# DNA Base Sequence Homology in *Rhizoctonia solani* Kühn: Inter- and Intragroup Relatedness of Anastomosis Group-9

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## ABSTRACT

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Isolates of anastomosis group-9 (AG-9) of *Rhizoctonia solani* are approximately 81% thiamine prototrophic and approximately 19% thiamine auxotrophic. These subgroups, or intraspecific groups (ISG), can be distinguished from one another by DNA hybridization techniques but not by hyphal anastomosis. DNA hybridization among isolates belonging within either group is 94% or higher, whereas hybridization among thiamine prototrophic and thiamine auxotrophic isolates ranges from 78 to 87%. Isolates of AG-9, regardless of thiamine requirement, show low DNA hybridization (15% or less) with isolates representing 10 AG and 15 ISG of *R. solani*, indicating the genetic isolation of AG-9. We propose that the two ISGs of AG-9 be designated AG-9 TP (thiamine prototrophic) and AG-9 TX (thiamine auxotrophic).

*Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk) is divided into subspecific groups based on the anastomosis behavior (11,13) of individual isolates. Hyphae of closely related isolates can anastomose when they confront one another, whereas hyphae of nonrelated isolates do not anastomose. Anastomosis reactions may range from fusion of walls and membranes of confronted hyphae, typical of self-anastomosis reactions, to no reaction observed between isolates of nonrelated groups. Intermediate reactions where walls (and perhaps membranes) connect but do not fuse, followed by the death of connecting and some adjacent cells, typically occur between members of the same anastomosis group (1,13).

Although there is disagreement about the formal taxonomic significance of the anastomosis group (AG), anastomosis has proved to be a simple and useful method of specifying subspecific groups within *R. solani*. Currently, *R. solani* is known to contain 12 anastomosis groups including AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-BI, AG-7, AG-8 (11), AG-9 (3), and AG-10 (12). Some of these anastomosis groups are further subdivided based on pathogenicity (AG-1, AG-2), colony morphology (AG-1), thiamine requirement (AG-2, AG-9), and/or DNA homology (AG-4, AG-6) into intraspecific groups (ISG) (11). The ISG is derived by combining anastomosis group affiliation with other descriptive characteristics such as those listed above.

Isolates of *R. solani* AG-9 have been recovered from soil and plant tissue at various locations in Alaska and one location in Oregon (3). This AG does not appear to aggressively attack established plants, but may be destructive to some plant species in the early stages of growth (2). Some isolates of AG-9 are prototrophic for thiamine, whereas others require thiamine for normal growth. This characteristic differentiates AG-9 from most other AG of *R. solani*, as thiamine requirement is a group-wide characteristic for all AG of *R. solani* except AG-2 and AG-9. Thiamine auxotrophic groups include AG-2-2, AG-5, and AG-BI.

Results of DNA/DNA reassociation kinetics studies have been reported on isolates representing most AG of *R. solani* (4–10). These studies have confirmed close relationships among isolates within most AG and more distant relationships among isolates of different AG. Similar studies by Vilgalys (15), in which genetic relationships among isolates were investigated with heterologous DNA/DNA hybridization techniques, are in agreement with the reports of Kuninaga and Yokosawa. This study with DNA/DNA reassociation kinetics was undertaken to: 1) determine the relationship of isolates of AG-9 with isolates representing other anastomosis groups of *R. solani* and 2) determine the relationship of thiamine prototrophic and thiamine auxotrophic isolates of AG-9.

## MATERIALS AND METHODS

Thiamine requirement was determined by growing the fungus in petri dishes containing Czapek-Dox agar with or without $10^{-5}$ M thiamine hydrochloride (14). Petri dishes containing the appropriate medium were seeded with 7-mm-diameter disks of mycelium representing the various isolates of *R. solani* AG-9. Dry weight determinations were made after 2 wk of growth at 20 C in the dark. Ten isolates of AG-9 were evaluated, including five prototrophs and five auxotrophs. Isolate V12M was collected from a potato stem, whereas all other isolates were collected from soil. All isolates originated from agricultural fields in south-central Alaska.

