Identification of Low-Virulence Strains of *Xanthomonas campestris* pv. *oryzae* from Rice in the United States

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ABSTRACT

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Rice plants exhibiting water-soaked lesions characteristic of a disease of bacterial etiology were observed on approximately 2,500 ha in Texas during the 1987 production season. Symptoms appeared following 20 days of nearly continuous rainfall that exceeded 50 cm at certain locations in the region. Lesions typically were associated with the leaf tips and edges, initially were water-soaked, and eventually appeared as bleached white to tan necrotic areas averaging 1–2 × 3–10 cm. Bacterial streaming was consistently associated with the vascular bundles of sectioned leaf specimens. *Xanthomonas campestris* pv. *oryzae* was recovered from plants exhibiting this symptom in 43 commercial fields in Texas and one field in Louisiana. Symptoms only were observed on recently introduced semidwarf cultivars derived from the suscept Taichung Native 1 (TN-1). Inoculation of 3-wk-old and 7-wk-old rice plants with bacteria isolated from lesions confirmed pathogenicity and reproduced symptoms observed in the field. Comparison of the causal organism with Asiatic strains of *X. c. oryzae* by standard biochemical tests, light and electron microscopy, fatty acid analysis, analysis of restriction fragment length polymorphisms, and reaction to monoclonal antibodies revealed numerous similarities but some consistent differences. Virulence of the U.S. strains on TN-1 was much reduced compared with strains from the Philippines. This is the first report of *X. c. oryzae* in the United States.

Additional keywords: bacterial leaf blight, *Oryza sativa*.

Bacterial leaf blight of rice (*Oryza sativa* L.) is caused by *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye. The pathogen is known to occur in all rice-growing areas of Asia (29) and recently has been reported from Australia (1) and West Africa (31). Lozano (21) reported the first occurrence of bacterial leaf blight in rice-growing countries in the Western Hemisphere including Colombia, Venezuela, and Bolivia in South America and Costa Rica, El Salvador, Honduras, Mexico, and Panama in Central America. Ou (28) also observed rice with symptoms of bacterial leaf blight in Ecuador and Panama.

In tropical Asia, yield loss in fields severely affected by bacterial leaf blight ranges from 20–30% and may approach 50% (32). The disease has become a major constraint on rice production in many regions of the world where nitrogen-responsive, semi-dwarf cultivars have been deployed without the incorporation of appropriate resistance (29).

The plant-to-plant spread of bacterial leaf blight is favored by intense wind-driven rainfall, which not only moves the bacterium, but also provides fresh injury sites along leaf edges that facilitate entry (26,29). The bacterium also may enter hydathodes (located along the edges of rice leaves), multiply in the epitheme, and finally gain access to major vessels (34). Colonization of the vascular system and subsequent lesion enlargement can proceed as rapidly as 2 cm/day when virulent Asiatic strains are inoculated into susceptible cultivars (4,10,24). Seedlings can become infected through broken root sites in transplanted rice, resulting in the kresek phase of the disease (33). This syndrome is rare in production systems where rice is direct seeded (29).

In June 1987, rice plants exhibiting water-soaked lesions characteristic of a disease of bacterial etiology were observed on approximately 2,500 ha in Texas representing 43 commercial fields. Isolations from such lesions resulted in recovery of three distinct groups of yellow-pigmented bacteria. Preliminary biochemical, serological, and genomic characterization and pathogenicity testing of each of these bacterial groups is presented.

MATERIALS AND METHODS

Isolation from diseased tissue. Water-soaked lesions and necrotic tissue from diseased leaves were hand sectioned perpendicular to vascular bundles and placed on glass slides. Sterile distilled water was applied to the cut surface. Tissue that exhibited bacterial streaming from the cut edge was surface sterilized and macerated in 12.5 mM potassium phosphate buffer, pH 7.0. Tissue and diluent were initially streaked to the following media: nutrient broth yeast extract agar (NBYA) (37), modified Silva-Buddenhagen medium (SB) (29), and Wakimoto's WFP medium (WFP) (29). All media were supplemented with cycloheximide at 40 μg/ml. Plates were incubated 4–5 days at 25 C and examined daily for colony formation. Individual colonies were picked and purified by the agar streak method. At least three single-colony isolations were made to ensure purity. Working strains were maintained on NBYA, and stored strains were kept at −20 C in NBY broth containing 10% glycerol.

