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

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http://hdl.handle.net/2324/24437
Characterization of Erwinia carotovora subsp. carotovora Strains on the Basis of Cellular Fatty Acid Composition

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(Received September 17, 2001 and accepted November 20, 2001)

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-hydroxyauric 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

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* Group and cluster analyses on the basis of fatty acid composition. See Fig. 1
* 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, KH₂PO₄ 0.5 g, NaCl 0.2 g, MgSO₄·7H₂O 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 methanlysis, 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

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<td>10.2±2.0</td>
<td>7.0–18.1</td>
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<td>0.7–5.7</td>
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<td>1.1±2.1</td>
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a Un–, unidentified
b n, number of strains
c SD, standard deviation

d | No. of strains | Thai (22) | Korean (23) | Japanese (44) | Group | Cluster | Euclidean distance |
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<td>8</td>
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**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.

REFERENCES


Smith, C. and J. A. Bartz 1990 Variation in the pathogenicity and aggressiveness of strains of Ecc isolated from different hosts. Plant Dis., 74: 505–509