Mycoscience (2006) 47:190–197
DOI 10.1007/s10267-006-0290-z

FULL PAPER

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Comparison of two fatty acid analysis protocols to characterize and differentiate *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici*

Received: August 12, 2005 / Accepted: January 28, 2006

Abstract The utility of fatty acid methyl ester (FAME) profiles for characterization and differentiation of isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* was investigated. Two fatty acid analysis protocols of the normal (MIDI) and a modified MIDI method were used for their utility. Only the modified MIDI method allowed a clear differentiation between *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicislycopersici*. FAME profiles using the modified MIDI method gave the most consistent and reproducible analyzed fatty acid data. Evaluation of the FAME profiles based on cluster analysis and principal-component analysis revealed that FAME profiles from tested isolates were correlated with the same vegetative compatibility groups (VCGs) compared to the same races in *F. oxysporum* f. sp. *lycopersici*. Results indicated that FAME profiles could be an additional tool useful for characterizing isolates and forma species of *F. oxysporum* obtained from tomato.

Key words Fatty acid analysis · *Fusarium* · Modified MIDI method · Tomato

Introduction

Fusarium oxysporum Schlechtend.: Fr. is a ubiquitous, asexual species complex (Kistler 1997). This fungus has highly diverse variants, including several zones of well-studied pathogenic formae speciales and races that cause wilt or root or crown rot diseases as well as ubiquitously present nonpathogenic strains (Armstrong and Armstrong 1981). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. and *F. oxysporum* f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker are pathogens that cause wilt and crown and root rot diseases, respectively, on tomato. Three races of *F. oxysporum* f. sp. *lycopersici* have been reported, and they are distinguished by their virulence to tomato cultivars that contain resistance genes (Stall 1961; McGrath et al. 1987). On the other hand, *F. oxysporum* f. sp. *radicis-lycopersici* was discovered and first reported in 1969 from Japan; it is a relatively newly recognized pathogen (Menzies and Jarvis 1994), and no race diversity has been reported.

Fungal diversity studies of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* have been compared with respect to vegetative compatibility group (VCG), mitochondrial DNA restriction fragment length polymorphisms (RFLPs), and nuclear DNA RFLPs (Elias and Schneider 1991; Katan et al. 1991; Elias et al. 1993; Marlatt et al. 1996; Rosewich et al. 1999). These studies revealed that different races in the same VCG are closely related whereas isolates of the same race in different VCGs are distinct from each other. The assignment of VCG is an indicator of genetic evolutionary origin, whereas race most likely evolves independently within different VCGs (Cai et al. 2003). Moreover, Kawabe et al. (2005) revealed that three evolutionary lineages of the tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici* were found among a worldwide sample of isolates based on phylogenetic analysis of the ribosomal DNA intergenic spacer region.

The qualitative and quantitative analysis of cellular fatty acids has been successfully used to characterize isolates of *F. oxysporum* f. sp. *vasinfectum* (Hering et al. 1999) and *F. oxysporum* B1 (Ellis et al. 2002). In their reports, a common protocol used for cellular fatty acid analysis was the MIDI method (Microbial Identification System; Microbial ID, Newark, DE, USA), which has been shown to give highly reproducible results for fungi (Miller and Berger 1985; Gudmestad et al. 1988). By slightly modifying the protocol used in the MIDI method, isolates *of Phytophthora infestans* (Mont.) de Bary and *Penicillium* spp. could be characterized and differentiated (Lopes da Silva et al. 1998; Larkin and Groves 2003). In contrast, Lanoiselet et al. (2005) demonstrated a comparison of two fatty acid analysis protocols [the MIDI and a modified MIDI method described by Matsumoto et al. (1996)] to characterize and

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Table 1. Isolates of *Fusarium* spp. used in this experiment

