Characterization, Virulence, and Genetic Variation of *Rhizoctonia solani* AG-9 in Alberta

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ABSTRACT

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A total of 130 *Rhizoctonia solani* AG-9 isolates were isolated from agricultural soils collected in 1992 and 1993 in central and northeastern Alberta. On potato dextrose agar, cultures developed a brown to tan mycelium and irregularly shaped, brown to dark brown sclerotia (0.5 to 2.0 mm), singly or in clumps. Number of nuclei per vegetative hyphal cell ranged from 3 to 17. Less than 20% of the isolates were thiamine auxotrophic. In a petri plate test, all isolates were virulent to canola, causing seed rot and seedling infection. In a greenhouse test, AG-9 isolates were mildly virulent to canola. Two isolates were further tested for virulence to 10 different crops. These isolates were highly virulent to cauliflower and moderately virulent to flax, causing significant pre-emergence damping-off. Isolates of AG-9 also were mildly virulent to canola in which emergence was reduced and root discoloration was observed. Isolates were avirulent to alfalfa, pea, tomato, wheat, barley, oat, and bromegrass, though slight root discoloration on seedlings of alfalfa, wheat, oat, and bromegrass was observed. Genetic variation of 12 isolates from Alberta and 3 from Alaska was analyzed by random amplified polymorphic DNA (RAPD) assay using different oligonucleotide primers. There was considerable variation within the *R. solani* AG-9 group; this suggests that AG-9, considered indigenous to Alaska, is present in a variety of environments and different geographic areas. It is a heterogeneous group and the genetic variation within the AG-9 group can be identified by the RAPD–polymerase chain reaction technique.

Rhizoctonia solani causes important diseases on most greenhouse and field-grown crops world-wide. It is a composite species, which can be divided into 13 anastomosis groups (AG) and several intraspecific subgroups (ISGs) on the basis of combinations of anastomosis, pathology, and morphology as well as molecular and biochemical characteristics (7,11,15,19). Virulence in AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-6, and AG-8 has been determined (1,2,4,13,14,16–18). AG-9 was first reported in the U.S. by Carling et al. (6) from potato plants and soil of potato fields in Alaska, and was later reported in Oregon (13). There is no report of the occurrence of AG-9 from any other country.

The genetic relatedness of isolates within AG-9 has been studied using the DNA hybridization technique (5), isozyme techniques (11), and the ribosomal DNA restriction fragment length polymorphism (RFLP) technique (20). Carling et al. (6) divided AG-9 into thiamine prototrophic and auxotrophic groups. Within these subgroups, isolates were genetically closely related according to the DNA hybridization technique (5). However, these differences could not be identified by RFLP (20) or isozyme techniques (11). The pathogenicity and distribution of AG-9 are not well known, and the importance of AG-9 in agriculture needs additional investigation.

In recent years, random amplified polymorphic DNA (RAPD) assay, a modified polymerase chain reaction (PCR) technique, has been increasingly used for the genetic study of plants and microorganisms. Compared with the RFLP technique, it has the advantages of speed and simplicity and does not require prior sequence information to generate a primer. It can amplify many different sequences spread randomly throughout the genome by using a series of different primers of random sequence. Genetic variation in isolates of *R. solani* AG-1 to AG-8 has been analyzed by the RAPD-PCR technique (7). It showed that all of the anastomosis groups and subgroups could be distinguished and there was considerable variation within some groups.

Rhizoctonia solani AG-9 was first isolated from soil samples collected in 1992 and 1993 from three locations in Alberta cropped with cereal, canola, and forages (9). The initial investigation was conducted to determine the effect of different rotation and tillage systems on Rhizoctonia damping-off and root rot of canola. Because this was the first time that isolates of *R. solani* AG-9 were found in Canada and the importance of these isolates in agriculture was unknown, further study was conducted on them.

The objectives of this study were to characterize isolates of *R. solani* AG-9 found in Alberta, determine their pathogenicity to selected crop plants, and detect the genetic variation within the group by means of the RAPD-PCR technique.

MATERIALS AND METHODS

Soil collection and isolation. Three identical crop rotation experiments involving barley, canola, and three other crops were set up at three locations (Viking, La Corey, and Alliance) in 1989 in Alberta to determine the effect of rotation and tillage on the diseases of canola and barley. In this study, only those plots involving canola and fallow in rotation with barley were used for soil collection to determine the effect of rotation and tillage on the population of *R. solani* in the soil. Soil was sampled to a depth of 15 cm from each plot in 1992 and 1993. Five subsamples were taken from each plot, mixed well to obtain a composite sample, and kept in a freezer (–14°C) until used for assay. Sixty-four soil samples were collected on 17 June 1992 from two locations (Viking and La Corey) and 96 samples on 8 June 1993 from three locations.