Fungal cultivation and preparation of whole-cell DNA was done as described previously (4). Analysis in CsCl/bisbenzimide density gradient centrifugation showed each DNA preparation to contain less than 3.4% mitochondrial DNA. Other methods associated with these DNA/DNA reassociation studies, including determination of the melting point, shearing of DNA, and determination of DNA fragment size, have been described previously (4). DNA/DNA reassociation was performed at 63 C (approximate melting point = 25 C), and sheared DNA samples (75–85 $\mu$g/ml) were dissolved in 5 × SSC (SSC = standard saline citrate: 150 mM NaCl, 15 mM sodium citrate, pH 7.0) with 20% dimethylsulfoxide to promote the reassociation rate. DNA similarity values were calculated by a previously reported equation (4).

The term *homology*, although retained in the title of this report in order to be consistent with earlier reports in this series (4–10), is replaced by the term *similarity* in the text. DNA/DNA reassociation kinetics establishes similarity in DNA but does not necessarily prove common evolutionary origin. The term *homology* is defined to specifically indicate common evolutionary origin.

## RESULTS

**Thiamine requirement.** The thiamine requirement was determined for 31 isolates of *R. solani* AG-9. Twenty-five isolates (81%) grew equally well on media with or without thiamine and were classified as thiamine prototrophs. Eight of these 25 isolates grew poorly but similarly on both media. Six of the 31 isolates, approximately 19%, grew luxuriantly on media containing thiamine but sparsely on media without thiamine. These six isolates were classified as thiamine auxotrophs. The performance of five prototrophs and five auxotrophs on media with or without thiamine is summarized in Table 1.

**DNA similarity within *R. solani* AG-9.** The results of hybridization among isolates of *R. solani* AG-9 are summarized in Table 2. Where different thiamine prototrophic isolates were hybridized, percent similarity ranged from 94 to 98%. Percent similarity between different thiamine auxotrophic isolates ranged from 92 to 99%. A lower percentage similarity, ranging from 78 to 87%, was observed when thiamine prototrophs were hybridized with thiamine auxotrophs, indicating a more distant genetic relationship between prototrophs and auxotrophs than within the two groups.

**DNA similarity between AG-9 and other AG of *R. solani*.** Percent similarity values for hybridization reactions between each of four isolates of AG-9, two thiamine prototrophs and two thiamine auxotrophs, and isolates representing 10 other AG and 15 other ISG of *R. solani* are summarized in Table 3. Hybridization values ranged from 2 to 15% and illustrate a distant relationship between isolates of AG-9 and isolates of other AG and ISG of *R. solani*.

## DISCUSSION

Previous studies by Kuninaga and Yokosawa (6) and Vilgalys (15) generally have shown a very high level of DNA hybridization among isolates that are members of the same anastomosis group. Isolates of AG-3, AG-5, AG-7, and AG-BI hybridized with other isolates within their respective groups at a rate of 91% or higher (6), indicating genetic homogeneity among isolates within these groups. Hybridization between isolates of different AG was 30% or less (6,15). Ranges of DNA hybridization values varied for different AG, and lower levels of hybridization have confirmed lack of homogeneity among isolates within AG-1, AG-2-1, and AG-2-2 (5,15). Before DNA hybridization studies, each of these three AG had been subdivided based on pathogenicity and/or colony morphology. The results of hybridization studies confirm the existence of genetic differences that corresponded to the well-known phenotypic differences. As mentioned earlier, these subgroups of anastomosis groups are sometimes called intraspecific groups (ISG) (11). Within AG-1, isolates of AG-1 IA (sasakii type) hybridized with other isolates within AG-1 IA at 98% or higher (4). Similarly, isolates of AG-1 IB (web blight type) hybridized with other isolates of AG-1 IB at 95.7% or higher. However, hybridization values between isolates of AG-1 IA and AG-1 IB ranged from 49.9 to 55.9%, indicating that the genetic distance between isolates of AG-1 IA and AG-1 IB is greater than between isolates within either ISG.

