A Repetitive DNA Sequence Differentiates *Xanthomonas campestris* pv. *oryzae* from Other Pathovars of *X. campestris*

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Two repetitive DNA sequences were detected in the genome of *Xanthomonas campestris* pv. *oryzae*. An EcoRI-HindIII restriction fragment (2.4 kilobases) containing one highly repetitive sequence was cloned (pJEL101). Approximately 81 copies of the repetitive sequence in pJEL101 were dispersed throughout the genome as determined by quantitative filter hybridizations. Repetitive sequences related to pJEL101 were detected in all strains of *X. c. pv. oryzae* from different geographic areas. Strains representing five of 25 other *X. campestris* pathovars appeared to contain multiple copies of a related element. Only *X. c. pv. oryzicola*, another rice pathogen, contained a relatively high copy number of pJEL101 sequences compared to *X. c. pv. oryzae*, although the hybridization banding pattern was different. The pJEL101 sequence is a useful probe for pathovar identification as well as strain and population analyses.

Additional keywords: restriction fragment length polymorphism.

*Xanthomonas campestris* pv. *oryzae* is the causal agent of bacterial leaf blight, a major rice disease prevalent throughout Asia. Traditionally, bacterial leaf blight is controlled by planting resistant rice cultivars. However, over time, virulent strains of *X. c. pv. oryzae* have been detected on the resistant cultivars (Mew 1987). In an effort to understand pathogenic variability, restriction fragment length polymorphism (RFLP) analysis of the genetic relationships of *X. c. pv. oryzae* strains was initiated. RFLP analysis has been used to study genetic relationships of bacterial genera, species, and pathovars (Cook et al. 1989; Denny 1988; Denny et al. 1988; Gabriel et al. 1988; Lazo et al. 1987). Our initial approach, however, was confounded because complex hybridization patterns, suggestive of repetitive DNA sequences, were observed when genomic cosmid clones were used as probes (Leach et al. 1988).

Although there are few reports of repetitive elements in *Xanthomonas* spp. (Bonas et al. 1989; Kearney and Staskawicz 1990), repetitive DNA sequences have been found in other bacterial genomes. Such sequences include the 11-base pair (bp) sequence involved in the uptake of DNA by *Haemophilus influenzae* (Smith et al. 1981), the 35-bp repetitive extragenic palindromic sequence of *Escherichia coli* and *Salmonella typhimurium* (Higgins et al. 1982; Stern et al. 1984), and transposable elements (insertion sequences, transposons, or transposing bacteriophages) ranging in size from 700 to greater than 10,000 bp (Appelbaum et al. 1985; Comai and Kosuge 1983; Flores et al. 1987; Iida et al. 1983; Kaluza et al. 1985; Kleckner 1981; McClafferty et al. 1988; Moglen and Oleson 1987; Ruvkun et al. 1982). Copy numbers of the repeated sequences vary from two to greater than 40 for insertion sequences (Iida et al. 1983) and up to 1,000 for the repetitive palindromic sequence (Stern et al. 1984). Although the function of most repetitive elements is not clear, some may be involved in adaptation by facilitating DNA rearrangements and altering gene expression (Iida et al. 1983; Kearney et al. 1988; Kleckner 1981; Ruvkun et al. 1982; Sapientza et al. 1982; Scordilis et al. 1987; Stern et al. 1984; Szabo and Mills 1984).

We report here the presence of highly repetitive DNA sequences (elements) in *X. c. pv. oryzae*. In addition, we describe the distribution of one element among strains of *X. c. pv. oryzae* and pathovars of *X. campestris* and the usefulness of that element as a probe for diagnosis of *X. c. pv. oryzae*.

