九州大学学術情報リポジトリ
Kyushu University Institutional Repository

Rapid Identification of Erwinia chrysanthemi Isolated from Soft Rotted Eggplant and Phalaenopsis sp. by Lipid and Fatty Acid Profiling

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

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

Ura, Hiroyuki
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

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

https://doi.org/10.5109/24326

出版情報：九州大学大学院農学研究院紀要. 44 (3/4), pp.257-263, 2000-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **44** (3·4), 257–263 (2000)

Rapid Identification of *Erwinia chrysanthemi* Isolated from Soft Rotted Eggplant and *Phalaenopsis* sp. by Lipid and Fatty Acid Profiling

Abu Ashraf Khan, Naruto Furuya, Hiroyuki Ura* and Nobuaki Matsuyama

Laboratory of Plant Pathology, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
(Received October 15, 1999 and accepted November 5, 1999)

Rapid identification of *Erwinia chrysanthemi* isolated from an eggplant and a *Phalaenopsis* sp. was conducted by lipid and fatty acid profiling. In lipid profiles, the eggplant and phalaenopsis strains as well as *E. chrysanthemi* pv. *chrysanthemi* were differentiated from *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* on the basis of the presence or absence of a spot at Rf 0.66, which was specific for *E. carotovora*. Further, marked differences were also observed in fatty acid composition of the strains. The ratios of the amount of lauric acid (12:0) and myristic acid (14:0) clearly differentiated eggplant and phalaenopsis strains and *E. chrysanthemi* pv. *chrysanthemi* from *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica*. Fatty acid profiles of the eggplant and phalaenopsis strains were identical with that of *E. chrysanthemi* pv. *chrysanthemi*. The results of lipid and fatty acid profiling indicated that the eggplant and phalaenopsis strains are *E. chrysanthemi*. Physiological and biochemical tests also confirmed the results. The lipid and fatty acid analysis will be a practical approach for differentiation and identification of *E. chrysanthemi* and *E. carotovora* at least presumptive level.

INTRODUCTION

Erwinia chrysanthemi, originally isolated from chrysanthemum by Burkholder *et al.* (1953) is a member of the pathogenic enterobacteria causing soft rot, stunting and wilting on a wide range of plants in different parts of the world. Though the physiological and biochemical characteristics are the basis of differentiation and identification of the pathogen (Cother and Powell, 1983; Ngwira and Samson, 1990), fatty acid profiling (Kori *et al.*, 1992), protein analysis (Moline, 1985; Uesugi *et al.*, 1990), DNA homology analysis (Brenner *et al.*, 1973) and PCR–RFLP analysis (Nassar *et al.*, 1996) have also been conducted to differentiate the bacterium from other enterobacteria.

Rapid identification of two strains of *E. chrysanthemi* isolated from an eggplant and a *Phalaenopsis* sp. by lipid and fatty acid profiling is described in this paper. Physiological and biochemical tests were also performed to confirm the results of the lipid and fatty acid profiling.

* Present address: Yame Regional Agriculture Extension Center, Fukuoka 834–0005, Japan

MATERIALS AND METHODS

Bacterial strains

Two strains isolated from a soft rotted eggplant and a *Phalaenopsis* sp. were included in this experiment. Other three strains, *Erwinia carotovora* subsp. *carotovora* ATCC 15713^T, *E. carotovora* subsp. *atroseptica* ATCC 33260^T and *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377 were used as reference strains.

Rapid extraction TLC for lipid profiles

The rapid extraction – TLC was conducted according to Khan and Matsuyama (1999b) for lipid profiling. One loopful of bacterial cells was gently suspended in 0.2 ml of chloroform – methanol – 0.3% NaCl solution (2:1:0.4, v/v/v) in a small glass vial and kept at least for 15 min at room temperature for lipid extraction. About 10 μl of lipid extract was spotted on the origin of a silica gel TLC plate. The plate was developed with chloroform – methanol – 0.2% $CaCl_2.2H_2O$ (55:35:8, v/v/v) for 1 hr. Lipid spots were detected by spraying ninhydrin followed by heating at 100 °C for 10 min and chromatograms were recorded by photocopy/photograph/computer (Adobe photoshop 3.0J).

