DNA Base Sequence Homology in *Rhizoctonia solani* Kühn V. Genetic Relatedness within AG-6

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国永史朗*・横沢菱三* : *Rhizoctonia solani* における DNA 塩基配列の相同性
V. 菌糸融合群第6群内における遺伝的類縁性

Abstract

DNA base sequence homology among AG-6 isolates of *Rhizoctonia solani* was determined by DNA-DNA reassociation kinetics. Five of 10 isolates examined shared high DNA homology ranging from 91.8 to 97.7%. This group was classified as AG-6 homogeneous group I (HG-I). DNA homology among the remaining 5 isolates was 55.4 to 66.2%. They showed only 47.5 to 62.9% DNA homology with AG-6 HG-I isolates. These isolates were designated AG-6 genotypic variation (GV) isolates. Each of them appeared to have gene structures that differed by nucleotide substitutions. The genetic divergence among AG-6 isolates was also clearly demonstrated by the electrophoretic patterns of proteins and appearances of the cultures. These results suggest that AG-6 consists of strains that are phylogenetically related but have significant genetic polymorphism. (Received December 23, 1983)

Key Words : *Rhizoctonia solani* (AG-6), DNA homology, genetic relatedness.

Introduction

Previous studies⁸⁻¹¹⁾ in this series of investigations reported that there are cases where all the isolates in a given anastomosis group (AG) of *Rhizoctonia solani* Kühn were genetically homogeneous. While this was demonstrated in the case of AG-2-1, AG-3, AG-5, AG-7 and AG-BI, it was shown that AG-1, AG-2-2 and AG-4 must be considered genetically heterogeneous groups.

AG-6 of *R. solani* is a new group detected in Japan¹²⁾. This group is widely distributed in non-cultivated soils¹²⁾. The present study attempts to clarify genetic relationship among isolates in this group on the basis of DNA-DNA reassociation kinetics.

Materials and Methods

Ten isolates of AG-6 selected to represent morphological types within the group were used. All were isolated from non-cultivated soils at various areas of Hokkaido¹²⁾. The characteristics of these isolates are given in Table 1.

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Table 1. Isolates of *Rhizoctonia solani* used

AG-6 isolate	Source ^{a)}	Geographic origin ^{b)}	Date
UBウ-1-A	Soil	Ubaranai, Abashiri-shi	June, 1976
HAM1-1	Soil	Hamamasumura, Hamamasu-gun	Aug, 1977
NTA3-1	Soil	Nakataiki, Hiroo-gun	June, 1977
AT2-1	Soil	Akkeshi, Akkeshi-gun	May, 1977
OHT1-1	Soil	Ohtakimura, Usu-gun	July, 1977
SO2-1	Soil	Soya, Wakkanai-shi	Aug, 1977
NKN2-1	Soil	Nukanai, Nakagawa-gun	June, 1977
KNB2-2	Soil	Konbu, Isoya-gun	July, 1977
HN1-1	Soil	Horonuka, Rumoi-gun	Aug, 1977
TSH1-1	Soil	Teshikaga, Kawakami-gun	May, 1977

a) Non-cultivated soils (Kuninaga & Yokosawa, 1978)¹²⁾.

b) In Hokkaido prefecture.

Methods for fungal cultivation, the preparation of DNAs, measurement of melting temperature (T_m) of DNAs, shearing of DNAs and the determination of DNA fragment size as well as for calculation of DNA homology values were described in previous papers^{7,8)}. DNA-DNA reassociation kinetics were observed in $5\times$ SSC containing 20 % DMSO at a temperature about 25 C below T_m . Methods for polyacrylamide gel electrophoresis of mycelial soluble proteins were also described previously¹¹⁾.

Results

Thermal denaturation of DNA of AG-6 isolates

The T_m of DNA from AG-6 isolates was examined as a measure for determining the

Fig. 1. Thermal denaturation profiles of DNA of AG-6 isolates of *Rhizoctonia solani* and *Escherichia coli* in $5\times$ SSC containing 20% DMSO. Bar designates T_m value.

Table 2. Thermal denaturation and base compositions of DNA of AG-6 isolates of *Rhizoctonia solani*

Isolate	T _m ^{a)} in 5×SSC + 20% DMSO	Base composition ^{b)} (GC mole %)
UBウ-1-A	87.9	46.1
HAM1-1	87.8	45.9 (41.4) ^{c)}
NTA3-1	87.8	45.9
AT2-1	87.6	45.4 (42.2) ^{c)}
OHT1-1	87.8	45.9
SO2-1	87.1	44.2 (41.8) ^{c)}
NKN2-1	86.7	43.2
KNB2-2	87.1	44.2
HN1-1	86.8	43.5
TSH1-1	86.9	43.7

a) The temperature corresponding to the midpoint of absorbance rise (Marmur & Doty, 1962)¹⁴⁾.

b) *E. coli* DNA (GC mole %=50) was used as a standard. The base composition of *R. solani* DNA was calculated on a equation modified from Marmur & Doty (1968)¹⁴⁾: GC mole %=50+ (T_m *R. solani* DNA-T_m *E. coli* DNA/0.41).

c) Base composition determined by chemical analysis (Kuninaga & Yokosawa, 1980)⁷⁾.

