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Reclassification of the Causal Agents of Bacterial Blight (*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.

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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 blight and bacterial leaf streak of rice, respectively.

Bacterial blight and bacterial leaf streak of rice are two important and well-known rice diseases (11). Bacterial blight 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.ry'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, L-rhamnose, lactose, raffinose,

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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[a]

Strain[b]	% of DNA binding to strain[c]:					
	LMG 5047^{T} [d]	LMG 795	LMG 6518	LMG 797	LMG 665	LMG 793
X. oryzae pv. *oryzae* LMG 5047^{T} (= PDDCC 3125^{T})	100					
X. oryzae pv. *oryzae* LMG 795 (= NCPPB 1153)	90	100				
X. oryzae pv. *oryzae* LMG 6518 (= IRN 235)	87		100			
X. oryzae pv. *oryzicola* LMG 797 (= NCPPB 1585)	80			100		
X. oryzae pv. *oryzicola* LMG 665 (= PDDCC 4654)		92		100	100	
X. oryzae pv. *oryzicola* LMG 793 (= NCPPB 1151)				96		100
X. campestris pv. *holcicola* LMG 736T2 (= NCPPB 2417)	44			46		
X. campestris pv. *coracanae* LMG 686 (= NCPPB 1786)	30					
X. campestris pv. *vasculorum* LMG 901 (= NCPPB 796)	38			53		
X. campestris pv. *phleipratensis* LMG 843 (= NCPPB 1837)				10		
X. campestris pv. *translucens* LMG 876 (= NCPPB 973)	10			12		
X. campestris pv. *undulosa* LMG 892 (= NCPPB 2821)				14		
X. campestris pv. *cerealis* LMG 679 (= NCPPB 1944)				15		
X. campestris pv. *hordei* LMG 737 (= NCPPB 2389)				17		
X. campestris pv. *poae* LMG 728 (= ATCC 33804)	8					
X. campestris pv. *campestris* LMG 568^{T} (= NCPPB 528^{T})	22			7		
X. axonopodis LMG 538T1^{T} (= NCPPB 457^{T})	34			42		
X. albilineans LMG 494^{T} (= NCPPB 2969^{T})				14		
X. fragariae LMG 706 (= F1 Benaki)				22		
X. populi LMG 5743^{T} (= CNBP 1817^{T})				19		
X. maltophilia LMG 958^{T} (= ATCC 13637^{T})				26		

[a] Data of Hoste and Gillis.
[b] 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.
[c] 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.
[d] T = type strain.

inulin, methanol, ethanol, *n*-propanol, ethanediol, *meso*-inositol, sorbitol, dulcitol, adenine, guanine, cytosine, thymine, sodium-potassium tartrate, sodium glyoxylate, 2-ketogluconic acid, tannic acid, *p*-hydroxybenzoic acid, hydroquinone, phloroglucinol, 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. Litmus milk 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. Esculin, Tween 40, and Tween 80 are hydrolyzed. H_2S 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 sodium dodecyl 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(= NCCPPB 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 phytopathologically 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 kresek 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 1585 = ICPB X0111 = 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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