Characterization of bacterial groups. Three distinct groups of bacterial strains were recovered from diseased rice. Thirty-two strains were examined for morphological, physiochemical, and biochemical features: 26 from group I and three each from groups II and III. Control strains of *X. c.* pv. *vesicatoria* (Doidge) Dye and *X. c.* pv. *campestris* (Pammel) Dowson also were included. Gram staining was performed on 48-hr cultures grown on nutrient agar (Difco Laboratories, Detroit, MI). Growth at 25, 28, 36, and 41 C was evaluated after 96 hr in shake cultures of nutrient broth (Difco). Oxidase tests were performed as described by Kovacs (19). Catalase activity, casein hydrolysis, starch hydrolysis, acetoin production, indole production, urease production, and nitrate reduction were determined as described by Dye (8). Gelatin hydrolysis, aesculin hydrolysis, phenylalanine deaminase activity, reduction of potassium tellurite (K_2TeO_3), acid formation from carbohydrates, growth on carbon sources, and oxidative-

fermentative tests were performed as described by Vera Cruz et al (36). Production of H_2S from L-cysteine was determined by the method of Dye (9). Pectinase activity was tested by the procedures of Beraha (6) and Hildebrand (12). Tolerance to triphenyl tetrazolium chloride was measured at 0.02 and 0.1% as described by Lovrekovich and Klement (20). All tests were performed at 28 C unless otherwise indicated. Hypersensitivity of tobacco (*Nicotiana tabacum* L. 'Hicks') was determined on 35-day-old plants by the method of Gitaitis et al (11).

Bacterial strains tentatively identified as potential *Xanthomonas* spp. were examined for flagellation by light microscopy (×1,000) with a silver impregnation stain (7) and by transmission electron microscopy (TEM). For TEM examination, a cell suspension (10^5 colony-forming units [cfu]/ml) was applied to carbon-backed, collodion-coated grids and allowed to incubate for 15 min. The grid was drained and cells were stained with phosphotungstate. Strains were grown on NBYA for 48 hr before treatment and suspended in sterile distilled water.

Absorbance spectra of pigments. Pigment extracts from 12 test strains (six of group I and three each of groups II and III) were spectrophotometrically analyzed and subjected to thin-layer chromatography as described by Irey and Stall (15). Bacterial cells were grown on nutrient agar for 48 hr at 28 C. The absorption spectrum of the pigment extracts was determined with a Beckman DU-64 recording spectrophotometer (Beckman Instruments, Palo Alto, CA).

Cellular fatty acid analysis. Total cellular fatty acids were analyzed by previously described methods (11,23,25,27). Twelve test strains, six of group I and three each of groups II and III were grown on trypticase soy broth agar (TSBA) and analyzed. Fatty acid profiles were computer matched to bacteria contained in the TSBA aerobic library (Microbial Identification System Software catalog, version 3.0, Microbial ID, Inc., Newark, DE). Groups also were compared with Asiatic strains of *X. c. oryzae* obtained from bacteria-blighted rice in the Philippines (strain PXO 61), Japan (JXO H75-304), and India (IXO 1), with three strains of *X. c. oryzicola* (Fang et al) Dye (BLS 175, BLS 292, and BLS 303) collected from Philippine rice exhibiting symptoms of the bacterial leaf streak disease (29), and with three strains of the bacterial brown blotch organism (22,36) obtained from J. Carlos Lozano (Centro Internacional Agricultura Tropical, Cali, Colombia) as CIAT 1171, 1173, and 1192.

Restriction fragment length polymorphisms. Genomic fingerprints of 12 bacterial strains obtained from symptomatic plants in this study (six of group I and three each of groups II and III) were analyzed. Groups were compared to Asiatic strains of *X. c. oryzae* (PXO 61, PXO 86, PXO 99, and 27 others) and *X. c. oryzicola* (BLS 175, BLS 292, and BLS 303). Strains also were compared with *X. c. translucens* (Jones et al) Dye (NEB 101, XT 110, and XT 115) and with the brown blotch organism (CIAT 1171, CIAT 1173, and CIAT 1192) from Colombia. Bacteria were maintained at 28 C on peptone sucrose agar.

DNA was extracted from bacterial cultures at late logarithmic growth phase. Extraction of total bacterial DNA was by a modification of the method of Owen and Borman (30). Modifications were that pelleted cells were first lysed at room temperature in a mixture containing 50 mM glucose, 25 mM Tris (pH 8.0), 10 mM ethylenediaminetetraacetic acid (EDTA), and 2 mg/ml lysozyme. In addition, after treatment with sodium dodecyl sulfate (SDS) (0.5%), RNase A (0.1 mg/ml), and protease (0.1 mg/ml), the lysate was extracted with an equal volume of phenol (saturated with 0.1 M Tris-HCl, pH 8.0), then phenol and chloroform-isoamyl alcohol (24:1), and finally chloroform-isoamyl alcohol (24:1).

DNA (10 μg) was digested to completion with *Eco*RI (1 unit enzyme per 2 μg of DNA) at 37 C for 2–3 hr in buffers provided by Bethesda Research Laboratories, Bethesda, MD. The DNA fragments (2 μg/well) were separated in 20 × 21.5 cm horizontal 0.7% agarose gels immersed in Tris-borate buffer (89 mM Tris-HCl, 89 mM boric acid, and 2 mM Na_2EDTA, pH 8.0) by electrophoresis at 2.5 v/cm for 24 hr. Fragments were stained with ethidium bromide (0.5 μg/ml) and visualized by ultraviolet irradiation. A 1-kilobase-pair ladder (Bethesda Research Laboratories) was included as a size standard. All strains were analyzed three times.