**MATERIALS AND METHODS**

**Plasmids and bacterial strains.** Plasmids used in this study are listed in Table 1.

The strains of *X. c. pv. oryzae* provided by A. Alvarez, University of Hawaii, Honolulu, were AXO-R3 from Australia; BXO-13 from Burma; TXO-R7 from Thailand; SLXO-CL1 from Sri Lanka; IXO-R33 from India; and JXO-R-48, JXO-H75304, JXO-T7133, JXO-H75373, and JXO-T7714 from Japan. Strains from Australia provided by L. Dietlof, Agricultural Research Laboratories, Inndooropilly, Queensland, Australia, included AXO-2028, AXO-2031, and AXO-2052. Strains from Colombia provided by J. Lozano, Centro Internacional de Agricultura Tropical, Cali, Colombia, were CXO-1185 and CXO-1186. Strains from India provided by K. Gupta, Punjab Agricultural University, Ludhiana, India, were IXO-1 and IXO-10. Strain N163 from Nepal was provided by T. Adhikari, National Rice Improvement Program, Kathmandu, Nepal. Strain IXO-B1 originating in Bangladesh was from this laboratory. Strains provided by C. Gonzalez, Texas A. & M. University, College Station, [bottom of page]
Table 1. Plasmids used in this study

<table>
<thead>
<tr>
<th>Designation</th>
<th>Description</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>pLAFR3</td>
<td>IncP, Te', Mob', cos</td>
<td>Staskawicz et al. 1987</td>
</tr>
<tr>
<td>pSA747</td>
<td>IncW, Km', Mob', cos</td>
<td>Tait et al. 1983</td>
</tr>
<tr>
<td>pUC18</td>
<td>CoE1 replication, Ap'</td>
<td>Norrander et al. 1983</td>
</tr>
<tr>
<td>pBluescript KS+</td>
<td>32-kb genomic clone from PX086R in pLAFR3</td>
<td>Stratagene, La Jolla, CA</td>
</tr>
<tr>
<td>pL86a</td>
<td>41-kb genomic clone from PX086R in pSA747</td>
<td>This study</td>
</tr>
<tr>
<td>pS86b</td>
<td>18-kb genomic clone from PX086R in pLAFR3</td>
<td>This study</td>
</tr>
<tr>
<td>pL86c</td>
<td>33-kb genomic clone from PX086R in pLAFR3</td>
<td>This study</td>
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<tr>
<td>pS86d</td>
<td>34-kb genomic clone from PX086R in pLAFR3</td>
<td>This study</td>
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<tr>
<td>pJEL101</td>
<td>2.4-kb EcoRI-HindIII fragment from pL86a, contains repetitive sequence</td>
<td>This study</td>
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<tr>
<td>pBS101</td>
<td>2.4-kb EcoRI-HindIII fragment from pJEL101 in pBluescript KS+</td>
<td>This study</td>
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<tr>
<td>pBS3-4</td>
<td>2.5-kb EcoRI fragment in pBluescript KS+, contains single copy avrR10 gene</td>
<td>Kelemu and Leach 1990</td>
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*Te', Km', and Ap' indicate resistance to tetracycline, kanamycin, and ampicillin, respectively, and kb, kilobase.

**Culture conditions.** Strains of *X. c. pv. oryzae* were maintained on peptone-sucrose agar (Tsuchiya et al. 1982) at 28°C. *E. coli* was maintained on Luria-Bertani agar (Miller 1972) at 37°C, and all other bacteria were maintained on nutrient agar (Difco Laboratories, Detroit, MI) at 28°C.

**DNA isolation.** Bacteria were grown in 15 ml of nutrient broth (Difco) for 15 hr at 28°C on a rotary shaker (200 rpm). Genomic DNA was extracted by a modification of the procedure of Owen and Borman (1987). Bacterial cells were pelleted by centrifugation (13,776 × g); resuspended in a mixture of 50 mM glucose, 25 mM Tris-Cl (pH 8.0), 10 mM Na2EDTA, 2 mg/ml freshly mixed lysozyme (total volume, 3.3 ml); and incubated at room temperature for 20 min. To each DNA sample, sodium dodecyl sulfate (SDS; 167 µl of a 10% solution) was added. After incubation for 10 min at 50°C, 134 µl of RNase A (2.5 mg/ml in 10 mM Tris-Cl, pH 7.6) was added, and the mixture was incubated at 37°C for 1 hr. Then, 170 µl of 0.5 M Na2EDTA was added, and the mixture was incubated for 10 min at 50°C. Proteinase K (50 µl of a 5 mg/ml solution in 10 mM Tris-Cl, pH 7.0) was added, and the mixture was incubated for 3-12 hr at 37°C. Each sample was then extracted with two equal volumes of phenol saturated with 0.1 M Tris-HCl (pH 8.0), one volume of a 1:1 phenol and chloroform-isoamyl alcohol (24:1) mixture, and one volume of chloroform-isoamyl alcohol. The DNA was precipitated with ethanol and redissolved in 500 µl of 10 mM Tris, pH 8.0, and 1 mM Na2EDTA. DNA concentrations were estimated spectrophotometrically.

