Characterization of *Rhizoctonia* spp. isolates collected from *Phaseolus vulgaris* in Puerto Rico[1,2]

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ABSTRACT

Thirteen isolates collected from soil samples, infected seeds, hypocotyls, leaves and pods of common beans (*Phaseolus vulgaris*) grown in the northwestern, southern and western areas of Puerto Rico were identified as *Rhizoctonia solani* (Rs), and one as binucleate *Rhizoctonia* sp. (Rb). All isolates were characterized by the nuclear condition of the hyphae cell and the anastomosis technique. Seven Rs isolates (54%) were classified into anastomosis group AG 4, and six (46%) were assigned to AG 1. The microsclerotic Rb isolate did not anastomose with any of the AG testers. The AG 4 isolates produced only microsclerotia. Five AG 1 isolates (36%) were macrosclerotic, and one was microsclerotic. The Rs isolates and the Rb isolate differed in mycelia radial growth after 48 h of incubation at 28° C on acidified potato dextrose agar. The sexual stage of the isolates was not observed throughout this research. Storing dried beet (*Beta vulgaris* L.) seeds colonized by *R. solani* for at least a year at 4° C in the dark seems to be a reliable method to ensure viability and virulence of the pathogen.

Key words: *Rhizoctonia solani, Thanatephorus cucumeris, Phaseolus vulgaris*, anastomosis, binucleate.

RESUMEN

Caracterización de aislados de *Rhizoctonia* spp. recolectados de *Phaseolus vulgaris* en Puerto Rico

Trece aislados de *Rhizoctonia* spp., recolectados en el noroeste, sur y oeste de Puerto Rico de muestras de suelo, semillas, hipocotilos, vainas y hojas infectadas de habichuela (*Phaseolus vulgaris* L.), se identificaron como *R. solani* (Rs) y uno como *Rhizoctonia* sp. binucleada (Rb). Todos los aislados se caracterizaron por la condición nuclear de las hifas y por la técnica de anastomosis. Siete aislados (54%) se clasificaron en el grupo de anastomosis GA 4, y seis (46%) en el GA 1. No hubo anastomosis con el ais-

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lado microesclerótico Rb al éste parearse con los repesentativos GA. Los aislados GA 4 produjeron solamente microesclerocios. Cinco aislados GA 1 (36%) fueron macroescleróticos y uno fue microesclerótico. El crecimiento radial micelial de los aislados Rs fue diferente al del aislado Rb luego de ser incubados por 48 h a 28° C en agar de papa dextrosada acidificada. Durante esta investigación no se observó el estado sexual de los aislados. El almacenamiento, por un año en la oscuridad a 4° C, de la semilla seca de remolacha (*Beta vulgaris* L.) colonizada por *R. solani* parece ser un método confiable para mantener la viabilidad y virulencia del patógeno.

INTRODUCTION

Rhizoctonia solani exists in nature in the form of diverse isolates or groups that differ in morphology, pathology, physiology and ecology (Ogoshi, 1987; Sneh et al., 1991). This variability provides this pathogen with a great potential for adaptability and survival (Baker, 1970). *Rhizoctonia solani* and its teleomorph is known to cause a large number of diseases in a wide range of species of plants under tropical and temperate climates (Baker, 1970; Ogoshi, 1987). This pathogen is capable of causing aerial blight diseases in the common bean (*Phaseolus vulgaris* L.) under moist and warm conditions (Cárdenas-Alonso, 1989; Galindo, 1982). Two *R. solani* isolates collected from bean leaves in Puerto Rico, and characterized as belonging to the interspecific group AG 1-IB, were first reported by Polanco et al. (1996). The objective of this research was to characterize 14 isolates of *Rhizoctonia* spp. associated with the common bean in Puerto Rico.