Serological affinities. The procedures used to produce the monoclonal antibodies have been described (3,5). Five monoclonal antibodies were used to differentiate the bacterial groups. XI and XII are genus-specific antibodies that react with numerous members of the genus *Xanthomonas*, including *X. c. oryzae*, but do not react with other taxa (2). Xco-1 is a pathovar-specific monoclonal antibody that reacts with 178 tested strains of *X. c. oryzae* from diverse geographical regions (5). Monoclonal antibody Xco-2 reacts with most, but not all, Asiatic strains of *X. c. oryzae* (5). Monoclonal antibody Xco-5 was generated from isolates of group I collected in this study. This monoclonal antibody was specific for isolates of *X. c. oryzae* collected in this study but failed to react with Asiatic isolates (5).

Formalinized bacterial cells washed three times in phosphate-buffered saline (PBS), resuspended in 0.05 M carbonate-bicarbonate buffer (pH 9.6), and adjusted to $A_{600nm} = 0.1$ were used for enzyme-linked immunosorbent assay (ELISA). Polyvinyl chloride 96-well plates (Costar, Cambridge, MA) were coated with 100 μl of the cell suspensions by drying in a 37 C circulating air incubator. The plates were blocked for 15 min with 5% BLOTTO (17) in PBS and washed once with borate buffer (pH 8.3). One hundred microliters of each of the following reagents (each diluted in a 1:3 dilution of 5% BLOTTO in borate buffer) were added sequentially: monoclonal antibody, 1:1000 rabbit anti-mouse globulin, and 1:1000 protein-A-horseradish peroxidase (Bio-Rad Laboratories, Richmond, CA). Plates were incubated 1 hr at room temperature and washed three times with borate buffer after the addition of each reagent. Finally, substrate consisting of 0.05% 5-amino-salicylic acid and 0.06% H_2O_2 in phosphate-EDTA buffer (3) was added, and after 1 hr, absorbance was measured at 450 nm with a Titertek Multiskan plate reader (Flow Laboratories, Inglewood, CA). Positive reactions had ELISA values (A_{450}) above 0.3, whereas negative reactions were in the range of 0–0.1.

Pathogenicity testing. The pathogenicity of strains was tested on susceptible seedling and adult rice cultivars. Seedling tests used the leaf-clipping method (18) to introduce bacteria into 21-day-old potted plants of the Lemont cultivar. Pots contained approximately 50 plants. Treatments were replicated three times. Inoculations were repeated twice. Inoculum was prepared by suspending cells from NBYA cultures 48–72 hr old in sterile distilled water to obtain 10^9 cfu/ml. Seedling reaction was evaluated after 7 and 14 days of incubation in the greenhouse. Bacteria were reisolated from the advancing margin of lesions, and identity was confirmed by fatty acid analysis.

Virulence of strains was assessed on 49-day-old plants of the rice cultivars TN-1, IR-8, and Lemont. The double-needle method of inoculation (4) was used to introduce bacteria into the three uppermost leaves of three plants in each pot. Asiatic strains (PXO-61 and PXO-86) were included for comparison. Treatments were replicated three times and arranged on a greenhouse bench in a completely random design. The experiment was repeated once. Virulence was determined on the basis of lesion length initiated by individual strains 14 days after inoculation.

RESULTS

Description of disease. Symptoms on diseased plants collected in the field were characterized by water-soaked lesions typically associated with the margins of fully developed leaves. Lesions were initially 5–10 mm wide and varied in length from 40 to 60 mm. On mature leaves, lesions measured 10–20 mm wide and varied in length from 40 mm up to 200 mm in rare cases. Affected tissue did not remain water-soaked but turned chlorotic yellow and then necrotic (white to light tan). After 5–7 days, a greyish growth of saprophytic fungi was visible on the surface of lesions. Early in development, lesions exhibited wavy margins (Fig. 1), but mature lesions were vein delimited to some extent and bounded by a reddish brown necrotic strip.

Symptoms were observed on plants in 43 commercial fields in six geographically separated counties in Texas. Isolations also were made from symptomatic tissue from one field in Louisiana. Symptoms were observed only on the recently released semidwarf cultivars Lemont (CI 475833) and Gulfmont (CI 502967).

Isolation and preliminary characterization. Symptomatic rice tissue consistently yielded three distinct groups of bacteria that produced yellow colonies on NBYA or SB medium. Groups II and III were apparent after 48-hr incubations, whereas group I strains were not visible until after 72–96 hr. Colonies of groups II and III were visible on WFP after 48 hr, but inconsistent results were obtained for isolation of group I strains on this medium. Strains of groups I and III produced mucoid colonies on NBYA or SB, whereas group II strains produced dry colonies on these media as well as on WFP. Representative strains of each group were selected for further characterization.