FAME isolate designation	Forma specialis/race and isolate no.	Source[a]	Origin	VCG[b]
Fusarium oxysporum f. sp. *lycopersici* race 1				
FAME-FOL-01	103036	MAFF	Mie, Japan	0032
FAME-FOL-02	103037	MAFF	Mie, Japan	0032
FAME-FOL-03	727501	MAFF	Japan	0030
FAME-FOL-04	KGFOL-23	M. Matsumoto	Kagoshima, Japan	0032
FAME-FOL-05	KGFOL-29	M. Matsumoto	Kagoshima, Japan	0032
FAME-FOL-06	FUFOL-9	M. Matsumoto	Fukuoka, Japan	0030
FAME-FOL-07	FUFOL-47	M. Matsumoto	Fukuoka, Japan	0030
FAME-FOL-08	CNPH27	Santos	Brazil	0030
F. o. f. sp. *lycopersici* race 2				
FAME-FOL-09	103038	MAFF	Ibaraki, Japan	0031
FAME-FOL-10	103039	MAFF	Ibaraki, Japan	0031
FAME-FOL-11	103042	MAFF	Tochiki, Japan	0031
FAME-FOL-12	103043	MAFF	Tochiki, Japan	0031
FAME-FOL-13	SUF803	SU	Japan	0031
FAME-FOL-14	TOFOL-81	M. Matsumoto	Tottori, Japan	0031
FAME-FOL-15	NGFOL-9	M. Matsumoto	Nagasaki, Japan	0031
FAME-FOL-16	CNPH23	Santos	Brazil	0030
F. o. f. sp. *lycopersici* race 3				
FAME-FOL-17	9701	FARC	Fukuoka, Japan	0033
FAME-FOL-18	F-1-1	FARC	Fukuoka, Japan	0033
FAME-FOL-19	MIFOL-16	M. Matsumoto	Miyazaki, Japan	0033
F. o. f. sp. *radicis-lycopersici*				
FAME-FORL-01	103044	MAFF	Gifu, Japan	NT
FAME-FORL-02	103045	MAFF	Gifu, Japan	NT
FAME-FORL-03	103046	MAFF	Gifu, Japan	NT
FAME-FORL-04	103047	MAFF	Kochi, Japan	NT
FAME-FORL-05	103048	MAFF	Kochi, Japan	NT
FAME-FORL-06	305937	MAFF	Kochi, Japan	NT
FAME-FORL-07	744008	MAFF	Fukuoka, Japan	NT
F. solani f. sp. *pisi*[c]				
FAME-FS-01	SUF654	SU	Japan	NT

FAME, fatty acid methyl ester; NT, not tested; VCG, vegetative compatibility group
[a] MAFF, microorganisms section of the gene bank in the Ministry of Agriculture, Forestry and Fisheries of Japan, Tsukuba, Ibaraki, Japan; SU, culture collection of fusarium in Shinshu University, Ueda, Nagano, Japan; FARC, Fukuoka Agricultural Research Center, Fukuoka, Japan
[b] VCG designations of isolates of 103036, 103037, 727501, CNPH27, 103038, 103039, 103042, 103043, SUF803, CNPH23, 9701, and F-1-1 were previously reported; results in this study agreed with previous VCG designations
[c] Control isolates of *F. solani* f. sp. *pisi* as used in Fig. 1 to Fig. 4

differentiate *Rhizoctonia oryzae* Ryker & Gooch and *R. oryzae-sativa* (Sawada) Mordue isolates from four countries and concluded that the MIDI method is insufficient to completely break the cell wall of these two species for the saponification step. The present study is intended to reveal the usefulness of a modified MIDI method for characterizing phytopathological properties by employing isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici*.

Materials and methods

Fungal isolates

Nineteen isolates of *F. oxysporum* f. sp. *lycopersici*, 7 isolates of *F. oxysporum* f. sp. *radicis-lycopersici*, and 1 isolate of *F. solani* f. sp. *pisi*, as a control, were used in this experiment (Table 1). All isolates were subcultured on potato dextrose agar (PDA) and incubated at 30°C. Plates were regularly checked for 1 month to confirm isolate identification and culture purity.

Pathogenicity test

Before using the tester isolates to conduct fatty acid analysis, greenhouse pathogenicity tests were performed with the differential tomato cutivars Ponderosa (no resistance), Okitsu no. 3 (resistant to race 1), and Walter (resistant to races 1 and 2) to confirm their pathogenicity to tomato plant. Bulk cells from 5-day-old cultures on a liquid media of potato dextrose broth were rinsed by centrifugation in deionized water. Conidial suspensions were adjusted to about 1×10^6 spores/ml. Tomato seedlings were grown in a vermiculite substrate (Vermiculite; Soil Improvement Materials, Dio Chemicals, Tokyo, Japan) for 2 weeks, until the first true leaf was fully expanded. The root dip method of inoculation was employed as described by Cai et al.

(2003). Race of *F. oxysporum* f. sp. *lycopersi*ci was decided by the disease severity assessment according to the method described by Marlatt et al. (1996).

VCG grouping

Nitrate-nonutilizing (nit) mutants were generated on PDA medium amended with 1.5% potassium chlorate (Puhalla 1985). Progeny mutants were phenotyped (*nit*1, *nit*2, or NitM) according to Correll et al. (1987). Vegetative compatibility was determined by pairing nit mutants on PDA medium and observing cultures for protoprophic growth periodically over a 2-week period. Heterokaryon formation between mutants was identified by the formation of a wild-type mycelium growth at the contact zone. At least two compatible NitM mutants from each of the isolates participated in the compatibility pairings with *nit*- or NitM-type mutants form other isolates in all possible combinations. The absence of wild-type growth between complementary nit mutants derived from the same parent isolate indicated alletic or vegetative self-incompatibility, whereas the absence of wild-type growth between nit mutants from different parent isolates indicated either noncomplementarity or inability to form a heterokaryon because of lack of vegetative compatibility (Katan et al. 1991). VCGs were numbered according to Kistler et al. (1988) and Katan (1999). The complementation pairings were carried out at least twice for each isolates with different nit mutants.