Bulked soil samples from each plot were sieved through a 9-mesh sieve to remove large particles. Then, 100 g of soil was sieved under running tap water through a 355-μm (42 mesh) sieve. Debris was collected from the sieve and placed on filter paper and air dried on a surface-sterilized bench of a transfer chamber overnight. Fifty clumps of debris were distributed evenly on five 9-cm petri plates (10 clumps/plate) containing a selective medium (10). All plates were arranged in a completely randomized design and incubated at 24°C without light for 48 h. *Rhizoctonia*-like isolates along the margins of each clump were identified with a light microscope (100×) according to the morphological characteristics of their mycelium (17). Hyphal tips from *Rhizoctonia*-like mycelium were transferred to potato

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dextrose agar (PDA) and purified cultures were stored at 4°C.

Anastomosis grouping. A total of 227 and 356 *Rhizoctonia*-like isolates were obtained from soil samples collected in 1992 and 1993, respectively. Isolates were paired with 10 tester isolates of *R. solani* (AG-1, AG-2-1, AG 2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, and AG-9, supplied by P. R. Verma, Agricultural Canada Research Station, Saskatoon) according to the procedure of Parmeter et al. (16). Three AG-9 tester isolates, originally from Alaska, were used in this study. PDA plugs, 4 mm in diameter, from the advancing margins of colonies of a tester strain and each unknown isolate were placed 3 to 4 cm apart on 2% water agar (WA) in 9-cm plastic petri plates. All plates were incubated at room temperature for about 2 days. When mycelia of two isolates overlapped slightly, the anastomosis points were observed under a microscope (100×). Anastomosis group was determined based on hyphal fusion between two isolates. Hyphal fusion at a minimum of five points was considered to be positive indication that both the isolates belonged to the same pathogenicity group. Killing reaction could be observed.

Table 1. Mycelial dry weight of 15 *Rhizoctonia solani* AG-9 isolates in glucose asparagine (GA) medium with or without thiamine hydrochloride (T) (10^{-5} M)

Isolate	Source	Mycelial dry weight (mg) GA-T	GA+T	GA+T/GA–T[z]
AG-9 (1)	Alaska	106	142	1.3
AG-9 (2) ATCC 62809	Alaska	93	129	1.4
AG-9 (3)	Alaska	120	162	1.4
3189-1	La Corey	109	141	1.3
3116-2	La Corey	13	8	0.6
3111-1	La Corey	163	165	1.0
3219-1	La Corey	21	65	3.1
3382-1	Viking	147	150	1.0
3322-2	Viking	15	169	11.3
3355-1	Viking	32	67	2.1
3381-1	Viking	22	159	7.2
3528-3	Alliance	158	157	1.0
3603-2	Alliance	167	168	1.0
3598-2	Alliance	7	125	17.9
3591-5	Alliance	18	14	0.8

[z] A ratio of >5 indicates the isolate requires thiamine.

Table 2. Virulence of *Rhizoctonia solani* AG-9 isolates to canola in petri plate and greenhouse tests

Location	Petri plate test: Number of isolates tested	Germination (%) day 3	Seedling infection (%) day 3	Greenhouse test: Number of isolates tested	Emergence (%) day 7	Survival (%) day 21
La Corey	37	90.7 c[y] (71–100)[z]	75.8 b (0–96)	10	94.5 ab (76–100)	94.4 ab (76–100)
Viking	12	95.9 b (84–100)	82.7 a (24–100)	7	92.6 b (68–100)	91.9 b (68–100)
Alliance	33	96.4 b (80–100)	82.0 a (44–100)	11	96.3 a (88–100)	96.4 a (88–100)
Check		98.5 a	0.0 c		98.7 a	98.7 a

[y] Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[z] Values in parentheses are ranges for isolate means for each location.