Table 3. DNA homology among isolates of *Rhizoctonia solani* AG-6

	AG-6 homogeneous group I isolates					AG-6 genotypic variation isolates				
	UBウ-1-A	HAM1-1	NTA3-1	AT2-1	OHT1-1	SO2-1	NKN2-1	KNB2-2	HN1-1	TSH1-1
UBウ-1-A	100 ^{a)}	93.3 ^{b)}	91.8	95.4	92.4	53.9	58.7	49.3	48.3	49.3
	HAM1-1	100	93.8	92.0	97.7	62.3	47.5	55.0	55.2	53.3
		NTA3-1	100	95.8	— ^{c)}	58.2	49.4	55.1	61.8	—
			AT2-1	100	—	57.4	56.0	60.8	51.4	—
				OHT1-1	100	60.8	62.4	53.9	62.9	—
					SO2-1	100	55.4	57.3	56.3	58.7
						NKN2-1	100	66.2	61.9	60.4
							KNB2-2	100	63.4	62.4
								HN1-1	100	59.4
									TSH1-1	100

a) Expected homology value.

b) Average of two or three determinations. All reactions of DNA-DNA reassociation kinetics were carried out in 5×SSC containing 20% DMSO at 61 C.

c) Not determined.

optimum temperature for DNA-DNA reassociation studies. Thermal denaturation was observed in $5\times$ SSC containing 20 % DMSO. Fig. 1 shows the thermal denaturation profiles of DNAs from representative isolates. Degrees of hyperchromicity of DNAs from AG-6 isolates ranged from 33.1 to 39.8 %. The T_m values and base compositions of DNAs are presented in Table 2. DNA base compositions of AG-6 isolates were distributed rather widely from 43.2 to 46.1 mole %.

DNA homology among AG-6 isolates

As shown in Table 3, the various DNA homology values were obtained from observations of 10 isolates in AG-6. High DNA homology values, 91.8 to 97.7 %, were observed among 5 isolates (UBウ-1-A, HAM1-1, NTA3-1, AT2-1 and OHT1-1). These isolates were classified as AG-6 homogeneous group I (HG-I). DNA homology values among the remaining 5 isolates (SO2-1, NKN2-1, KNB2-2, HN1-1 and TSH1-1) were 55.4 to 66.2

Fig. 2. Reassociation kinetics of the mixture of DNA from isolates of *Rhizoctonia solani* AG-6. All reactions were carried out in $5\times$ SSC containing 20% DMSO at 61 C.

Fig. 3. Reassociation kinetics of the mixture of DNA from isolates of *Rhizoctonia solani* AG-6. All reactions were carried out in $5\times$ SSC containing 20 % DMSO at 61 C.

%. These were designated as AG-6 genotypic variation (GV) isolates and showed 47.5 to 62.9 % homology with the isolates in AG-6 HG-I. Profiles of DNA reassociation kinetics among representative isolates in AG-6 are shown in Fig. 2 and 3.

Protein similarity among AG-6 isolates

As shown in Fig. 4, all the AG-6 isolates showed a common protein band intensely stained at Ef 0.3. There was also a marked similarity of protein bands at other Ef

Fig. 4. Electrophoretic profiles of mycelial proteins from AG-6 isolates of *Rhizoctonia solani*.

Fig. 5. Cultural appearance of AG-6 isolates of *Rhizoctonia solani* grown on potato dextrose agar for 14 days at 28 C.

AG-6 HG-I isolates—top row (left to right): OHT1-1, UBウ-1-A and HAM1-1.

AG-6 GV isolates—bottom row (left to right): NKN2-1, TSH1-1 and HN1-1.

values among isolates in AG-6 HG-I. There were definite differences in banding patterns between AG-6 HG-I and AG-6 GV isolates. Also, in contrast to protein patterns of isolates in AG-6 HG-I, there was strong protein polymorphism among AG-6 GV isolates.

