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Characterization of *Erwinia carotovora* subsp. *carotovora* Strains on the Basis of Cellular Fatty Acid Composition

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Eighty-seven strains of *Erwinia carotovora* subsp. *carotovora* (Ecc) isolated from various host plants in several geographic regions of Asia during different years, were characterized by their fatty acid compositions. All strains contained, in decreasing order of amount present, lauric acid (12:0), palmitic acid (16:0), palmitoleic acid (16:1 *cis* 9), vaccenic acid (18:1 *cis* 11) and one unidentified fatty acid (Un–3). Also present in some but not all strains were capric acid (10:0), myristic acid (14:0), 3-hydroxylauric acid (12:0 3-OH), stearic acid (18:0), linoleic acid (18:3 *cis* 9,12) and four unidentified fatty acids (Un–1, Un–2, Un–4 and Un–5). In cluster analysis based on the fatty acid composition, the Ecc strains were composed of two groups (I and II). When the strains were grouped according to their geographical origins, some relationships were found. All Korean and most of Thai strains belonged to the group II, while Japanese isolates belonged to the both groups.

INTRODUCTION

The plant pathogenic enterobacterium *Erwinia carotovora* subsp. *carotovora* (Ecc) belongs to the soft rot group of erwinias and has the ability to infect a number of plant species, including several economically important crops. Previous attempts to characterize Ecc strains on the basis of pathogenicity and biochemical characteristics (Lelliot and Dickey, 1984; Smith and Bartz, 1990), serology (De Boer *et al.*, 1987) and molecular biology (Darrasse *et al.*, 1994) have shown that there is a great deal of variability in this subspecies.

Fatty acid analysis has become a valuable taxonomic tool in classification of plant pathogenic bacteria (Moss, 1981). Some groups of bacteria have profiles sufficiently different from each other to permit differentiation based on a few calculated parameters, such as percentages of individual fatty acids. The fatty acid analyses of *E. carotovora* have been described (De Boer and Sasser, 1986; Wells and Moline, 1991). However, little is known about detailed characterization of Ecc strains in Asian areas. In this study, we examine the cellular fatty acid compositions of Ecc strains isolated from Thailand, Korea and Japan by gas–liquid chromatography.

MATERIALS AND METHODS

Bacterial strains

Relevant characteristics of Ecc strains used in this study are listed in Table 1. All the

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Table 1. Origins and groupings of *E. carotovora* subsp. *carotovora* strains by fatty acid composition

Strain	Source	Isolated from	Year isolated	Group[a)]	Cluster[a)]
014-2	Thailand	Cauliflower	1980	I	B
014-9	Thailand	Cauliflower	1980	II	E
131-1	Thailand	Bell pepper	1980	II	G
168-7	Thailand	Chinese cabbage	1980	II	G
435-2	Thailand	Lettuce	1982	II	G
435-6	Thailand	Lettuce	1982	II	E
462-53-1	Thailand	Tomato	1982	II	G
473-1	Thailand	Chinese cabbage	1982	II	G
475-1	Thailand	Hot pepper	1982	II	E
476-4	Thailand	Bird chili	1982	II	E
476-7	Thailand	Bird chili	1982	II	E
479-2	Thailand	Coriander	1982	II	E
485-5	Thailand	Cabbage	1982	II	G
486-4	Thailand	Sweet pepper	1982	I	A
486-5	Thailand	Sweet pepper	1982	II	E
486-7	Thailand	Sweet pepper	1982	II	E
486-8	Thailand	Sweet pepper	1982	II	E
489-4	Thailand	Cabbage	1982	II	E
489-5	Thailand	Cabbage	1982	II	E
493-1	Thailand	Potato	1982	II	E
493-3	Thailand	Potato	1982	II	G
493-5	Thailand	Potato	1982	I	A
N7101	Japan	Sweet pepper	1971	II	E
N7109	Japan	Cauliflower	1971	II	E
N7116	Japan	Cabbage	1971	II	G
N7127	Japan	Carrot	1971	I	A
N7128	Japan	Celery	1971	I	A
N7129	Japan	Radish	1971	I	A
N7131	Japan	Tomato	1971	I	A
N7135	Japan	Tomato	1971	II	G
N7157	Japan	Chinese cabbage	1971	I	A
Ku7514	Japan	Water melon	1975	I	A
Sr79-33-3	Japan	Potato	1979	I	A
S8488	Japan	Sunflower	1984	I	C
1B	Japan	Shallot	1982	II	G
190	Japan	Carrot	1984	II	E
43	Japan	Cabbage	1984	I	A
645ar	Japan	Chinese cabbage	1960	I	A
Ar13	Japan	Chinese cabbage	1960	II	G
20	Japan	Chinese cabbage	1970	I	A
EH8504	Japan	Cucumber	1985	I	A
EH8510	Japan	Cucumber	1985	I	A
EH8514	Japan	Cucumber	1985	I	A
EH8519	Japan	Cucumber	1985	II	G
B1	Japan	Broccoli	1985	II	G
K1	Japan	Radish	1985	II	E