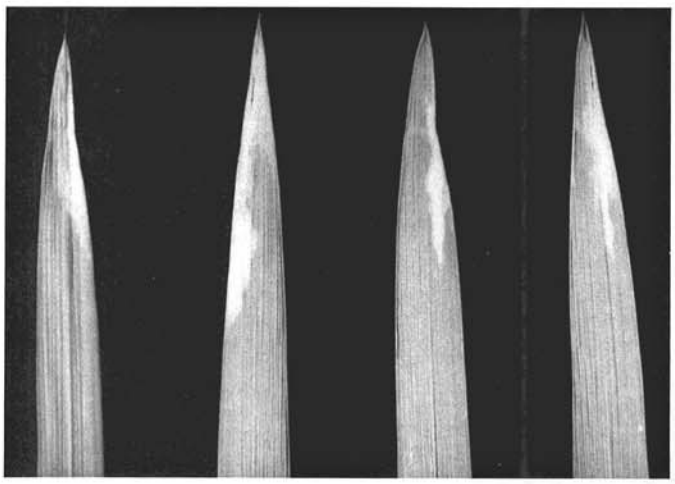

Fig. 1. Rice leaves of the cultivar Lemont affected by bacterial leaf blight caused by *Xanthomonas campestris* pv. *oryzae*. Leaves are approximately 55 days old and were collected from a field near El Campo, TX.

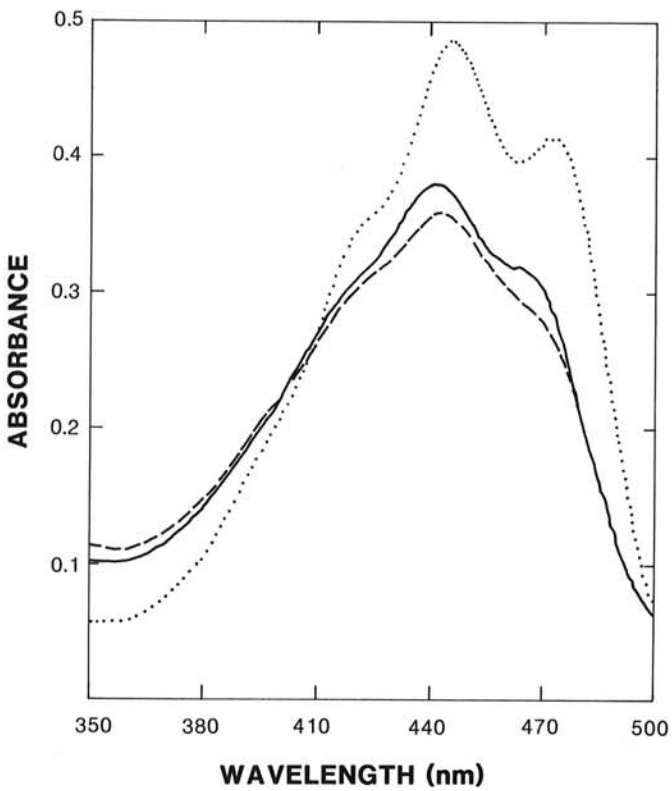

Fig. 2. Absorption spectra of crude pigment extracts from gram-negative, yellow-pigmented bacteria isolated from rice. Spectra are representations of group I strain X1-8 as *Xanthomonas campestris* pv. *oryzae* (solid line), group II strain X1-2 as *Pseudomonas paucimobilis* (dotted line), and group III strain X1-1 as *Xanthomonas* sp. (dashed line).

Strains of all three groups were gram-negative rods and motile by means of a single polar flagellum. The biochemical characteristics of all three groups were generally those associated with members of the genus *Xanthomonas* or *Pseudomonas* and were uniform for strains within groups (Table 1). In addition, all strains formed acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-mannose, glucose, sucrose, and trehalose. Groups II and III did not cause hypersensitivity in tobacco, but group I strains and a control strain of *X. c. vesicatoria* did.

Analysis of pigments. The absorption maxima of crude pigment extracts from bacterial groups I and III was 443 nm (Fig. 2). Thin-layer chromatography showed migration of the pigments at retardation factor (R_f) values of 0.43–0.48 (mean 0.46). Absorbance peaks for group II strains occurred at 472 and 445 nm with a slight inflection at 420 nm (Fig. 2). The pigments migrated with an R_f value of 0.75.

