Isolation of Anastomosis Group 5 of *Rhizoctonia solani* from Potato Field Soils in Maine

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ABSTRACT

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Nine of 50 isolates of *Rhizoctonia solani* obtained from potato field soils in Maine were found to be members of anastomosis group (AG) 5. All nine isolates anastomosed readily with the tester for AG 5. No hyphal fusion was observed between these isolates and any of the testers for AGs 1, 2, 3, or 4. In pathogenicity trials under greenhouse conditions, eight of the nine AG 5 isolates tested produced sunken brown lesions on cultivar Katahdin potato stems; the ninth isolate appeared to be nonpathogenic.

Additional key words: *Solanum tuberosum, Thanatephorus cucumeris.*

The importance of the anastomosis group (AG) concept to the study of the pathology and ecology of *Rhizoctonia solani* Kühn is well established (2). With the exception of AG BI (17), the anastomosis groups in this species are genetically isolated and differ from one another in pathological and cultural characteristics (2,4,18,19,21). Therefore, determination of anastomosis groupings is an essential part of any research undertaken with isolates of this fungus. Parmeter et al (19) found that 93% of 138 isolates of *R. solani* from a wide range of host plants and geographic locations could be classified as belonging to one of four anastomosis groups. Since that time, the majority of plant pathogens identified as *R. solani* have been assigned to one or another of these four groups (2). In 1972, Ogoshi described two additional groups, AG 5 and AG 6, in Japan (18). Abe and Tsuboki (1), also in Japan, reported that 3% of the isolates of *R. solani* obtained from lesions on potato plants and sclerotia on potato tubers were members of AG 5. Results of other work in Japan (16) demonstrated that 8.5% of the isolates of *R. solani* obtained from noncultivated soils were members of AG 5.

In the summer of 1982, soilborne isolates of *R. solani* were obtained from potato fields in Maine as part of a study on the association between the content of double-stranded ribonucleic acid (dsRNA) and cytoplasmic hypovirulence in this fungal pathogen. In 1983, we presented a summary of the above survey, which was the first published report on the occurrence of AG 5 isolates outside of Japan (3). Also in 1983, while this paper was in review, Grisham and Anderson (10) reported on the isolation of

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two nonpathogenic AG 5 isolates from carrots in Minnesota. The presence of members of AG 5 among naturally occurring isolates of *R. solani* as well as their pathogenicity on potato or tomato stems are reported in this study.

MATERIALS AND METHODS

Isolation of *R. solani* from soil. Soil samples were collected at random from a number of locations within each of 25 potato fields in southern, central, and northern Maine. Samples from a single field were pooled, and aliquots of soil were plated by means of a pellet soil sampler (11) onto a selective medium for *Rhizoctonia* spp. (15). The soil assay plates were incubated at room temperature (23 ± 2 C) for 24–48 hr, then mass transfers of mycelia from colonies resembling *R. solani* were made to acidified potato-dextrose agar (APDA). Mass transfers of mycelium from contaminant-free APDA plates were then made to potato-dextrose yeast extract agar (PDYA) slants in screw-cap culture tubes for storage at room temperature. Hyphal-tip isolation was avoided in order to prevent loss of any dsRNA that might be present in the isolates (5).

Identification of isolates of *R. solani*. To distinguish between binucleate fungi resembling *Rhizoctonia* and multinucleate *R. solani*, nuclei in young (<7-day-old) vegetative cells from cultures of *Rhizoctonia* grown on PDYA were stained with 0.5% aniline blue in lactophenol combined with an acidified wetting agent (12). Isolates determined to be *R. solani* were then tested to determine their anastomosis groupings by pairing each isolate with two tester isolates from each of AG 1, AG 2-1, AG 2-2, AG 3, and AG 4, and with one tester from AG 5 according to established procedures (13,19).

