Mycoscience (2005) 46:319–324
DOI 10.1007/s10267-005-0253-9

FULL PAPER

Masaru Matsumoto

Analysis of whole cellular fatty acids and anastomosis relationships of binucleate *Rhizoctonia* spp. associated with *Ceratobasidium cornigerum*

Received: February 21, 2005 / Accepted: June 28, 2005

Abstract Previous research has demonstrated that whole cellular fatty acids analysis is a useful tool for identifying and establishing taxonomic relationships between anastomosis groups (AGs) and related *Rhizoctonia* isolates. In this experiment, the composition of fatty acid of 28 isolates of teleomorph genus *Ceratobasidium cornigerum*, consisting of binucleate *Rhizoctonia*, AG-A, AG-B(o), AG-C, AG-P, and AG-Q, was evaluated using gas chromatography. Eleven fatty acids identified, i.e., myristic, pentadecanoic, palmitic, 2-hydroxypalmitic, palmitoleic, heptadecanoic, 9-heptadecenoic, stearic, oleic, linoleic, and linolenic acids, were present in isolates of AG-A, AG-B(o), AG-C, AG-P, and AG-Q. The major fatty acids, palmitic, oleic, and linoleic acids, were common in all isolates, constituting 87.1% to 94.7% of the whole cellular fatty acids identified. Isolates within the same AG were closely clustered, whereas isolates from different AGs were clearly and distinctly clustered based on average linkage cluster analysis of whole cellular fatty acids. Principal-component analysis generated from all fatty acids also confirmed the divergent separation of the 5 AGs of binucleate *Rhizoctonia*.

Key words *Ceratobasidium* · Fatty acid analysis · *Rhizoctonia*

Introduction

Plant pathogenic organisms, collectively known as *Rhizoctonia* DC., are important plant-associated fungi that have been widely distributed and consist of large and complex groups (Cubeta and Vilgalys 2000). Species of *Rhizoctonia* contain a high level of genetic diversity in morphology, pathology, and physiology. The anamorph genus of *Rhizoctonia* is heterogeneous and connected with the teleomorph genera, including *Thanatephorus* Donk and *Ceratobasidium* D.P. Rogers (Anderson 1982). The close relationship between *Thanatephorus* and *Ceratobasidium* was reported, based on septal pore and teleomorph characteristics (Muller et al. 1998).

The most convenient system for classification of fungi within the *Rhizoctonia* complex is based on the phenomenon of hyphal anastomosis grouping (AG) (Ogoshi 1987; Carling 1996). Hyphal anastomosis, defined as a manifestation of somatic or vegetative compatibility between isolates, is observed among isolates belonging to the same anastomosis group. Currently, multinucleate *Rhizoctonia* species have been differentiated into 14 AGs of genus *Thanatephorus*, designated as AG-1 to AG-13 (Ogoshi 1987; Sneh et al. 1991; Carling et al. 2002). A similar classification method to that of binucleate *Rhizoctonia* species has been assigned to 14 and 7 AGs of the genus *Ceratobasidium*, designated as AG-A to AG-S isolated in Japan and as CAG-1 to CAG-7 isolated in North America, respectively (Burpee et al. 1980a; Ogoshi 1987; Sneh et al. 1991).

Many species of *Ceratobasidium* have been described in association with isolates of binucleate *Rhizoctonia*, including *C. cornigerum* (Bourdot) D.P. Rogers (Burpee et al. 1980b), *C. setariae* (Sawada) Oniki, Ogoshi & T. Araki, *C. gramineum* (Ikata & T. Matsuura) Oniki, Ogoshi & T. Araki (Oniki et al. 1986), and *C. oryzae-sativae* P.S. Gunnell & R.K. Webster (Gunnell 1986). Because the genus relates to the binucleate *Rhizoctonia*, the speciation of *Ceratobasidium* may parallel relationships between *Thanatephorus cucumeris* (A.B. Frank) Donk and *Rhizoctonia solani* J.G. Kühn. For example, isolates of *C. cornigerum* were assigned to five AGs designated AG-A, AG-B(o), AG-C, AG-P, and AG-Q based on the hyphal anastomosis phenomenon (Sneh et al. 1991). Isolates of AG-A are isolated from widespread hosts and soils belonging to *R. candida* W. Yamam, *R. endophytica* H.K. Saksena & Vaartaja var. *endophytica*, *R. fragariae* S.S. Husain & W.E. Mckee, and *R. ramicola* W.A. Weber &

