

Assessment of genetic variability and strain identification of *Xanthomonas oryzae* pv. *oryzae* using RAPD-PCR and *IS1112*-based PCR

V. S. Gupta^{†,*}, M. D. Rajebhosale[†], M. Sodhi[‡], Sukhwinder Singh[‡], S. S. Gnanamanickam[#], H. S. Dhaliwal[‡] and P. K. Ranjekar[†]

[†]Plant Molecular Biology Unit, Division of Biochemical Sciences, National Chemical Laboratory, Pune 411 008, India

[‡]Biotechnology Centre, Punjab Agricultural University, Ludhiana 141 004, India

[#]Centre for Advanced Studies in Botany, University of Madras, Chennai 600 025, India

Sixteen isolates of *Xanthomonas oryzae* pv. *oryzae* (*X.o.* pv. *oryzae*) representing different geographical locations in India along with 2 isolates from the Philippines were analysed using polymorphic RAPDs. The primers OPA-03, OPA-04, OPA-10, OPA-11, OPK-7, OPK-12 and OPK-17 generated simple, specific and reproducible fingerprint patterns, indicating usefulness of RAPD markers in differentiating *X.o.* pv. *oryzae* isolates. At a similarity index of 0.5 the isolates were grouped into only 2 clusters, suggesting that these primers are not very efficient in grouping isolates. Primers PJEL1 and PJEL2, used in insertion sequence *IS1112*-based PCR, also generated specific and reproducible fingerprint patterns for the same set of the *X.o.* pv. *oryzae* isolates. Based on the RAPD-PCR (seven primers) and *IS1112*-PCR (two primers) data, at a similarity of 0.57, sixteen out of 18 isolates were grouped into 5 different clusters and 2 isolates were loosely grouped with them. The data using RAPD-PCR and *IS1112*-based PCR approaches revealed their potential in rapid identification of isolates, in assessment of genetic variation in the Indian pathogen population and in generating unique DNA fragments specific to 8 isolates of *X.o.* pv. *oryzae*.

BACTERIAL blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*¹ (*X.o.* pv. *oryzae*) has become an increasingly important problem affecting rice production worldwide. Breeding rice varieties with genetic resistance for bacterial blight is an effective and economical strategy for the control of disease and is an important component of rice improvement programme². However, it is a matter of concern that the widespread use of only a few resistance genes might accelerate the evolution of new pathogenic races leading to outbreak of this disease, causing serious losses in crop production³. In fact virulent strains of *X.o.* pv. *oryzae* causing the bacterial leaf blight have already been detected on the resistant cultivars of rice². Since host population influences the

genetic diversity and population structure of the pathogen, it is important to identify the causal pathogen strains and isolate accurately in a plant pathogen system⁴⁻⁶. Furthermore, *X.o.* pv. *oryzae* is seed-borne⁷, and hence international tracking of the pathogen population would be useful for quarantine programmes.

The development of the polymerase chain reaction (PCR) technique⁸ has provided a highly sensitive method, where a few nanograms of template DNA are enough to obtain simple and reproducible amplification patterns from complex genomic DNA⁹. The amplification reaction using random oligomeric primers produces RAPD (randomly amplified polymorphic DNA) fragments which are extensively used to detect genetic variation in various eukaryotes and prokaryotes. In recent years RAPD-PCR approach has been shown to be useful in classifying a number of microbial strains and species^{10,11}, including various *Xanthomonas* spp. such as *Xanthomonas albilineas*¹², *X. fragariae*¹³, *X. maltophilia*¹⁴ and *X. campestris* pv. *pelargonii*¹⁵. RAPD-PCR has also been used to identify races of *Gremmeniella albertina*¹⁶, ZG groups of *Rhizoctonia solani*¹⁷, geographically distinct populations of *Phytophthora megasperma*¹⁸ and different populations and species of arbuscular mycorrhizal fungi¹⁹.

The repeat probe pJEL101, isolated from *X.o.* pv. *oryzae*, has already been shown to be a potential DNA marker. It could differentiate *X.o.* pv. *oryzae* isolates from other *Xanthomonas* pathovars²⁰, and could also be used to assess genetic diversity in Philippino²¹ and Asian isolates²². Further the repeat element pJEL 101 is found to contain an insertion element, *IS1112*, with a relatively high copy number (about 80 copies). Outwardly-directed primers complementary to *IS1112*, designated as pJEL1 and pJEL2, are shown to have the potential in pathotyping of *X.o.* pv. *oryzae*²³. Another PCR-based approach, namely rep-PCR has also been shown to be useful in measurement of haplotypic variation in *X.o.* pv. *oryzae*²⁴.