Table 1. Continued

Strain	Source	Isolated from	Year isolated	Group	Cluster
K2	Japan	Radish	1985	II	G
MAFF[b)]106567	Japan	Cucumber	1985	II	E
MAFF 301049	Japan	Eggplant	1948	I	D
MAFF 301053	Japan	Radish	1957	I	D
MAFF 301282	Japan	Melon	1976	II	G
MAFF 301394	Japan	Cabbage	1971	I	D
MAFF 301396	Japan	Carrot	1971	II	E
MAFF 301399	Japan	Elephant's foot	1971	II	E
MAFF 301404	Japan	Parsley	1973	I	D
MAFF 301891	Japan	Sweet pepper	1971	II	E
MAFF 301905	Japan	Tobacco	1971	II	E
MAFF 301917	Japan	Cauliflower	1971	I	D
MAFF 301937	Japan	Mulberry	1974	II	E
MAFF 301941	Japan	Ginger	1974	II	E
MAFF 302107	Japan	Japanese angelica tree	1988	I	D
MAFF 302773	Japan	Garlic	1983	I	D
MAFF 311115	Japan	Calla	1994	I	D
MAFF 810035	Japan	Lettuce	1980	II	E
Ecc1/95	Korea	Wasabi	1995	II	E
Ecc2/95	Korea	Chicory	1995	II	F
Ecc3/95	Korea	Potato	1995	II	F
Ecc4/95	Korea	Chinese cabbage	1995	II	G
Ecc5/95	Korea	Chinese cabbage	1995	II	F
Ecc6/95	Korea	Potato	1995	II	G
Ecc1/96	Korea	Chinese cabbage	1996	II	G
Ecc2/96	Korea	Chinese cabbage	1996	II	G
Ecc3/96	Korea	Chinese cabbage	1996	II	G
Ecc4/96	Korea	Chinese cabbage	1996	II	G
Ecc5/96	Korea	Chinese cabbage	1996	II	G
Ecc6/96	Korea	Wasabi	1996	II	G
Ecc7/96	Korea	Onion	1996	II	E
Ecc8/96	Korea	Crisphead lettuce	1996	II	E
Ecc9/96	Korea	Radish	1996	II	E
Ecc11/96	Korea	Potato	1996	II	G
Ecc12/96	Korea	Cucumber	1996	II	E
Ecc13/96	Korea	Pumpkin	1996	II	E
Ecc14/96	Korea	Potato	1996	II	G
Ecc1/97	Korea	Pepper	1997	II	G
Ecc2/97	Korea	Calla	1997	II	G
Ecc3/97	Korea	Potato	1997	II	G
Ecc1/98	Korea	Cactus	1998	II	E

[a)] Group and cluster analyses on the basis of fatty acid composition. See Fig. 1

[b)] MAFF, Ministry of Agriculture, Forestry and Fisheries Genebank, Japan

strains were stored in freezing medium (skim milk 100 g, sodium glutamate 10 g, distilled water 1 liter) at −70 °C. When required, all strains were grown on MGY agar (mannitol 10.0 g, L-glutamic acid 2.0 g, KH_2PO_4 0.5 g, NaCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, yeast extract 0.25 g, agar 15.0 g, distilled water 1 liter, pH 7.0) (Keane *et al.*, 1970) at 28 °C for 2 days.

Preparation of the samples

Extraction of whole cellular fatty acids was conducted by the method of Gudmestad *et al.* (1988) with slight modifications. Bacterial strains were cultured for 2 days on King's B agar. Portions (approximately 5 mg, fresh weight) of cells were 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. After methanolysis, one ml of water was added and the FAMEs were extracted with petroleum ether by shaking. The solvent phase was washed with equal volume of 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. Samples were stored at −20 °C.

Analysis of cellular fatty acids

Two microliters of the concentrate was injected into a 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. Under these conditions, methyl esters were separated in about 15 min. Fatty acids were identified by comparison of retention times with those of 21 purified reference standards. Euclidean distance among the strains based on fatty acid composition was assessed with average linkage cluster analysis procedure using the statistic package software SYSTAT.

RESULTS AND DISCUSSION

Fatty acid profiles can be affected by culture conditions, physiological age of cells, and experimental factors in the laboratory (Casano *et al.*, 1986). KB medium was selected for this study because the greater diversity of fatty acids detected in cells grown on KB than in cells grown on TSA medium (Wells and Moline, 1991).

Vaccenic acid (18:1 *cis* 11), palmitoleic acid (16:1 *cis* 9), palmitic acid (16:0), lauric acid (12:0), and one unidentified fatty acid (Un-3) were present in all the 87 strains. Capric acid (10:0), myristic acid (14:0), 3-hydroxylauric acid (12:0 3-OH), stearic acid (18:0), linoleic acid (18:3 *cis* 9,12) and four unidentified fatty acids (Un-1, Un-2, Un-4 and Un-5) were found in some but not all strains. De Boer and Sasser (1986) reported that the Ecc and Eca could be differentiated by some of the calculations based on the ratio of 12:0/14:0 and 16:0/12:0. Values for these ratios of <3.71 and >4.87, respectively, were used as benchmarks for Eca strains, while the contrasting values, >3.71 and <4.87 were used as benchmarks for Ecc strains. In our study, all strains except one strain (S8488) were below 3.1 for the ratio of 16:0 divided by 12:0. However, 24 out of 87 strains were not accordance with the ratio of 12:0 divided by 14:0 for Ecc. These results indicated that the intermediate strains could be distinguished from both typical Ecc and Eca in fatty acid composition.