**Cloning of DNA fragments.** A cosmid library of strain PX086R DNA constructed by ligation of partially digested EcoRI fragments into pSa747 was described previously (Kelemu and Leach 1990). A second library of Sau3A partial fragments was constructed from DNA of strain PX086R in pLAFR3 as described by Swanson et al. (1988). Subcloning procedures and plasmid analysis and isolation procedures were as described in Maniatis et al. (1982).

**RFLP analysis.** Bacterial DNA (10 µg) was digested to completion with EcoRI (2 units per microgram of DNA)
at 37°C for 2–3 hr in buffers provided by Bethesda Research Laboratories (BRL, Gaithersburg, MD) or Promega (Madison, WI). The DNA fragments (2–3 μg per well) were separated in 0.7% agarose gels immersed in Tris-borate buffer (89 mM Tris-HCl, 89 mM boric acid, and 2 mM Na2EDTA, pH 8.0) by electrophoresis. A 1-kilobase (kb) ladder (BRL) was included in gels as a size standard.

Transfer of DNA fragments from agarose gels onto nylon membranes, Southern hybridizations, and high-stringency washes were done as described by the manufacturer of the GeneScreen Plus membrane (Du Pont Co., Wilmington, DE). Plasmid preparations were labeled with [32P]CTP using a nick translation kit (BRL). Blots were prehybridized at 65°C C for 3–4 hr in a solution composed of 0.1% SDS, 50 mM sodium phosphate buffer (pH 7.0), 1.0 M NaCl, and 300 μg/ml denatured salmon sperm DNA. For hybridization, denatured labeled probe DNA (10^6 cpm/ml) was added directly to the prehybridization solution, and the blot was incubated at 65°C C for 18 hr. After hybridization, the blot was washed three times at 65°C C in 2× SSC (20× SSC contains 3 M NaCl and 0.3 M Na2-citrate) containing 0.1% SDS and 5 mM PB and then three times in 0.5× SSC containing 0.1% SDS and 3 mM PB. Autoradiographic exposures were at ~80°C C using Cronex film (Du Pont) and a Du Pont Cronex Hi-Plus intensifying screen.

To compare strains of X. c. pv. oryzae and X. c. pv. oryzicola, the presence or absence of hybridization bands corresponding to DNA fragments of 6 kb or less was scored for each strain. Comparisons were only made within individual blots. The proportion of mismatched fragments was calculated for all pairwise combinations of strains as described by Denny et al. (1988).

**Deletion analysis of the repetitive sequence in pJEL101.** The 2.4-kb EcoRI–HindIII fragment contained in pJEL101 was cloned into pBluescript KS+ (Stratagene, La Jolla, CA) to construct pBS101 and subjected to deletion mutagenesis by digestion with exonuclease III as described by Ausubel et al. (1989). To visualize the presence of the repetitive sequences, deleted plasmids were digested with BamHI (to linearize plasmids containing deletions from the HindIII side of the insert) or HindIII (to linearize plasmids containing deletions from the EcoRI side of the insert). Linearized plasmids were then separated by electrophoresis, transferred to nylon membranes, and probed with 32P-labeled pBS66 (Table I).