M. Matsumoto (✉)
Institute of Tropical Agriculture, Kyushu University,
6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
Tel. +81-92-642-3072; Fax +81-92-642-3077
e-mail: mmatsu@agr.kyushu-u.ac.jp

Table 1. Isolates of *Ceratobasidium cornigerum* used in this experiment

Gas chromatography isolate designation	AG	Original designation	Host or habitat
FAP001	AG-A	AH-1	*Arachis hypogaea*
FAP002	AG-A	BP-1	*Brassica campestris*
FAP003	AG-A	GM-2	*Glycine max*
FAP004	AG-A	SN-4	Soil
FAP005	AG-A	C-538	*Solanum tuberosum*
FAP006	AG-A	SN-1	Soil
FAP007	AG-A	BN-1	*Cucumis sativus*
FAP008	AG-B(o)	C-431	*Oryza sativa*
FAP009	AG-B(o)	POER-2	Unknown
FAP010	AG-B(o)	C-302	Unknown
FAP011	AG-B(o)	W159	*Setaria* sp.
FAP012	AG-B(o)	W157	Unknown
FAP013	AG-B(o)	CFM1	*Brassica oleracea*
FAP014	AG-C	Ao-1-2	Soil
FAP015	AG-C	GS-3	*Goodyera schlechtendaliana*
FAP016	AG-C	Ka-1-1	Soil
FAP017	AG-C	STC-26	Soil
FAP018	AG-C	STC-37	Soil
FAP019	AG-C	70B	Soil
FAP020	AG-P	C-578	*Camellia sinensis*
FAP021	AG-P	C-579	Soil
FAP022	AG-P	C-582	Soil
FAP023	AG-P	C-583	Soil
FAP024	AG-P	C-584	Soil
FAP025	AG-Q	C-620	*Cynodon dactylon*
FAP026	AG-Q	C-626	*Cynodon dactylon*
FAP027	AG-Q	C-670	*Cynodon dactylon*
FAP028	AG-Q	RK3	*Zoysia tenuifolia*
FAP029[a]	AG-1 IA	Cs-Ka	*Oryza sativa*

[a] Control isolates of *Rhizoctonia solani* as used in Figs. 1 and 2

D.A. Roberts, AG-B(o) isolates belong to AG-B and are able to anastomose with AG-Ba and AG-Bb at low frequencies. Isolates of AG-C are also obtained from many hosts and soils, similar to AG-A, but belong to the single species of *R. globularis*. Isolates of AG-P are isolated from tea plant and soils in Japan. Isolates of AG-Q are obtained from bermudagrass and have been discussed with their pathological relationship to AG-D (Tanaka et al. 1994; Toda et al. 1999).

One of the most rapidly developing methods is analysis of quantities and qualitative differences in total cellular fatty acid compositions in *Rhizoctonia* species (Stevens Johnk and Jones 1992; Matsumoto et al. 1996). A common protocol used for fatty acid analysis is the MIDI system (Microbial Identification System; Microbial ID, Network, DE, USA), by which highly reproducible results have been obtained in *R. solani* AG-1, AG-2, AG-3, and AG-4 (Stevens Johnk and Jones 1993, 1994, 2001; Stevens Johnk et al. 1993). Moreover, the modified MIDI method was used and described the utility of differentiation of different AGs within *R. solani* (Matsumoto et al. 1996; Matsumoto and Matsuyama 1999) and binucleate *Rhizoctonia* species of AG-Ba, AG-Bb, and AG-D (Priyatmojo et al. 2002a,b). On the other hand, variation of fatty acid compositions within isolates of same species or same subgroups was reported by Jabaji-Hare (1988).