Cultivation of fungi

The fungal isolates were grown in 100 ml potato dextrose broth (BD; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) in 200-ml Erlenmeyer flasks. Flasks were inoculated with three 12-mm-diameter disks of mycelia that were grown on PDA (Difco) amended with 25 mg/l streptomycin. The flasks were then rotated for 7 days at 140 rpm at 25°C. The mycelium was washed in sterile deionized water, excess water was removed by lightly pressing with filter paper, and the agar plugs were removed. The mycelium was lyophilized and stored at −15°C until use.

Extraction of fatty acid methyl ester and analysis

Two methods of a sample preparation protocol according to Miller and Berger (1985), hereafter referred to as the MIDI method, and Matsumoto et al. (1996) and Lanoiselet et al. (2005), hereafter referred to as the modified MIDI method, were used. Although a modified MIDI method closely resembles the MIDI method, its essential difference is a 3-h saponification step compared with the 30-min step in the MIDI method. A modified MIDI method was employed using a 3-h saponification step, and the remainder followed the normal protocol.

Thirty milligrams of lyophilized mycelia was placed with 0.5 ml reagent 1 (45 g NaOH, 150 ml methanol, 150 ml H_2O) in a capped heat-resistant glass tube. Saponification of fatty acid ethyl ester (FAME) was conducted by heating the tube for 30 min at the MIDI method and then for 3 h at the modified MIDI method in an oven at 100°C. The tubes were then cooled down quickly to room temperature in a water bath. For methylation of the free fatty acids, 1 ml reagent 2 (325 ml 6N HCl, 275 ml methanol) was added. The tubes were shaken and transferred to a heating oven at 80°C for 10 min. After cooling down to room temperature, 2 ml reagent 3 (200 ml *n*-hexane, 200 ml *tert*-butyl methyl ether) was added to each sample. After shaking for 10 min, the aqueous phase was discarded. The organic phase was washed with 30 ml reagent 4 (10.8 g NaOH in 900 ml H_2O) and shaken for 5 min. The organic phase was added to an equal volume of distilled water, shaken vigorously, and transferred to a new tube. The sample was then added with 0.5 mg anhydrous sodium sulfate to remove water from the organic phase. The organic phase was concentrated with nitrogen gas and stored in a refrigerator at 4°C until the analysis was performed.

FAME was analyzed using a gas liquid chromatograph (Shimadzu GC17A) equipped with a flame ionization detector (FID) 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 FID temperature was maintained at 180°C. The column temperature was initially programmed at 160°C and increased by 1°C/min to a final temperature of 250°C with split. FAMEs were identified by comparing their retention times with those of authentic materials containing straight chain saturated and unsaturated fatty acids from C10 to C20 (Sigma-Aldrich Co., Tokyo, Japan). To determine the composition of each fatty acid from its peak on the gas chromatogram, the peak area (PA) was calculated based on the data from the height of the peak (h) and retention time (RT) and shown as the formula PA = 2.507 h RT. Triplicate determinations were made for each isolate.

Data evaluation

Data for isolates of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *racidis-lycopersici* were subjected to an analysis of variance (ANOVA). The variability among isolates from different races of *F. oxysporum* f. sp. *lycopersici* 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 relativity among isolates based on fatty acid compositions was assessed with principal-component and cluster analyses (nearest neighbor method, squared Euclidean distance) using StatPartner (ver. 2.0; NEC Software, Tokyo, Japan).

Results

Total cellular fatty acid analysis: MIDI method.

A total of ten fatty acids were detected in the 27 isolates tested using the MIDI method (Table 2). Significant differences ($P < 0.05$) in FAMEs were observed among three races of *F. oxysporum* f. sp. *lycopersici* and from the

Table 2. Total cellular fatty acid profiles of isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* determined by the normal Microbial identification system (MIDI) method

Fungi	Composition of fatty acids (%)									
	14:0	15:0	16:0	16:1 ω7c	17:0	17:1 ω8c	18:0	18:1 ω9c	18:2 ω6,9c	20:1 ω11c
F. o. f. sp. *lycopersici* race 1	0.99 bc	0.45 a	15.99 a	0.96 a	0.21 c	0.49 b	9.17 c	25.57 b	44.29 a	1.87 b
F. o. f. sp. *lycopersici* race 2	1.05 c	0.46 a	16.89 a	1.03 a	0.20 c	0.52 b	7.70 b	27.11 bc	43.62 a	1.46 a
F. o. f. sp. *lycopersici* race 3	0.80 a	0.54 c	18.57 b	1.14 b	0.14 a	0.36 a	8.64 c	23.02 a	44.80 a	1.99 c
F. o. f. sp. *radicis-lycopersici*	0.94 b	0.49 b	20.86 c	1.01 a	0.18 b	0.48 b	4.45 a	27.73 c	42.31 a	1.54 a

Values in each column followed by the same letter are not significantly different for Waller–Duncan *K* ratio *t* test, $P = 0.05$

