A Comparison of DNA Base Compositions among Anastomosis Groups in *Rhizoctonia solani* Kühn

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国永史朗*・横沢菱三* : 菌糸融合群を異にする *Rhizoctonia solani*
菌の GC 含量比較

Abstract

Base composition of DNAs of 30 isolates of *Rhizoctonia solani* Kühn was compared. The relationships between the base composition of DNA and each anastomosis group or cultural type were investigated.

The range of guanine plus cytosine (GC) content of *R. solani* DNA was 40.9 to 49.3 mole %, and its average was 45.3 mole %. The GC contents of the isolates within each anastomosis group were almost same. Little difference of GC content was observed between the sasakii type (IA) and the web-blight type (IB) in AG-1. Furthermore GC contents of the rush type (IIIB) and the root rot type (IV) in AG-2-2, were quite similar. While a significant difference was observed between the potato type (IV) in AG-3 and the root rot type (IV).

AG-1 showed the highest GC content (avg. 48.8 mole %) among all of the anastomosis groups, followed by AG-4 (avg. 47.3 mole %), AG-3 (avg. 47.1 mole %), AG-5 (avg. 45.2 mole %), AG-2-2 (avg. 44.6 mole %), AG-2-1 (avg. 43.7 mole %), AG-6 (avg. 41.8 mole %) and AG-BI (avg. 41.4 mole %).

These results suggest that each anastomosis group must be regarded as a genetically independent unit.

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Introduction

It has been well documented that *Rhizoctonia solani* Kühn involves physiologically,^{19,23)} biochemically,¹⁶⁾ serologically¹⁾ and ecologically^{30,31)} different groups. Some researchers^{18, 20, 21, 22)} have attempted to divide this fungus to some groups with anastomosis behaviours. Japanese isolates were divided into six anastomosis groups (AGs): AG-1 (sasakii type and web-blight type), AG-2-1 (winter crops type), AG-2-2 (rush type and root rot type), AG-3 (potato type), AG-4 (praticola type) and AG-5¹⁸⁾. Recently two new AGs, AG-6 and AG-BI, were obtained from non-cultivated soils¹⁰⁾. The suggestion has been made that the physiological and morphological similarity of isolates within the same AG must be greater than that among different AGs^{11, 18, 23)}.

Determination of the base composition of deoxyribonucleic acid (DNA), expressed as mole percent of guanine plus cytosine (GC content), has been well established as one of

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the most useful approaches to bacterial taxonomy^{9,12,13,15}). However this method has scarcely been used for fungal taxonomy. DNAs of fungi were analyzed by Uryson & Belozersky²⁷), Vanyushin *et al.*²⁸) and by Storck.²⁴) Storck & Alexopoulos²⁶) analyzed the DNAs of 322 species of fungi and suggested that there might be a relationship between the GC content of fungal DNA and taxonomic and phylogenetic data.

Of plant pathogenic fungi, GC content of *Phytophthora infestans* is the first report on the DNA composition⁶). Recently Chang & Wu³) have reported comparative GC contents of 63 isolates of *R. solani*.

Table 1. The isolates used

Isolate	Source	Geographic origin	Anastomosis group	Cultural type
R 71	Rice, Sheath	Tochigi	AG-1 ^{a)}	IA, sasakii type ^{b)}
CS-Ka	Rice, Sheath	Hokkaido	"	"
A-10	Rice	Hiroshima	"	"
OO 1-7	Soil	Hokkaido	"	IB, web-blight type
K-18	Sugar beet, Petiole	Hokkaido	"	"
SFBV-1	Sugar beet, Seedling	Tochigi	"	"
R123	Radish, Seedling	Aichi	AG-2-1	II, winter crops type
SHM 1-2	Soil	Hokkaido	"	"
RPS-4	Pea, Seedling	Gunma	"	"
B 52	Sugar beet, Petiole	Okayama	AG-2-2	IIIB, rush type
B 60	Sugar beet, Petiole	Okayama	"	"
C-112	Mat rush, Stem	Kumamoto	"	"
R 88	Soil	Kanagawa	"	IV, root rot type
BR-2	Sugar beet, Root	Hokkaido	"	"
48 HDTN	Sugar beet, Root	Hokkaido	"	"
ST 3-1	Potato		AG-3	IV, potato type
ST4-1	Potato, Sclerotia	Hokkaido	"	"
ST 6-3	Potato, Sclerotia	Hokkaido	"	"
RR 5-2	Sugar beet, Root	Hokkaido	AG-4	IIIA, praticola type
Chr-3	Chrysanthemum, Stem	Tochigi	"	"
R 101	Cabbage, Seedling	Kanagawa	"	"
TE 1-5	Soil	Hokkaido	AG-5	
R3P	Potato, Sclerotia	Hokkaido	"	
K-7	Sugar beet	Hokkaido	"	
AT 2-1	Soil	Hokkaido	AG-6	
HAM 1-1	Soil	Hokkaido	"	
SO 2-1	Soil	Hokkaido	"	
SH 1-2	Soil	Hokkaido	AG-BI	
KA 1-1	Soil	Hokkaido	"	
KO 1-1	Soil	Hokkaido	"	

a) Anastomosis group (Ogoshi, 1976)

b) Cultural type (Watanabe & Matsuda, 1966)

To examine genetical and evolutionary relationships among each anastomosis groups in *R. solani*, DNAs base compositions of their isolates were compared.

