Characteristics of the whole cell fatty acid profiles of *Pseudomonas corrugata*

**Fatty acids of P. corrugata**

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**Abstract**

The fatty acid methyl ester (FAME) profiles of eighty strains of *Pseudomonas corrugata* from different geographic origins have been studied. Gas chromatographic profiles were obtained. The use of hexane/methyl-tert butyl ether (MTBE) (1:1) for the extractions improves the yield of hydroxy-FAMEs used to identify *P. corrugata* as compared to hexane alone. The analysis of the extracts with hexane/MTBE showed that many strains do not have the characteristic FAMEs of *P. corrugata* (3-hydroxydodecenoic acid, 3-hydroxytetradecanoic acid, 3-hydroxyhexadecanoic acid and two unknown additional fatty acids). These differences among strains seemed to be related to bacterial dissociation, associated with changes in morphological aspect of the colonies due to subculture. The comparison of profiles of wrinkled and smooth colonies isolated from ten strains confirmed the differences among those in specific FAMEs. Therefore, the FAME profiles are a useful tool for the identification of *P. corrugata* when the bacteria have not been subcultured in vitro for a long time. Multivariate analyses of data showed that four clusters can be observed supporting the heterogeneity of the strains of *P. corrugata*.

**Abbreviations:** FAME – fatty acid methyl ester; MTBE – methyl tert-butyl ether.

**Introduction**

*Pseudomonas corrugata* Roberts and Scarlett (1981) is the etiological agent of tomato pith necrosis and is also reported as the responsible for a similar disease in pepper (López et al., 1994) and chrysanthemum (Fiori, 1992). It seems to be a ubiquitous bacterium also isolated from soil (Scortichini, 1990), water (Scarlett et al., 1978), alfalfa symptomless plants (Lukezic, 1979), wheat rhizosphere (Ryder & Borrett, 1990) and rice plants (Van Outryve et al., 1992) for which no rapid identification methods have been described.

*P. corrugata* forms wrinkled colonies on medium rich in glucose, but this character is not stable since, under repeated culturing, the colonies can become smooth (Lukezic, 1979). There are some biochemical differences between wrinkled and smooth forms of *P. corrugata* (Siverio et al., 1993).

Fatty acid analysis is a useful tool for the identification and classification of bacterial plant pathogens (Miller & Berger, 1985; Stead, 1988; Sasser, 1990; Stead et al., 1992). Most species of the genus *Pseudomonas* can be easily identified (Ikemoto et al., 1978; Oyaizu & Komagata, 1983; Takikawa, 1990; Janse, 1991a; Janse, 1991b; Stead, 1992), even though there are some problems with overlapping profiles, specially to distinguish pathovars (Stead, 1991; Stead, 1992). The whole cell fatty acid profile of *P. corrugata* has been precisely described by Stead (1992) as well as those of the plant pathogenic *Pseudomonas*. This profile has the characteristic peaks of the *Pseudomonas* rRNA group I. Besides, there are significant amounts of
five additional fatty acids (3-hydroxydodecanioic acid, 3-hydroxytetradecanoic acid, 3-hydroxyhexadecanoic acid, and two unknown additional fatty acids) with less than 2% of the whole profile peak area that allows to distinguish P. corrugata from other Pseudomonas (Stead, 1992). However, the fatty acid profiles of some strains identified as P. corrugata in Spain and obtained after extractions with hexane did not have these five characteristic fatty acids (López et al., 1994).

This research undertakes the study of the fatty acid methyl ester (FAME) profiles of P. corrugata with a wide collection of strains from different origins. The main differences observed among strains of P. corrugata and between wrinkled and smooth forms are described and the usefulness of the FAME profiles in the diagnosis of this bacterium is discussed.

Materials and methods

**Bacterial strains.** Table 1 lists the 80 strains of P. corrugata used in this study, identification of which had been previously confirmed (Siverio et al., 1993). They were routinely grown on King’s B medium (King et al., 1954) and peptone yeast glucose agar (PYGA: bactopeptone, 5 g/l; yeast extract, 5 g/l; glucose, 10 g/l; and agar, 20 g/l) and stored at -70 °C in glycerol (25% v:v). The latter medium was used to observe their colony morphology. Wrinkled and smooth colonies of the strains J.374, 903PD, 8894, 1.1.3, 1.2.3, 14.1, 29.1.r, 2445, 2451 and 313, grown on PYGA, were isolated to assess differences between fatty acid profiles of both forms.