A partial relationship between the fatty acid compositions and geographical origins was found. Mean percentage of unidentified fatty acid (Un–5) for Korean strains were significantly higher than that for Thai and Japanese strains (Table 2). In cluster analysis

Table 2. Percentage of fatty acids in 87 strains of *E. carotovora* subsp. *carotovora* isolated from Thailand, Korea and Japan

Fatty acid	Thai (n=22)[b]		Korean (n=23)		Japanese (n=42)	
	Relative % present		Relative % present		Relative % present	
	Range	Mean±SD[c]	Range	Mean±SD	Range	Mean±SD
10:0	0.0–11.6	3.9±3.2	0.0–12.9	4.2±3.5	0.0–18.0	4.5±4.6
12:0	6.3–15.7	10.2±2.0	7.0–18.1	11.4±2.7	2.1–20.2	10.5±2.8
14:0	0.7–5.7	2.6±1.2	0.0–9.2	1.7±1.9	0.0–10.6	2.2±2.3
Un–1[a]	0.0–9.8	1.1±2.1	0.0–1.5	0.1±0.4	0.0–2.5	0.3±0.6
16:0	10.7–22.4	17.4±2.9	9.2–19.9	14.3±3.1	10.2–31.8	19.5±4.6
16:1 *cis* 9	15.2–26.2	19.4±2.8	14.2–25.0	18.3±2.8	4.3–33.7	22.7±5.8
Un–2	0.0–2.4	0.5±0.6	0.0–3.6	0.2±0.7	0.0–2.4	0.3±0.5
12:0 3–OH	0.0–1.7	0.5±0.5	0.0–6.3	0.3±1.3	0.0–2.1	0.3±0.5
18:0	0.0–0.9	0.3±0.2	0.0–4.3	0.3±0.9	0.0–2.2	0.5±0.7
Un–3	5.1–15.3	9.0±2.4	4.6–11.2	7.0±1.9	1.6–22.7	10.3±3.7
Un–4	0.0–0.5	0.2±0.2	0.0–1.6	0.2±0.4	0.0–1.7	0.2±0.3
18:3 *cis* 9,12	0.0–2.5	0.3±0.5	0.0–0.3	0.0±0.1	0.0–9.5	0.2±1.9
18:1 *cis* 11	17.5–46.6	34.2±7.3	28.5–53.4	39.9±6.4	6.9–51.7	28.2±11.1
Un–5	0.0–1.5	0.3±0.4	0.0–8.9	2.2±2.4	0.0–3.1	0.3±0.7

[a] Un–, unidentified
[b] n, number of strains
[c] SD, standard deviation

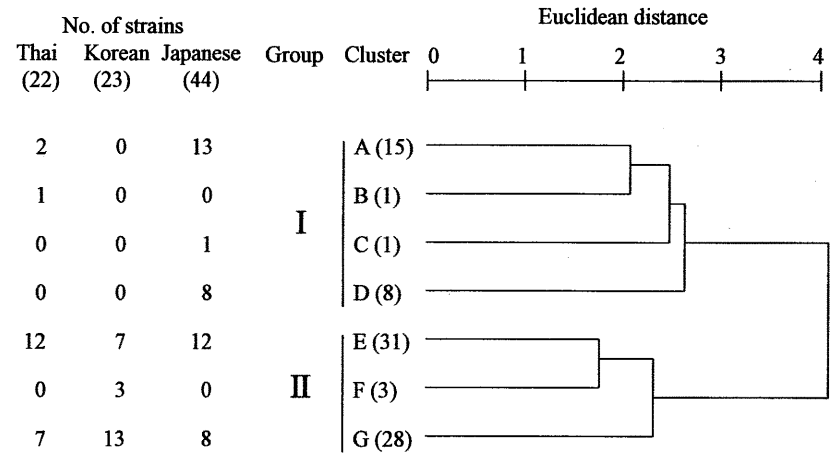

Fig. 1. Relationships among 87 strains of *E. carotovora* subsp. *carotovora* on the basis of fatty acid composition. Groups and clusters are indicated on the left. Numbers of strains are in parentheses.

based on fatty acid composition, the Ecc strains were composed of two groups (I and II) and seven clusters (A–G). Most of strains used in this study belonged to cluster A, E and G. All Korean and most of Thai strains belonged to the group II, while Japanese isolates belonged to the both groups (Fig. 1).

Further studies with larger numbers of strains and analysis under different cultural conditions will be required to understand relationships among geographic origins of the pathogen.

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