Fatty acid analysis. Profiles of the methyl esters of fatty acids from whole-cell preparations were distinct for each of the three bacterial groups (Table 2). Fatty acids from strains of group I were predominantly C16 and C17 fatty acids. Lesser amounts of 18 other fatty acids were produced by strains of this group grown on TSBA media. Strains in group I could not be fit with significant probabilities to bacteria contained in the TSBA aerobic

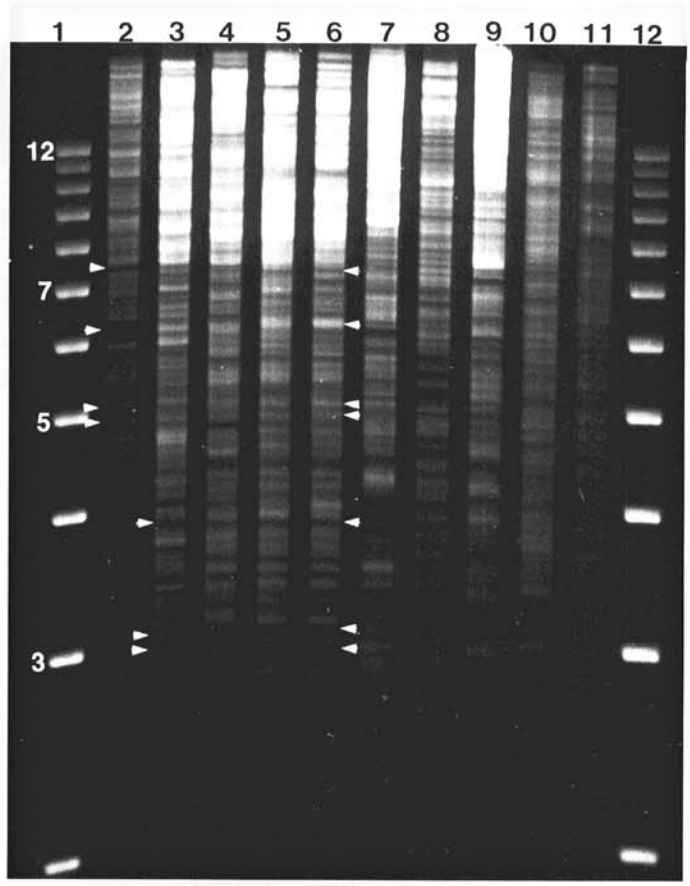

Fig. 3. Comparison of restriction fragment length polymorphisms in *Eco*RI-digested total DNA. Lanes 2 and 3, *Xanthomonas campestris* pv. *oryzae* (PXO-86, PXO-61 from the Philippines); lanes 4–6, group I strains (X1-7 from Texas, X1-8 from Texas, and X8-1A from Louisiana); lane 7, a group II strain (X1-2); lane 8, the brown blotch organism (CIAT 1173); lane 9, a group III strain (X1-1); lane 10, *X. c.* pv. *oryzicola* (BLS175); lane 11, *X. c.* pv. *translucens* (NEB101). Lanes 1 and 12 are 1 kilobase (kb) ladder size standards. The arrows indicate the pattern of bands (6.2, 5.2, and 5.1 kb) and spaces (7.4, 4.0, 3.2, and 3.1 kb) diagnostic for *X. c.* pv. *oryzae*. Approximately 2.0 μg DNA per well was loaded in lanes 3–11. In lane 2, about 1.0 μg per well was loaded to enhance visibility of the space at 7.8 kb.

library, which includes *X. albilineans* (Ashby) Dowson, *X. axonopodis* Starr and Garces, and 24 pathovars of *X. campestris*, but does not include *X. c. oryzae* or *X. c. oryzicola*.

The fatty acid compositions of three known strains of *X. c. oryzae* from Asia (Philippines, Japan, India) were very similar to the group I strains recovered from rice in the United States (Table 2). The fatty acid compositions of Asiatic strains of *X. c. oryzae* were distinctly different from the 24 pathovars of *X. campestris* contained in the TSBA library, particularly in the low amounts of C15:0 fatty acids, but showed strong similarities to three Asiatic strains of *X. c. oryzicola* (Table 2).

The fatty acid composition of group II strains exhibited similarity indices of 0.52–0.67 to the TSBA library-generated profile of *Pseudomonas paucimobilis* Holmes et al. A total of nine fatty acids were identified in whole-cell preparations of strains in group II. The majority of the fatty acids in group II strains (65.1%) chromatographed as C18:1 cis 11 (Table 2). Three additional major peaks were identified as C14:0 2-OH, C16:0, and C16:1 cis 9 fatty acids.

Strains of the organism that causes bacterial brown blotch of rice in South America (22,36) were identified as *P. paucimobilis* by fatty acid analysis but they had a low similarity index (0.17) to the library profile for this species. Fatty acid profiles of the brown blotch organism did appear similar to group II strains (Table 2).

Group III strains could not be fit with significant probabilities to any of the taxa contained in the TSBA library. Strains in this group possessed the most complex fatty acid profile of the three groups studied. Forty-two fatty acids were resolved. Eight major peaks accounted for 80.6% of the fatty acids identified (Table 2). C15:0 iso and C15:0 anteiso fatty acids accounted for 30.1 and 5.5%, respectively, of the total composition of the strains

TABLE 1. Morphological and biochemical characteristics of gram-negative, yellow-pigmented bacterial groups isolated from diseased rice

