Trial for Rapid Identification of Pathogens from Blasted Pear Blossoms and Rotted Radish Leaves by the Direct Colony TLC and Whole Cellular Fatty Acid Analysis

Khan, Abu Ashraf
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

Furuya, Naruto
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

Matsumoto, Masaru
Institute of Tropical Agriculture, Kyushu University

Matsuyama, Nobuaki
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/24277

出版情報：九州大学大学院農学研究院紀要. 43 (3/4), pp.327-335, 1999-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **43** (3·4), 327–335 (1999)

Trial for Rapid Identification of Pathogens from Blasted Pear Blossoms and Rotted Radish Leaves by the Direct Colony TLC and Whole Cellular Fatty Acid Analysis

Abu Ashraf Khan, Naruto Furuya, Masaru Matsumoto* and Nobuaki Matsuyama

Laboratory of Plant Pathology, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
(Received October 23, 1998 and accepted November 6, 1998)

Rapid identification of phytopathogenic bacteria isolated from blasted pear blossoms and rotted radish leaves was tried by the direct colony TLC and whole cellular fatty acid analysis. All the 22 reference strains of fluorescent pseudomonads exhibited similar chromatograms with those of the pear and radish strains at the direct colony TLC. A dendrogram of strains based on fatty acid compositions showed that all pathovars of *P. syringae*, *P. viridiflava* and the pear and radish strains were closely related with lauric acid and palmitoleic acid as their major fatty acids. On the other hand, *P. marginalis*, *P. fluorescens* and *P. aeruginosa* predominantly containing an unidentified fatty acid, were clustered separately. The results of the direct colony TLC and fatty acid analysis suggested that the pear and radish strains belonged to *P. syringae* or *P. viridiflava*. Physiological and biochemical tests of the pear and radish strains confirmed that pear and radish strains were *P. syringae* and *P. viridiflava*, respectively. The direct colony TLC and/or whole cellular fatty acid analysis in combination with some selected physiological and biochemical tests will be convenient and practical approach for rapid identification of phytopathogenic bacteria.

INTRODUCTION

Bacterial pathogens are generally identified by physiological and biochemical tests. But all these tests are laborious and time–consuming. Therefore, development of rapid identification methods for phytopathogenic bacteria has been emphasized. Fatty acid analysis of bacterial cells is important tool for classification and identification of bacteria (De Boer and Sasser, 1986; Kori *et al.*, 1992; Roy, 1988; Stead, 1992). The fatty acid data are more useful for identification of bacteria when they are used in combination with selected conventional tests (Wallace *et al.*, 1988).

The direct colony thin layer chromatography (TLC) for rapid detection and identification of bacterial lipids was invented by Matsuyama *et al.*, (1986) and applied successfully for rapid differentiation of phytopathogenic bacteria (Matsuyama *et al.*, 1993a,b,c; Matsuyama and Furuya, 1993d; Matsuyama, 1995a,b). Practical use of this method for identification of phytopathogenic bacteria from diseased plants is in progress.

In this report, we describe the results of practical application of the direct colony TLC and whole cellular fatty acid analysis by GLC for identification of two bacterial strains, which were isolated from blasted pear (*Pyrus serotina*) blossoms and rotted radish

* Institute of Tropical Agriculture, Kyushu University, Fukuoka 812–8581, Japan

(*Raphanus sativus*) leaves. Simultaneously, the conventional physiological and biochemical tests were also conducted for confirmation of the results of TLC and GLC methods.

MATERIALS AND METHODS

Bacterial strains

Two strains were isolated from blasted pear blossoms and rotted radish leaves at Fukuoka Prefecture and used in this study. Other strains of fluorescent pseudomonads used as reference strains are listed in Table 1.

Table 1. List of fluorescent pseudomonads used as reference.