The objective of the present study is to determine whether whole cellular fatty acid analysis could be used to distinguish teleomorph *C. cornigerum* with anamorph binucleate *Rhizoctonia* species, including AG-A, AG-B(o), AG-C, AG-P, and AG-Q. Thus, a modified MIDI system for accommodating use of *Rhizoctonia* species was created to determine qualitative and quantitative differences in these AG isolates of *C. cornigerum*.

Materials and methods

Fungal isolates and culture conditions

Twenty-eight isolates of *C. cornigerum*, including 7 isolates of AG-A, 6 isolates of AG-B(o), 6 isolates of AG-C, 5 isolates of AG-P, and 4 isolates of AG-Q, were tested (Table 1). AG typing of *Rhizoctonia* isolates was carried out according to the methods described by Sneh et al. (1991). As a control, 1 isolate of *R. solani* AG-1 IA was also used in this experiment. All isolates were subcultured on a slant culture of potato dextrose agar (PDA) amended with 50 mg/l streptomycin sulfate (Sigma, St. Louis, MO, USA) at 25°C in the dark. These cultures were regularly inspected for 2 weeks to confirm isolate identification and culture purity.

Cellular fatty acid analysis

Samples for fatty acid analysis were prepared by culturing each isolate on PDA amended with 50 mg/l streptomycin

Table 2. Percentage composition of cellular fatty acids of *Ceratobasidium cornigerum* after 5 days culturing on potato dextrose broth

Anastomosis group	Percentage of fatty acids (%)										
	14:0	15:0	16:0	16:1 *cis*-9	16:0 2OH	17:0	17:1 *cis*-9	18:0	18:1 *cis*-9	18:2 *cis*-9,12	18:3 *cis*-9,12
AG-A	0.77 b	0.11 a	11.75 ab	0.36 b	0.47 b	0.16 c	0.40 a	2.32 a	18.66 ab	63.77 c	2.04 b
AG-B(o)	0.48 a	0.76 d	10.01 a	1.13 c	0.54 b	0.00 a	0.28 b	2.79 ab	21.64 b	63.01 c	1.16 a
AG-C	1.09 c	0.19 b	13.13 b	0.19 a	0.34 a	0.28 d	0.11 a	5.67 c	30.42 c	45.38 a	1.16 a
AG-P	0.72 d	0.66 c	16.21 c	0.46 b	0.33 a	0.00 a	0.10 a	9.36 c	13.56 a	57.41 b	2.23 b
AG-Q	1.45 b	0.96 e	19.22 d	1.10 c	0.84 c	0.09 b	0.37 c	3.42 b	27.50 c	44.08 a	0.35 a
AG-1IA[a]	1.31	0.75	9.42	1.80	0.01	0.67	1.40	0.80	10.70	72.14	1.00

Values in each column followed by the same letter are not significantly different for Waller–Duncan *K*-ratio *t* test, $P = 0.05$
[a]Control of root isolates of *R. solani* AG-1 IA as used in Figs. 1 and 2

sulfate in the dark at 25°C for 5 days. Three mycelial disks each 15 mm in diameter from actively growing hyphae were placed in 25 ml potato dextrose broth (Difco, Detroit, MI, USA) in a 100-ml Erlenmeyer flask amended with 50 mg/l streptomycin sulfate and incubated without shaking at 25°C for 5 days in the dark. The mycelia were then washed in sterile distilled water, filtrated in a vacuum, lyophilized, and stored at –20°C until used.