Materials and Methods

Isolates. Thirty isolates of *R. solani* selected from each anastomosis group^{10,18)} and cultural type³¹⁾ were used. The details of these isolates are given in Table 1.

Cultivation of fungi. The inoculum of each isolate was prepared by homogenizing a mycelial mat previously cultured on potato dextrose (PD) broth for 5 days. The mycelial homogenate was then inoculated on PD broth. After 5 days of incubation at 25 C, mycelial mats were collected and washed twice with a saline-EDTA solution (0.15 M NaCl, 0.1 M ethylenediaminetetraacetate, pH 8.0), and were used for DNA isolation.

DNA extraction and purification. Extraction and purification diagram of *R.*

Fig. 1. Isolation procedure of *R. solani* DNA.

a) 0.15 M NaCl-0.1 M EDTA (pH 8.0)

b) 0.015 M NaCl-0.0015 M citrate (pH 7.0)

c) 9 vols. of 0.015 M NaCl-0.0015 M citrate plus 1 vol. of 3 M acetate-0.001 M EDTA

solani DNA is shown in Fig. 1. DNA was extracted from mycelium powder which was ground in liquid nitrogen²⁾. The procedure used for DNA purification is a combination of the methods described by Cheng & Sueoka⁵⁾, Villa & Storck²⁰⁾, and Marmur¹⁴⁾. α -Amylase and pronase E (Kaken chemical, LTD) were used respectively for the removal of proteins and polysaccharides from a DNA sample. Finally purified DNA was obtained by using the isopropyl alcohol precipitation technique¹⁴⁾. The DNA usually appeared in fibrous form, and was collected by spooling onto a glass stirring rod.

Determination of DNA base composition. Base composition of DNA was determined by chemical analysis⁴⁾. After hydrolysis of the DNA sample with 98–100 % formic acid for 30 minutes at 175 C, DNA bases were separated by paper-chromatography using isopropyl alcohol-hydrochloride-water as a solvent system^{32,33)}. Each spot detected

Table 2. Chemically analyzed base composition of *R. solani* DNA

Isolate	Base composition of DNA (mole %)				Purine	Adenine+Thymine
	Adenine	Guanine	Cytosine	Thymine	Pyrimidine	Guanine+Cytosine
R 71	25.5	24.3	25.0	25.2	0.99	1.03
CS-Ka	25.8	24.0	24.8	25.4	0.99	1.05
A-10	25.3	24.7	24.6	25.4	1.00	1.03
OO 1-7	25.8	23.6	24.4	26.2	0.98	1.08
K-18	24.9	24.0	25.1	26.0	0.96	1.03
SFBV-1	25.6	24.0	24.5	25.9	0.98	1.06
R 123	27.1	22.6	22.7	27.6	0.99	1.21
SHM 1-2	28.8	21.3	21.5	28.4	1.00	1.34
RPS-4	28.8	21.7	21.4	28.1	1.02	1.32
B 52	27.4	22.6	22.3	27.7	1.00	1.23
B 60	27.5	22.0	22.0	28.5	0.98	1.27
C-112	27.4	22.4	22.2	28.0	0.99	1.24
R 88	27.3	22.1	23.2	27.4	0.98	1.21
BR-2	28.2	21.6	22.3	27.9	0.99	1.28
48HDTN	27.5	22.3	22.3	27.9	0.99	1.24
ST3-1	25.7	23.6	23.4	27.3	0.97	1.13
ST4-1	26.4	23.4	23.2	27.0	0.99	1.15
ST6-3	26.2	23.9	24.0	25.9	1.00	1.09
RR5-2	25.5	23.5	23.6	27.4	0.96	1.12
Chr-3	25.5	23.9	24.1	26.5	0.98	1.08
R 101	26.8	23.3	23.6	26.3	1.00	1.13
TE 1-5	27.2	22.9	22.6	27.3	1.00	1.20
R3P	26.7	22.7	22.0	27.8	1.01	1.25
K-7	27.8	22.4	22.0	27.8	1.01	1.25
AT 2-1	29.2	20.3	21.9	28.6	0.98	1.37
HAM 1-1	29.4	20.1	21.3	29.2	0.98	1.42
SO 2-1	29.0	20.8	21.0	29.2	0.99	1.39
SH 1-2	30.1	20.3	20.7	28.9	1.02	1.44
KA 1-1	29.6	20.7	21.6	28.1	1.01	1.36
KO 1-1	29.1	20.1	20.8	30.0	0.97	1.44

with UV lamp was eluted with 0.01N HCl. The eluted bases were identified, and the amount of each was determined from its ultraviolet absorption spectrum by use of a spectrophotometer (MPS-5000, Shimadzu co. LTD).