Rate of growth and cultural characteristics. Isolates were tested for rates of radial growth at 18 C and 24 C on PDYA in 9-cm-diameter plastic petri dishes that had been centrally inoculated with 5-mm-diameter disks of mycelium from the actively growing margins of 2- or 3-day-old cultures on PDYA. Five replicate plates per isolate were used in a randomized complete block design. Plates were incubated in the dark at 18 and 24 C, and colonies were measured at 24, 48, and 72 hr along two diameters at right angles to one another. Color and texture of mycelium, zonation, and number and distribution of sclerotia were determined in PDYA cultures that were 2–3 wk old.

Pathogenicity tests. Isolates identified as members of AG 5 were tested for pathogenicity on potatoes grown in pots in the greenhouse. Inoculum was prepared on moistened wheat grain in glass petri dishes (10 g of wheat grain plus 20 ml of distilled water per dish), which were autoclaved twice at 24-hr intervals, inoculated with plugs of mycelium from cultures grown on PDYA, and incubated at 25 C for 10 days. Plastic pots (14 cm in diameter) were filled with a moistened sterile (autoclaved) greenhouse potting mix (peat moss, vermiculite, perlite, and sand) to which the wheat inoculum (10 g per pot) was added and mixed to a depth of 5 cm. Alternatively, mycelial plugs from PDYA cultures were used as inoculum. Three to five pots were inoculated with each isolate.

