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MELINDA SULLIVAN
APHIS
USDA/APHIS/PPQ/CPHST Suite 108
2301 Research Blvd
Ft Collins, CO 80526

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DELIVERY: E-mail Post to Web: melinda.j.sullivan@aphis.usda.gov
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Reclassification of the Causal Agents of Bacterial Bight (Xanthomonas campestris pv. oryzae) and Bacterial Leaf Streak (Xanthomonas campestris pv. oryzicola) of Rice as Pathovars of Xanthomonas oryzae (ex Ishiyama 1922) sp. nov., nom. rev.

J. SWINGS, M. VAN DEN MOOTER, L. VAUTERIN, B. HOSTE, M. GILLIS, T. W. MEW, AND K. KERSTERS

Laboratorium voor Microbiologie en microbiële Genetica, Rijksuniversiteit, Ledeganckstraat, 35, B-9000 Ghent, Belgium, and International Rice Research Institute, Plant Pathology Department, Manila, Philippines

On the basis of phenotypic, genotypic, and chemotaxonomic data, Xanthomonas oryzae (ex Ishiyama 1922) sp. nov., nom. rev. is proposed; this species comprises Xanthomonas oryzae pv. oryzae (Ishiyama 1922) comb. nov. and Xanthomonas oryzae pv. oryzicola (Fang, Ren, Chen, Chu, Faan, and Wu 1957) comb. nov., the causal agents of bacterial bight and bacterial leaf streak of rice, respectively.

Bacterial bight and bacterial leaf streak of rice are two important and well-known rice diseases (11). Bacterial bight on rice is caused by Xanthomonas campestris pv. oryzae (Ishiyama 1922) Dye 1978 (5) and has been known in Japan for over a century (11). The discovery and description of bacterial leaf streak, which is caused by Xanthomonas campestris pv. oryzicola (Fang, Ren, Chen, Chu, Faan, and Wu 1957) Dye 1978 are more recent (6). In this paper we summarize all of the available data indicating that the present classification of these xanthomonads is unsatisfactory, and we propose that they should be reclassified as pathovars of Xanthomonas oryzae (ex Ishiyama 1922) sp. nov., nom. rev. (7).

Both pathovars belong to the genus Xanthomonas. DNA-rRNA hybridizations (4) have confirmed that both X. campestris pv. oryzae and X. campestris pv. oryzicola belong to the genus Xanthomonas, in a separate rRNA branch of the gamma group of the Proteobacteria (12).

The phenotypic features of both organisms (15) fit the phenotypic description of the genus Xanthomonas as given in Bergey's Manual of Systematic Bacteriology (2).

The DNA base composition (guanine-plus-cytosine content) is 64.6 mol% for X. campestris pv. oryzae and 65 mol% for X. campestris pv. oryzicola (15). These values fall within the guanine-plus-cytosine content range of the genus Xanthomonas (63 to 71 mol%) (2).

Neither pathovar belongs to any previously described Xanthomonas species. On the basis of an extensive phenotypic study of 295 characteristics of all Xanthomonas species and 119 X. campestris pathovars (a total of 266 strains), eight phena were differentiated within the genus Xanthomonas; these phena corresponded to X. albilineans, X. axonopodis, X. campestris, X. fragariae, "X. graminis," X. maltophilia, "X. populi," and a group of 10 X. campestris pv. oryzae strains (14).

The fatty acid profiles of X. campestris pv. oryzae and X. campestris pv. oryzicola were very similar, but there were differences in the presence of 12:0 iso3OH, 12:0 3OH, and 15:0 anteiso fatty acids. Both profiles were very different from those of all other X. campestris pathovars investigated (13).

Comparisons of sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein patterns allowed Kersters et al. (8) to separate X. campestris pv. oryzae and X. campestris pv. oryzicola from each other and from X. albilineans, X. axonopodis, "X. populi," X. fragariae, and X. campestris pv. campestris, as well as from various X. campestris pathovars isolated from grasses and cereals.

Six strains of X. campestris pv. oryzae and X. campestris pv. oryzicola were genotypically highly related (average level of DNA binding, 91%) (Table 1), which means that they constitute one species. The low DNA binding values in the rest of Table 1 indicate that neither the type strains of X. campestris and the five other species tested nor the other X. campestris pathovars of grasses and cereals are related to the X. campestris pv. oryzae-X. campestris pv. oryzicola group at the species level. The level of relatedness that seems to exist between X. oryzae and X. axonopodis needs further investigation. The results of all of the widely different methods described above support the creation of a separate species.