Bacterial species	Isolate	Source
Pseudomonas syringae		
pv. *syringae*	ATCC 19310^T	ATCC
pv. *oryzae*	MAFF 301538	NIAR
pv. *tabaci*	PA–28	KTES
pv. *tabaci*	Ku–7102	AKU
pv. *lachrymans*	1319	NIAS
pv. *lachrymans*	1321	"
pv. *mori*	P–23	"
pv. *pisi*	MAFF 301211	NIAR
pv. *pisi*	MAFF 301213	"
pv. *theae*	MAFF 750001	"
pv. *coronafaciens*	MAFF 301314	"
pv. *phaseolicola*	MAFF 301616	"
pv. *japonica*	MAFF 301163	"
pv. *atropurpurea*	MAFF 301307	"
pv. *morsprunorum*	MAFF 301444	"
pv. *striafaciens*	P–71	NIAS
pv. *myricae*	MAFF 301464	NIAR
pv. *tomato*	MAFF 301593	"
P. viridiflava	MAFF 301130	"
P. marginalis	2153	NIAS
P. fluorescens	ATCC 13525^T	ATCC
P. aeruginosa	P–45	NIAS

ATCC: American Type Culture Collection.
NIAR: National Institute of Agrobiological Resources, Tsukuba, Japan.
KTES: Kagoshima Tobacco Experiment Station, Japan.
AKU: Faculty of Agriculture, Kyushu University, Fukuoka, Japan.
NIAS: National Institute of Agricultural Sciences, Tokyo, Japan.
Type culture: Small T at the shoulder of isolate number indicates type culture.

Direct colony TLC

Each strain was cultured on a slant of King's B medium (Eiken Chem. Co.) at 30 °C for 3 days. One loopful of bacterial colony was pasted directly on a TLC plate (Merck Co.) and dried completely. The plate was then developed with chloroform–methanol (2:1,v/v) for 10 min and dried. Bacterial cells were scraped out and again developed to the same direction with chloroform-methanol–water (60:25:4, v/v/v). The amino lipids were detected by spraying with ninhydrin followed by heating at 100 °C for 10 min. The chromatograms were documented by photocopy (Matsuyama *et al.*, 1993a,b,c; Matsuyama and Furuya, 1993d; Matsuyama, 1995a,b).

GLC analysis

Extraction of whole cellular fatty acids was conducted by the method of Gudmestad *et al.* (1988) with slight modification. Bacteria were grown in 523 broth (Kado and Heskett, 1970) at 30 °C for 48 hr by shaking. Five mg of lyophilized cells was methylated with 0.5 ml 5% HCl–methanol at 100 °C for 3 hr in a sealed glass tube to obtain fatty acid methyl ester (FAME) derivatives. The content was cooled at room temperature and transferred to a new eppendorf tube. Then, FAMEs were added with 0.5 ml of distilled water and petroleum ether and centrifuged at 5000 rpm for 5 min. The solvent phase was collected in a new eppendorf tube, washed with 0.5 ml distilled water to remove HCl and dehydrated by mixing with 0.5 g anhydrous sodium sulfate. The organic phase was concentrated by blowing nitrogen gas. FAMEs were analyzed by GLC chromatograph (Shimadzu C–R–7A Plus) equipped with HR–SS–10 capillary column. The column and injection–port temperature were maintained at 180 °C and 250 °C, respectively, and the flow rate of nitrogen gas was 50 ml/min. Average values of fatty acid composition were used to differentiate the strains. Relative similarities among the strains based on fatty acid composition were assessed with average linkage cluster analysis procedure using the statistics package software SYSTAT.

Physiological and biochemical tests

To test physiological and biochemical properties of the pear and radish strains the authentic methods reported by various researchers were applied as follows: Gram stain and levan formation (Schaad, 1988), production of fluorescent pigment on King's B medium (King *et al.*, 1954), oxidase tests (Kovacs, 1956), potato soft rot (Lelliot *et al.*, 1966), arginine dehydrolase (Thornley, 1960), tobacco hypersensitive reaction (Klement and Goodman, 1967). Carbon source utilization tests were also conducted by adding each carbon source (0.1% wt/vol) to the mineral base medium of Ayers *et al.* (1919). Carbon stock solutions were sterilized by autoclaving, except for the solutions sensitive to heat, which were sterilized by filtration. A suspension of bacterial cells grown on 523 slant was streaked onto each test medium, incubated at 30 °C and evaluated periodically for 21 days. The results on the minimal medium without any addition of carbon source were used as controls.