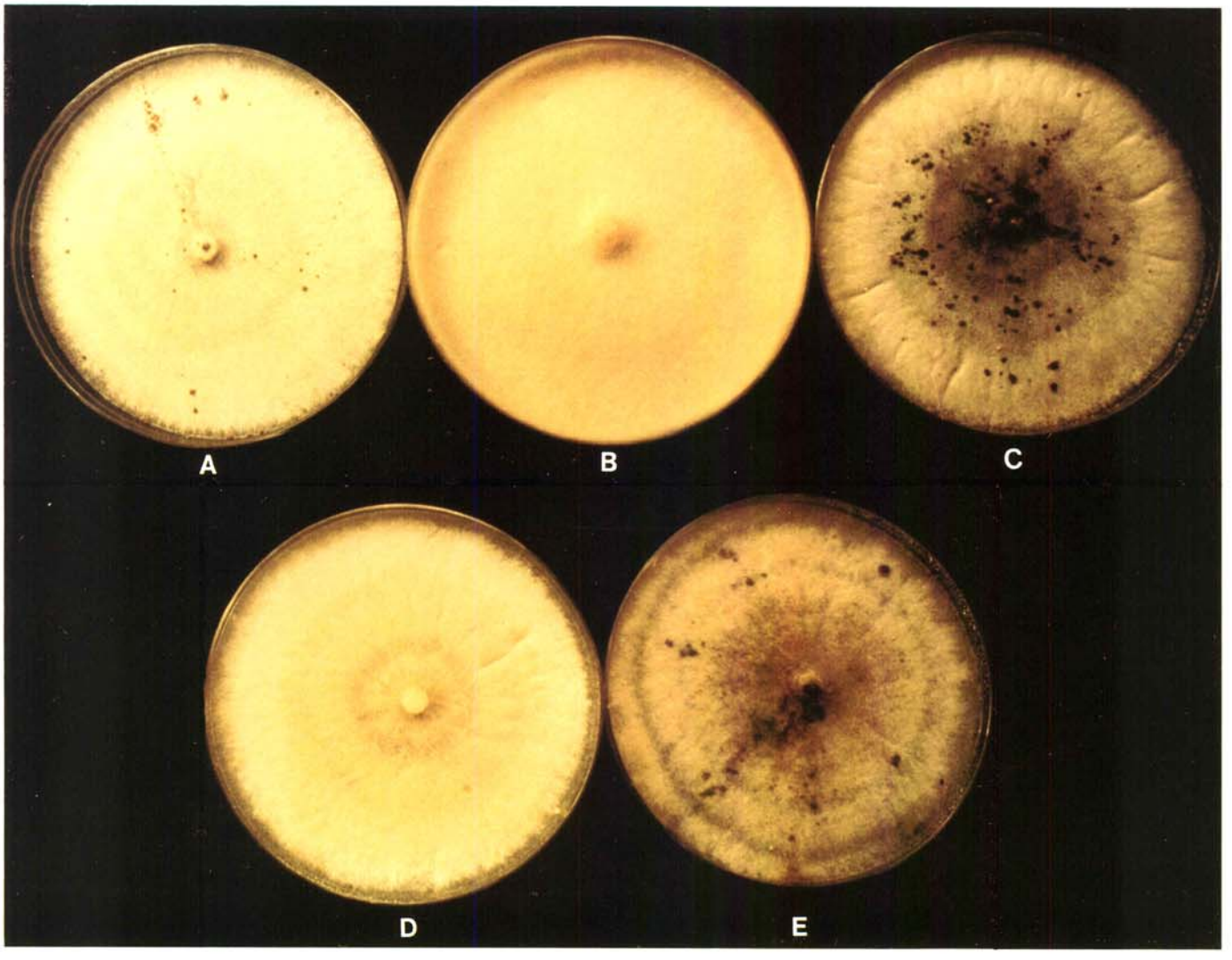

Fig. 1. Cultural characteristics of isolates of AG 5 grown for 2 wk on potato-dextrose yeast extract agar. **A,** Isolate #441 from Japan; **B;** isolates Rs 1 from Maine showing "unusual" yellow color; **C–E,** other AG 5 isolates recovered from soil in Maine: **C,** Rs 5, **D,** Rs 53, **E,** Rs 59.

Tubers of *Solanum tuberosum* L. 'Katahdin' were surface-sterilized in 2% (v/v) formaldehyde for 5 min and allowed to break dormancy at room temperature. Single-eye seed pieces were cut from tubers with a melon-ball cutter and allowed to suberize in a moist chamber at room temperature for 2 wk before planting. Immediately after the AG 5 inoculum or uninoculated control wheat grain was added to the pots as described above, one to three seed pieces were planted in each pot at a depth of ~5 cm. Soil temperature was maintained at 24 ± 4 C, and pots were watered as needed.

Three weeks after planting, emerged and nonemerged sprouts plus remains of seed pieces were harvested and examined for lesions. Isolations were made from the margins of all stem lesions by surface-sterilizing 5-mm cubes of tissue in 0.525% (v/v) sodium hypochlorite and plating them on acidified PDYA. Isolates of *Rhizoctonia* growing from the tissue were transferred to PDYA and then paired in anastomosis tests with tester isolates from AG 3 and AG 5 for positive identification.

Pathogenicity tests on tomato, *Lycopersicum esculentum* L. 'Rutgers,' were also conducted as described above.