**Quantitative slot blot.** The copy number of the repetitive DNA element in the genome of X. c. pv. oryzae was estimated by a modification of the quantitative dot blot procedure described by Pruitt and Meyerowitz (1986). DNA from X. c. pv. oryzae was diluted with salmon sperm DNA such that the total DNA concentration remained at 10 μg/ml. A 50-μl sample from each dilution was sheared and denatured by adding 50 μl of 0.6 M NaOH and heating at 50°C C for 20 min. The mixture was then chilled on ice. Immediately before filtering, 400 μl of 20× SSC with 75 mM HCl was added to each tube, and 200-μl samples were then filtered slowly (15–30 sec per 100-μl sample) through a nitrocellulose membrane (Bio-Dot blotting media, Bio-Rad Laboratories, Richmond, CA) equilibrated in 20× SSC using a Bio-Slot microfiltration apparatus (Bio-Rad). The wells were each rinsed with 20× SSC. The filter was cut into strips containing 24 slots each (from each of two dilution series) and baked for 4 hr at 80°C C in a vacuum oven. Hybridizations and autoradiography were as described above. The intensity of hybridization was measured by scanning densitometry.

Identical sets of membranes were hybridized with a clone in pBluescript KS+ containing a 2.5-kb single copy sequence (pBS8, Table I) or with the clone containing the repetitive sequence (pJEL101). To obtain relative copy numbers of complementary sequences for each probe per genome, values from densitometric scans for duplicate dilution series were averaged and plotted against the quantity of genomic DNA on the filter. Lines were fitted by linear regression analysis, and the slopes were calculated from the linear portion of the curves. The number of copies

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**Fig. 1.** Autograms of identical Southern blots after hybridization with 32P-labeled clones pL66a (A) and pS86d (B). Lane a, 1-kilobase ladder; lanes b and c, genomic DNA of Xanthomonas campestris pv. oryzae PXO61 and PXO68, respectively; lane d, pL86a; lane e, pS86b; lane f, pL86c; lane g, pS86d; lane h, pJEL101; lane i, pSa747; and lane j, pLAFR3. DNA samples were digested with EcoRI before electrophoresis and transfer. pJEL101 was digested with EcoRI and HindIII.
per genome was determined using the following equation:

\[
\text{copy no./genome} = \text{slope a/slope b} \\
\quad \times \frac{\text{specific activity } b}{\text{specific activity } a} \\
\quad \times \frac{\text{mol. wt. insert of } b}{\text{mol. wt. insert of } a}
\]

where \(a = \text{probe pJEL101}\) and \(b = \text{probe pBS8-4}\). Copy numbers are the average values of two experiments.

**RESULTS**

**Identification of repetitive DNA sequences.** Initially, randomly selected clones from genomic libraries of *X. c. pv. oryzae* were used as hybridization probes in Southern blots of EcoRI-digested DNA from *X. c. pv. oryzae*. For comparative purposes, the blots (shown in Fig. 1) were repeated to include a subclone of one repetitive sequence (pJEL101, Table 1, described in more detail below). The vectors (pLAFR3 or pSa747) hybridized with each other but not with genomic DNA from *X. c. pv. oryzae* (data not shown). Many of the cosmid clones (data are presented for two examples, pL68a and pS68d; Table 1) hybridized to more fragments in digests of total genomic DNA from *X. c. pv. oryzae* than expected from the complexity of the individual clones. Clone pL68a contained six EcoRI fragments (sizes 25.0, 11.0, 11.0, 5.5, 1.6, and 1.0 kb, Fig. 1A, lane d). However, numerous bands were detected in total genomic DNA (Fig. 1A, lanes b and c). Clone pS68d contained nine EcoRI fragments (sizes 15.0, 11.4, 9.2, 4.9, 2.3, 1.9, 1.6, 1.0, and 0.8 kb, Fig. 1B, lane g) and also provided a complex band pattern with genomic DNA (Fig. 1B, lanes b and c). The hybridization patterns from both pL68a and pS68d were more complicated than would be expected for partial digestion of the genomic DNA. In addition, probes of single copy sequences (for example, pBS8-4, Table 1) yielded the expected single band patterns using the same DNA preparations (data not shown). Thus, the results were consistent with the presence of one or more repetitive sequences in the two genomic clones.