Test	Group I *Xanthomonas campestris* pv. *oryzae*	Group II *Pseudomonas paucimobilis*	Group II *Xanthomonas* sp.
Morphology			
Shape	Rod	Rod	Rod
Flagella	Single/Polar	Single/Polar	Single/Polar
Colonies	Mucoid/96 hr	Dry/48 hr	Mucoid/48 hr
Growth at:			
25 C	+	+	+
28 C	+	+	+
36 C	+	+	+
41 C	−	+	−
Oxidase test	−	+	−
Oxidative metabolism	+	+	+
Catalase activity	+	+	+
Hydrolysis of:			
Aesculin	+	+	+
Casein	+	−	+
Gelatin	−	−	+
Potato starch	+	−	+
Rice starch	+	−	+
Growth on sole carbon source as:			
Fructose	+	+	+
Glycerol	−	+	+
Trehalose	+	+	+
D-xylose	+	+	+
Acetoin production	−	+	−
Indole production	−	−	−
Urease production	−	−	−
K_2TeO_3 reduction	−	−	−
Nitrate reduction	−	−	−
H_2S from L-cysteine	+	+	+
Pectinase activity	−	−	+
Phenylalanine deaminase activity	−	−	−
Tolerance to triphenyl tetrazolium chloride at:			
0.02%	−	+	+
0.10%	−	−	−

TABLE 2. Fatty acid composition of three gram-negative, yellow-pigmented bacterial groups isolated from diseased rice compared with fatty acid composition of two pathovars of *Xanthomonas campestris* and the bacterial brown blotch organism

Strains	Fatty acids (%)[a]														
	C11:0 iso	30H C11:0 iso	30H C12:0	30H C13:0 iso	C14:0 iso	C14:0	20H C14:0	C15:0 iso	C15:0 anteiso	C16:0 iso	C16:0	C16:1 cis 9	C17:1 iso	C17:0 iso	C18:1 cis 11
Group I (*X. c.* pv. *oryzae*)	3.2	1.7	2.4	3.7	0.0	0.7	0.0	3.5	0.6	0.4	19.4	24.2	10.1	14.7	1.8
Group II (*Pseudomonas paucimobilis*)	0.0	0.0	0.0	0.0	0.0	0.6	7.4	0.0	0.0	0.0	15.0	9.4	0.0	0.0	65.1
Group III (*Xanthomonas* sp.)	5.1	1.7	3.1	1.4	4.0	2.4	0.0	30.1	5.5	5.9	7.6	18.1	1.6	4.2	0.5
X. c. oryzae (Asiatic)	3.3	2.6	3.4	3.2	0.0	0.5	0.0	3.0	0.3	0.1	21.1	27.1	7.2	13.4	1.5
X. c. pv. *oryzicola* (Asiatic)	3.5	2.3	2.4	4.3	0.0	1.1	0.0	5.1	1.6	0.5	18.7	28.4	8.4	11.6	1.8
Brown blotch organism	0.0	0.0	0.0	0.0	0.0	0.8	8.2	0.0	0.0	0.0	13.3	16.3	0.0	0.0	60.0

[a] Values represent the means of three strains except in group I, in which they represent the means of six strains.

tested. The amounts and ratios of these fatty acids are characteristic of pathovars of *X. campestris* (11,23). The TSBA software library identified strains of group III as *X. campestris* but with a single match similarity index of less than 0.05.

Restriction fragment length polymorphisms. The fragments generated by *Eco*RI digestion of genomic DNA resulted in complex but reproducible banding patterns after electrophoresis through agarose gels. *Eco*RI digests of DNA from bacterial groups I, II, and III produced fragment patterns that were distinct from one another (Fig. 3). The pattern of bands and spaces produced by strains of group I were very similiar to one another but were not identical (Fig. 3).

A pattern of seven bands or spaces was consistently present in the 30 isolates of *X. c. oryzae* examined from the Philippines, Japan, and India. This pattern consisted of fragments of 6.2, 5.2, and 5.1 kilobases (kb) with clear spaces at about 7.4, 4.0, 3.2, and 3.1 kb equivalents (Fig. 3). Of 21 pathovars of *X. campestris* compared, banding patterns of strains of group I most closely aligned with strains of *X. c. oryzae*. Analysis of restriction fragment lengths showed clear differences between group I strains and other potential rice pathogens including *X. c. oryzicola*, the bacterial brown blotch organism (22), and *X. c. translucens* from wheat (Fig. 3).

Reaction to monoclonal antibodies. All strains of group I reacted with monoclonal antibody Xco-1, which reacts with more than 178 known strains of *X. c. oryzae* from diverse geographic origins (5). Group I strains react differentially to monoclonal antibody Xco-2 (Table 3) demonstrating the presence of serotypes within the group I population. Strains of group II failed to react with monoclonal antibodies specific for strains of *X. c. oryzae* or with the genus-specific monoclonal antibodies X1 and X11 (Table 3). Strains in group III reacted with the genus-specific monoclonal antibodies X1 and X11 but failed to react with any of the three monoclonal antibodies that are pathovar specific for *X. c. oryzae* (Xco-1, Xco-2, or Xco-5).