RESULTS AND DISCUSSION

The pear and radish strains showed identical TLC chromatograms with those of the

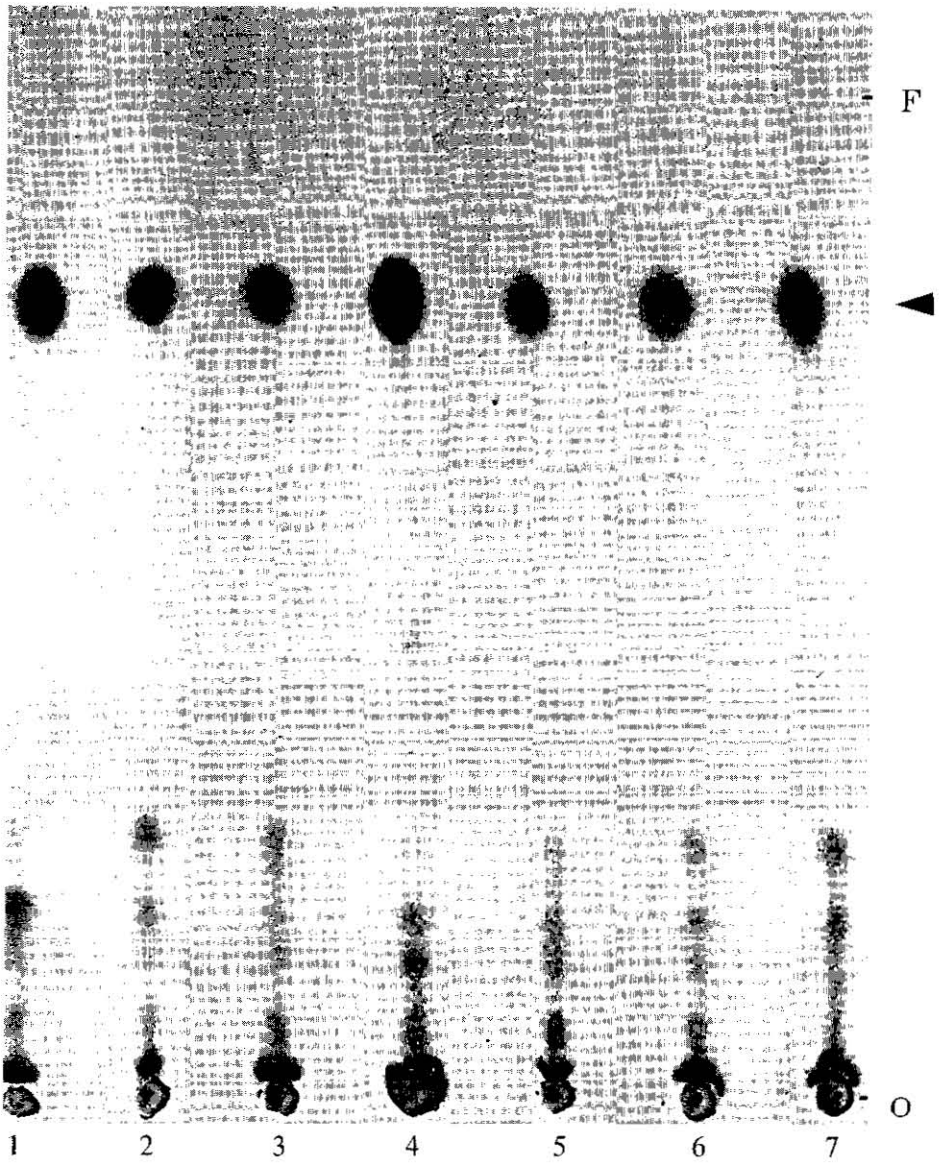

Fig. 1. TLC chromatograms of lipids from pear and radish strains and other reference strains by the direct colony TLC[a].

1. *P. marginalis* 2153
2. Radish strain
3. *P. viridiflava* MAFF 301130
4. *P. syr.* pv. *syringae* ATCC 19310^T
5. Pear strain
6. *P. syr.* pv. *tomato* MAFF 301593
7. *P. syr.* pv. *tabaci* Ku–7102

O : Origin, F: Solvent front

Arrow head indicates the common spot

[a] The direct colony TLC method is described under materials and methods.