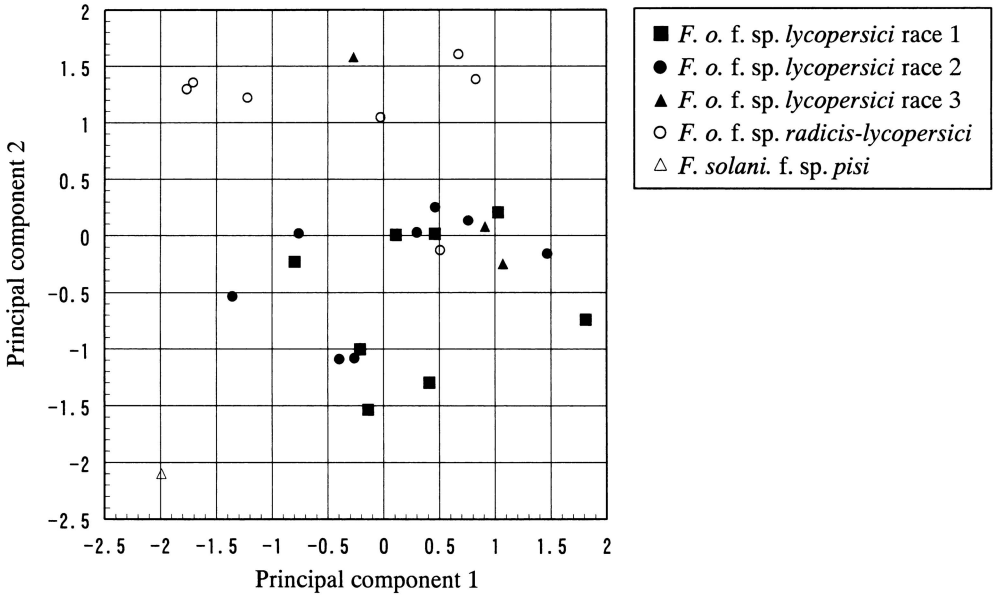

Fig. 1. Relationship of fatty acid compositions among isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* as represented by plots of the first two principal-component analyses using normal MIDI (Microbial Identification System) method

different forma specialis of *F. oxysporum* isolates for seven of the ten fatty acids detected when using this method. In both *F. oxysporum* isolates, 16:0, 18:0, 18:1 ω9c, and 18:2 ω6,9c fatty acids accounted for more than 90% of FAMEs. Fatty acid 18:2ω6,9c was the most dominant fatty acid composition in both *F. oxysporum* isolates, although qualitative differences were observed. Other fatty acids, 16:0, 18:0, and 18:1 ω9c, also showed qualitative differences among three races of *F. oxysporum* f. sp. *lycopersici* and formae speciales of *F. oxysporum* of tomato wilt pathogens.

Principal-component analysis revealed that *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* were present in relatively distinct groups; however, dispersion of cellular fatty acid composition was observed within the same *F. oxysporum* isolates belonging to the same formae speciales (Fig. 1). The first two of the ten principal components of this analysis accounted for 91.6% of the variation in the data. Principal components 1 and 2 accounted for 72.2% and 19.4% of the variation, respectively. Whole cellular fatty acid compositions of 27 isolates tested were compared by conducting average linkage cluster analysis based on the ten fatty acids detected. The dendrogram produced from the detected fatty acids showed that the clustering was unrelated to races, formae speciales of *F. oxysporum* of tomato pathogens, and VCGs (Fig. 2).

Total cellular fatty acid analysis: modified MIDI method

Twelve fatty acids were detected in the 27 isolates tested when using the modified MIDI method (Table 3). There was significant difference ($P < 0.05$) in FAMEs between three races of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* isolates for 11 of 12 fatty acids detected when using this method. By observing the modified MIDI method, 16:0, 18:0, 18:1 ω9c, and 18:2 ω6,9c fatty acids accounted for more than 95% of FAMEs in all isolates tested. The most predominant fatty acid was differed in isolates of *F. oxysporum* f. sp. *lycopersici*, and *F. oxysporum* f. sp. *radicis-lycopersici*, and their quantitative differences were observed. The fatty acid of 18:2 ω6,9c was contained in isolates of *F. oxysporum* f. sp. *lycopersici* race 1 (38.2%), race 2 (43.1%), and race 3 (37.2%), and 18:1 ω9c was also contained in isolates of *F. oxysporum* f. sp. *radicis-lycopersici* (39.4%), respectively. The fatty acids 16:0 and 18:0 were also dominantly contained in *F. oxysporum* f. sp. *lycopersici* race 1 (17.8%) and race 3 (14.2%), respectively.