Within AG-2, isolates of AG-2-1, AG-2-2 IIIB, or AG-2-2 IV hybridized at 98.1% or higher with other isolates from their respective groups (5). However, hybridization between isolates of AG-2-2 IIIB and AG-2-2 IV ranged from 68.6 to 71.5%, and hybridization between AG-2-1 and either AG-2-2 IIIB or AG-2-2 IV ranged from 37.6 to 49.4%, again indicating greater genetic variation between than within ISG and confirming heterogeneity within AG-2.

DNA hybridization studies also revealed previously unknown heterogeneity in AG-4 and AG-6 (7,8). Isolates of the two homogeneous groups (HG-I and HG-II) within AG-4 hybridized at high levels (greater than 88.5%) with isolates from their respective groups but at lower levels (30.9–47.9%) between groups. Existence of these subgroups of AG-4 was confirmed in similar studies by Vilgalys (15). AG-6 was found to contain two subgroups; HG-I, a homogeneous group with hybridization values of 91.8% or higher among its isolates, and GV, an extremely heterogeneous subgroup with hybridization values of 55.4–66.2% among its isolates. Hybridization values of isolates between AG-6 HG-I and isolates of AG-6 GV ranged from 47.5 to 62.9%. This heterogeneity within AG-6 revealed by DNA hybridization studies is not easily or reliably detected by anastomosis comparisons (Carling and Leiner, *unpublished*).

*R. solani* AG-9 is a heterogeneous anastomosis group similar to AG-1, AG-2, AG-4, and AG-6 in that it contains two readily identifiable ISG. These ISG are identifiable phenotypically by thiamine requirement, and now genetically by DNA hybridization. Hybridization percentages are 92% or higher within the thiamine prototrophic and thiamine auxotrophic subgroups, but drop to 68–87% between subgroups. We propose that these ISG be designated AG-9 TP (thiamine prototrophic) and AG-9 TX (thiamine auxotrophic).

Isolates of *R. solani* AG-9, whether AG-9 TP or AG-9 TX,

TABLE 1. Thiamine requirements of isolates of *Rhizoctonia solani* AG-9

| Isolate | Source | Mycelial dry weight (mg)[a] | | Ratio |
|---|---|---|---|---|
| | | − Thiamine | + Thiamine | +/− thiamine |
| Prototrophs: | | | | |
| KHP15 | soil | 210 | 232 | 1.11 ± 0.02 |
| S5B2 | soil | 198 | 224 | 1.13 ± 0.04 |
| V12M | potato stem | 212 | 220 | 1.04 ± 0.03 |
| S21[b] | soil | 204 | 208 | 1.02 ± 0.01 |
| BS24 | soil | 31 | 28 | 0.89 ± 0.13 |
| Auxotrophs: | | | | |
| S4R1 | soil | 16 | 191 | 17.68 ± 12.24 |
| KHP7 | soil | 4 | 223 | 59.25 ± 8.75 |
| S4R4 | soil | 31 | 218 | 7.33 ± 1.46 |
| S9R1 | soil | 3 | 222 | 90.31 ± 4.76 |
| BS4 | soil | 36 | 177 | 5.71 ± 2.07 |

[a] Mycelial dry weight figures are the average of at least three replications. Ratios are ± standard deviation.
[b] S21 = ATCC 62804.

TABLE 2. DNA similarity among thiamine prototrophic and thiamine auxotrophic isolates of *Rhizoctonia solani* AG-9

| Isolate | Thiamine prototrophs | | | | | Thiamine auxotrophs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S21 | V12M | S5B2 | BS24 | KHP15 | S9R1 | S4R1 | S4R4 | KHP7 | BS4 |
| S21 | 100 | 97[a] | 98 | 96 | 97 | 84 | 86 | 87 | 82 | 81 |
| V12M | ··· | 100 | 96 | 94 | 95 | 81 | 79 | 82 | 83 | 84 |
| S5B2 | ··· | ··· | 100 | 98 | nd[b] | 81 | 80 | 82 | 84 | 78 |
| BS24 | ··· | ··· | ··· | 100 | nd | 78 | 83 | 78 | 86 | 79 |
| KHP15 | ··· | ··· | ··· | ··· | 100 | nd | 86 | nd | nd | 86 |
| S9R1 | ··· | ··· | ··· | ··· | ··· | 100 | 97 | 92 | 96 | 97 |
| S4R1 | ··· | ··· | ··· | ··· | ··· | ··· | 100 | 99 | 98 | 96 |
| S4R4 | ··· | ··· | ··· | ··· | ··· | ··· | ··· | 100 | nd | nd |
| KHP7 | ··· | ··· | ··· | ··· | ··· | ··· | ··· | ··· | 100 | 95 |
| BS4 | ··· | ··· | ··· | ··· | ··· | ··· | ··· | ··· | ··· | 100 |