Pathogenicity testing. Strains in group I caused a curling of seedling leaves after 7 days and chlorosis and necrosis after 14 days. Bacterial streaming associated with the vascular bundles was evident from sectioned preparations of these specimens well in advance of symptoms. Strains in groups II and III were not pathogenic on seedlings of the semidwarf cultivar Lemont (Table 4).

Inoculation of 49-day-old rice plants with representative strains of the three bacterial groups produced results similar to the seedling reactions. Asiatic strains of *X. c. oryzae* were capable of causing symptoms affecting more than 50% of the entire leaf blade in less that 14 days. By comparison, strains in group I produced limited symptoms on the susceptible cultivars Lemont and TN-1 (Table 4). Strains in group I produced significantly ($P = 0.05$) shorter lesions on IR-8, whereas both Philippine strains were virulent on this cultivar (Table 4). Group II and III strains did not cause measurable lesions after 14 days on any of the cultivars tested (Table 4).

DISCUSSION

Bacteria recovered from diseased rice tissue included three distinct gram-negative, yellow-pigmented groups. The absorbance spectra and pigment R_f values of both group I and group III strains agree with published values for xanthomonadins (15,35), whereas those of group II strains are similar to those of *P. paucimobilis* (13,16) and other gram-negative, yellow-pigmented bacteria that are not *Xanthomonas* spp. (15). Strains of group I were capable of reproducing symptoms of diseased rice as observed in commercial fields, exhibited morphological and biochemical features consistent with *X. c. oryzae* from other rice-growing regions (8,21,29,36), and, for the present, are named as such based on "the host plant from which first isolated" convention (35).

Fatty acid analysis demonstrated major differences between group I strains and other *Xanthomonas* spp. and 24 pathovars of *X. campestris* contained in the TSBA library. Fatty acid analysis also demonstrated similarities between group I strains and the strains of *X. c. oryzae* and *X. c. oryzicola* obtained from Asiatic sources (which were not contained in the 3.0 version of the library). When we supplemented the fatty acid analysis with genomic comparisons, serological reactions, and symptoms of the disease as they appeared on host plants, we were able to clearly differentiate between group I strains and *X. c. oryzicola*, the causal agent of bacterial streak disease (29).

Group I strains were consistently less virulent to rice than two strains of *X. c. oryzae* from the Philippines. Virulence, as measured by lesion length on susceptible hosts, also was low compared to numerous strains of *X. c. oryzae* from other Asiatic sources (Leach, *unpublished*).

Bacterial leaf blight again appeared in Texas and Louisiana rice in 1988. The 1988 production season was characterized by below-normal rainfall during the late vegetative to early reproductive growth stages of the crop. As in 1987, the disease appeared to be associated only with semidwarf cultivars and to

TABLE 3. Enzyme-linked immunosorbent assay reaction to monoclonal antibodies of gram-negative, yellow-pigmented bacterial groups isolated from diseased rice

	Monoclonal antibody[a]				
Strain designation	X 1	X 11	Xco-1	Xco-2	Xco-5
Group I (*Xanthomonas campestris* pv. *oryzae*)					
X1-5	+	+	+	+	+
X1-7	+	+	+	+	+
X1-6	+	+	+	−	+
X1-8	+	+	+	−	+
X1-10	+	+	+	−	+
X8-1A	+	+	+	−	+
Group II (*Pseudomonas paucimobilis*)					
X1-2	−	−	−	−	−
X1-3	−	−	−	−	−
X1-4	−	−	−	−	−
Group III (*Xanthomonas* sp.)					
X1-1	+	+	−	−	−
X1-9	+	+	−	−	−
X4-4A	+	+	−	−	−

[a] Monoclonal antibodies X 1 and X 11 are genus specific for *Xanthomonas* spp. Xco-1 and Xco-2 are pathovar-specific antibodies produced to a Philippine strain of *X. c. oryzae*. Xco-5 is an antibody produced from a fusion of Texas strain X1-5.

TABLE 4. Pathogenicity of gram-negative, yellow-pigmented bacteria isolated from diseased rice to seedling and adult rice plants

	Seedling reaction[a]	Adult plant reaction[b]		
Test organisms		TN-1	IR-8	Lemont
Group I (*Xanthomonas campestris* pv. *oryzae*)				
Six strains	+	3.9	0.2	2.4
Group II (*Pseudomonas paucimobilis*)				
Three strains	−	0.0	0.0	0.0
Group III (*Xanthomonas* sp.)				
Three strains	−	0.0	0.0	0.0
X. c. oryzae				
Two Philippine strains	ND[c]	11.5	8.5	11.4

[a] Three-week-old cultivar Lemont seedlings inoculated at 10^9 colony-forming units/ml with leaf-clip method. Reactions were scored positive if wilting and necrosis of inoculated leaves was evident after 7 days of incubation.

[b] Seven-week-old adult plants inoculated at 5×10^9 by double-needle inoculation method. Adult plant reactions were scored as lesion length (cm) after 14 days of incubation. Group I strains produced significantly shorter lesions on cultivar IR-8 than on cultivar TN-1 or Lemont. Other cultivar interactions were not significant according to least significant difference test ($P = 0.05$).