Table 2. Percentage composition of whole cellular fatty acids in pear and radish strains and fluorescent pseudomonads used as reference.

Bacterial species	Fatty acid composition									
	Lauric	3–OH Capric	Palmitic	Un[a]–1	Palmitoleic	3–OH Lauric	Oleic	Un–2	Un–3	2–OH Palmitic
Pear strain	17.11	12.56	13.71	13.37	18.34	13.19	3.69	0.00	4.64	3.36
Radish strain	18.58	8.44	12.52	9.72	25.83	9.63	9.05	0.00	0.00	6.20
P. syr. pv. *syringae* ATCC 19310[T]	22.88	10.14	12.71	9.56	20.89	9.32	6.46	0.39	0.26	7.34
P. syr. pv. *oryzae* MAFF 301538	27.22	14.90	10.12	10.71	13.30	9.56	2.94	0.35	0.44	10.44
P. syr. pv. *mori* P–23	21.05	13.17	10.04	11.83	17.11	11.54	4.68	0.45	0.81	9.28
P. syr. pv. *tabaci* PA–28	25.53	15.72	8.12	10.47	12.58	11.39	3.22	0.17	0.51	11.25
P. syr. pv. *tabaci* Ku–7102	26.27	14.11	8.73	11.54	10.56	10.16	3.68	0.83	1.04	12.64
P. syr. pv. *theae* MAFF 750001	19.41	13.81	8.67	12.45	19.18	11.78	3.42	0.46	0.13	10.66
P. syr. pv. *lachrymans* NIAS 1321	24.25	10.97	10.03	11.05	18.76	10.17	3.65	0.26	0.88	9.95
P. syr. pv. *lachrymans* NIAS 1319	27.28	11.84	10.01	11.32	15.30	9.89	3.21	0.37	0.38	10.65
P. syr. pv. *pisi* MAFF 301211	20.77	9.44	13.64	9.01	25.39	7.40	6.29	0.42	0.14	7.47
P. syr. pv. *pisi* MAFF 301213	16.91	13.16	12.97	13.37	22.53	11.83	4.93	0.00	0.51	3.77
P. syr. pv. *phaseolicola* MAFF 301616	18.43	13.12	11.13	11.36	20.21	10.79	5.32	0.00	0.00	9.62
P. syr. pv. *japonica* MAFF 301163	18.07	12.09	11.82	11.73	20.85	12.38	4.69	0.12	0.92	7.30
P. syr. pv. *atropurpurea* MAFF 301307	22.56	11.99	9.21	13.73	17.33	12.11	3.18	0.34	0.64	8.88
P. syr. pv. *coronafaciens* MAFF 301314	21.70	11.61	10.35	11.57	18.57	9.77	3.12	0.30	0.00	12.98
P. syr. pv. *morsprunorum* MAFF 30144	22.32	13.75	13.79	12.53	14.89	10.12	3.57	0.26	1.75	7.00
P. syr. pv. *myricae* MAFF 301464	16.74	12.78	13.79	15.34	14.86	13.99	5.16	0.00	3.18	4.13
P. syr. pv. *tomato* MAFF 301593	18.39	14.24	12.33	13.25	16.87	13.33	3.40	0.00	3.67	4.51
P. syr. pv. *striafaciens* P–71	18.37	12.83	14.46	12.40	18.20	11.61	5.62	0.00	1.80	4.70
P. viridiflava MAFF 301130	18.81	9.24	11.66	10.18	25.32	10.65	7.79	0.00	0.00	6.34
P. marginalis 2153	8.26	12.26	14.27	27.43	15.12	11.63	4.44	0.22	3.76	2.57
P. fluorescens ATCC 13525[T]	9.45	15.35	15.09	26.12	11.50	12.27	4.84	0.00	2.14	3.20
P. aeruginosa P–45	12.18	15.40	11.48	19.21	9.71	13.47	15.35	0.16	0.58	2.43

[a]Un–, Unidentified

reference strains of fluorescent pseudomonads used in this study (Fig. 1). Only a single common spot (arrow-head in figure) which appears on the chromatograms of gram-negative bacteria except for *Agrobacterium* spp. was detected. A similar type TLC chromatogram was reported earlier for *P. syringae* (Matsuyama and Furuya, 1993d) and the chromatographic profiles suggested that the pear and radish strains belong to *P. syringae* or related species.