Table 3. Virulence of two isolates of *Rhizoctonia solani* AG-9[y]

Crop	Isolate	Emergence (%) day 7	Survival (%) day 21	Symptomless plants (%) day 21
Canola	3381-1	95 a[z]	93 a	73 a
	3528-3	100 a	98 a	90 b
	Check	100 a	100 a	100 b
Cauliflower	3381-1	30 a	30 a	25 a
	3528-3	35 a	35 a	30 a
	Check	95 b	95 b	95 b
Flax	3381-1	45 a	45 a	35 a
	3528-3	68 ab	68 ab	55 ab
	Check	75 b	75 b	75 b

[y] Data for seven other crops that are not susceptible to isolates of AG-9 are not listed.
[z] Means in a column within each crop followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Cultural characteristics. Isolates of *R. solani* AG-9 were grown on PDA plates in the dark at room temperature for 2 to 4 weeks. The morphology of the mycelial growth and shape and size of sclerotia were then examined.

Nuclear staining. Twelve isolates from Alberta (3189-1, 3116-2, 3111-1, 3219-1, 3382-1, 3322-2, 3355-1, 3381-1, 3528-3, 3603-2, 3598-2, and 3591-5), four from each location (La Corey, Viking, and Alliance), and three identified isolates from Alaska—AG-9(1), AG-9(2), and AG-9(3)—were examined by the safranin O staining method (21) to determine the number of nuclei present in vegetative hyphal cells. Microscope slides were coated with 2% WA and placed on moistened filter paper in petri plates. Individual isolates were inoculated onto the slides, incubated at room temperature for 2 days, then stained with a mixture of 0.5% safranin O and 3% KOH and examined immediately under a microscope (400×); 50 observations were made for each isolate.

Thiamine requirement test. Twenty-nine isolates of *R. solani* AG-9 were grown on 2% WA for 4 days, and then transferred to glucose asparagine medium (GA) (14) with or without thiamine hydrochloride (10^{-5} M). After incubation at room temperature in the dark for 2 weeks, mycelial dry weight was determined. Three replicated plates for each treatment and each isolate were used. The test was repeated once.

Pathogenicity test. Eighty-two AG-9 isolates from 1993 soil samples were tested for pathogenicity to canola (*Brassica napus* L. 'Westar') in petri plates, and 28 of these isolates were also tested in a greenhouse.

Petri plate test. A PDA plug (3 mm in diameter) bearing mycelium of an isolate was transferred to 2% WA in a 9-cm petri plate. All inoculated plates were incubated at room temperature for 3 to 5 days until the mycelium covered most of the plate. Twenty-five surface sterilized (10 min in 1% NaOCl solution) canola seeds (cv. Westar) were placed on each inoculated plate. Four replicated plates of each isolate were arranged in a completely randomized design and incubated at room temperature for 3 days (23). Seed germination and seedling infection were then recorded. Seeds on WA plates without fungus served as control. Fifteen isolates chosen at random (five isolates per location) were retested on canola with the same method.

Greenhouse test. *Rhizoctonia solani* AG-9 isolates were grown on autoclaved barley grain at 25°C in the dark for 2 weeks, air dried, and then stored at 4°C until used. Twenty-five surface-sterilized canola seeds (cv. Westar) or 10 seeds of barley (*Hordeum vulgare* L. 'Galt') were placed on a packed surface of autoclaved soil mixture (soil/sand/vermiculite/peat moss, 1:1:1:1) in a 4-inch-diameter plastic pot and covered with 100 ml of inoculum-soil mixture containing 100 infested barley

grains per liter of soil (22). Four replicated pots were used for each isolate. Seeds covered with sterilized soil served as a control. All pots were arranged in a randomized complete block design in a plastic tray in a greenhouse with day and night temperatures of 18 and 10°C, respectively, and 16 h of light a day. Pots were watered from the bottom by filling the tray and then draining the excess water after the soil surface became wet. Emergence was recorded 7 days after planting and survival 21 days after planting. Five representative isolates from each location were retested on canola and barley in a greenhouse.

The host range of two AG-9 isolates (3381-1 and 3528-3) was determined in a greenhouse on canola (cv. Westar), barley (cv. Galt), alfalfa (*Medicago sativa* L. 'Peace'), oat (*Avena sativa* L.), wheat (*Triticum aestivum* L. 'North Star'), pea (*Pisum sativum* L. 'Redley'), bromegrass (*Bromus* sp.), cauliflower (*B. oleracea* L. var. *botrytis* L. 'Early Snowbird'), flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.). Ten seeds were planted in each plastic pot. Four replicated pots for each treatment were arranged in a randomized complete block design. The test was repeated once.

Fifteen isolates of AG-9, including three isolates from Alaska, were further tested for their pathogenicity on cauliflower and flax. Ten seeds were planted per pot. Four replicated pots for each treatment were arranged in a randomized complete block design.