MATERIALS AND METHODS

Rhizoctonia spp. were collected in the hot rainy summers of 1995 and 1996 from the soil and infected hypocotyls, leaves, pods and seeds of common bean plants grown in northwestern, southern and western areas of Puerto Rico (PR). Affected bean tissues and seeds were washed under running tap water, surface-sterilized for 1 to 2 min in 5% sodium hypochlorite solution, rinsed with tap water, and then plated on acidified potato dextrose agar (aPDA). Fifty grams of moist soil (50% moisture-holding capacity) was taken from each soil sample and mixed with 1 g of autoclaved beet (*Beta vulgaris* L.) seeds in 10-cm-diameter Petri dishes. After 48 h of incubation at 28° C, seeds were recovered in a No. 14 sieve, washed with running water, blotted on a paper towel and placed on aPDA (Papavizas et al., 1975). The isolates obtained were preserved on colonized dried beet seeds and stored in sterilized screw-capped glass vials at 4° C in the dark (Olaya, 1992).

Disks of active growing *Rhizoctonia* isolates from 48-hour-old cultures were cut and placed in the center of aPDA Petri dishes and incubated at 28° C. Radial growth was determined after 48 h.

Colony characteristics, such as color, and size and distribution of sclerotia on each aPDA plate, were determined after 12 days of incubation at 28° C under continuous light. Sclerotia were collected and measured with an ocular micrometer. The sclerotia sizes ranging from 0.5 mm to 1 mm were considered microsclerotic. Those more than 1 mm in size were considered macrosclerotic. The clean slide technique reported by Kronland and Stanghellini (1988) was used to observe nuclear and anastomosis conditions. The AGs of the PR isolates were determined by pairing the unknown isolates with seven known representative AG testers provided by Dr. Gilberto Olaya (Department of Plant Pathology, Cornell University, Geneva, NY). A 5-mm diameter disk containing the PR isolate was removed from 2-day-old culture and placed on one end of a clean glass slide. A 5-mm diameter disk of a known AG tester was placed 2 cm apart on the other end. The slide was incubated in a moist chamber at 28° C for 24 to 28 h. After the hyphae from two disks overlapped, the slide was removed from the moist chamber. Mycelia adjacent to disks were cut and agar plugs removed from the slide. The area of the overlapping hyphae was treated with safranin O (0.5% water solution) and 3% KOH (Bandoni, 1971) and covered with a coverslip. The stained area was observed under the light microscope to determine the number of nuclei per cell and the anastomosis of the unknown isolate. This process (perfect hyphal fusion) was repeated at least three times with each of the testers.

RESULTS AND DISCUSSION

Eleven isolates of *Rhizoctonia* spp. were collected from soil samples and infected seeds and bean tissues in Isabela and Salinas during the summer of 1995, and three from Aguada in 1996. Five isolates were collected from soil samples (38% of the total isolates), three from seeds (21%), two from leaves (14%), two from hypocotyls (14%) and two from pods (14%) (Table 1).

All isolates were identified according to the mycelia morphology as reported by Parmeter and Whitney (1970) and Ogoshi (1985). The mycelia color of the isolates varied in intensity from pale to dark brown. After 12 days of incubation at 28° C, all isolates produced dark brown sclerotia, which varied in size, number and distribution on the surface of the aPDA plate (Figure 1). Nine isolates (64% of the total isolates) were microsclerotic and five (36%) were macrosclerotic in aggregates. Three to nine nuclei per vegetative cell were found in the multinucleate isolates (Figure 2), and two nuclei per cell (binucleate) were observed in only one isolate. This character is important in the identification of *Rhizoctonia* spp. (Parmeter and Whitney, 1970). One

TABLE 1.—*Characteristics of* Rhizoctonia *spp. isolates collected from three different sites in Puerto Rico during the summers of 1995 and 1996.*