The procedure followed for the extraction of fatty acids from fungal mycelial cells was according to the method of Matsumoto et al. (1996) with slight modifications. Fatty acids extracted from 30 mg lyophilized mycelia were methylated with 0.5 ml 5% (v/v) HCl-methanol at 100°C for 3 h in a capped heat-resistant glass tube. The resultant fatty acid methyl esters (FAMEs) were cooled to room temperature, diluted with 1 ml distilled water, and extracted with 2 ml *n*-hexane by shaking. Tubes were then centrifuged at 15000 *g* for 10 min. The solvent (organic) phase was transferred to a new glass tube, washed with an equal volume of distilled water to remove HCl, and dehydrated by mixing with 0.5 mg anhydrous sodium sulfate. The solvent was centrifuged with nitrogen gas. Samples were stored at –20°C.

FAMEs were analyzed using a gas liquid chromatograph (Shimadzu GC17A) equipped with a flame ionization detector and a 0.25 mm × 50 m HR-SS-10 capillary column. Nitrogen was used as the carrier gas maintained at the pressure of 95 kPa. The column temperature was maintained at 180°C. The injection temperature was initially programmed at 160°C and increased by 1°C/min to a final temperature of 250°C. FAMEs were identified by comparing their retention times with those of authentic materials containing straight-chain saturated fatty acids from C10 to C20 (Sigma, Tokyo, Japan). To major the composition of each fatty acid from its peak on the gas chromatogram, peak area (PA) was calculated based on the data from height of peak (h) and retention time (RT), shown as the formula (PA = 2.507 h RT) (Carroll 1961). Triplicate determinations were made for each isolate.

Data evaluation

Data for teleomorph *C. cornigerum* including AG-A, AG-B(o), AG-C, AG-P, and AG-Q isolates were subjected to an analysis of variance (ANOVA). The variability among isolates from different AGs was assessed with respect to both individual and overall fatty acid compositions by the Waller–Duncan *K*-ratio *t* test ($K = 100$, $t = 0.05$). In addition, the amount of variability and relatedness among isolates based on fatty acid composition was assessed with principal-component and cluster analyses (nearest neighbor method, squared Euclidean distance) using StatPartner (Ver. 2.0 NEC Software, Tokyo, Japan).

Results

Cellular fatty acid analysis

Eleven fatty acids in total were detected in 28 isolates of *C. cornigerum* belonging to five different AGs: AG-A, AG-B(o), AG-C, AG-P, and AG-Q (Table 2): myristic (14:0), pentadecanoic (15:0), palmitic (16:0), 2-hydroxypalmitic (16:0 2OH), palmitoleic (16:1 *cis*-9), heptadecanoic (17:0), 9-heptadecenoic (17:1 *cis*-9), stearic (18:0), oleic (18:1 *cis*-9), linoleic (18:2 *cis*-9,12), and linolenic (18:3 *cis*-9,12). Among the fatty acids detected, palmitic, oleic, and linoleic acids were the major fatty acids found in all isolates, comprising 87.1% to 94.7% of the cellular fatty acids identified. Heptadecanoic acid was detected in isolates of AG-A, AG-C, and AG-Q but not in isolates of AG-B(o) and AG-P. Stearic acid comprised the majority in all isolates, especially constituting 9.4% of cellular fatty acid in isolates of AG-P.

Although total cellular fatty acid composition among isolates of teleomorph *C. cornigerum*, with the exception of undetected heptadecanoic acid in AG-B(o) and AG-P, were qualitatively similar, quantitative divergence was observed (see Table 2). ANOVA indicated that there were significant differences ($P = 0.05$) among isolates within each