In GLC analysis, ten fatty acids were detected and quantified as to bacterial strains used in this study. Among them, lauric acid, 3-OH capric acid, palmitic acid, palmitoleic acid, 3-OH lauric acid, oleic acid, 2-OH palmitic acid and one unidentified fatty acid (unidentified-1) were common in all strains including the pear and radish strains. *P. marginalis*, *P. fluorescens* and *P. aeruginosa* predominantly contained an unidentified fatty acid (unidentified-1) when compared with the compositions of *P. syringae* pathovars, *P. viridiflava* and pear and radish strains. Whereas lauric acid and palmitoleic acid were predominant in *P. syringae* pathovars, *P. viridiflava*, and pear and radish strains. The fatty acid composition also revealed that *P. aeruginosa* was different from *P. marginalis* and *P. fluorescens* for higher oleic acid content (Table 2). These results show that *P. syringae* pathovars and *P. viridiflava* can be clearly differentiated from *P. marginalis*, *P. fluorescens* and *P. aeruginosa* by their fatty acid compositions.

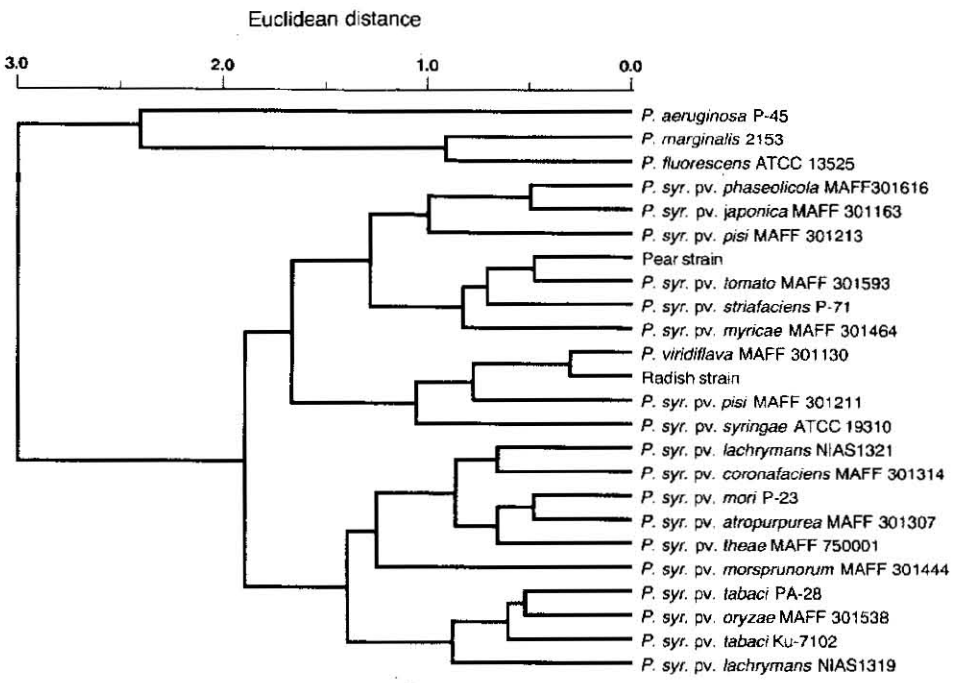

Fig. 2. Dendrogram of cluster analysis on pear and radish strains and other fluorescent pseudomonads used as refefence.

Table 3. Physiological and biochemical tests for pear and radish strains and fluorescent pseudomonads.