Isolate code[1]	Location	Isolate source	Sclerotia size[2]	Radial growth (cm/48 h)	Nuclear condition[3]	Anastomosis group[4]
Rb 001	Isabela	Soil	Micro	2.6	B	nd
Rs 002	Isabela	Seeds	Micro	1.6	M	4
Rs 003	Isabela	Soil	Micro	2.9	M	4
Rs 004	Isabela	Soil	Micro	3.4	M	4
Rs 005	Isabela	Soil	Micro	1.6	M	4
Rs 006	Isabela	Seeds	Micro	2.0	M	4
Rs 007	Salinas	Hypocotyls	Micro	2.2	M	4
Rs 008	Salinas	Hypocotyls	Micro	2.7	M	4
Rs 009	Isabela	Pods	Macro, A	1.4	M	1
Rs 010	Isabela	Pods	Macro, A	2.0	M	1
Rs 011	Isabela	Leaves	Macro, A	2.3	M	1
Rs 012	Aguada	Leaves	Macro, A	1.0	M	1
Rs 013	Aguada	Soil	Micro	4.1	M	1
Rs 014	Aguada	Seeds	Macro, A	3.5	M	1

[1]Rb = binucleate *Rhizoctonia* sp.; Rs = *Rhizoctonia solani*

[2]Microsclerotic = 0.5 mm to 1 mm; macrosclerotic = > 1 mm; A = aggregate.

[3]B = binucleate; M = multinucleate.

[4]Anastomosis group = AG; nd = not determined.

isolate was binucleate (Rb 001). The multinucleate isolates (Rs 002 to Rs 014) were identified as *R. solani* type, and the binucleate Rb 001 as binucleate *Rhizoctonia* sp. that fitted the criteria of Parmeter and Whitney (1970) for *R. solani*.

The classification of *Rhizoctonia* spp. is based on the cytomorphology of hyphae and the morphology of cultures, affinities for hyphal anastomosis or fusion, and more recently DNA base sequence homology (Sneh et al., 1991). *Rhizoctonia solani* is considered a single species divided into anastomosis groups (AGs) and intraspecific groups (ISGs) (Ogoshi, 1987). According to Sneh et al. (1991), isolates of *R. solani* (teleomorph or sexual stage: *Thanatephorus cucumeris*) consist of 20 AGs, including ISGs. The binucleate *Rhizoctonia* spp. (Rb) fungi (*Ceratobasidium* spp. and *Tulasnella calospora*), have been separated into 21 AGs (Sneh et al., 1991). The isolate Rb 001 did not anastomose with any of the seven known AG testers. Those of *R. solani* showed positive anastomosis reaction (perfect hyphal fusion). Seven isolates (54% of the total isolates) were grouped into AG 4, and six (46%) into AG 1 (Table 1). The AG isolates characterized in this research were confirmed by Dr. Olaya. The determination of AGs among isolates of *Rhizoctonia* has be-