[a] Similarity values are expressed as percentages and represent the averages of two or three replications.
[b] nd = not determined.

TABLE 3. DNA similarity among isolates of *Rhizoctonia solani* AG-9 and isolates representing other intraspecific groups of *R. solani*

| Intraspecific group[a] | Isolates | Percentage similarity with AG-9 isolate | | | |
|---|---|---|---|---|---|
| | | S21 | V12M | S9R1 | S4R1 |
| AG-1 (IA) | CS-Ka | 9[b] | 4 | 11 | 7 |
| AG-1 (IB) | 001-7 | 12 | nd[c] | 8 | nd |
| AG-1 (IC) | PS-1 | 6 | 3 | 15 | 8 |
| AG-2-1 | R123 | 7 | 7 | 5 | 6 |
| AG-2-2 (IIIB) | B60 | 10 | nd | 8 | nd |
| AG-2-2 (IV) | BR-2 | 13 | 8 | 9 | 9 |
| AG-3 | ST3-1 | 9 | 4 | 7 | 10 |
| AG-4 HG-I | Chr-3 | 3 | 2 | 9 | 4 |
| AG-4 HG-II | HI 521-21 | 7 | nd | 8 | nd |
| AG-5 | TMA1-4 | 6 | 8 | 4 | 9 |
| AG-6 HG-I | NTA3-1 | 13 | 9 | 10 | 11 |
| AG-6 GV | NKN2-1 | 9 | 7 | 6 | 4 |
| AG-7 | 1556 | 6 | 2 | 5 | 2 |
| AG-8 | A21 | 2 | 7 | 3 | 9 |
| AG-BI | AI1-4 | 11 | 6 | 8 | 10 |

[a]Intraspecific grouping is based upon anastomosis, colony morphology, pathogenicity, thiamine requirement, and/or DNA homology (12).
[b]Similarity values are expressed as percentages and represent the averages of two replications.
[c]nd = not determined.

hybridize at very low rates with isolates representing 15 other ISG of *R. solani*. This confirms the genetic isolation of AG-9 previously indicated by the absence of any anastomosis reaction between isolates of AG-9 and other AG of *R. solani*. Slightly higher rates of hybridization (from 15 to 30%) would indicate the possibility of anastomosis to a limited extent (bridging) with other AG (10). Bridging is not known to occur in isolates of AG-9, and levels of hybridization of 15% or less confirm that bridging does not occur.

Vilgalys and Gonzalez (16) recently reported on the occurrence of certain restriction fragment length polymorphisms (RFLPs) in ribosomal DNA of *R. solani*. Eighty-seven isolates representing 11 AG and 15 ISG, including thiamine prototrophic and thiamine auxotrophic (isolates 114 Rs and 116 Rs) isolates of AG-9, were evaluated in their study. Vilgalys and Gonzalez did not observe any obvious relationship between the RFLP patterns in isolates of AG-9 and their thiamine requirement. However, they also did not observe relationships between RFLP patterns and ISGs within AG-1, AG-2, AG-4, or AG-6. It is not known why RFLP patterns did not relate more closely to ISGs. However, the particular enzymes used by Vilgalys and Gonzalez did not detect the genetic differences responsible for the various phenotypic characteristics, including anastomosis, pathogenicity, colony morphology, and thiamine requirement, used to define ISGs in *R. solani*. Future searching for RFLPs, using additional enzymes, as well as other molecular studies, may answer these questions.

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