All data were analyzed by means of the SAS GLM program and Duncan's multiple range test for mean comparison (SAS Institute, Cary, NC).

Genetic variation determination. Fifteen *R. solani* AG-9 isolates (the same isolates used to determine the number of nuclei) were used to determine the genetic relatedness of isolates within the AG-9 group by the RAPD-PCR technique. Isolates were transferred from stock cultures into liquid V8 juice medium in jars (40 ml/jar), and incubated at room temperature for 1 week. The mycelial mat was washed twice with sterile double distilled water and dried on Whatman filter paper. Genomic DNA of *R. solani* AG-9 isolates was purified following the procedure described by Ashktorab and Cohen (3) and quantified using spectrophotometry. The following 10 primers were used for PCR reactions:

R1,5′-GTCCATTCAGTCGGTGCT-3′
USP,5′-GTAAAACGACGGCCAGT-3′
R28,5′-ATGGATCCGC-3′
T7,5′-GTAATACGACTCACTATAG-3′
C7,5′-GAATGCCTTCCAAGCCGGT-3′
N6,5′-CCACCATGATATTCGGCAAG-3′
N8,5′-GAGTACGTGCTCGCTCGATG-3′
E4,5′-TGGGTCGACGGATC-3′
C3,5′-GGTGCCACGAGTAATC-3′
C13,5′-CCAGTCTTCGTAGAGAATCG-3′

PCR reactions were carried out in a volume of 30 µl containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 3 mM DTT (dithiothreitol), 200 µM each of dNTPs, 1 µM primer, 1.25 U *Taq* DNA polymerase, and 50 ng genomic DNA. The thermal cycling profile for primers R28 and E4 consisted of 40 cycles of 94°C for 50 s, 35°C for 60 s, and 72°C for 2 min 30 s. For primers R1 and T7, it consisted of five low stringency cycles of 94°C for 60 s, 42°C for 1 min 20 s, and 72°C for 2 min 30 s, and 30 high stringency cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 2 min 30 s. For all the other primers, the cycling profile consisted of 10 low stringency cycles of 94°C for 60 s, 40°C for 60 s, and 72°C for 2 min 30 s, and 30 high stringency cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 2 min 30 s. In addition, all the profiles were preceded by an initial denaturation at 94°C for 2 min and followed by a final extension at 72°C for 3 min. DNA amplification was performed in a GenAmp System 9600 (Perkin-Elmer Cetus, Norwalk, CT). The amplified products were electrophoresed in 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light.

A similarity matrix was constructed from the RAPD-PCR patterns. The similarity coefficient F between two isolates =

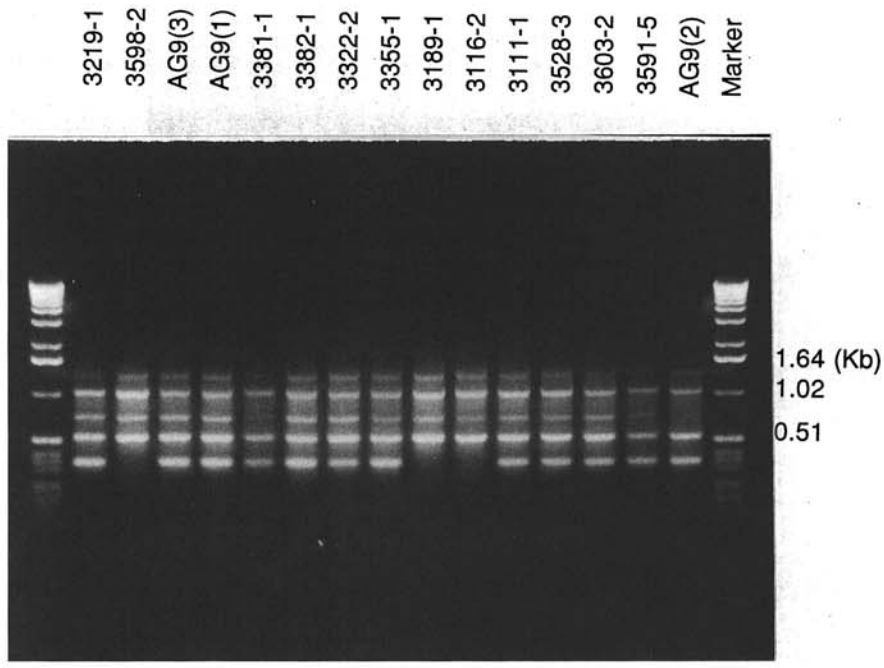

Fig. 1. Fingerprint patterns for isolates of *Rhizoctonia solani* AG-9 generated by random amplified polymorphic DNA–polymerase chain reaction with primer USP.