Clone pL68a hybridized with three EcoRI fragments in pS68d (Fig. 1A, lane e). The three hybridizing fragments represented vector pSa747 and two fragments of genomic origin (Fig. 1A, lane e). Since pS68d was generated by ligation of partial EcoRI fragments into pSa747, digestion of the clone yielded one vector fragment. Similarly, pL68a hybridized with the pSa747 fragment of pS68d (Fig. 1A, lane g). Probe pS68d hybridized with two bands of pL68a that contained the two vector arms of pLAFR3 (23.0 and 5.5 kb; Fig. 1B, lane d). Hybridization of pS68d with pS68b was due to the common vector fragment of pSa747 (Fig. 1B, lane e). Probe pS68d did not hybridize to clone pJEL101 containing the repetitive sequence (Fig. 1B, lane h). Since pS68d did not hybridize with genomic sequences in pS68b (Fig. 1B, lane e) and pL68a did not hybridize to genomic fragments from pS68d (Fig. 1A, lane g), we determined that pL68a and pS68d contained different repetitive elements.

**Isolation of a X. c. pv. oryzae repetitive element.** The clone pL68a was digested with EcoRI and HindIII, transferred to nylon membranes, and probed with pL686 (pL686 [Table 1], when used to probe genomic DNA, resulted in a pattern similar to that revealed by pL68a). A 2.4-kb EcoRI–HindIII fragment, which contained the region of hybridization, was identified and subcloned into pUC18. When the subclone (pJEL101) was used as a probe of total genomic DNA from two strains of *X. c. pv. oryzae*, a complex signal pattern similar to the pattern obtained with pL68a was observed (compare lanes b and c of Figs. 1A and 2). The genomic fragments in pS68d that were detected with pL68a were also detected with pJEL101 (Fig. 2, lane e). Clone pJEL101 also hybridized with pLAFR3 (Fig. 2, lane j), presumably through sequences shared between pUC18 and pLAFR3 since pLAFR3 contains the HaelII fragment of pUC18 (Staskawicz et al. 1987). The pUC18 vector does not hybridize with genomic DNA from *X. c. pv. oryzae* (data not shown). Clone pJEL101 did not hybridize to the pSa747 sequences (Fig. 2, lane i) or to any genomic fragments of pS68d (Fig. 2, lane g). Only two bands were detected in pL68a with pJEL101 (Fig. 2, lane d), and these were the same bands that hybridized with pS68d (Fig. 1B, lane d), indicating that the repetitive element was near the vector cloning site. Only the 5.4-kb fragment from pL68a hybridized with the EcoRI–HindIII fragment isolated from pJEL101 (data not shown).

A further test to confirm the difference of the element(s) in pJEL101 and pS68d was performed by probing identical genomic DNA filters with each clone. DNA from strain PXO86Ri was digested with BamHI, EcoRI, and HindIII and probed with pJEL101 and pS68d. The lanes representing the same enzyme digest were then aligned to compare the patterns from each probe. Where individual bands could be distinguished (bands less than 6 kb, Fig. 3), the majority of bands detected with both probes were unique in each of the three enzyme treatments.

To further characterize the distribution of the pJEL101 repetitive sequence in the genome of *X. c. pv. oryzae*, the clone was used as a probe against plasmid DNA from *X. c. pv. oryzae* and randomly selected cosmid clones of genomic DNA from *X. c. pv. oryzae*. The subclone did not hybridize with plasmid pPXO112 from strain PXO112 or plasmid pPXO124 from strain PXO124 (data not shown).