Principal-component analysis revealed that the dispersion of FAMEs was at a low level within each same formae speciales of *F. oxysporum* of the tomato wilt pathogens, but strictly distinct groups were formed among different races or VCGs of *F. oxysporum* f. sp. *lycopersici* (Fig. 3). The first

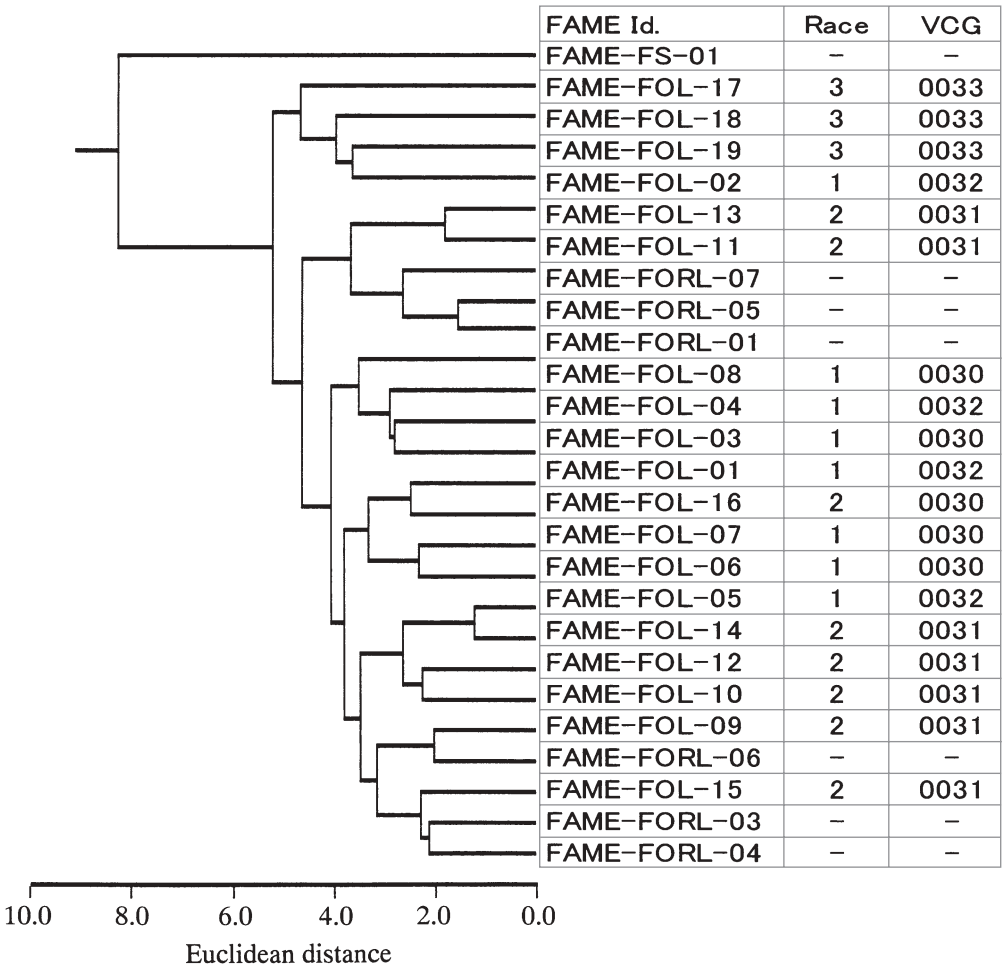

Fig. 2. Dendrogram showing relationships among 27 isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* based on the analysis of fatty acid methyl ester (FAME) profiles using the normal MIDI method (nearest neighbor, squared Euclidean distance)

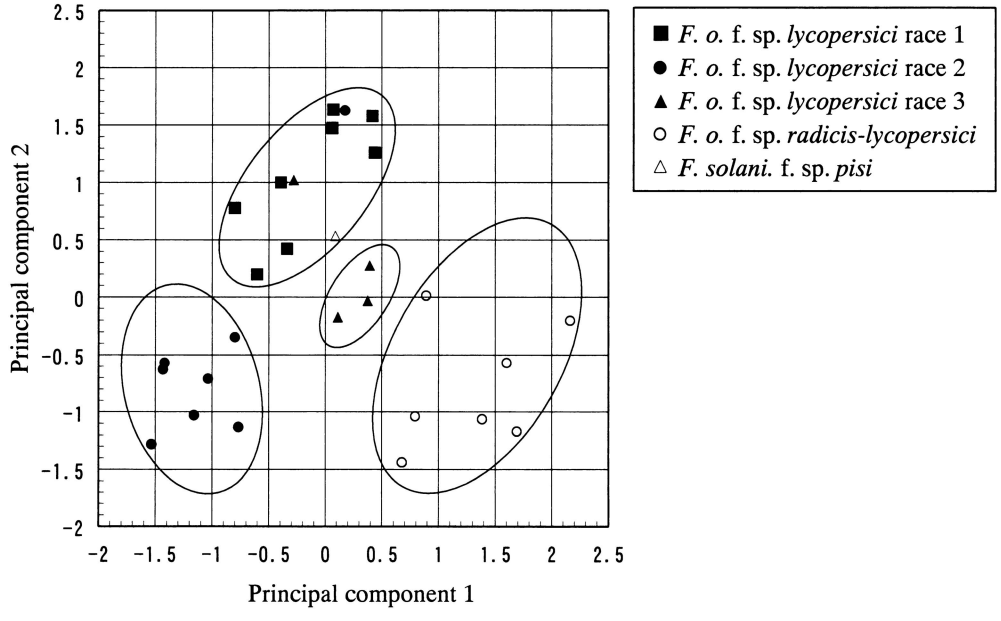

Fig. 3. Relationship of fatty acid compositions among isolates of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* as represented by plots of the first two principal-component analyses using the modified MIDI method