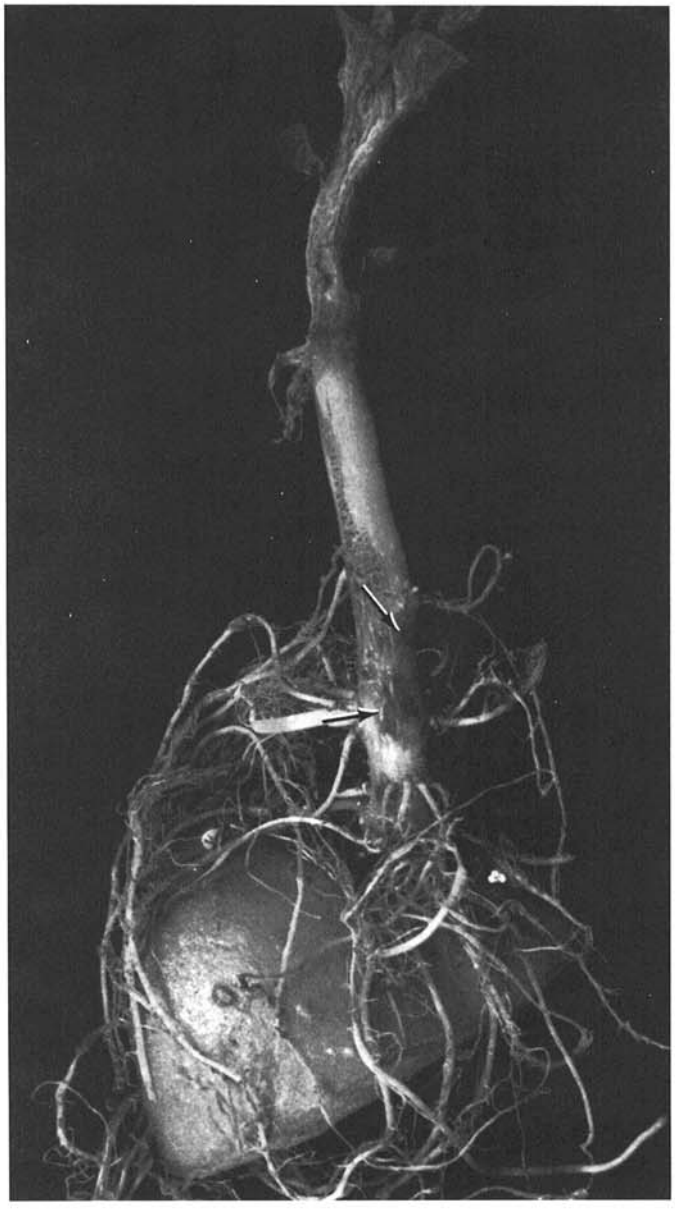

Fig. 2. Lesions on cultivar Katahdin potato stems caused by infection with the AG 5 isolate Rs 10 of *Rhizoctonia solani*.

RESULTS

Identification of isolates of *R. solani*. Sixty fungal isolates with hyphal branching typical of *Rhizoctonia* spp. were isolated. One of these subsequently failed to grow in culture. Of the remaining 59 isolates, nine were binucleate. The remaining 50 isolates possessed multinucleate cells with dolipore septa and were identified as *R. solani*.

Nine isolates anastomosed readily with the AG 5 tester (Table 1). The AG 5 tester, originally obtained from A. Ogoshi in Japan and designated as isolate #441, was donated by E. E. Butler. Since only one AG 5 tester was available initially, one of the nine isolates (Rs 10) was selected as a second tester and was paired with the other eight isolates in a test that confirmed that these are all members of the same anastomosis group. In addition, no hyphal fusions were observed between these isolates and any of the testers for AGs 1 through 4.

The remaining 41 isolates included representatives from each of the other four anastomosis groups, as well as four isolates that failed to anastomose with any tester (Table 1).

Growth rate. Table 1 shows growth rate (millimeters per day) for the interval between 24 and 48 hr at 18 and 24 C. A number of AG 1 isolates reached a radial growth diameter >90 mm at 24 C, before 72 hr. Thus, growth rate data for the 48- to 72-hr interval were not included in the statistical analysis. At 24 C, isolates of AG 1 as a group grew significantly ($P = 0.01$) faster than those in the other four groups, whereas AG 4 grew faster ($P = 0.01$) than AGs 2, 3, and 5 (Table 1). Although AG 1 grew faster than AG 4 at 18 C, the difference was not significant ($P = 0.01$). At 18 C, isolates in AGs 2, 3, and 5 grew more slowly ($P = 0.01$) than those in AGs 1 or 4.

Cultural characteristics. Eight of the nine AG 5 isolates produced mycelium that was mostly appressed to the surface of the agar. The PDYA cultures of six of these isolates were either cream-colored or light tan, and the remaining two were darker tan to brown (Fig. 1). The eight isolates usually showed some zonation on PDYA. Three of these isolates produced very few or no sclerotia in culture. The remaining five isolates produced sclerotia mainly in the center of the culture with a few scattered toward the edge of the plate. The sclerotia were dark chocolate brown.