Cultural appearance of AG-6 isolates

As shown in Fig. 5, AG-6 HG-I and AG-6 GV were easily distinguished from each other in terms by colony characteristics on potato dextrose agar (PDA) after 14 days. AG-6 HG-I isolates (OHT1-1, UBウ-1-A and HAM1-1) had similar characteristics, as follows: abundant sclerotia, uniform in size, 1.0 to 1.5 mm diam., often aggregated into compounded sclerotia, mycelioid or wooly surface and change of colour to yellowish-brown in PDA. In contrast, AG-6 GV isolates (NKN2-1, TSH1-1 and HN1-1) showed the following appearances: few or abundant aerial hyphae; concentric zonation in some isolates: generally few or no sclerotia; change of colour to dark brown in PDA.

Discussion

These studies suggest that AG-6 contains isolates that are genetically homogeneous (here classified as AG-6 HG-I). It also contains isolates having lower DNA homology with each other as well as with the isolates in AG-6 HG-I. The latter heterogeneous isolates were designated as AG-6 genotypic variation (GV) isolates. However, the possibility cannot be excluded that further studies, dealing with large numbers of isolates outside AG-6 HG-I, may detect some forming homogeneous groups different from AG-6 HG-I.

DNA homology between AG-6 HG-I and AG-6 GV isolates ranged from 47.5 to 62.9 %, whereas, that within AG-6 GV was from 55.4 to 66.2 %. These results suggest that AG-6 is a genetically polymorphic group composed of strains with partial phylogenetical relatedness.

The present experiments suggest that each of AG-6 GV isolates examined possesses a different gene structure arising from particular nucleotide substitutions^{6,13)} or DNA rearrangements^{2,5)} involving about 45 % of the entire molecule of DNAs. This is the unique feature of AG-6, not observed in other AGs of *R. solani*. This suggests that genetic polymorphism in AG-6 has increased the ability for survival of this widely distributed group¹²⁾.

Previous studies¹¹⁾ showed that the relationship among AG-4 isolates as determined on the basis of DNA homology agrees well with that seen in electrophoretic pattern of proteins. In the present study, homologous electrophoretic pattern was presented in AG-6 HG-I. In contrast, polymorphic protein patterns were observed in AG-6 GV isolates that are genetically divergent. These results may be expected from the fact that the primary structure of proteins of organisms is defined by the nucleotide sequences of their DNAs. Our experiments suggest that electrophoretic patterns of total soluble proteins (enzymes) from mycelia may be a useful measure of the relatedness of isolates in AGs of *R. solani*.

In the present study, we also found a relatively wide range of DNA base compositions (GC contents) which may be explained on the basis of the great total variation of

isolates in AG-6. On the average, AG-6 GV isolates showed slightly lower GC contents than isolates in AG-6 HG-I. The observation of DNA homology values further suggests that AG-6 GV may be a variant group in AG-6 HG-I. If so, the diversification is accompanied by a progressive decrease in GC content.

Storck and Alexopoulos¹⁵⁾ suggested that the evolution of fungi is usually accompanied by a progressive increase of GC content. Conversely, Freese⁴⁾ and Sueoka¹⁶⁾ concluded from their mathematical models of DNA base changes by mutation that there is nothing justifying the assumption of unidirectional increase in GC contents during the process of diversification. A final appraisal of the phylogenetical significance of DNA base compositions in *R. solani* may require an accumulation of further information about DNA repetitive sequences^{1,3,17)} and DNA rearrangement^{2,5)} of fungal genomes.

The authors are grateful to Dr. A. Ogoshi, Hokkaido University, and Dr. R. Baker, Department of Botany and Plant Pathology, Colorado State University, for critical reading of this manuscript.

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和 文 摘 要

Rhizoctonia solani における DNA 塩基配列の相同性

V. 菌糸融合群第6群内における遺伝的類縁性

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Rhizoctonia solani の菌糸融合群第6群 (AG-6) 内における菌株間の遺伝的類縁性を検討するため、DNA-DNA 再会合反応速度解析に基づき、DNA 塩基配列の相同性を比較した。その結果、AG-6 は遺伝的に均一な菌株の一群と様々な遺伝的分化を示す菌株とから成ることがわかった。前者を AG-6 homogeneous group I (HG-I)、後者を AG-6 genotypic variation (GV) 菌株とした。AG-6 HG-I 菌株間相互の DNA 相同性は91.8%~97.7%、AG-6 GV 菌株間相互では55.4%~66.2%であった。また AG-6 HG-I 菌株と AG-6 GV 菌株間では47.5%~62.9%であった。AG-6 菌株間の遺伝的な多様性は菌体可溶性蛋白電気泳動パターンおよび培養型比較結果からもよく示された。以上の結果は、AG-6 は遺伝的多型を示す群であり、系統的には互いに近縁な菌株から成ることを示唆する。