Table 3. Total cellular fatty acid profiles of isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f.sp. *radicis-lycopersici* determined by the modified MIDI method

Fungi	Composition of fatty acids (%)										
	14:0	15:0	16:0	16:0 2OH	16:1 ω7c	17:0	17:1 ω8c	18:0	18:0 3OH	18:1 ω9c	18:2 ω6,9c	20:1 ω11c
F. o. f. sp. *lycopersici* race 1	0.77 d	0.41 a	17.79 c	0.10 a	0.86 d	0.15 a	0.52 d	10.72 b	0.70 d	28.16 b	38.20 b	1.63 b
F. o. f. sp. *lycopersici* race 2	0.56 c	0.83 d	13.37 a	0.10 a	0.82 c	0.24 b	0.41 c	12.92 c	0.50 b	24.60 a	43.62 c	2.05 c
F. o. f. sp. *lycopersici* race 3	0.36 b	0.62 c	14.94 b	0.11 a	0.68 b	0.28 c	0.35 b	14.17 d	0.20 a	29.44 b	37.23 b	1.60 b
F. o. f. sp. *radicis-lycopersici*	0.29 a	0.48 b	14.10 ab	0.10 a	0.51 a	0.39 d	0.30 a	8.35 a	0.60 c	39.41 c	34.46 a	1.02 a

Values in each column followed by the same letter are not significantly different for Waller–Duncan *K* ratio *t* test, $P = 0.05$

2 of the 12 principal components of this analysis accounted for 95.6% of the variation in the data. Principal components 1 and 2 accounted for 83.3% and 12.3% of the variation, respectively. FAME profiles of 27 isolates tested were compared by conducting average linkage cluster analysis based on the 12 fatty acids detected. The dendrogram produced from the detected fatty acids showed that the isolates of VCG 0032 and VCG 0030 of *F. oxysporum* f. sp. *lycopersici* were independently clustered, with a Euclidean distance of 1.5 and 1.6, respectively. Furthermore, they were closely clustered among race 1 isolates [except for the isolates of FAME-FOL-16 (race 2, VCG 0030)] with a distance of 4.9. Isolates of race 2 (VCG 0031) and race 3 (VCG 0033) were independently clustered with each other, with a Euclidean distance of 1.8 and 2.0, respectively, and they were closely clustered with a distance of 3.8. Isolates of *F. oxysporum* f. sp. *radicis-lycopersici* were closely clustered, with a distance of 2.0, and they were furthermore clustered with *F. oxysporum* f. sp. *lycopersici* with a distance of 5.9 (Fig. 4).

Discussion

Lanoiselet et al. (2005) reported that the total cellular fatty acid analysis from *Rhizoctonia* spp. using the modified MIDI method permitted differentiation between isolates of *R. oryzae* and *R. oryzae-sativae*. By comparing the normal MIDI method and the modified MIDI method, significant differences ($P < 0.05$) in fatty acid composition were observed for both *R. oryzae* and *R. oryzae-sativae* isolates. In the present study, our data supported that FAMEs profiles using the modified MIDI method permitted the differentiation of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* isolates. Furthermore, when the two protocols were compared, significant differences ($P < 0.05$) were observed within the different races and/or VCGs of *F. oxysporum* f. sp. *lycopersici*. The modified MIDI method introduced consistent and reproducible data of fatty acid composition between samples of the same isolates compared to the normal MIDI method (data not shown).

In this experiment, we noticed that the essential difference between the MIDI method and the modified MIDI method was the saponification step, which is 2.5 h longer for the modified MIDI method. In both protocols, the saponification is initiated to break the fungal cell wall to expose the phospholipid bilayer membrane. In previous studies, 30-min saponification is the standard time for fungal fatty acid extraction and has been introduced successfully to characterize and classify *Penicilium* spp. (Lopes da Silva et al. 1998) and *Phytophthora infestans* (Larkin and Groves 2003). However, our results suggested that a 30-min saponification step is inadequate for breaking fungal mycelial cells and spore cells of *Fusarium oxysporum* to expose the entire phospholipid bilayer membrane. A similar phenomenon was also observed by Lanoiselet et al. (2005) to differentiate isolates of *R. oryzae* and *R. oryzae-sativae* us-