One AG 5 member (Rs 1) produced a thick, fluffy mat of aerial mycelium which was distinctly yellow in 1- to 2-wk-old cultures on PDYA (Fig. 1). The yellow color gradually darkened to light brown as the culture aged. No sclerotia were produced on the culture surface, but a few were embedded in the agar. Although there was variation in color and sclerotial production among the AG 5 isolates described here, the group as a whole was distinctly different

TABLE 1. Classification of isolates of *R. solani*, obtained from soil of potato fields in Maine, into anastomosis groups and growth rate at 18 C and 24 C

Anastomosis group	Number of isolates	Location[y]	Growth rate (mm/day)[z]	
			18 C	24 C
1[w]	5	S,C,N	30 a	43 a
2[x]	8	C,N	17 b	21 c
3	11	S,C,N	19 b	24 c
4	13	S,N	26 a	34 b
5	9	S,C,N	18 b	23 c
Unknown	4	N		
Binucleate	9	C,N		
Total	59			

[w] Four AG 1 isolates were of the sasakii type, and one was of the microsclerotial type.

[x] AG 2 isolates anastomosed more efficiently with the AG 2 type 1 testers than with those of type 2 used in this study.

[y] Regions of the state where isolates from a particular group were collected: S = southern Maine; C = central Maine; N = northern Maine.

[z] Values represent means for isolates pooled by anastomosis groups. Five replicate plates were used per isolate. Means in a column followed by the same letter are not signficantly different ($P = 0.01$) according to Duncan's multiple range test.

from most of the isolates in the other four groups collected in this study.

The five members of AG 1 included one isolate of the microsclerotial type (web-blight pathogens) and four of the sasakii type (sheath-blight pathogens) (2). Both of these types were distinctive in culture and were not likely to be confused with isolates from any of the other anastomosis groups. The AG 2 isolates showed a greater amount of variation in culture than any other groups, ranging in color from light tan to brown, and in production of sclerotia from few to many. Concentric zones of growth appeared commonly. The 11 AG 3 isolates consistently displayed brown to dark brown mycelium and abundant production of brown sclerotia. The AG 4 cultures were white and velvety when younger than 7 days old, but gray or brownish-gray, thick, and velvety when 2–3 wk old. Very few sclerotia were produced on the surface of these AG 4 cultures.

Pathogenicity tests. Sunken brown lesions were produced on Katahdin potato stems inoculated with eight of the AG 5 isolates from Maine and with the AG 5 tester isolate from Japan (Fig. 2). Similar results were obtained when plugs of mycelium from PDYA cultures were used as inoculum. No lesions were produced on stems inoculated with wheat grain or an agar plug alone. Isolates of *R. solani* obtained from the lesions induced by each of the eight cultures were paired in anastomosis tests with an AG 3 and an AG 5 tester for positive identification. All isolates of *R. solani* obtained from the lesions were positively identified as belonging to AG 5, indicating that members of this group were capable of infecting potato stems. Results of similar pathogenicity tests on cultivar Rutgers tomato seedlings indicated that all but two of the AG 5 isolates were capable of causing lesions on tomato stems. Relatively little damping-off of tomato seedlings was observed.

DISCUSSION

Growth rate (millimeters per day) and appearance in culture of the isolates of *R. solani* used in this study were in good agreement with previous descriptions of the five AGs (18,21). AG 1 has been shown to grow significantly faster than any other AG at similar temperature regimes, whereas AG 4 grows faster than AGs 2, 3, and 5 (18).