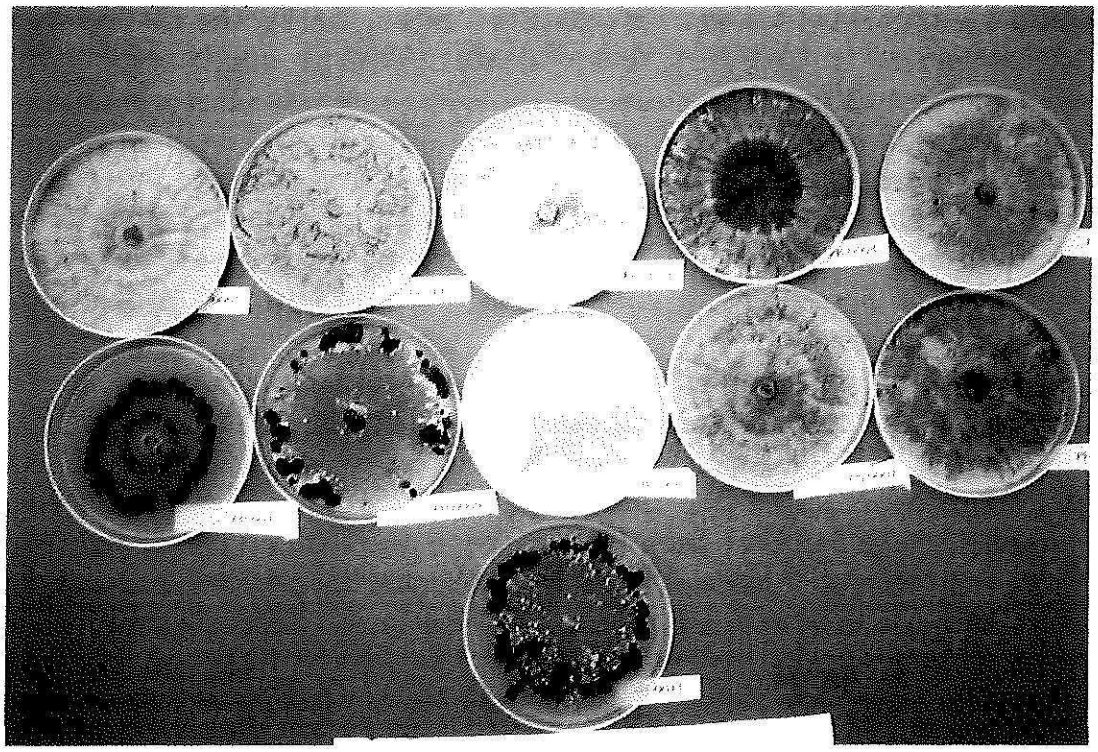

FIGURE 1. Representative cultures of binucleate *Rhizoctonia* sp. (No. 0001) (top left) and *Rhizoctonia solani* (isolates No. 0002 to No. 0011). Observed sclerotia size and their distribution on aPDA plates after 12 days of incubation under continuous light.