GLC for fatty acid profiles

Whole cellular fatty acid analysis was conducted followed by Khan *et al.* (1999a). Bacterial cells were shake–cultured in 523 broth (Kado and Heskett, 1970) at 30 °C for 48 hr. Five milligrams of the lyophilized cells was methylated with 0.5 ml of 5% HCl–methanol at 100 °C for 3 hr in a sealed glass tube. The fatty acid methyl ester (FAME) derivatives were extracted with 0.5 ml petroleum ether. The solvent phase was washed with 0.5 ml distilled water and dehydrated by mixing with 0.5 g anhydrous sodium sulfate. The organic phase was concentrated by nitrogen gas blowing and subjected to gas liquid chromatography (GLC), Shimadzu GC–17A equipped with HR–SS–10 column. The column and injection–port temperatures were maintained at 180 °C and 250 °C, respectively, and the flow rate of nitrogen gas was 50 ml/min. Fatty acids were identified by the comparison of retention time with the standard. Relative similarities among the strains based on the fatty acid composition were assessed with average linkage cluster analysis procedure using the statistics package software SYSTAT.

Physiological and biochemical tests

Gram stain, oxidase, catalase, potato soft rot, glucose fermentation, acetoin production, nitrate reduction, gelatin liquefaction, urease production, indole test, sensitivity to erythromycin, gas from glucose, methyl red test, lecithinase, reducing substance from sucrose and phosphatase tests were conducted followed by the standard methods (Mc Clung and Toabe, 1947; Hugh and Leifson, 1953; Kovacs, 1956; Lelliot *et al.*, 1966; Dye, 1968; Suslow *et al.*, 1982; Schaad, 1988). Acid production from various organic substrates was tested by Dye's method (Dye, 1968). Utilization of organic acids was determined by streaking one loopful bacterial suspension on the medium of Ayer's *et al.* (1919) supplemented with 0.1% of the substrates. The growth of bacteria was observed after 7, 14 and 21 days of incubation at 30 °C.

RESULTS AND DISCUSSION

As can be seen in Fig. 1, chromatograms of the eggplant and phalaenopsis strains as well as *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377 were different from those of *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC 33260^T. A benchmark spot at Rf 0.66 (arrow in the figure) was observed in *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC 33260^T, while absent in the eggplant and phalaenopsis strains as well as *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377. Matsuyama and Furuya (1993) reported that distinct differences were observed between chromatographic profiles of *E. carotovora* subsp.

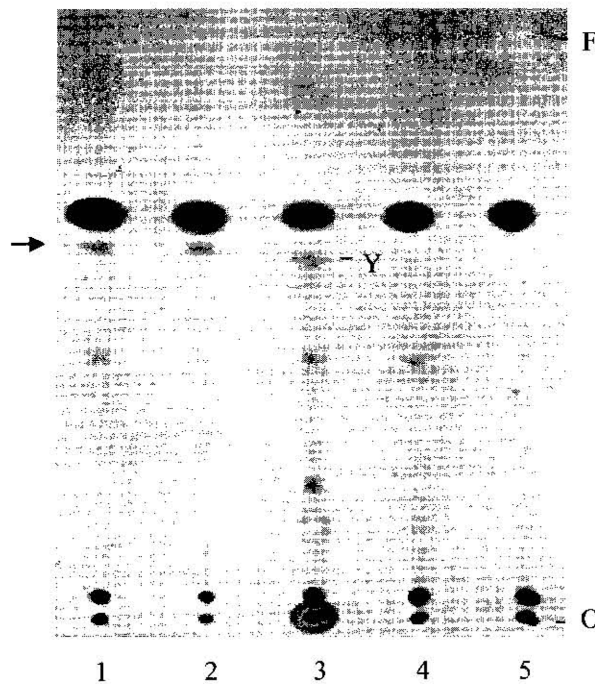

Fig. 1. TLC chromatograms of lipids from eggplant and phalaenopsis strains as well as reference strains by the rapid extraction–TLC.
1. *E. carotovora* subsp. *carotovora* ATCC 15713^T
2. *E. carotovora* subsp. *atroseptica* ATCC 33260^T
3. *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377
4. Eggplant strain
5. Phalaenopsis strain

O: origin, F: solvent front, arrow indicates the benchmark spot for *E. carotovora* and Y: indicates a yellow spot for *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377.

carotovora and *E. chrysanthemi* by the direct colony TLC, and a characteristic spot at Rf 0.57 was always detected on the chromatograph of *E. carotovora* subsp. *carotovora*. The results of the present experiment agreed well with the previous findings. A yellow spot (Y in the figure) was observed in *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377, while absent in the other strains (Fig. 1). This spot at Rf 0.62 was pigment produced by this strain. Therefore, this spot was not considered. These results suggested that the eggplant and phalaenopsis strains would be *E. chrysanthemi* but not *E. carotovora*.