Tests	Strain[a)]																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Gram stain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fluorescent pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tobacco HR	+	+	+	Chl.	+	+	+	+	chl.	chl.	chl.	+	+	+	+	Chl.	Chl.	Chl.	+	Chl.	Chl.	+	−	−
Potato soft rot	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Oxidase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Levan	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Arginine dihydolase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Growth at 41 °C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	−	+	+	+	−	+	+	+
Esculin hydrolysis	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−
Tyrosinase activity	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−
Utilization of:																								
Adonitol	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−	−	−	+	+	−
Erythritol	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	+	+	−
Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Sucrose	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Trehalose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−
Cellobiose	−		−	−	−	−	−		−	−	−	−	−			−	−	−	−	−	−	−	−	−
D-Xylose	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−
Betaine	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+
L-Histidine	+	+	−	+	−	−	−	−	+	+	+	−	−	+	+	−	+	+	+	+	−	+	+	+
DL-Homoserine	−		−	−	+	+	−		−	−	−	−	−	−		−	−	−	−	−	−	−	−	−
Beta-alanine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Anthranilate	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	+	+
L-Ascorbate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L-Tartrate	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−
D-Tartrate	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−			
2-Ketoglutarate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Propionate	+	+	−	−	−	−	−	−	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+	+
Quinate	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
L-Lactate	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−		−	+	−	−	+	+	+
Trigonelline-HCl	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−

+, Positive; −, Negative; Chl., Chlorosis

a) 1-Pear strain; 2-Radish strain; 3-*P. syr.* pv. *syringae* ATCC19310^T; 4-*P. viridiflava* MAFF 301130; 5-*P. syr.* pv. *pisi* MAFF 301211; 6-*P. syr.* pv. *pisi* MAFF 301213; 7-*P. syr.* pv. *lachrymans* NIAS 1319; 8 *P. syr.* pv. *lachrymans* NIAS 1321; 9-*P. syr.* pv. *tabaci* PA-28; 10-*P. syr.* pv. *tabaci* Ku-7102; 11-*P. syr.* pv. *morsprunorum* MAFF 301444; 12-*P. syr.* pv. *coronafaciens* MAFF 301314; 13 -*P. syr.* pv. *oryzae* MAFF 301538; 14 -*P. syr.* pv. *atropurpurea* MAFF 301307; 15-*P. syr.* pv. *japonica* MAFF 301163; 16-*P. syr.* pv. *theae* MAFF 750001; 17-*P. syr.* pv. *mori* P-23; 18-*P. syr.* pv. *tomato* MAFF 301593; 19-*P. syr.* pv. *striafaciens* P-71; 20-*P. syr.* pv. *myricae* MAFF 301464; 21-*P. syr.* pv. *phaseolicola* MAFF 301616; 22-*P. marginalis* 2153; 23-*P. fluorescens* ATCC 13525^T; 24-*P. aeruginosa* P-45.

The dendrogram analysis based on fatty acid composition formed two major clusters (Fig. 2). The first group consisted of *P. marginalis*, *P. fluorescens* and *P. aeruginosa* whereas the second group contained *P. syringae* pathovars, *P. viridiflava*, and pear and radish strains. The latter group was again divided into five sub-clusters. In these sub-clusters, pear strain grouped with *P. syr.* pv. *tomato* MAFF 301593, *P. syr.* pv. *striafaciens* P-71 and *P. syr.* pv. *myricae* MAFF 301464 and radish strain clustered with *P. viridiflava* MAFF 301130, *P. syr.* pv. *pisi* MAAFF 301211 and *P. syr.* pv. *syringae* ATCC 19310 (Fig. 2). Previously, Stead (1992) described three well defined profile-types in fluorescent pseudomonads, represented by *P. syringae*, *P. aeruginosa*, and *P. fluorescens*, where all plant pathogenic species tended to have profiles very similar to the profile of *P. syr.* pv. *syringae*, though some differences were observed among some taxa. This is also supported by the results of the present study. Hence, it is evident from the results of fatty acid analysis that the pear and radish strains belong to *P. syringae* or *P. viridiflava*.

Bacteriological properties of the pear and radish strains were examined in order to confirm the results of TLC and GLC analyses. As summarized in Table 3, they were gram-negative, positive to fluorescent pigment production on King's B medium and caused hypersensitive reaction on tobacco leaves. They were negative in the following tests: oxidase activity, arginine dihydrolase activity and growth at 41 °C. Further, the other characteristics of pear and radish strains verified that they belong to *P. syringae* and *P. viridiflava*, respectively.

Although the determination of the pathovar of *P. syringae* was not possible, rapid identification of phytopathogenic bacteria using direct colony TLC and fatty acid analysis in combination with some selected physiological and biochemical tests is more convenient and practical.