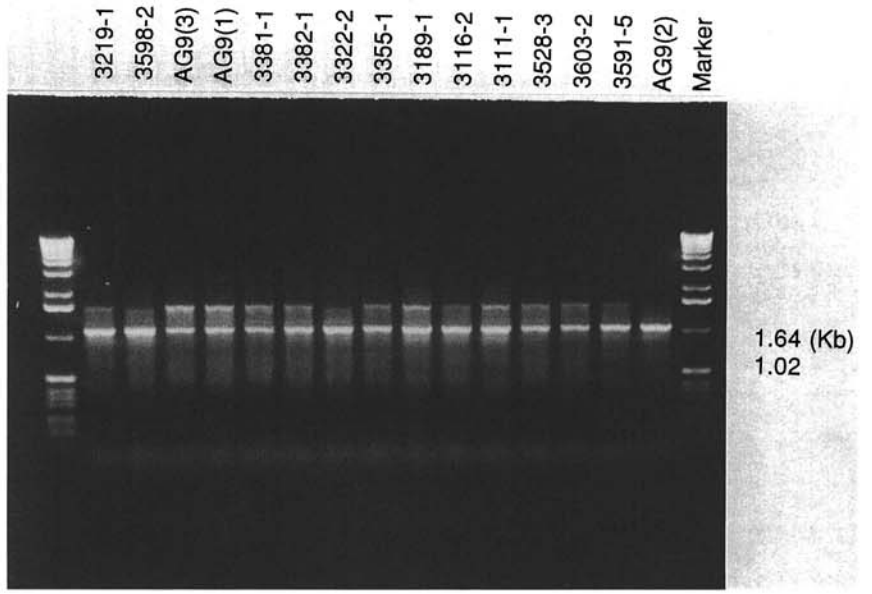

Fig. 2. Fingerprint patterns for isolates of *Rhizoctonia solani* AG-9 generated by random amplified polymorphic DNA–polymerase chain reaction with primer R1.

$2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of common fragments between the two isolates, and $N_x + N_y$ is the total of fragments for the two isolates (12).

RESULTS

A total of 130 isolates of *R. solani* AG-9 were identified. Also, AG-2-1, AG-4, and *Rhizoctonia*-like binucleate isolates were identified from the soil samples.

Isolate characterization. Isolates of AG-9 growing on PDA were white to light tan initially and turned dark brown within 3 weeks. Most isolates showed concentric zones of appressed and aerial mycelium. Small to medium-sized (0.5 to 2 mm in diameter), irregularly shaped, brown to dark brown sclerotia were produced singly or in clumps. Sclerotia were distributed over the surface of the agar but mainly were found on the center around the original inoculum source.

Multinucleate hyphal cells were clearly stained by safranin O. Number of nuclei per cell among 15 isolates tested ranged from 3 to 17 with an average of 5.3 to 7.4.

In 29 isolates tested for thiamine requirement, five (17%) isolates were thiamine auxotrophic, having a +T/–T ratio of more than 5.0 (Table 1). Ten isolates grew poorly but similarly in both media.

Virulence. In petri plate tests, all isolates of AG-9 demonstrated some virulence to canola (Table 2). Within 3 days of canola seeds being placed on the surface of the mycelium, some seeds were colonized by the mycelium, rotted quickly, and did not germinate. Most germinating seeds became infected and showed distinct discoloration. In the greenhouse tests, however, there was considerable variation among the isolates in virulence on canola. Isolates of AG-9 from two of the three locations did not significantly reduce emergence and survival. No relationship in virulence could be established between the petri plate and greenhouse tests on canola; isolates of AG-9 that showed a certain degree of virulence on canola in petri plate tests were not as virulent in the greenhouse tests (Table 2).

In the host range test, isolates of *R. solani* AG-9 were highly virulent to cauliflower and moderately virulent to flax, but not to eight other crop species (Table 3). The percent emergence at day 7 and percentage of symptomless plants at day 21 after seeding and inoculation were significantly lower for cauliflower and flax compared with the control. Root discoloration was observed on canola, barley, alfalfa, and bromegrass plants, but no significant differences were observed in percent emergence and percent survival compared with the control.