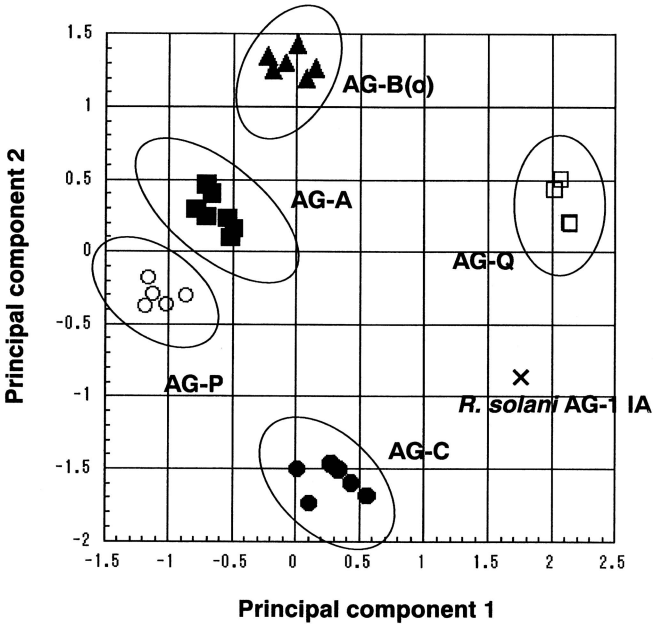

Fig. 1. Variability in whole cellular fatty acid composition among isolates of five binucleate *Rhizoctonia* spp. associated with *Ceratobasidium cornigerum*. Plot of the first two principal components (principal components 1 and 2) was derived from the percentage composition of fatty acids, representing AG-A (■), AG-B(o) (▲), AG-C (●), AG-P (○), AG-Q (□), and AG-1 IA (×)

AG belonging to *C. cornigerum*. ANOVA followed by mean separation, using the Waller–Duncan *K*-ratio *t* test, indicated that there were significant differences ($P = 0.05$) among AGs in the 11 fatty acids detected. The percentage composition of fatty acids of isolates of AG-A and AG-B(o) tended to be similar to the profiles of the percentage composition of linoleic acid. Additionally, the percentage compositions of linoleic acid also showed similar profiles between isolates of AG-C and AG-Q.

Principal-component analysis generated from all fatty acids detected (11 fatty acids) showed a close relationship among isolates with in a single AG but divergence among the five different AGs belonging to the teleomorph *C. cornigerum* (Fig. 1). The first 2 of the 11 principal components of this analysis accounted for 64.5% of the variation in the data. Principal components 1 and 2 accounted for 32.6% and 31.9% of the variation, respectively.

Whole cellular fatty acid compositions of 28 isolates tested were compared by conducting average linkage cluster analysis based on the 11 fatty acids detected (Fig. 2). The dendrogram produced from the detected fatty acids showed that the isolates within a single AG expected for AG-B(o) were clustered within a Euclidean distance of less than 1.0. Isolates of AG-A and AG-B(o) were closely clustered with a Euclidean distance of 1.85. They were clustered further from isolates of AG-C, with a Euclidean distance of 1.95. Isolates of AG-P were clustered with isolates of AG-A, AG-B(o), and AG-C, with a Euclidean distance of 2.1, and isolates of AG-Q were clustered with isolates of AG-A, AG-B(o), AG-C, and AG-P, with a Euclidean distance of 2.5.

Discussion

In this experiment, extraction of whole cellular fatty acids was performed based on the modified MIDI method arranged by Matsumoto et al. (1997). This method can affect all cellular fatty acid compositions by arranging extraction conditions based on differences of media and incubation periods. Although many researchers who reported on the classification and identification of *Rhizoctonia* spp. have used fatty acid analysis, the data of fatty acid compositions obtained showed quantitative and qualitative variations. Therefore, although published data on fatty acid compositions seem to be reliable, direct comparison of these data is difficult. Based on the aforementioned point, cultural conditions for the extraction of whole cellular fatty acids were set by using potato dextrose broth and 5-day cultured mycelia.