Both pathogens clearly belong to the genus Xanthomonas but cannot be assigned to the species X. campestris, X. fragariae, X. albilineans, X. axonopodis, "X. populi," or X. maltophilia. The data described above support transfer of both pathogens to a new species, for which we propose the name Xanthomonas oryzae (Ishiyama 1922) nom. rev.

Description of Xanthomonas oryzae (Ishiyama 1922) sp. nov., nom. rev. Xanthomonas oryzae (o.r.y’zae. Gr. n. oryza, rice; M. L. gen. n. oryzae, pertaining to rice). The description of X. oryzae is the same as the description of the genus. The phenotypic characteristics described below are based on the data of Vera Cruz et al. (15) and Van den Mooter (Ph.D. thesis, Rijksuniversiteit, Ghent, Belgium, 1984). Cells are straight rods, 0.4 to 0.8 by 1.5 to 2.9 μm. Gram negative. Motile by means of a single polar flagellum. Cells occur singly, in pairs, or sometimes in chains. Filaments may occur. Obligately aerobic. Catalase present. Indole formation, 2-ketogluconate formation, urease, egg yolk hydrolysis, nitrate reduction, and oxidase are all negative. Carbon sources are used oxidatively and never fermentatively. Starch is hydrolyzed after 7 days. Growth occurs on D-xylose, D-glucose, D-fructose, D-galactose, cellobiose, sucrose, trehalose, sodium fumarate, sodium L-lactate, sodium L-malate, sodium oxaloacetate, and sodium succinate, but not on L-arabinose, D-ribose, D-ribose, lactose, raffinose,
TABLE 1. DNA-DNA hybridization between X. oryzae strains and strains of X. campestris pathovars from grasses and cereals or type strains of Xanthomonas species*

<table>
<thead>
<tr>
<th>Strain*</th>
<th>% of DNA binding to strain**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMG 5047†</td>
</tr>
<tr>
<td>X. oryzae pv. oryzae LMG 5047† (= PDDCC 3125†)</td>
<td>100</td>
</tr>
<tr>
<td>X. oryzae pv. oryzae LMG 795 (= NCPPB 1153)</td>
<td>90</td>
</tr>
<tr>
<td>X. oryzae pv. oryzae LMG 6518 (= IRN 235)</td>
<td>87</td>
</tr>
<tr>
<td>X. oryzae pv. oryzicola LMG 797 (= NCPPB 1585)</td>
<td>80</td>
</tr>
<tr>
<td>X. oryzae pv. oryzicola LMG 665 (= PDDCC 4654)</td>
<td></td>
</tr>
<tr>
<td>X. oryzae pv. oryzicola LMG 793 (= NCPPB 1151)</td>
<td></td>
</tr>
<tr>
<td>X. campestris pv. kholcicola LMG 736T (= NCPPB 2417)</td>
<td>44</td>
</tr>
<tr>
<td>X. campestris pv. coranocianae LMG 686 (= NCPPB 1786)</td>
<td>30</td>
</tr>
<tr>
<td>X. campestris pv. vasconilum LMG 901 (= NCPPB 796)</td>
<td>38</td>
</tr>
<tr>
<td>X. campestris pv. phleipratensis LMG 843 (= NCPPB 1837)</td>
<td></td>
</tr>
<tr>
<td>X. campestris pv. translacens LMG 876 (= NCPPB 973)</td>
<td>10</td>
</tr>
<tr>
<td>X. campestris pv. undulosa LMG 892 (= NCPPB 2821)</td>
<td>14</td>
</tr>
<tr>
<td>X. campestris pv. cerea LMG 679 (= NCPPB 1944)</td>
<td>15</td>
</tr>
<tr>
<td>X. campestris pv. hordei LMG 737 (= NCPPB 2389)</td>
<td>17</td>
</tr>
<tr>
<td>X. campestris pv. pisi LMG 728 (= ATCC 33804)</td>
<td>8</td>
</tr>
<tr>
<td>X. campestris pv. campestris LMG 568† (= NCPPB 258†)</td>
<td>22</td>
</tr>
<tr>
<td>X. axonopodis LMG 538T† (= NCPPB 457†)</td>
<td>34</td>
</tr>
<tr>
<td>X. albilineans LMG 494† (= NCPPB 2969†)</td>
<td></td>
</tr>
<tr>
<td>X. fragariae LMG 706 (= F1 Benaki)</td>
<td></td>
</tr>
<tr>
<td>X. populi LMG 574† (= CNBP 1817†)</td>
<td>19</td>
</tr>
<tr>
<td>X. maltophilia LMG 988† (= ATCC 13637†)</td>
<td></td>
</tr>
</tbody>
</table>