Differences in virulence to cauliflower and flax were observed within the 15 isolates that were used for the PCR test. The percent emergence among isolates ranged from 45 to 90% on cauliflower and from 50 to 83% on flax. The percent survival 21 days after inoculation ranged from 5 to 67% on cauliflower and 45 to 80% on flax. Within the three isolates from Alaska, isolate AG9(2) (ATCC 62809) was more virulent on cauliflower and flax than the other two isolates, and had a slower growth rate on PDA, WA, and GA.

Genetic variation. DNA of 15 AG-9 isolates was purified in amounts ranging from 296 µg/ml to 1,209 µg/ml. In PCR reactions with 10 primers, no amplification products were observed for primer T7. Primers USP, C7, N6, and C3 gave multiple products in the 0.34 to 1.64 kb size range, and R1 gave very few products but very clear fingerprint patterns (Figs. 1 to 5). With these five primers, all isolates had one or two fragments in common for each primer. The remaining primers, R28, N8, C13, and E4, gave very few or faint bands. Primer USP yielded three polymorphic DNA fragment patterns. Based on the DNA fingerprint for primer USP, isolates could be divided into three groups: Group 1 included isolates AG-9(1), AG-9(3), 3219-1, 3382-1, 3322-2, 3355-1, 3111-1, 3528-3, and 3603-2; Group 2 included isolates 3598-2, 3189-1, and 3116-2;

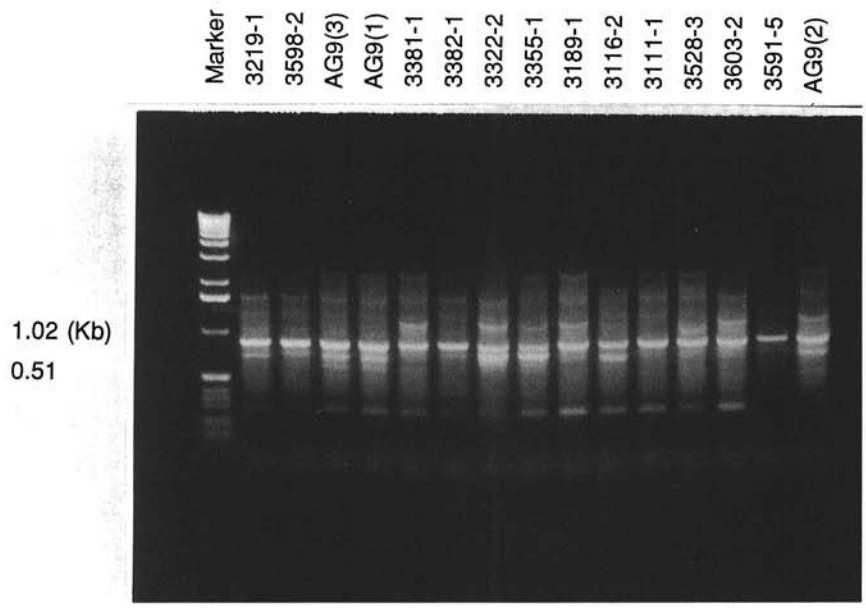

Fig. 3. Fingerprint patterns for isolates of *Rhizoctonia solani* AG-9 generated by random amplified polymorphic DNA–polymerase chain reaction with primer C7.