In the present study, our fatty acid analysis revealed that each of the AGs belonging to the same telemorph *C. cornigerum* was obviously divergent based on the differentiation of fatty acid compositions and the closely related hyphal anastomosis behavior. Especially, isolates of AG-C, AG-P, and AG-Q showed AG-specific profiles of fatty acid composition, and these profiles were closely correlated to the anastomosis behavior. A similar phenomenon has also been reported in the study of fatty acid analysis of the telemorph genera *Tanatephorus* and *Waitea* Warcup & P.H.B. Talbot. Our results suggest that whole cellular fatty acid analysis can explain the correlation between anastomosis behavior and profiles of fatty acid compositions.

Isolates of AG-A consist of several anamorphs, *R. candida*, *R. endophytica* var. *endophytica*, *R. fragariae*, and *R. ramicola*, according to classification based on differences in the host plant. In the previous study, fatty acid analysis revealed that isolates of AG-A can be divided into two subgroups, AG-A(I) and AG-A (II) (Matsumoto and Seint 2003). In this experiment, the results of average linkage cluster analysis showed that isolates of AG-A were clustered within a single AG, with a Euclidean distance of less than 1.0 and similar profiles to fatty acid compositions of AG-A (I). However, the existence of subgroups [AG-A (I) and AG-A (II)] based on molecular biological techniques, such as restriction fragment length polymorphism (RFLP), rDNA-internal transcribed spacer (ITS), and random amplified polymorphic DNA (RAPD) analysis, were not reported. Therefore, additional taxonomic research concerning AG-A subgroups or consisting of different species will be necessary for resolving the species complexity by using large numbers of AG-A isolates.

It is interesting that isolates of AG-A and AG-B(o) were closely related by principal-component analysis and average linkage cluster analysis based on the whole cellular fatty acid compositions, especially linoleic acid. This result agrees with that of Gonzalez et al. (2001), who reported the phylogenetic relationships of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorph using ribosomal RNA systematics.