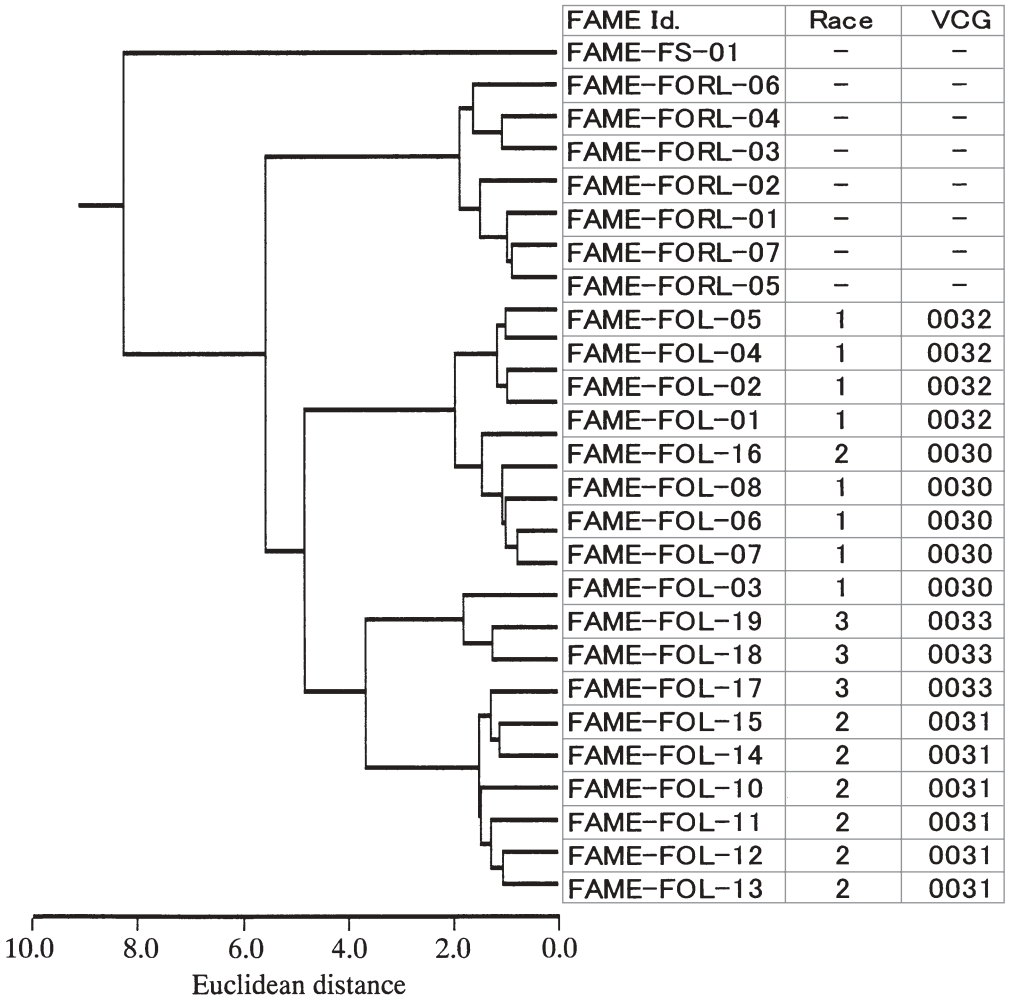

Fig. 4. Dendrogram showing relationships among 27 isolates of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* based on the analysis of FAME profiles using the modified MIDI method (nearest neighbor, squared Euclidean distance)

ing whole cellular fatty acid analysis using the modified MIDI method.

As has been shown in other fatty acid studies (Hering et al. 1999), isolates of *F. oxysporum* f. sp. *vasinfectum* were classified into five groups and identical patterns were observed in isolates of race 3 and race 5 and in isolates of race 2 and race 6. However, fatty acid analysis in combination with random amplified polymorphic DNA (RAPD) study and VCGs relationships were not fully understood in isolates of *F. oxysporum* f. sp. *vasinfectum.* In the present study, whole cellular fatty acid analysis revealed that isolates of VCG 0030 were consistent in the groups of similar FAME profiles (four isolates of race 1 and one isolate of race 2). Moreover, isolates of VCG 0030 and VCG 0032, belonging to race 1, presented similar FAME profiles and closely clustered with each other (see Fig. 4). These finding are in agreement with phylogenetic analysis carried out by Mes et al. (1999) and Kawabe et al. (2005), as isolates of VCG 0030 and of VCG 0032 had a close phylogenetic relationship. However, our FAME profiles examined in this experiment were restricted locality because of limited isolates obtained mostly in Japan and used except for 2 Brazil isolates (VCG 0030 of race 1 and race 2). Therefore, it should be mentioned that the analysis of whole cellular fatty acids requires large number of isolates obtained from worldwide locations reflecting to various races and VCGs in case of *Fusarium oxysporum*.

Our FAME profiles examined in this experiment suggested that isolates of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* could be characterized and differentiated at subspecies levels. Moreover, FAME profiles also indicated that characterization of races, VCGs, and other phylogenetic phenotypes in the case of *F. oxysporum* f. sp. *lycopersici* should be discussed because other taxonomic and phylogenetic criteria should be mandatory to characterize these fungi more accurately.

It is likely that the optimum extraction protocols such as isolates of *Fusarium oxysporum* need to be established for whole cellular fatty acid analysis by modifying the normal MIDI method. Finally, adoption of a uniform practice for extraction protocols will be required for the successful application of fatty acid analysis to fungal classification and identification.