* Data of Hoste and Gillis.
† Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CNBP, Collection Nationale des Bactéries Phytopathogènes, Angers, France; IRN, isolated record number; LMG, Collection of the Laboratory for Microbiology, Gent, Belgium; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England; PDDCC, Culture Collection of Plant Disease Division, Auckland, New Zealand.
** The degrees of DNA binding were determined spectrophotometrically from the initial renaturation rates by using the method of De Ley et al. (3). The optimal renaturation temperature was 81.3°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7). The values are the means of at least two experiments.
†† Type strain.

Inulin, methanol, ethanol, n-propanol, ethanediol, mesoinositol, sorbitol, dulcitol, adenine, guanine, cytosine, thymine, sodium-potassium tartrate, sodium glyoxalate, 2-ketogluconic acid, tannic acid, p-hydroxybenzoic acid, hydroquinone, phosphogluconic acid, resorcinol, amygdalin, arbutin, or esculin. Acid is formed from D-glucose, D-fructose, D-galactose, cellobiose, and trehalose but not from D-ribose. L-rhamnose, salicin, sorbitol, meso-inositol, dulcitol, adonitol, maltose, lactose, or inulin. Lactose is never acidified. No growth occurs at 4 or 35°C or in the presence of 3% NaCl. Weak growth occurs at 32 and 10°C. Esccula, TWEEN 40, and TWEEN 80 are hydrolyzed. H₂S is formed. Growth is inhibited in media containing 0.001% (wt/vol) tetracycline hydrochloride, 0.005% chloramphenicol, 0.001% novobiocin, or 0.001% doxycycline, but not in media containing 0.001% trimethoprim or 0.005% metronidazole.

X. oryzae can be differentiated phenotypically from X. campestris by the following two features, for which X. oryzae is negative and X. campestris is positive: lecithinase activity and growth on sodium-propionate as a carbon source. The most reliable way to differentiate X. oryzae from other Xanthomonas species is by using sodium dodcyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins (8), fatty acid profiles (13), and specific monoclonal antibodies (1) or by DNA-DNA hybridization.

The guanine-plus-cytosine values range from 64.6 to 65 mol% . The neotype strain is strain PDDCC 3125 (= NCPPB 3002 = Dye YK9 = Rao X08 = LMG 5047).

X. oryzae contains the causal agents of bacterial blight and leaf streak of rice. The two groups constitute a single DNA cluster and species in which no other X. campestris pathovar of grasses or cereals belongs (Table 1). A DNA-DNA binding value of 86% is too high to create a subspecies for each group. Nevertheless, the two pathovars are phylogenetically and phenotypically so clearly different that we propose to keep them as separate pathovars within X. oryzae sp. nov., nom. rev. We propose the two pathovars described below.

Xanthomonas oryzae pv. oryzae (Ishiyama 1922) comb. nov. causes leaf blight or streak symptoms on rice (11). Bacterial blight occurs in Asia, the Americas, Africa, and Australia and in tropical and temperate climates. The pathovar reference strain is strain PDDCC 3125 (= NCPPB 3002 = Dye YK9 = Rao X08 = LMG 5047). Currently six pathogenic races are identified in the Philippines on the basis of differential cultivars (9, 10), but many more are suspected. No correlation was found between phenotype or protein electrophoretic fingerprinting on the one hand and the pathogenic races on the other hand (15).

Xanthomonas oryzae pv. oryzicola (Fang, Ren, Chen, Chu, Faan, and Wu 1957) comb. nov. causes bacterial leaf streak symptoms on rice. It is widely distributed in tropical Asia. The pathovar reference strain is strain PDDCC 5743 (= NCPPB 2015 = ICPB X011 = Hayward B2115 = LMG 797). No pathogenic races have been distinguished.

Differentiation between X. oryzae pv. oryzae and X. oryzae pv. oryzicola is possible by (i) the symptoms caused on rice (11), (ii) a few phenotypic features (15), (iii) polyacrylamide gel electrophoresis protein fingerprints (8, 15), (iv) fatty acid profiles (13), and (v) pathovar-specific monoclonal antibodies (1).

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LITERATURE CITED