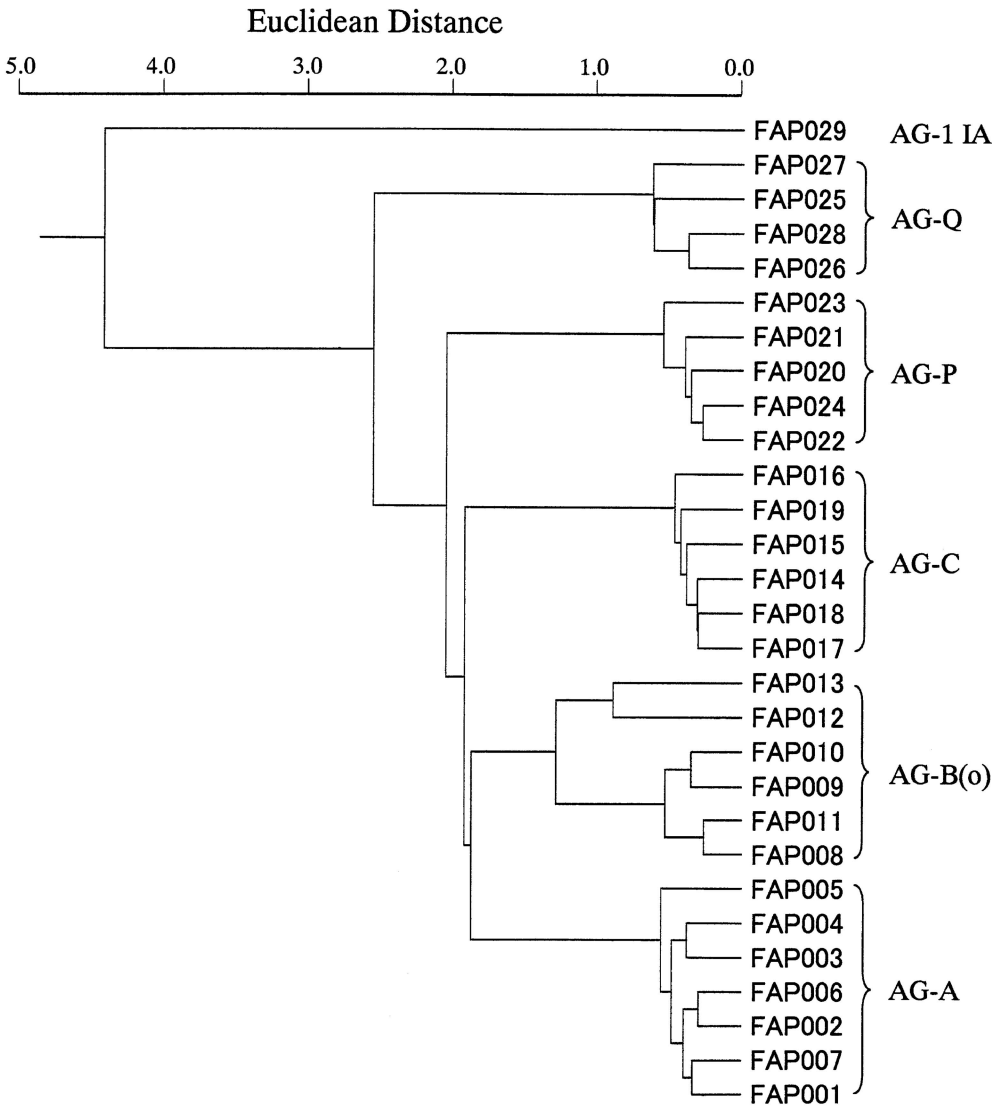

Fig. 2. Dendrogram showing relationships among 28 isolates of five *Rhizoctonia* spp. associated with *Ceratobasidium cornigerum* based on the percentage composition of total cellular fatty acid (nearest neighbor, squared Euclidean distance)

Toda et al. (1999) reported that isolates of AG-Q, a causal agent of bermudagrass disease, can anastomose with isolates of AG-D and should be identified as the AG-D group. In this experiment, we did not use the isolates of AG-D and could not identify the whole cellular fatty acids composition of AG-D. Therefore, additional AG-D isolates should be tested for fatty acid analysis to clarify the relationship between AG-D and AG-Q.

In conclusion, the present study suggests that cellular fatty acid analysis is useful for differentiating teleomorphs of *Ceratobasidium cornigerum* belonging to AG-A, AG-B(o), AG-C, AG-P, and AG-Q. Additional binucleate *Rhizoctonia* isolates containing several telemorph *Ceratobasidium* species should be tested to further assess their variability in fatty acid composition.

References

Anderson NA (1982) The genetics and pathology of *Rhizoctonia solani*. Annu Rev Phytopathol 20:329–374

Burpee LL, Sanders PL, Cole H Jr, Sherwood RT (1980a) Anastomosis groups among isolates of *Ceratobasidium cornigerum* (Bourd.) Rogers and related fungi. Mycologia 72:689–701

Burpee LL, Sanders PL, Cole H Jr, Sherwood RT (1980b) Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing four anastomosis groups. Phytopathology 70:843–846

Carling DE (1996) Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In: Sheh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 37–47

Carling DE, Baird RE, Gitaitis RD, Brainard KA, Kuninaga S (2002) Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology 92:893–899

Carroll KK (1961) Quantitative estimation of peak areas in gas-liquid chromatography. Nature (Lond) 191:377–378

Cubeta MA, Vilgalys R (2000) *Rhizoctonia* In: Ledergerg J (ed) Encyclopedia of microbiology, vol 4. Academic Press, San Diego, pp 109–116

Gonzalez D, Carling DE, Kuninaga S, Vilgalys R, Cubeta MA (2001) Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. Mycologia 93:1138–1150

Gunnell PS (1986) Characterization of the teleomorphs of *Rhizoctonia oryzae-sativae*, *Rhizoctonia oryzae*, and *Rhizoctonia zeae*, and the effect of cultural practices on aggregate sheath spot of rice, caused by *Rhizoctonia oryzae-sativae*. Ph D thesis, University of California, Davis