Ten kinds of fatty acids were detected in GLC analysis of the FAMEs of the strains. Among them, lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1 *cis* 9), stearic acid (18:0), oleic acid (18:1 *cis* 9) and *cis* vaccenic acid (18:1 *cis* 11) were common in all strains. The eggplant and phalaenopsis strains showed identical fatty acid profiles with those of *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377, and these were distinctly different from those of *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC 33260^T. Higher amount of lauric acid (12:0) and lower amount of myristic acid (14:0) were characteristically detected in *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC

Table 1. Percentage composition of whole cellular fatty acids in eggplant and phalaenopsis strains as well as reference strains.

Bacterial strain	Fatty acid composition									
	12:0	14:0	10:0 3–OH	16:0	16:1 *cis* 9	18:0	18:1 *cis* 9	Un–1	18:1 *cis* 11	18:3 *cis* 6, 9, 12
E. carotovora subsp. *carotovora* ATCC 15713^T	14.12	4.96	0	17.20	30.39	1.16	7.53	0	24.61	0
E. carotovora subsp. *atroseptica* ATCC 33260^T	13.74	4.52	0	18.02	27.16	0.68	5.73	0	30.13	0
E. chrysanthemi pv. *chrysanthemi* NCPPB 377	4.60	6.02	4.99	16.97	20.88	1.15	5.08	2.67	35.6	1.99
Eggplant strain	5.34	8.83	7.19	10.37	22.46	0.82	2.49	1.28	39.72	1.46
Phalaenopsis strain	3.30	9.61	3.50	20.26	19.97	1.08	4.42	2.53	34.19	1.09

Un–, unidentified

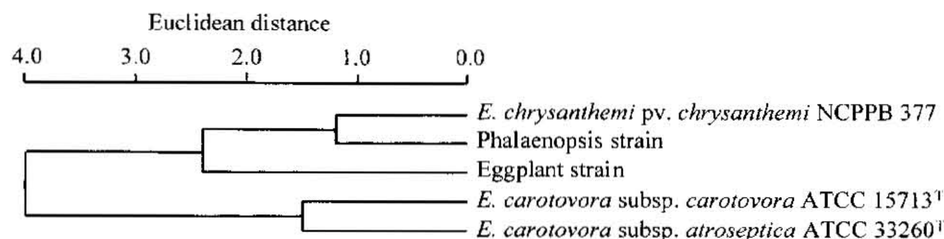

Fig. 2. Dendrogram of cluster analysis on eggplant and phalaenopsis strains as well as reference strains.

Table 2. Physiological and biochemical properties of eggplant and phalaenopsis strains as well as reference strains.

Property	Present strain		Reference strain		
	Eggplant strain	Phalaenopsis strain	*E. chrysanthemi* pv. *chrysanthemi* NCPPB 377	*E. carotovora* subsp. *carotovora* ATCC 15713ᵀ	*E. carotovora* subsp. *atroseptica* ATCC 33260ᵀ
Gram stain	–	–	–	–	–
Oxidase	–	–	–	–	–
Catalase	+	+	+	+	+
Potato soft rot	+	+	+	+	+
Glucose fermentation	+	+	+	+	+
Acetoin production	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+
Urease production	–	–	–	–	–
Indole test	+	+	+	–	–
Sensitivity to erythromycin	+	+	+	–	–
Gas from glucose	+	+	+	–	–
Methyl red test	–	–	–	+	+
Lecithinase	+	+	+	–	–
Phosphatase	+	+	+	–	–
Reducing substances from sucrose	–	–	–	–	+
Acid production from:					
Mannitol	+	+	+	+	+
Dulcitol	–	–	–	–	–
Sorbitol	–	–	–	–	–
Adonitol	–		–	–	–
Inositol	–	–	–	+	–
Inulin	–	–	–	+	+
Salicin	+	+	+	+	+
Maltose	–	–	–	–	–
Cellobiose	+	+	+	+	+
Lactose	–	–	–	+	+
Xylose	+	+	+	+	+
L–Arabinose	+	+	+	+	+
Ribose	+	+	+	+	+
Raffinose	+	+	+	+	+
Mellibiose	+	+	+	+	+
Mannose	+	+	+	+	+
Trehalose	–	–	–	+	+
L–Rhamnose	+	+	+	+	+
Melezitose	–	–	–	–	–
α-Methyl glucoside	–	–	–	–	+
Utilization of:					
Malonate	+	+	+	–	–
D–Tartrate	–	–	–	–	–
Galacturonate	+	+	+	+	+
Na–Citrate	+	+	+	+	+

+, positive; –, negative

33260^T. However, this trend was *vice versa* in the case of the eggplant and phalaenopsis strains as well as *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377 (Table 1). These results showed that *E. carotovora* will be differentiated clearly from *E. chrysanthemi* by the fatty acid profiles. In particular, the ratio of lauric acid (12:0) and myristic acid (14:0) will be useful marker. Similar observation was reported earlier by Kori *et al.* (1992). Furthermore, 3–hydroxy capric acid (10:0 3–OH), linolenic acid (18:3 *cis* 6, 9, 12) and one unidentified fatty acid (unidentified – 1) were not recorded in *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC 33260^T but observed in the eggplant and phalaenopsis strains as well as *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377 (Table 1).

