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Erwinia chrysanthemi: description of two new biovars (bv 8 and bv 9) isolated from kalanchoe and maize host plants

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Summary — The bacterial phytopathogen Erwinia chrysanthemi may attack a wide spectrum of host plants. The species is officially divided into 6 pathovars on the basis of their host range. An alternative biovar classification which was based on biochemical criteria was proposed independently of pathogenicity (table I). Two new biovars are described in the present study. Biovar 8 contains 4 maize strains originating from India, France and the USA. Biovar 9 contains 3 kalanchoe strains from Denmark, France and Switzerland (table II). The 2 new biovars were found to differ from bvs 3 and 7 by the arginin dihydrolase test (table III). The validity of using the biovar system instead of the official pathovar classification for E chrysanthemi is discussed. Particularly, it takes into account the diversity of the species that occurs in more than 50 host plants.

Erwinia chrysanthemi / maize / kalanchoe / biovar / identification


Erwinia chrysanthemi / maïs / kalanchoe / biovar / identification

INTRODUCTION

Erwinia chrysanthemi (Burkholder et al, 1953) is a phytopathogenic bacterium which induces soft-rot and wilting. The bacterium attacks a wide range of host-plants, and occurs in many areas of the world (Bradbury, 1984). In phytobacteriology, infra-subspecific epithets were chosen as “pathovars”, terms currently used to designate organisms on the basis of their host range (Young et al, 1978). E chrysanthemi was first divided into 4 pathovars according to the host of origin: pv (pathovar) chrysanthemi from Chrysanthemum morifolium, pv dieffenbachiae from Dieffenbachia spp, pv parthenii from Parthenium argentatum and pv zeae from Zea mays. Then 2 more pathovars were added: pv dianthicola from Dianthus sp and pv paradisiaca from Musa paradisiaca. The 6 pathovars are listed in the last Bergey’s Manual of Systematic Bacteriology (Lelliott and Dickey, 1984) with the mention that “the relationship between pathogenicity, phenotypic properties and serological reactions of strains of the pathovars” is “not entirely clear”.

Since strains of E chrysanthemi have now been isolated from more than 50 plant species (Bradbury, 1984), it seems difficult to maintain the practice of naming the bacteria after the plant they come from. In the case of pathogenic differences between the isolates, host specificity seems difficult to prove (Dickey, 1981; Janse and Ruissen, 1988). However, physiological (biochemical) testing revealed differences between E chrysanthemi strains (Hildebrand et al, 1978; Samson and Nassan-Agha, 1978; Dickey, 1979; Dickey and Victoria, 1980; Thomson et al, 1981).
The biochemical differences have led to the classification into biovars (Samson et al, 1987) ie subdivisions of the bacterial species that could group all the strains showing the same biochemical profile. Seven biovars have been described using 10 biochemical tests (see table I). The present study adds 2 more biovars to the Erwinia chrysanthemi biovar system.

**MATERIALS AND METHODS**

**Bacteria**

The bacteria were isolated from 2 host-plants: Kalanchoe blossfeldiana and Zea mays (see table II). The strains were cultivated on LPA slants (yeast extract 3 g/l, peptone 5 g/l, agar 15 g/l, without glucose) that could be kept several months. Stocks of the strains were procured by freeze-drying.

**Characterization**

The identity of the strains was established by the criteria adopted in Bergey’s Manual (Lelliott and Dickey, 1984). The tests, performed according to Lelliott and Stead (1987) unless otherwise specified were: Gram reaction by KOH solubilization (Suslow et al, 1982), pectate degradation (Sutton’s medium; modified by Bonnet, 1973), oxidation/fermentation of glucose in Hugh and Leifson medium, gas production from d-glucose, starch hydrolysis, nitrate reduction, indole production from tryptophan, malonate utilization in ARJ medium (Ayers et al, 1919) by indicator shift (bromothymol blue), and lecithin hydrolysis on egg yolk medium.

**Biovar criteria**

The criteria for biovar definition (Samson et al, 1987) were as follows: growth at 39 °C, anaerobic degradation of arginine (ADH) according to Moeller (1955), inulin assimilation in phenol red peptone water; other carbon sources were tested by acidification/alkalinisation of ARJ liquid medium (bromothymol blue) mixed with 0.3% of the carbohydrate: α(-)-arabinose, 5-ketogluconate, mannitol, melibiose, raffinose and α(-) tartrate. Cis-aconitate was discarded because of the variability of the result for the same strain.

**API galleries**

Some criteria can be obtained by using API galleries (La Balme-les-Grottes, 38390 Montalieu Vercieu, France) such as arginine dihydrolase, indole, mannitol and melibiose from the API 20E, and α(-)-arabinose, 5-ketogluconate, inulin, mannitol, melibiose and raffinose from API 50CHE, provided a longer period of time is used than specified (4 to 6 days).

**RESULTS**

The strains tested were identified as E chrysanthemi based on the negative Gram reaction, glu-
cose fermentation, nitrate reduction, Sutton poly-
pectate acidification and liquefaction, no starch
hydrolysis, gas production from d-glucose, indole
production from tryptophan, malonate alkaliniza-
tion and lecithin hydrolysis.

When the biovar criteria were examined, none
of the strains studied corresponded to any of the
7 previously described biovars. The 4 Zea mays
strains, which show the same new profile, are
grouped in the biovar 8 category, while the Ka-
lanchoe strains, displaying other common char-
acteristics, are contained in the biovar 9 (see ta-
ble III).