REFERENCES

Ayers, S. H. P. Rupp and W. T. Johnson 1919 A study of the alkali-forming bacteria in milk. *U. S. Dept. Agric. Bull.*, 782

De Boer, S. H. and H. Sasser 1986 Differentiation of *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* on the basis of cellular fatty acid composition. *Can. J. Microbiol.*, **32**: 796–800

Gudmestad, N. C., P. J. Henningson and W. M. Bugbee 1988 Cellular fatty acid composition of strains of *Corynebacterium michiganense* subsp. *sepedonicum* from potato and sugar beet. *Can. J. Microbiol.*, **34**: 716–722

Kado, C. I. and M. G. Heskett 1970 Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology*, **60**: 969–976

King, E. O., M. K. Ward and D. E. Raney 1954 Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.*, **44**: 301–307

Klement, A., and R. Goodman 1967 The hypersensitive reaction to infection by bacterial plant pathogens. *Ann. Rev. Phytopathol.*, **5**: 17–44

Kori, Y., N. Furuya, K. Tsuno and N. Matsuyama 1992 Differentiation of *Erwinia chrysanthemi* and *E. carotovora* subsp. *carotovora* by the cellular fatty acid analysis. *J. Fac. Agr., Kyushu Univ.*, **37**: 173–178

Kovacs, N. 1956 Identification of *Pseudomonas pyocyana* by the oxidase reaction. *Nature*, **178**: 703

Lelliott, R. A., E. Billing and A. C. Hayward 1966 A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J. Appl. Bacteriol.*, **29**: 470–489

Matsuyama, N., I. H. Mian, A. M. Akanda and N. Furuya 1993a Application of the direct colony TLC method for identification of phytopathogenic bacteria. *J. Fac. Agr., Kyushu Univ.*, **37**(3, 4): 281–285

Matsuyama, N., I. H. Mian, A. M. Akanda and N. Furuya 1993b Rapid identification of phytopathogenic bacteria by the direct colony thin-layer chromatography (1). *Proc. Assoc., Pl. Prot. Kyushu*, **39**: 60–64

Matsuyama, N., I. H. Mian, A. M. Akanda and N. Furuya 1993c Comparative studies on thin-layer chromatograms of lipids from various phytopathogenic bacteria. *Ann. Phytopath. Soc. Japan*, **59**: 528–534

Matsuyama, N. and N. Furuya 1993d Application of the direct colony TLC for identification of phytopathogenic bacteria (II) Chromatographic profile of *Erwinia* and *Pseudomonas* spp. *J. Fac. Agr., Kyushu Univ.*, **38**(1, 2): 89–95

Matsuyama, N. 1995a Trial for rapid identification of phytopathogenic bacteria by HPLC and the direct colony TLC. *J. Fac. Agr., Kyushu univ.*, **40**(1, 2): 87–91

Matsuyama, N. 1995b Application of the direct colony TLC for identification of phytopathogenic bacteria (III). Distinction of the pseudomonads in the rRNA-homology group II (*Burkholderia* spp.). *J. Fac. Agr., Kyushu Univ.*, **40**(1, 2): 189–196

Matsuyama, T., M. Sogawa, K. Kaneda and I. Yano 1986 Rapid detection and identification of bacterial lipids by direct colony thin-layer chromatography. *Proc. of 23rd Int. Symp. of Adv. in Chrom., Chiba, Japan*, pp 127–128

Roy, M. A. 1988 Use of fatty acids for the identification of phytopathogenic bacteria. *Plant Disease*, **72**: 460.

Schaad, N. W. 1988 Laboratory guide for identification of plant pathogenic bacteria. 2nd Edition. *The Bacteriology Committee of the American Phytopathologyical Society*, pp 164

Stead, D. E. 1992 Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *Int. J. Syst. Bacteriol.*, **42**: 281–295

Thornley, M. J. 1960 The differentiation of *Pseudomonas* from other gram negative bacteria on the basis of arginine metabolism. *J. Appl. Bacteriol.*, **1**: 37–52

Wallace, P. L., D. G. Hollis, R. E. Weaver and C. W. Moss 1988 Cellular fatty acid composition of *Kingella* species, *Cardiobacterium hominis*, and *Eikennella corodens*. *J Clin. Microbiol.*, **26**: 1592–1594