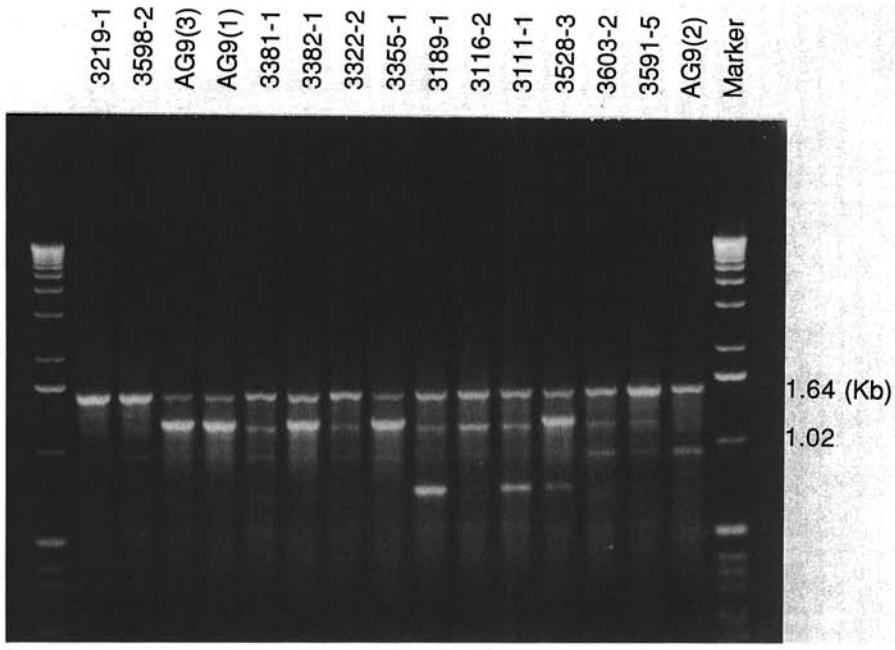

Fig. 4. Fingerprint patterns for isolates of *Rhizoctonia solani* AG-9 generated by random amplified polymorphic DNA–polymerase chain reaction with primer N6.

Group 3 included 3381-1, 3591-5, and AG-9(2).

The similarity matrix derived from combined data of Figures 1 through 5 is shown in Table 4. Within the AG-9 group, the similarity coefficients are all higher than 50%. It is clear that two isolates from Alaska, AG-9(1) and (3), are identical (F = 100%), but not so closely related to the third, AG-9(2), (F = 74%). Two isolates from Alberta, 3219-1 and 3382-1, are closely related to AG-9(1) and AG-9(3) (F > 90%). Isolate AG-9(2) from Alaska is closely related to 3322-2 (F = 89%), which is a thiamine auxotrophic isolate. Isolate 3381-1 is closely related to 3603-2 and 3591-5 (F = 92%).

DISCUSSION

In Canada, isolates of *R. solani* AG-9 were found in agricultural soils in Alberta. The isolates had characteristics similar to those of AG-9 isolates reported from Alaska. The number of nuclei also corresponded with that reported by Carling et al. (6). Carling et al. (6) first reported the isolation of *R. solani* AG-9 from potato plants and soil of potato fields, but they could not establish the pathogenicity of AG-9 isolates on potato and eight other crops. They suggested that the isolates of AG-9 may be generally a nonpathogenic anastomosis group in soil. In petri plate tests, all AG-9 isolates could colonize canola seeds and caused seed rot and seedling infection 3 days after inoculation. In greenhouse tests, however, these isolates did not significantly reduce percent emergence or percent survival of canola and barley.

Isolates of AG-9 were avirulent to cereal crops, forages, field pea, and tomato, supporting the conclusion of Carling et al. (6) that isolates in this anastomosis group are soil saprophytes. This hypothesis is also supported by studies that found that the population of AG-9 in soil, averaged over three locations, was not significantly affected by rotation and tillage systems (9). However, isolates of AG-9 were highly virulent to cauliflower and moderately virulent to flax in this study, suggesting that *R. solani* AG-9 may cause significant damage in cauliflower-growing areas. It is possible that AG-9 may be a pathogen on some other related species, including cruciferous weeds, and could exist in various soil environments. More isolates of AG-9 were found at La Corey and Alliance than at Viking. In the virulence test of 15 isolates, there were significant differences in virulence on cauliflower and flax among the isolates. It is not known if these are due to environmental effects or genetic variation.

Isolates of AG-9 can be subdivided into two groups based on their thiamine requirement. More than 80% of the isolates were thiamine prototrophic and less than 20% thiamine auxotrophic. This is in agreement with the results reported by Carling et al. (6).

There was a considerable genetic variation within the *R. solani* AG-9 group as detected by RAPD-PCR technology. The similarity coefficients from combined data based on fingerprint patterns derived by primers USP, R1, C7, N6, and C3 ranged from 67 to 100% within 15 AG-9 isolates tested. However, there were no specific DNA patterns for the isolates from different biogeographical origins. Two isolates from Alaska had a pattern similar to those of seven Alberta isolates detected by means of primer USP. Two Alberta isolates (3219-1 and 3382-1) were closely related to these two Alaska isolates, as their similarity coefficients were higher than 90%. Also, no specific relationship was found between the DNA fingerprint and thiamine requirement of AG-9 isolates. Three isolates from the thiamine auxotrophic group did not always show the same pattern detected by different primers, perhaps because of the particular primers used. Carling and Kuninaga (5) proposed two ISGs of AG-9 (TP and TX) based on a DNA