Results

Base composition of R. solani DNA

Base composition of DNAs of 30 *R. solani* isolates is shown in Table 2. The sum of the purine bases (adenine plus guanine) was close to the sum of the pyrimidine bases (cytosine plus thymine), so DNA structures obtained were considered as typical double strand. The ratio of adenine plus thymine (AT) to guanine plus cytosine (GC) of examined isolates was in the range of 1.03 to 1.44.

GC contents of each anastomosis group and each cultural type

R. solani isolates were found to have a GC content ranging from 40.9 to 49.3 mole % (Table 3). The average GC content was 45.3 mole %. Fig. 2 shows the range of GC content of each anastomosis group and cultural type. In all isolates of one anastomosis group or one cultural type, almost the same GC contents were shown. The isolates of AG-1 (sasakii type) showed the highest average GC content, followed by AG-4 (praticola type), AG-3 (potato type), AG-5, AG-2-2 (rush type and root rot type), AG-2-1 (winter crops type), AG-6 and AG-BI. Details of GC contents of each anastomosis group or cultural type were as follows:

AG-1 (IA, sasakii type and IB, web-blight type): AG-1 showed a GC content ranging from 48.0 to 49.3 mole % (average 48.8 mole %). This group had the highest GC content among all anastomosis groups or cultural types. The average GC contents of the sasakii type and of the web-blight type were 49.1 and 48.5 mole %, respectively. Both cultural types showed similar DNA base composition.

AG-2-1 (II, winter crops type): AG-2-1 had a GC content ranging from 42.8 to 45.3 mole % (average 43.7 mole %). The widest intragroup variation of GC content was observed in this group.

AG-2-2 (IIIB, rush type and IV, root rot type): AG-2-2 had a GC content ranging

Table 3. GC content of DNA preparations from *R. solani*

Anastomosis group	Isolate	Mole percent guanine plus cytosine
AG-1	R 71	49.3
	CS-Ka	48.8
	A-10	49.3
	OO 1-7	48.0
	K-18	49.1
	SFBV-1	48.5
AG-2-1	R 123	45.3
	SHM 1-2	42.8
	RPS-4	43.1
AG-2-2	B 52	44.9
	B 60	44.0
	C-112	44.6
	R 88	45.3
	BR-2	43.9
	48HDTN	44.6
AG-3	ST3-1	47.0
	ST4-1	46.6
	ST6-3	47.9
AG-4	RR5-2	47.1
	Chr-3	48.0
	R 101	46.9
AG-5	TE 1-5	45.5
	R3P	45.6
	K-7	44.4
AG-6	AT 2-1	42.2
	HAM 1-1	41.4
	SO 2-1	41.8
AG-BI	SH 1-2	41.0
	KA 1-1	42.3
	KO 1-1	40.9
Range		40.9-49.3
Average		45.3

Fig. 2. Range of GC content values of anastomosis group and cultural type of *R. solani*. Arrow indicates the average GC content of each group.

from 43.9 to 45.3 mole % (average 44.6 mole %). No significant difference between AG-2-1 and AG-2-2 was observed. The average GC contents of the rush type and of the root rot type were almost the same 44.5 and 44.6 mole %, respectively.

AG-3 (IV, potato type): AG-3 had a GC content ranging from 46.6 to 47.9 mole % (average 47.1 mole %). The potato type and the root rot type, belongings of different anastomosis groups, were significantly different in GC contents.

AG-4 (IIIA, praticola type): AG-4 had a GC content ranging from 46.9 to 48.0 mole % (average 47.3 mole %). The average GC content of this group was very close to AG-3 and ranked next to AG-1.

AG-5: AG-5 had a GC content ranging from 44.4 to 45.6 mole % (average 45.2 mole %). The GC content of this group was close to that of AG-2-2.

AG-6: AG-6 had a GC content ranging from 41.4 to 42.2 mole % (average 41.8 mole %). Intragroup variation of this group was remarkably lower than others.

AG-BI: AG-BI had a GC content ranging from 40.9 to 42.3 mole % (average 41.4 mole %). The GC content of this group was the lowest among all anastomosis groups, but relatively close to that of AG-6.