DISCUSSION

Two new biovars that differ from the 7 previously
described biovars were found. They differed only
in the ADH test. Biovar 8 is a biovar 3 that could
be ADH positive, and biovar 9 is a biovar 7 that
appears to react as ADH negative. However, the
ADH test must not be considered as a variable
test. When it is performed according to Moeller
(1955), ie in the presence of 0.05% glucose
(which allows better growth) and pyridoxal phos-
phate (which promotes the decarboxylase activi-
ty), the results are consistent for a given strain.
This method was successfully applied to some Erwinia by Zherebilo and Gvozdyak (1976). It
proved to be more sensitive in our laboratory for
fermentative bacteria than Thornley’s test
(1960).

To allow comparisons between laboratories,
we would recommend the use of previously des-
cribed methods. We also designate reference
strains for the new biovars: CFBP 1447 (NCPPB
2546) for bv8, and CFBP 1805 (Dinesen EKI) for
bv9.

Table II. Origin of the bacteria. a: CFBP (French Collection of Phytopathogenic Bacteria), INRA Angers, France; b:
National Collection of Plant Pathogenic Bacteria, ADAS Harpenden, Great Britain.

<table>
<thead>
<tr>
<th>Strain No</th>
<th>Host plant</th>
<th>Yr of isolation</th>
<th>Country</th>
<th>Author’s No</th>
<th>NCPPB equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1447</td>
<td>Zea mays</td>
<td>1969</td>
<td>India</td>
<td>Payak (17)</td>
<td>2546</td>
</tr>
<tr>
<td>1528</td>
<td>Zea mays</td>
<td>1966</td>
<td>USA</td>
<td>Kelman (W3.20/SR80)</td>
<td>2541</td>
</tr>
<tr>
<td>1531</td>
<td>Zea mays</td>
<td>1966</td>
<td>USA</td>
<td>Kelman (W1.1/SR62)</td>
<td>–</td>
</tr>
<tr>
<td>1596</td>
<td>Zea mays</td>
<td>1974</td>
<td>France</td>
<td>Samson (237-3)</td>
<td>–</td>
</tr>
<tr>
<td>1805</td>
<td>Kalanchoe blossfeldiana</td>
<td>1977</td>
<td>Denmark</td>
<td>Dinesen (EK II)</td>
<td>–</td>
</tr>
<tr>
<td>2598</td>
<td>Kalanchoe blossfeldiana</td>
<td>1985</td>
<td>Switzerland</td>
<td>Grimm (531)</td>
<td>–</td>
</tr>
<tr>
<td>2982</td>
<td>Kalanchoe blossfeldiana</td>
<td>1988</td>
<td>France</td>
<td>Samson (SH 170-1)</td>
<td>–</td>
</tr>
</tbody>
</table>

Table III. Description of biovars 8 and 9. T: the strains 1447 and 1805 are chosen as references for the new biovars,

<table>
<thead>
<tr>
<th>Biovars</th>
<th>Strain No</th>
<th>39 °C</th>
<th>ADH</th>
<th>d-Arabinose</th>
<th>5-ketoogluconate</th>
<th>Inulin</th>
<th>Mannitol</th>
<th>Melibiose</th>
<th>Raffinose</th>
<th>d-Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>bv8</td>
<td>1447</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1528</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1531</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1596</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1805</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>bv9</td>
<td>2598</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2982</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Erwinia chrysanthemi, new biovars
The classification of the kalanchoe strains into a new biovar is consistent with the results of Dinesen (1979) who described his strains pathogenic to kalanchoe as defined by ADH, melibiose, raffinose and d(-)arabinose negative tests. He concluded that the strains he tested did not fit in the 3 biovars known at that time. Similarly, Janse and Ruissen (1988), applying the biovar system to classify the E chrysanthemi isolated from the Netherlands, found that their own kalanchoe isolates could belong to biovar 7, but with a negative ADH. It thus seems that the creation of biovar 9 is enhanced.

The question of relationships between host plants and biovars has again arisen. The majority of the maize strains studied belong to either biovar 3 (Samson and Nassan-Agha, 1978; Dickey, 1979 whose subdivision IV is equivalent to bv3), or to biovar 8 (this study). But biovar 3 is not restricted to maize strains, since it harbour isolates from many other plants: Aechmea sp, Aglaonema, Ananas, Chrysanthemum morifolium, Cyclamen, Dieffenbachia, Dracaena, Euphorbia sp, Ipomoea sp, Musa sp, Pelargonium, Phalaenopsis, Philodendron, Saintpaulia, Syngonium. Biovar 9 was created for the 3 kalanchoe strains studied. The fact that these strains were from different origins, such as Denmark, Switzerland and France, leads us to hypothesize that biovar 9 could be linked to the host of origin. However, one Dianthus sp strain from the Netherlands may belong to the same biovar (Janse and Ruissen, 1988).

In fact, with 9 criteria that may be plus or minus, one could mathematically expect 29 = 512 combinations. Up to now, 9 biovars have been discovered. It seems evident that if other biovars were found in nature, they would probably be less numerous than 512. The biovar system is therefore proposed to classify E chrysanthemi isolates independently of their host. In the case of biochemical differences being detected between the strains, it only means that the strains are "biochemical variants". The taxonomic value of such variants must be estimated on wide collections of bacteria. Such a need was expressed during an EPPO conference held in 1985 in Wageningen, on "the new diagnostic techniques in plant protection". The biovar distribution would give a biological structure to the diversity of this bacterial species. In the Netherlands, 41 strains were found to belong to 3 biovars (Janse and Ruissen, 1988). In our laboratory, a study of almost 200 strains is being carried out isolated from 27 host plants and originating from 5 continents (Samson et al, 1990). The aim is to confirm the respective weight of each biovar, in order to propose a true taxonomical subdivision of E chrysanthemi species instead of the confusing pathovar classification.

ACKNOWLEDGMENTS

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Erwinia chrysanthemi, new biovars


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