Dendrogram based on the fatty acid composition formed two clusters. The eggplant and phalaenopsis strains were grouped with *E. chrysanthemi* pv. *chrysanthemi* NCPPB 377. On the other hand, *E. carotovora* subsp. *carotovora* ATCC 15713^T and *E. carotovora* subsp. *atroseptica* ATCC 33260^T formed another cluster (Fig. 2). These results indicated that the eggplant and phalaenopsis strains are *E. chrysanthemi*.

Physiological and biochemical properties of the eggplant and phalaenopsis strains showed that they were positive in catalase, potato soft rot, glucose fermentation, acetoin production, nitrate reduction, gelatin liquefaction, indole production, sensitivity to erythromycin, gas from glucose, lecithinase and phosphatase tests. They were negative in Gram staining, oxidase, urease production, methyl red test and production of reducing substances from sucrose. The results of the tests for acid production and utilization of carbon source summarized in Table 2 confirmed that the eggplant and phalaenopsis strains are *E. chrysanthemi*. This experiment proved the effectiveness of lipid and fatty acid profiling for identification of phytopathogenic bacteria at least presumptive level.

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

Brenner, D. J., A. G. Steigerwalt, G. V. Miklos and G. R. Fanning 1973 Deoxyribonucleic acid relatedness among erwiniae and other *Enterobacteriaceae*: the soft–rot organisms (genus *Pectobacterium* waldee). *Int. J. Syst. Bacteriol.*, **23**: 205–216

Burkholder, W. H., L. A. McFadden and A. W. Dimock 1953 A bacterial blight of chrysanthemums. *Phytopathology*, **53**: 522–526

Cother, E. J. and V. Powell 1983 Physiological and pathological characteristics of *Erwinia chrysanthemi* isolates from potato tubers. *J. Appl. Bacteriol.*, **54**: 37–43

Dye, D. W. 1968 A taxonomic study of the genus *Erwinia*. I. The "amylovora" group. *N. Z. J. Sci.*, **11**: 590–607

Hugh, R. and E. Leifson 1953 The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram–negative bacteria. *J. Bacteriol.*, **66**: 24–26

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

Khan, A. A., N. Furuya, M. Matsumoto and N. Matsuyama 1999a 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. *J. Fac. Agr., Kyushu Univ.*, **43**: 327–335

Khan, A. A. and N. Matsuyama 1999b A rapid extraction – TLC method for differentiation of *Burkholderia* spp., *Ralstonia solanacearum, Herbaspirillum rubrisubalbicans* and *Pseudomonas syringae* pathovars. *J. Fac. Agr., Kyushu Univ.*, **44**: 49–58

Kori, Y., N. Furuya, K. Tsuno and N. Matsuyama 1992 Differentiation of *Erwinia chrysanthemi* and *E.*

carotovora subsp. *carotovora* by 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. and N. Furuya 1993 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**: 89–95

Mc Clung, L. S. and R. Toabe 1947 The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. *J. Bacteriol.*, **53**: 139–147

Moline, H. E. 1985 Differentiation of soft rotting bacteria with two–dimensional polyacrylamide gel electrophoresis. *Phytopathology*, **75**: 549–553

Nassar, A., A. Darrasse, M. Lemattre, A. Kotoujansky, C. Dervin, R. Vedel and Y. Bertheau 1996 Characterization of *Erwinia chrysanthemi* by pectinolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR–amplified fragments of *pel* genes. *Appl. Environ. Microbiol.*, **62**: 2228–2235

Ngwira, N. and R. Samson 1990 *Erwinia chrysanthemi*: description of two new biovars (bv. 8 and bv. 9) isolated from kalanchoe and maize host plants. *Agronomie*, **10**: 341–345

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

Suslow, T. V., M. N. Schroth and M. Isaka 1982 Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology*, **72**: 917–918

Uesugi, C. H., K. Tsuchiya, K. Tsuno, N. Matsuyama and S. Wakimoto 1990 SDS–polyacrylamide gel electrophoretic profiles of membrane proteins associated with host of origin in *Erwinia chrysanthemi strains. Ann. Phytolpathol. Soc. Jpn.*, **56**: 597–604