Discussion

The difference of base sequence reflects on the diversity of phenotypic characteristics and GC content is similar in morphologically related organisms^{9,12,13,15}.

The GC content of DNA from species in Myxomycetes, Phycomycetes, Ascomycetes, and Basidiomycetes ranges from 34 to 63 mole %^{24,27,28}. A difference between minimum and maximum GC content decreases from classes and subclasses to genera and species^{24,26}.

In more than 80 % of the species of tested fungi, GC content diversity was less than 5 mole %²⁶⁾. *Fusarium*^{8, 26)} (eight species) and *Chaetomium globosum*^{26, 29)}, found to have variable characteristics within one clone or one species, showed a relatively narrow GC content range, contrary to expectation. But in a few cases, such as *Thamnidium elegans* and *Helminthosporium speciferum*, GC content variation within a species was more than 10 mole %²⁶⁾. From all evidence available at present, Storck & Alexopoulos²⁶⁾ stated that the intraspecific variation in GC content of fungus is generally less than 10 mole %.

Using a spectrophotometric method⁷⁾, Chang & Wu³⁾ reported that GC content of DNA from 63 isolates of *R. solani* ranged from 35.72 to 52.20 mole %. In the present experiment, GC content range of 30 isolates was from 40.9 to 49.3 mole %. As shown by these results, *R. solani* had a wide intraspecific variation in GC content, as compared with many other fungi.

In present study, GC contents of isolates within the same anastomosis group were quite similar, and we could not detect any difference of GC content between the sasakii type (IA) and the web-blight type (IB) in AG-1, or between the rush type (IIIB) and the root rot type (IV) in AG-2-2. These facts suggest good homogeneity among isolates within the same anastomosis group. This result corresponds to biochemical¹⁶⁾ and serological¹⁾ studies of *R. solani*. Matsuyama et al¹⁶⁾ reported that non-specific esterase zymograms of same anastomosis group gave almost identical pattern. Adams & Butler¹⁾ have recently reported that the serologic groups corresponded to the anastomosis groups. Sherwood²³⁾, and Ogoshi¹⁸⁾ also described that ecological and morphological characteristics might be common to isolates of the same anastomosis group.

From these facts, we suggest that the wide intraspecific variation in GC content observed in *R. solani* is attributed only to a total variation of eight genetically independent anastomosis groups.

In this experiment, of eight anastomosis groups in *R. solani*, AG-1 (sasakii type) showed the highest GC content. It is well established that AG-1^{30, 31)} have the physiologically and ecologically different characteristics and the pathogenic specificities in comparison with those of other anastomosis groups. Storck & Alexopoulos²⁶⁾ suggested that the evolution of fungi could have been accompanied by a progressive increase in the GC content of DNA. The increment in GC content accompanying evolution is also found among bacteria for the Actinomycetes^{9, 26)} and also among intragenetic variations of Yeasts^{17, 25, 26)}. Whether or not AG-1 is considered as the most evolutionary group of other anastomosis groups, should be elucidated by further studies.

Our results showed close GC content values between different anastomosis groups, i. e., AG-3 and AG-4, AG-2-2 and AG-5, or AG-6 and AG-BI. But these two anastomosis groups are different in some ecological and physiological characteristics^{11, 18, 23)}. Further studies will be conducted.

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和文摘要

菌糸融合群を異にする *Rhizoctonia solani* 菌のGC含量比較

・ 国永史朗・横沢菱三

Rhizoctonia solani の同一菌糸融合群内または培養型内の菌株の遺伝的類縁性を知る手段として、菌体からDNAを抽出し、その塩基組成を測定した。*R. solani* 30菌株のDNAのGC (Guanine plus Cytosine) 含量は40.9~49.3 mole %の範囲にあり平均45.3 mole %であった。同一菌糸融合群内の菌株のGC含量は相互に近い値を示した。また、培養型を異にしても同じ菌糸融合群に属するもの、すなわちAG-1の *sasakii* type と web-blight type の間、あるいはAG-2-2の rush type と root rot type の間でGC含量には大差

が認められなかった。逆に同一培養型とされ菌糸融合群の異なるもの、すなわち root rot type (IV) と potato type (IV) の間で GC 含量は明らかに異なった。菌糸融合群のうち、AG-1 は最も高い GC 含量 (平均 48.8 mole %) を示し、次いで AG-4 (平均 47.3 mole %), AG-3 (平均 47.1 mole %), AG-5 (平均 45.2 mole %), AG-2-2 (平均 44.6 mole %), AG-2-1 (平均 43.7 mole %), AG-6 (平均 41.8 mole %), AG-BI (平均 41.4 mole %) であった。以上のことから *R. solani* の同一菌糸融合群内の菌株は、遺伝的に非常に近縁であることが示唆された。