Jabaji-Hare SH (1988) Lipid and fatty acid profiles of some vesicular-arbuscular mycorrhizal fungi: contribution to taxonomy. Mycologia 80:622–629

Matsumoto M, Matsuyama N (1999) Grouping of isolates in AG 2 of *Rhizoctonia solani* by total cellular fatty acid analysis. Mycoscience 40:35–39

Matsumoto M, Seint SA (2003) Analysis of whole-cellular fatty acids for binucleate *Rhizoctonia* species associated with AG-A. Bull Inst Trop Agric Kyushu Univ 26:45–52

Matsumoto M, Furuya N, Takanami Y, Matsuyama N (1996) A study of fatty acid analysis as a new taxonomical tool for differentiating *Rhizoctonia* spp. J Fac Agric Kyushu Univ 40:279–286

Matsumoto M, Furuya N, Matsuyama N (1997) Characterization of *Rhizoctonia* spp., causal agents of sheath diseases of rice plant by total cellular fatty acids analysis. Ann Phytopathol Soc Jpn 63:149–154

Muller WH, Stalpers JA van Aelst AC, VanderKrift TP, Boekhout T (1998) Field emission gun-scanning electron microscopy of septal pore caps of selected species in the *Rhizoctonia* s.l. complex. Mycologia 90:170–179

Ogoshi A (1987) Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia* Kuhn. Ann Rev Phytopathol 25:125–143

Oniki M, Ogoshi A, Araki T (1986) *Ceratobasidium setariae*, *C. cornigerum*, and *C. gramineum*, the teleomorphs of the pathogenic binucleate *Rhizoctonia* fungi from gramineous plants. Trans Mycol Soc Jpn 27:147–158

Priyatmojo A, Yamauchi R, Naito S, Kageyama K, Hyakumachi M (2002a) Comparison of whole-cell fatty acid compositions of isolates of *Rhizoctonia solani* AG 2 from Tobacco and Tulip, AG 2-1 and AG-BI. J Phytopathol 150:283–288

Priyatmojo A, Yamauchi R, Kageyama K, Hyakumachi M (2002b) Whole-cell fatty acid composition to characterize and differentiate isolates of *Rhizoctonia* species associated with turfgrass diseases in Japan. J Gen Plant Pathol 68:1–7

Sneh B, Burpee L, Ogoshi A (1991) Identification of *Rhizoctonia* species. APS Press, St. Paul, MN

Stevens Johnk J, Jones RK (1992) Determination of whole-cell fatty acids in isolates of *Rhizoctonia solani* AG-1 IA. Phytopathology 82:68–72

Stevens Johnk J, Jones RK (1993) Differentiation of population s of AG 2-2 of *Rhizoctonia solani* by analysis of cellular fatty acids. Phytopathology 83:278–283

Stevens Johnk J, Jones RK (1994) Comparison of whole-cell fatty acid compositions in intraspecific groups of *Rhizoctonia solani* AG 1. Phytopathology 84:271–275

Stevens Johnk J, Jones RK (2001) Differentiation of three homogeneous groups of *Rhizoctonia solani* anastomosis group 4 by analysis of fatty acids. Phytopathology 91:821–830

Steven Johnk J, Jones RK, Shew HD, Carling DE (1993) Characterization of populations of *Rhizoctonia solani* AG 3 from potato and tobacco. Phytopathology 83:854–858

Tanaka A, Kitabayashi H, Tani T, Ogoshi A (1994) A pathogen causing a patch so-called "elephant footprint" on zoysia grasses. Ann Phytopathol Soc Jpn 60:344 (abstract)

Toda T, Hyakumachi M, Suga H, Kageyama K, Tanaka A, Tani T (1999) Differentiation of *Rhizoctonia* AG-D isolates from turfgrass into subgroup I and II based on RFLP analysis of the ITS region from rDNA, and on RAPD analysis. Eur J Plant Pathol 105:835–846