Eighteen percent of the isolates of *R. solani* collected in this survey of potato fields in Maine were members of AG 5. This was unexpected because there were no reports of the presence of this group outside of Japan at the time this study was completed. While this paper was in review, Grisham and Anderson (10) reported the isolation of two nonpathogenic AG 5 cultures from carrots. Since the majority of plant pathogens identified as *R. solani* are thought to be members of AG 1, 2, 3, or 4 (2), it is not surprising that most studies on soil populations of *R. solani* associated with various crops have been concerned with only those four groups, and tests to identify anastomosis groupings have not included testers from AG 5. This is probably the major reason there have been few reports on this group.

AG 5 is probably widespread in Maine as indicated by the fact that isolates of this group were obtained from fields in southern, central, and northern Maine (Table 1). It has been reported that both AG 3 and AG 5 are associated with formation of sclerotia on potato tubers (1). If that is the case in Maine, the presence of seedborne inoculum of this group would probably have assured its spread throughout all potato growing areas of the state. Although the pathogenicity tests reported here show clearly that members of AG 5 are capable of infecting potato stems, the extent to which this occurs on potatoes in the field is not known. The importance of AG 5 in the development of Rhizoctonia disease on potatoes in Maine can only be determined by studying isolates from naturally occurring infections in the field at various times during the growing season.

The soilborne isolates of *R. solani* described in this study were originally collected for the purpose of studying a potential relationship between dsRNA content and virulence. Since this was not intended to be an exhaustive survey of the soil populations of *Rhizoctonia* spp., firm conclusions about the relative frequency of occurrence of the various AGs in potato fields cannot be drawn from these data. However, it is interesting that, in spite of the limited sample size, the collection of isolates included members of five different AGs, as well as binucleate Rhizoctonialike fungi. With the exception of a single AG 5 culture (Rs 1), all of the soilborne isolates of *R. solani* in this study were pathogenic, to a varying degree, on appropriate hosts (2) (*unpublished*). Two AG 4 isolates, a highly virulent isolate (Rs 4) and a hypovirulent isolate (Rs 33) on cultivar Topcrop beans, were used to inoculate Katahdin potato. Interestingly, both AG 4 isolates induced superficial lesions on potato stems. The lesions produced by Rs 4 were more conspicuous than those caused by Rs 33 (D. H. Zanzinger, *unpublished*). In general, data reported in the literature (6,7,9,13,20) indicate that the AGs isolated most frequently from a particular soil are those pathogenic to the host crop planted in that soil. The data reported suggest that potato fields in Maine support a greater diversity of AGs than other cultivated soils for which relevant information is available. The reasons for the existence of this diversity, and its implications on the effectiveness of crop rotation as a means of managing Rhizoctonia disease of potato warrant further investigation.

Abe and Tsuboki (1) found that isolates from AGs 2, 3, and 5 were pathogenic on stems and stolons of potatoes and that AGs 3 and 5 were associated with formation of sclerotia on tubers. However, of 273 isolates of *R. solani* they collected from diseased potato plants and sclerotia on tubers, 96% were in AG 3. Only 3% were in AG 5, and about 1% were in AGs 1 and 2. In contrast, Chang and Tu (6) found that 39.8% of diseased potato plants sampled were infected with AG 3, 38.9% with AG 4, 11.1% with AG 2, and 9.3% with AG 1. Chand and Logan (6) in Northern Ireland found that of 182 isolates of *R. solani* from tuberborne sclerotia, 95.6% belonged to AG 3, and the remainder to AG 2 type 1. The AG 3 isolates were more virulent to potato than those of AG 2 type 1. Also, a recent report (14) from the International Potato Center in Peru indicates that all isolates from infected potatoes grown in the San Ramon and Huancayo valleys belong to AG 4. It is not known what roles the various anastomosis groups play in the development of the Rhizoctonia disease of potatoes in Maine, but it seems likely that isolates from all of the five anastomosis groups may play a role in the system involving potato plants and *R. solani*, depending upon the prevailing environmental (abiotic and biotic) conditions. This would explain the diversity of anastomosis groups occurring in soils cropped intensively with potatoes, such as those sampled in this survey, and the marginal control provided by rotation (8).

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