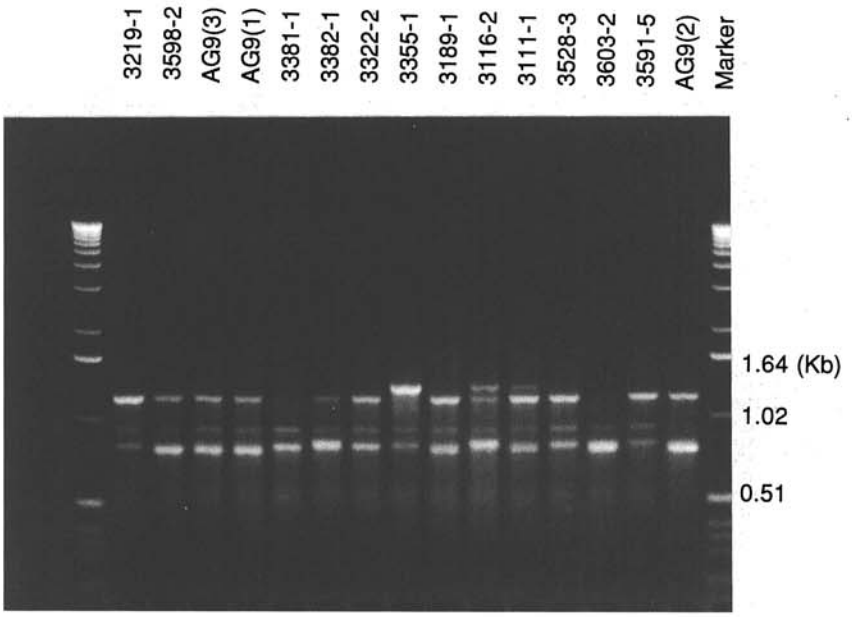

Fig. 5. Fingerprint patterns for isolates of *Rhizoctonia solani* AG-9 generated by random amplified polymorphic DNA–polymerase chain reaction with primer C3.

Table 4. Similarity coefficients of combined data from Figures. 1, 2, 3, 4, and 5 expressed as percentage

	Similarity (%)													
Isolate	3598-2	AG9(3)	AG9(1)	3381-1	3382-1	3322-2	3355-1	3189-1	3116-2	3111-1	3528-3	3603-2	3591-5	AG9(2)
3219-1	92	90	90	80	89	83	83	79	90	90	83	86	85	85
3598-2		82	82	67	80	82	74	77	89	79	74	77	75	83
AG9(3)			100	74	93	87	87	83	87	84	87	83	82	74
AG9(1)				74	93	87	87	83	87	84	87	83	82	74
3381-1					80	82	82	77	74	79	82	92	92	75
3382-1						86	86	89	86	90	93	89	88	72
3322-2							87	83	87	84	87	90	82	89
3355-1								83	87	84	87	90	74	74
3189-1									83	80	97	86	77	77
3116-2										90	80	83	82	74
3111-1											90	87	86	71
3528-3												90	82	74
3603-2													85	77
3591-5														75
AG9(2)														

hybridization study. Within ISG groups, the similarity was higher than 90%, whereas between subgroups the similarity was lower than 87%. Vilgalys and Gonzalez (20) did not observe any obvious relationship between the RFLP patterns in isolates of AG-9 and their thiamine requirement. Also, the difference could not be detected by means of cloned DNA probes in RFLP analysis (8) or isozyme technique (11). The patterns from the primers used here also did not clearly distinguish the thiamine auto- and auxotrophic subgroups or isolates that differed in virulence on cauliflower and flax. However, since considerable variation within the AG-9 isolates was observed here, screening additional primers may identify such polymorphic DNA fragments.

The *F* values for the isolates in our study are much higher than those from Duncan et al. (7). This may be because only isolates of AG-9 were used in our study. The genetic relatedness is much closer for isolates within anastomosis groups than between groups. It may also depend on the primers used. This latter effect could be minimized by using many primers. The primers T7, R1, and R28, which gave good DNA products in the study by Duncan et al. (7), did not give similar results in our study although similar PCR amplification profiles were used. Primer T7 could not yield any products for AG-9 as it could for ZG3 to ZG5 in the experiment by Duncan et al. (7). These results indicate that specific primers are needed for detecting anastomosis groups within species. All isolates detected by five primers had one or two fragment(s) in common, indicating that the anastomosis grouping has a genetic basis. This is in agreement with the report by Duncan et al. (7).

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