**Extraction of FAMEs.** Bacteria were grown for 24 h at 28 °C on Trypticase Soy Broth Agar (TSBA: Trypticase Soy Broth (BBL # 11768), 30 g/l, plus Bactoagar (Difco # 0140), 15 g/l) and ca. 40 mg (wet weight) of cells were harvested. In order to obtain about the same total amount of cells, the total peak area was controlled afterward in the chromatographic profiles. FAMEs were obtained as described by Miller and Berger (1985). The extractions were performed twice with each strain: one with hexane/methyl-tert butyl ether (MTBE) (1:1) and the other with hexane alone. Extractions were performed in batches of 25–30 strains, simultaneously. To evaluate the variation due to culture and chromatographic conditions five strains were repeated four times for each extraction procedure. The extracts were analysed with the Microbial Identification System (MIS, Hewlett-Packard model 5898), controlled with the Microbial Identification System software (MIDI, Microbial ID, Inc., Newark, DE, USA) and using the Aerobe library (the Aerobe TSBA database, version 3.7, January 1993). The MIS includes a gas chromatograph with a 25 m × 0.2 mm 5% methylphenyl silicone fused silica capillary column (H2 as carrier gas), a flame-ionization detector, an automatic sampler, an integrator and a computer. It

<table>
<thead>
<tr>
<th>Location</th>
<th>Source1</th>
<th>Strains2</th>
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</thead>
<tbody>
<tr>
<td>France</td>
<td>L. Gardan</td>
<td>83, 83.4</td>
</tr>
<tr>
<td>Germany</td>
<td>S. Kön</td>
<td>374; 1,375; 1,609</td>
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<td></td>
<td>K. Naumann and E. Griesbach</td>
<td>V,45; Da.do.2</td>
</tr>
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<td>Italy</td>
<td>M. Scotichini</td>
<td>Pel,86; 903FS; 903PD; 903T</td>
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<tr>
<td>Japan</td>
<td>H. Kuwata and K. Okawa</td>
<td>Cl; E1; F1; G1</td>
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<td>New Zealand</td>
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<td>Spain</td>
<td>IVIA</td>
<td>536.1.1; 536.6.2; 536.7.1; 536.10.2; 542.1.1; 588.2.1; 588.3.1; 614.1; 614.4.1; 614.5.3; 632.2; 632.5; 712.2a; Ps.Cor.1; T6; T7; 1.1.3; 1.1.6; 1.2.3; 2.1; 5.4; 9.2; 12.3; 14.1; 14.4; 14.6; 1113.2; 1113.5; 29.1.r; 29.1.1; 592.4.4; 592.5.4 (Capsicum annuum)</td>
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<tr>
<td>Sweden</td>
<td>K. Olsson and P. Persson</td>
<td>53; 54</td>
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<tr>
<td>Switzerland</td>
<td>J. Vogelsanger and R. Grimm</td>
<td>6; 113; 490; 501; 580</td>
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<tr>
<td>United Kingdom</td>
<td>NCPPB</td>
<td>2445; 2447; 2449; 2450; 2451; 2455; 2456; 2457; 2458; 2903</td>
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<tr>
<td>United States</td>
<td>W. P. Bond and L. L. Black</td>
<td>Pс.2; Pс.3; Pс.11</td>
</tr>
<tr>
<td></td>
<td>J. B. Jones</td>
<td>JPс.3; JPс.4</td>
</tr>
<tr>
<td></td>
<td>F. L. Lukezic</td>
<td>792. 299; 313 (Medicago sativa)</td>
</tr>
</tbody>
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1 ICMP, International Collection of Microorganisms from Plants, Auckland, New Zealand; IVIA, Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain. NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, Britain. 2 Unless otherwise noted, the host was Lycopersicon esculentum.