[c] Not determined.

cause less than 1% yield reduction using the critical point model of Reddy et al (32) to estimate loss. It would appear from observations over the last two seasons that the occurrence of bacterial leaf blight in U.S. rice will not be severely limited by climatic events. Efforts to develop effective control measures would seem prudent if the organism demonstrates the ability to shift to greater virulence.

Questions arise as to the origin of this low-virulence form of *X. c. oryzae*. We felt that the sudden appearance of this new disease could have been the result of its introduction on seed. It also could have been introduced from Central America by the tropical weather system that affected the Gulf Coast during June 1987. Another possibility is that it is an indigenous organism, expressing its limited virulence on the recently increased semidwarf acreage under conditions favorable for its development and spread.

Differences in serological reactions and other stable physiological traits within a collection of 26 strains of this organism from the United States confirm the existence of at least two distinct subpopulations. These subpopulations could be identified by serological reactions to the pathovar-specific monoclonal antibody XCO-2 (5) or with bacteriocin typing (Gonzalez, *unpublished*). Subpopulations were geographically dispersed and became evident when comparisons were made between collections of strains from separate fields. Strains recovered when multiple isolations were made from different plants within the same field were always of the same subpopulation type. This is interpreted as evidence that the group I strains may represent an indigenous population that has existed for a sufficient period of time to evolve into two distinguishable subpopulations. It also suggests that the organism may be disseminated from a local source.

Our evidence suggests that the U.S. strains are indigenous and that they were not introduced from South America by the tropical depression that affected the Texas Upper Gulf Coast in June 1987. Strains of the bacterial leaf blight pathogen collected in Colombia (21,36) were obtained from J. Carlos Lozano as CIAT 1185 and CIAT 1186. The CIAT isolates are comparable in virulence to Asiatic strains of *X. c. oryzae* (Leach, *unpublished*) and do not react to the monoclonal antibody (Xco-5) specific for the group I strains (5). Fatty acid profiles of these strains fit a Library Generation Software profile (Microbial ID) made from 34 Asiatic strains of *X. c. oryzae* but not a profile made from 36 group I strains collected in this study (Jones, Barnes, Gonzalez, and Leach, *unpublished*).

The organism might have been newly introduced to the area by seed transmission. However, during 1987, we did not observe a relationship between planting seed source and the subsequent development of bacterial leaf blight in a particular field. The 44 affected fields identified in 1987 were planted from a total of 15 separate seed lots (Jones, *unpublished*). In many cases, affected and unaffected fields were observed on the same farm in fields that had been planted from the same seed lot. After harvest, 89 commercial seed lots including 21 from affected fields were examined for the presence of *X. c. oryzae* on the seed using ELISA and monoclonal antibody Xco-1. In all cases, tests failed to detect *X. c. oryzae*, but strains of bacterial groups II (*P. paucimobilis*) and III (*Xanthomonas* sp.) were frequently recovered (5, and Jones, Barnes, Alvarez, and Benedict, *unpublished*). This information suggests that the distribution of the disease in 1987 could not be ascribed to seed transmission.

Strains of bacteria belonging to group III were the most frequently recovered bacteria in this study both from leaf tissue and seed. This organism has morphological and physiological features consistent with the genus *Xanthomonas*. It was not pathogenic to rice under our test conditions but was always found in association with symptomatic tissue. The lack of pathogenicity would preclude its inclusion in this taxon according to some authors (35). The role of this organism in the development of bacterial leaf blight is not understood. The organism may suppress symptoms, as has been described for *Erwinia herbicola* (Löhnis) Dye (14), it may exacerbate them, or it may have no effect at all. Pectolytic *Xanthomonas* spp. have recently been described as epiphytic inhabitants of plant surfaces (11,23).

Numerous investigators (26,29) report difficulty in isolating *X. c. oryzae* because of the presence of "fast-growing, yellow bacteria." We suggest that bacterial strains in group III (*Xanthomonas* sp.) may represent an epiphyte common to rice culture and, in conjunction with group II isolates, may be responsible for this difficulty. These organisms also may contribute to the reasons why the literature conflicts regarding seed transmission of bacterial leaf blight (29).

Epidemiological investigations of bacterial leaf blight of rice also have been hampered by the frequent isolation of "gram-negative, yellow bacteria." The use of fatty acid analysis, the use of monoclonal antibodies, and the identification of a lytic phage specific only to the group I strains of *X. c. oryzae* (Gonzalez, *unpublished*) have served as effective tools to distinguish *X. c. oryzae* from other bacteria and also to distinguish between the Asiatic strains and the U.S. strains of the organism. This latter distinction remains of great concern because the effectiveness of quarantine measures directed against the introduction of highly virulent Asiatic strains of *X. c. oryzae* are as important to the U.S. rice industry today as before the discovery of the low-virulence strains.

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