Acknowledgment This experiment was supported in part by the Special Coordination Funds for Promoting Science and Technology of the Science and Technology Agency of the Japanese Government.

References

Armstrong GM, Armstrong JK (1981) Formae speciales and races *of Fusarium oxysporum* causing wilt diseases. In: Nelson PE, Toussoun TA, Cook RJ (eds) *Fusarium*: diseases, biology, and taxonomy. Pennsylvania State University Press, University Park, PA, pp 391–399

Cai G, Gale LR, Schneider RW, Kistler HC, Davis RM, Elias KS, Miyao EM (2003) Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. Phytopathology 93:1014–1022

Correll JC, Klittich CJR, Leslie JF (1987) Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility test. Phytopathology 77:1640–1646

Elias KS, Schneider RW (1991) Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici.* Phytopathology 81:159–162

Elias KS, Zamir D, Lichtman-Pleban T, Katan T (1993) Population structure of *Fusarium oxysporum* f. sp. *lycopersici:* restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. Mol Plant-Microbe Interact 6:565–572

Ellis RJ, Geuns Jan MC, Zarnowski R (2002) Fatty acid composition from an epiphytic strain of *Fusarium oxysporum* associated with algal crusts. Acta Microbiol Pol 51:391–394

Gudmestad NC, Henningson PJ, Bugbee WM (1988) Cellular fatty acid comparison of strains of *Corynebacterium michiganense* subsp. *sepedomicum* from potato and sugar beet. Can J Microbiol 34:716–722

Hering O, Nirengerg HI, Kohn S, Deml G (1999) Characterization of isolates of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans., races 1–6, by cellular fatty acid analysis. J Phytopathol 147:509–514

Hering O, Nirengerg HI, Kohn S, Deml G (1999) Characterization of isolates of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans., race 1–6, by cellular fatty acid analysis. Phytopathology 147:509–514

Katan T (1999) Current status of vegetative compatibility groups in *Fusarium oxysporum*. Phytoparasitica 27:51–64

Katan T, Zamir D, Sarfatti M, Katan J (1991) Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Phytopathology 81:255–262

Kawabe M, Kobayashi Y, Okada G, Yamaguchi I, Teraoka T, Arie T (2005) Three evolutionary lineages of tomato wilt pathogen, Fusarium oxysporum f. sp. lycopersici, based on sequences of ISG, MAT1, and pg1, are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. J Gen Plant Pathol 71:263–272

Kistler HC (1997) Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87:474–479

Kistler HC, Alabouvette C, Baayen RP, Bentley S, Brayford D, Coddington A, Correll J, Davoussi, MJ, Elias K, Fernandez D, Gordon TR, Katan T, Kim HG, Leslie JF, Martyn RD, Migheli Q, Moore NY, O'Donnell K, Ploetz RC, Rutherford MA, Summerell B, Waalwijk C, Woo S (1988) Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 88:30–32

Lanoiselet VM, Cother EJ, Cother NJ, Ash GJ, Harper JDI (2005) Comparison of two total cellular fatty acid analysis protocols to differentiate *Rhizoctonia oryzae* and *R. oryzae-sativae*. Mycologia 97:77–83

Larkin RP, Groves CL (2003) Identification and characterization of isolates of *Phytophthora infestans* using fatty acid methyl ester (FAME) profiles. Plant Dis 87:1233–1243

Lopes da Silva T, de Sousa E, Pereira PT, Ferrao AM, Roseiro JC (1998) Cellular fatty acid profiles for the differentiation of *Penicillium* species. FEMS Microbiol Lett 164:303–310

Marlatt ML, Correll JC, Kaufmann P, Cooper PE (1996) Two genetically distinct populations of *Fusarium oxysporum* f. sp. *lycopersici* race 3 in the United States. Plant Dis 80:1336–1342

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

McGrath DJ, Gillespie G, Vawdrey L (1987) Inheritance of resistance to *Fusarium oxysporum* f. sp. *lycopersici* divides race 2 and 3 of *Lycopersicon pennellii*. Aust J Agric Res 38:729–733

Mes JJ, Weststeijn EA, Herlaar F, Lambalk JJM, Wijbrandi J, Haring MA, Cornelissen BJC (1999) Biological and molecular characterization of *Fusarium oxysporum* f. sp. *lycopersici* divides race 1 isolates into separate virulence groups. Phytopathology 89:156–160

Miller L, Berger T (1985) Bacteria identification by gas chromatography of whole cell fatty acids. Hewlett-Packard application note 228–241. Hewlett-Packard, Avondale, PA

Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can J Bot 63:179–183

Rosewich UL, Pettway RE, Katan T, Kistler HC (2003) Population genetic analysis corroborates dispersal of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida to Europe. Phytopathology 89:623–630

Stall RE (1961) Development of Fusarium wilt on resistant varieties of tomato caused by a strain different from race 1 isolates of *Fusarium oxysporum* f. *lycopersici*. Plant Dis Rep 45:12–15