Idaho
13	*S. tuberosum* (stolon)	Idaho
6	*S. tuberosum* (stem)	Idaho
1	Soil	Idaho
1	*S. tuberosum*	Minnesota
1	*S. tuberosum* (stem)	Nebraska
1	*S. tuberosum*	New Zealand
1	*S. tuberosum* (tuber)	Washington

(*continued on next page*)

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Table 1. *(continued from preceding page)*

Isolates: group[a] and number	Host	Accession or geographic origin
R. solani AG-4 (white)[b]		
1	*Gossypium hirsutum*	Arizona
1	*Beta vulgaris*	ATCC 10177
1	*Beta vulgaris*	ATCC 14007
1	...	ATCC 14011
1	...	Australia
2	Soil	California
2	*Aster* sp.	California
1	*Ponderosa pine*	California
2	*Pinus* sp.	California
7	*Gossypium hirsutum*	California
4	*Phaseolus* sp.	California
2	*Spinacia oleracea*	California
2	*Medicago sativa*	California
2	*Daucus carota*	California
2	*Pisum sativum*	California
2	*Howea* sp.	California
1	*Beta vulgaris*	California
1	...	CBS[c]
1	*Picea glauca*	Canada
1	*Pinus* sp.	Canada
2	*Pinus bankiana*	Canada
1	...	Canada
1	*Beta vulgaris*	England
5	Soil	Idaho
2	*Medicago sativa*	Minnesota
1	*Lespedeza stipulacea*	North Carolina
1	*Medicago sativa*	North Carolina
1	*Pinus* sp.	North Carolina
R. solani-like (white)[b]		
1	*Oryza sativa*	CBS
1	*Fragaria* sp.	California
2	...	California

[a] Identification based on hyphal anastomosis with tester strains.
[b] Colony color on Stewart's medium.
[c] American Type Culture Collection.
[d] Host unknown.
[e] Centraalbureau voor Schimmelcultures.

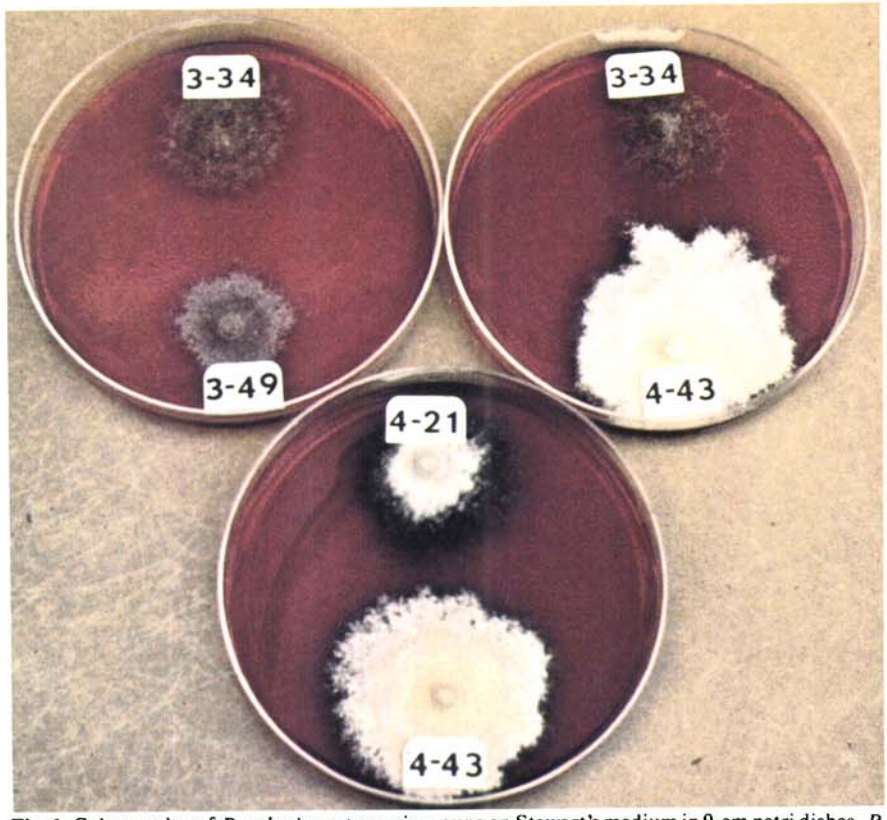

Fig. 1. Colony color of *R. solani* anastomosis groups on Stewart's medium in 9-cm petri dishes. *R. solani* AG-3 (3-34 and 3-49) is uniquely brown, whereas isolates of AG-4 (4-21 and 4-43) are white.

fungi were studied (Table 1). Tester strains of confirmed anastomosis groupings were supplied by A. R. Weinhold (Department of Plant Pathology, University of California, Berkeley, CA).

Every isolate of *R. solani* was tested for anastomosis with the tester strains according to Parmeter et al (7), with some modifications. Each isolate grown on Difco PDA was paired with three different tester strains on cellophane strips (4.5 cm^2) resting on 2% water agar (WA) in 9-cm petri dishes. To promote hyphal growth, the cellophane strips were dipped in soft PDA (13 g/L) before transferring to the WA. The inoculated dishes were incubated until hyphae from the different isolates overlapped (2–3 days at 25 C in dark), then the strips were removed and examined for hyphal anastomosis. Isolates that morphologically resembled *R. solani* but were binucleate were not tested for AGs.

To distinguish AG-3 isolates on the basis of colony appearance, 5-mm-diameter disks from the margins of 4-day-old PDA cultures of every isolate were transferred onto PDA and Stewart's medium (11). Two plastic plates per isolate (two to three disks per plate) were used and, after incubating for 4 days at 25 C in the dark, examined for colony appearance (Fig. 1). Although various characteristics of the colonies were examined on both media, color was the best character for differentiating AG-3 isolates. Most isolates were tested twice.

RESULTS AND DISCUSSION

The test for hyphal anastomosis identified 11 AG-1, 14 AG-2, 50 AG-3, and 51 AG-4 isolates of *R. solani* (Table 1). Confirming preliminary results, all AG-3 isolates on Stewart's medium were consistently brown, whereas all other *R. solani* AG-types and *R. solani*-like isolates were white (Table 1, Fig. 1). Because of a slow growth rate, some isolates of AG-2 and AG-3 had to be incubated for 7 days before evaluation.

After 4 days on PDA, most isolates of *R. solani* were various shades of brown. Many AG-3 isolates on PDA were dark brown after 14 days of incubation, but during this period, the appearance of AG-3 isolates on PDA was not consistently different relative to all other *Rhizoctonia* species. On Stewart's medium, however, the mycelium of most AG-3 colonies was sparce and fleecy, whereas AG-4 mycelium was dense and matted. These characteristics, however, were also present in other AG-types and could not be used to distinguish AG-3 isolates.

Stewart's medium appears useful as a tool to differentiate AG-3 from other *Rhizoctonia* isolates. Although results are supported by a large and representative collection of isolates, it is possible that exceptions might occur.

Separating AG-3 isolates from numerous *Rhizoctonia* isolates with minimum

labor is the main advantage of this procedure. Hyphal anastomosis might also be used as a complementary procedure. Studies of *R. solani* as a pathogen of potatoes in field soil may be greatly facilitated by this method.

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