![Fig. 2. Southern blot analysis of Xanthomonas campesiris pv. oryzae total DNA and genomic clones with 32P-labeled p.JEL101. Lane a, 1-kilobase ladder; lanes b and c, genomic DNA from strains PXO11 and PXO86Ri of X. c. pv. oryzae, respectively; lane d, pL68a; lane e, pS68d; lane f, pL686; lane g, pS68d; lane h, pJEL101; lane i, pSa747; and lane j, pLAFR3. DNA samples were digested with EcoRI before electrophoresis and transfer. pJEL101 was digested with EcoRI and HindIII.](image-url)
On the other hand, pJEL101 hybridized with 20 separate EcoRI-HindIII fragments in 17 of 20 clones tested from a PXO86^Rf genomic library in pSa747 (data not shown). The 20 cosmid clones were considered random because restriction analysis revealed different DNA fragmentation (data not shown) and characterization of the pSa747 library revealed no bias toward any particular clones (Kelemu and Leach 1990).

Radiolabeled pS86b, which also contained sequences that hybridized to the repetitive element, was used to probe a series of deletion derivatives generated from the EcoRI-HindIII fragment of pJEL101 (Fig. 4). Derivatives lacking about 1 kb from the EcoRI side of the fragment did not hybridize with pS86b, indicating that the repetitive element contained in pJEL101 was located near the EcoRI site and was approximately 1 kb (Fig. 4, lanes 1 and m). Since all deletions starting from the HindIII side of the EcoRI-

\[ \text{HindIII fragment hybridized with pS86b (Fig. 4, lanes a-f), we could not determine if the EcoRI site was contained within the repetitive element or if the element ended just upstream of the EcoRI site.} \]

An estimate of the sequence copy number represented by the repetitive element in pJEL101 was obtained from quantitative filter hybridization experiments. Filters containing serial dilutions of genomic DNA from X. c. pv. oryzae were treated separately with radioactively labeled pJEL101 and a single copy sequence from X. c. pv. oryzae, pBS8-4 (Table 1). After correction for the specific activity and length of hybridizing DNA of the different probes, the amount of hybridization of genomic DNA to pJEL101 and pBS8-4 was compared. Assuming the repetitive element was 1.0 kb (from deletion data), we determined that approximately 81 copies were present in the genome of X. c. pv. oryzae.

\[ \text{Distribution of the pJEL101 sequence in the genus Xanthomonas. To assess the use of the pJEL101 repetitive sequence as a diagnostic tool, the presence or absence of the sequence in 30 different strains of X. c. pv. oryzae, 25 other pathovars of X. campestris, and two different species of Xanthomonas (X. albilineans and X. fragariae) was determined. Hybridizations were performed with genomic DNA from strains of X. c. pv. oryzae obtained from diverse geographic origins. With the exception of strains from the United States, 30 or more bands from EcoRI-digested total DNA from strains of X. c. pv. oryzae could be distinguished after hybridization with pJEL101 (Fig. 5, lanes a, b, and g-o). Although signal differences were evident, the patterns were similar in their complexity, and numerous common subgroups of bands (band complexes) were apparent. Strains from the United States also contained a repetitive element (Fig. 5, lanes c, e, and f). However, hybridization of DNA from strain UXO-X-1-7 from Texas yielded only 11 bands (Fig. 5, lane c). The DNA of strains from Louisiana contained only nine hybridizing fragments and had a pattern different from the UXO-X-1-7 pattern (compare lanes c, e,}
and f of Fig. 5). Therefore, the number of fragments containing pJEL101-related sequences was considerably lower when compared to strains of X. c. pv. oryzae from other areas. A bacterium identified as a Xanthomonas spp., which was isolated from rice but was not pathogenic to it, did not hybridize with pJEL101 (Fig. 5, lane d).

pJEL101 also hybridized with DNA digests from eight of 25 pathovars of X. campestris. Of the strains with DNA that hybridized, only X. c. pv. oryzicola contained a quantity of bands similar to that observed in X. c. pv. oryzae (Fig. 6, lanes m and n). The number of bands was estimated to be less than 25 from visual inspection of the filter, and thus, the actual number of copies in each strain was likely to be fewer than the copies found in strains of X. c. pv. oryzae (81), with the exception of the strains from the United States. DNA from X. c. pv. holicola (Fig. 6, lanes c and d), X. c. pv. vasculorum (Fig. 6, lanes i and j), X. c. pv. phleipratensis (Fig. 6, lanes k and l), and X. c. pv. pelargonii (not shown) also hybridized to pJEL101 in a manner indicative of a repetitive sequence, although the number of bands in each case was below 10. One or two bands also were detected in DNA from some other pathovars (X. c. pv. vesicatoria, lanes o and p of Fig. 6; X. c. pv. secalis and X. c. pv. undulosa, not shown).