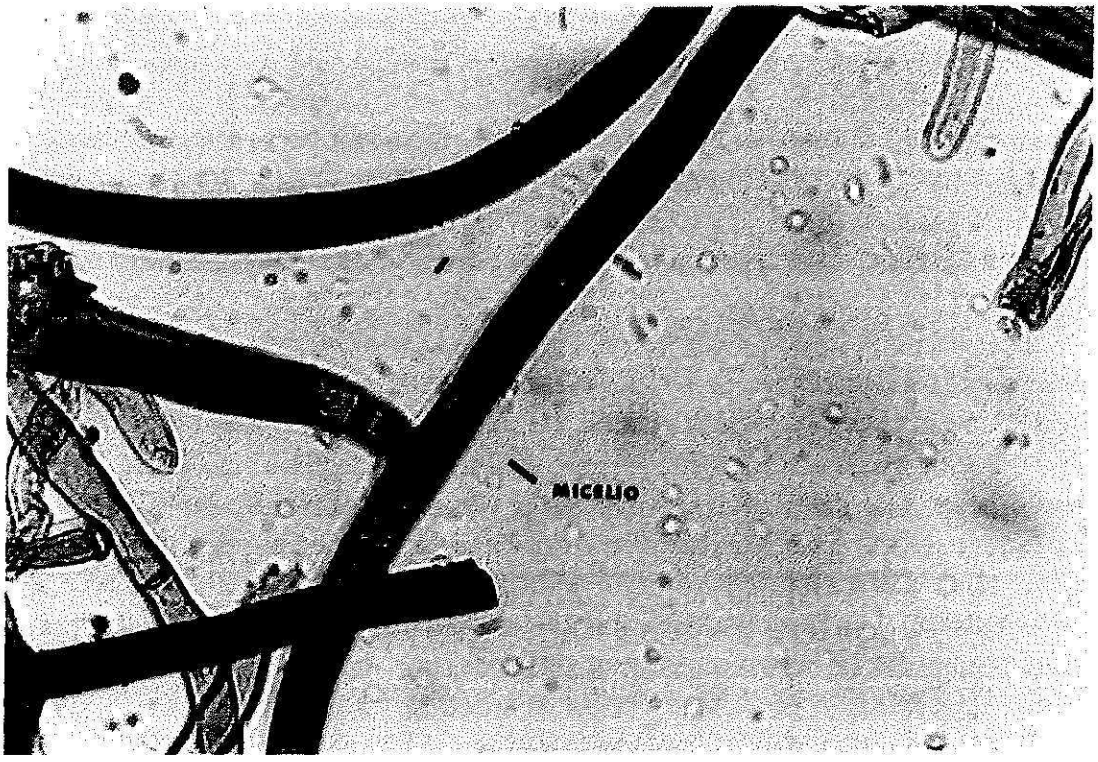

FIGURE 2. Hyphae of *Rhizoctonia solani* (Rs) showing multiple nuclei stained with Safranin O after 24 h of incubation.

come an important taxonomic tool (Sneh et al., 1991). The Rb 001 was microsclerotic. In the AG 4, seven isolates (50%) produced micro-; five AG 1 isolates (36%) were macrosclerotic, and one was microsclerotic.

Isolates *R. solani* (Rs 002 to Rs 014) and the Rb 001 isolate differed in radial growth when incubated on aPDA for 48 h at 28° C. The radial growth of the AG 4s ranged from 1.6 to 3.4 cm, and those of the AG 1s from 1.0 to 4.1 cm. The radial growth of Rb 001 for this period was 2.6 cm (Table 1). The mycelium colonized all the surface of aPDA plates after 72 h of incubation.

A diverse population of *R. solani* exists affecting common bean for level of pathogenicity according to the anastomosis group of the population. Ogoshi (1987) reported that Rs AG 4 are mainly isolated from plants of the families Chenopodiaceae, Leguminosae, and Solanaceae, whereas the Rs AG 1 are from Leguminosae and Gramineae. Several authors reported AG 1 isolates that were collected from leaves, hypocotyls, pods, and seeds of common bean and lima bean (Bolkan and Ribeiro, 1985; Cárdenas-Alonso, 1989; Dillard, 1987; Galindo, 1982). Galindo (1982) reported that isolates belonging to the AG 1, AG 2 and AG 4 were capable of infecting common bean leaves and hypocotyls in the greenhouse. Isolates of AG 4 have been collected from common bean seeds in the Dominican Republic (Godoy et al., 1992). AG 4 isolates obtained from soil, seeds and hypocotyls were pathogenic to leaves and hypocotyls of common bean and lima bean (Summer, 1985; Bolkan and Ribeiro, 1985).

We conducted no pathogenicity test of the Rb 001 obtained from soil samples taken in Isabela bean fields. Some AG isolates of binucleate *Rhizoctonia* sp. have been reported as pathogenic, avirulent or weakly pathogenic in common bean (Burpee et al., 1980; Olaya, 1992) and snap bean (Summer, 1985). Gutiérrez and Torres (1990) found that one isolate of Rb obtained from soil inhibited in vitro the mycelial growth of *R. solani*.

It is known that *T. cucumeris* and its anamorph are capable of causing aerial blight diseases on common bean under moist and warm conditions (Galindo, 1982; Olaya, 1992). No morphological characteristics (hymenia, basidia, sterigmata and basidiospores) of *T. cucumeris* have been observed throughout this research. Specific substrate and environmental factors (relative humidity, temperature, light and aeration) that influence teleomorph formation of *Rhizoctonia* spp. are not understood. Certain conditions are required for the fungus fruiting, and these differ among species and among isolates. The teleomorph state has been reported on bean tissues in the field (Galindo, 1982; Cárdenas-Alonso, 1989). Kotyla (1947) and Parmeter et al. (1969) have induced basidiospore formation of *R. solani* under laboratory and greenhouse conditions.

Storing dried beet seeds colonized by *R. solani,* for at least one year at 4° C in the dark, seems to be a reliable method to ensure viability and virulence of the pathogen. Other successful methods using dried cereal grains have been reported (Sneh et al., 1991).

This study will provide important information to those interested in this ubiquitous group of fungi. They are significant soilborne plant pathogens and they also cause diseases of roots, stems and aerial parts of the common bean crop in Puerto Rico.

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