Hybridization of pJEL101 to DNA from seven additional strains of X. c. pv. oryzicola resulted in patterns as complex as those previously observed with strains BLS175 and BLS256 (Fig. 7). The RFLP patterns of the nine strains appeared different from the patterns for strains of X. c. pv. oryzae. The proportion of mismatched fragments calculated for all pairwise combinations of strains of X. c. pv. oryzae vs X. c. pv. oryzicola ranged from 0.84–0.93 as compared to 0.08–0.36 for comparisons of strains of X. c. pv. oryzae on the same blot. The proportion of mismatched fragments calculated for all pairwise combinations of the nine strains of X. c. pv. oryzicola ranged from 0.08–0.69, whereas combinations of X. c. pv. oryzae from different geographic areas (Fig. 5, excluding those from the United States) ranged from 0.03–0.57. Thus,
although the repetitive element was fairly polymorphic within EcoRI-digested DNA from each pathovar, the two pathovars could clearly and easily be distinguished by hybridization patterns with pJEL101.

**DISCUSSION**

Hybridization between total genomic DNA and individual genomic clones revealed the presence of two highly repetitive sequences in *X. c. pv. oryzae*. The two elements, contained in pJEL101 and pS86d, are distinct elements because genomic sequences of the two clones did not cross-hybridize and, in addition, hybridization patterns with genomic DNA digests were different. Repetitive sequences of pJEL101 were detected in all strains of *X. c. pv. oryzae* and some other pathovars of *X. campestris*.

The discovery of repetitive elements is not unique to *X. c. pv. oryzae*. More noteworthy is the prevalence of repetitive sequences in the genome of *X. c. pv. oryzae*. For example, pJEL101 hybridized to 85% of the pSa747 clones of PX086K tested, indicating that the element is distributed throughout the genome. Clone pS86d contains a different repetitive sequence, indicating that repetitive sequences are ubiquitous components of the genome of *X. c. pv. oryzae*.

The repetitive element occupies about 1 kb of the EcoRI-HindIII fragment in pJEL101, based on hybridization analysis of deleted mutants. However, a more detailed analysis is necessary to determine if the whole repetitive element is contained within pJEL101. Since there is no clear indication of the borders of repetitive elements, sequence analysis of several different clones containing related elements will be required.

Quantitation of the slot blot hybridization provided an estimate of 81 copies of the element in pJEL101 per genome. The copy number (≈ 81) and size (≈ 1.0 kb) of the pJEL101 element are most similar to the copy number (2-40) and size (0.7-10 kb) of insertion sequences (Appelbaum et al. 1985; Comai and Kosuge 1983; Flores et al. 1987; Iida et al. 1983; Kaluza et al. 1985; Kleeckner et al. 1988; McLaflery et al. 1987; Mogen and Olsen 1987; Ruvkun et al. 1982), although there is no evidence that the pJEL101 element transposes. The copy number of the repetitive sequence(s) in pS86d appears to be similar to that of the element in pJEL101, based on Southern hybridizations.

All strains of *X. c. pv. oryzae* tested from various geographic areas hybridized extensively with pJEL101. The fact that all strains of *X. c. pv. oryzae* contain the repetitive element in high copy indicates the evolution of the pathovar as a distinct clonal population. The exceptional strains were all from recent collections from the United States (Jones et al. 1989). Fatty acid profiles, physiological and biochemical tests, restriction endonuclease analysis, serological identification, host range, and symptoms on rice grouped the strains from the United States more closely to *X. c. pv. oryzae* than to other pathovars of *X. campestris*. The strains from the United States, however, are not as aggressive to rice as strains from Asia. Strains from Asia and the United States also can be differentiated by monoclonal antibodies generated to strains from the United States. Hybridization with pJEL101 provides additional evidence of differences between the strains at the genomic level.

The 125 pathovars of *X. campestris* are differentiated from one another on the basis of host range and symptoms on a particular host (Bradbury 1984; Leyns et al. 1984). For example, *X. c. pv. oryzae* is differentiated from other pathovars of *X. campestris* by its specificity to rice and from *X. c. pv. oryzicola* by the difference in symptoms induced on rice (blight vs streak). *X. c. pv. oryzae* cannot be identified solely on the basis of biochemical or physiological tests (Vera Cruz et al. 1984). Examination of pathovars of *X. campestris* showed that the pJEL101 sequence could be used as a probe for identification of *X. c. pv. oryzae* by RFLP analysis. Only one other species (*X. c. pv. oryzicola*) possessed a complex hybridization pattern, and the pattern was distinct from that of all strains of *X. c. pv. oryzae*. The high complexity of the signal for *X. c. pv. oryzicola* was intriguing because it is also a rice pathogen. The presence in high copy of the pJEL101 element in all the strains of *X. c. pv. oryzicola* prompts

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**Fig. 7. Southern blot analysis of genomic DNA from Xanthomonas campestris pv. oryzae.** The probe was 32P-labeled pJEL101. Strains PX086K and PX061 of *X. c. pv. oryzae* (lanes a and b, respectively). Strains of *X. c. pv. oryzicola*: BLS303 (lane c), BLS298 (lane d), BLS295 (lane e), BLS292 (lane f), BLS290 (lane g), BLS288 (lane h), BLS286 (lane i), BLS179 (lane j), and BLS175 (lane k). Genomic DNA was digested with EcoRI.
speculation that the pathovar is evolutionarily more closely related to X. c. pv. oryzae than to other pathovars. The differences in hybridization patterns and copy numbers of the element between the X. c. pv. oryzae and X. c. pv. oryzicola, however, suggest a clear divergence of the two populations.

The pJEL101 clone could possibly serve for diagnosis of strains from other pathovars of X. campestris. Nine strains of X. c. pv. oryzicola were found to have characteristic band patterns. Strains of X. c. pv. holcicola, X. c. pv. vascularum, and X. c. pv. phleiprastesis showed similar or identical patterns within the same pathovar designation; no two pathovars had the same pattern. Although pathovar-specific patterns were present, more strains of each pathovar from various geographic locations need to be examined. There was no correlation between type of host plant (monocot vs dicot) and hybridization with pJEL101. It is not known if strains of other pathovars whose DNA hybridized with pJEL101, other than X. c. pv. oryzicola, are pathogenic to rice. Additional sequences of pJEL101 that are not included in the 1-kb repetitive sequence of X. c. pv. oryzae may have hybridized with the DNA from other X. campestris pathovars.

Repetitive sequences provide an alternative strategy for RFLP analysis of genetic relationships among strains of X. c. pv. oryzae. Their dispersed nature allows examination of many segments of the genome at one time. Repetitive sequences may also provide sensitive probes for pathovar diagnosis due to the high copy number. However, the presence of related sequences in other pathovars of X. campestris precludes the use of pJEL101 in dot hybridization procedures (Gilbertson et al. 1989). Other repetitive sequences and specific sequences in the pJEL101 element are currently being examined for pathovar specificity.

Further characterization of the repetitive sequences is required to determine what type or class of repetitive DNA is present in pJEL101. However, the high copy number of the pJEL101 element, the ubiquity of the sequence, the variability of hybridization patterns among strains of the same pathovar, and the presence of a similar element in a related rice pathogen (X. c. pv. oryzicola) compel speculation that the pJEL101 sequence, as well as others, may be involved in genetic adaptation of strains to diverse rice cultivars.

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LITERATURE CITED