In this laboratory, studies were initiated to examine the Indian isolates of *X.o.* pv. *oryzae* at a molecular level. Since very little information was available regarding the molecular pathotyping of Indian isolates, this study was considered to be important in assisting the national rice breeding efforts in relation to bacterial blight disease. In a previous report, potential of various multilocus probes, like pV47, (TG)₁₀ and pBS101 and a gene probe *avrXa10* was shown in analysing the Indian pathogen population²⁵. All these probes needed Southern hybridization, which is time consuming and hence there was a need for a rapid technique such as PCR-based approach which could be more efficient and also sensitive.

In this report, we demonstrate the utility of RAPD-PCR and *IS1112*-based PCR in generating specific genomic fingerprints and their potential in assess-

*For correspondence. (e-mail: Vidya@ems.ncl.res.in)

RESEARCH COMMUNICATIONS

ing genetic variation in Indian isolates of *X.o. pv. oryzae*.

The Indian isolates were obtained from various sources in the form of pure single-cell isolates (Table 1). All the isolates were routinely subcultured on MGYB (malt extract 0.3%, glucose 1%, yeast extract 0.3%, peptone 0.5%) agar slants and were stored as paraffin oil stocks at 4°C and as glycerol stocks at -70°C. The race grouping of *X.o. pv. oryzae* used in the present study was based on virulence tests with differential rice varieties IR8, IR20, IR1545-339, Cas 209 and DV 85 which contain the bacterial blight resistance genes *Xa-11*, *Xa-4*, *Xa-5*, *Xa-10* and both *Xa-5* and *Xa-7*, respectively². For all the PCR reactions, total DNA from bacterial isolates was extracted by a procedure of Rajebhosale *et al.*²⁵. In the present study RAPD-PCR was performed by using a set of two series of primers, namely OPA and OPK (Operon Technologies Inc, USA) each comprising twenty primers. Reaction mixtures for PCR amplification were prepared in a final volume of 25 µl, overlaid with 25 µl of mineral oil. The reaction mixture contained 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.001% gelatin, 100 µM each of dATP, dCTP, dGTP and dTTP, 5 pmol of primer, 20 ng of genomic DNA and 0.3 U Taq polymerase. PCR amplification was performed in a Perkin Elmer Cetus Thermocycler by using following cycles: initial stage at 95°C for 5 min, 45 cycles each of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min and a final soak at 4°C. The amplified products were electrophoresed on a 1.4% agarose gel in 1X

TAE buffer, stained with ethidium bromide and photographed on a UV transilluminator. All the amplification reactions were performed more than thrice for each isolate/primer combination and only those primers giving reproducible patterns were used for scoring.

A total of 16 Indian isolates along with two Philippine isolates of *X.o. pv. oryzae* were examined for their RAPD-PCR patterns on agarose gel electrophoresis. In all, forty random primers from OPA and OPK series were used to amplify the bacterial DNA in PCR reactions. Four primers (OPA-03, OPA-04, OPA-10 and OPA-11) from OPA series and three primers (OPK-07, OPK-12 and OPK-17) from OPK series generated polymorphic and reproducible patterns in three different replicates. All these polymorphic primers yielded multiple DNA amplification products, the sizes of which ranged from approximately 0.1 to 3.0 kb. Both the intense as well as faint bands were scored for calculating similarity index values, so as to maximize the number of scorable characters and minimize the statistical errors²⁶.

Figure 1 depicts a representative electrophoretic pattern of RAPD-PCR amplified products from *X.o. pv. oryzae* Indian isolates using primer OPK-12. As seen in the figure, majority of amplification products are in the form of strong and well-defined bands in the range of ~ 2.5 kb to ~ 0.3 kb. The electrophoretic profile with this primer is highly distinct and polymorphic, generating isolate-specific DNA fingerprint pattern. A few isolates such as IXoCI, PXo86 and IXo3813 (lanes 1, 4 and 18) show presence of many closely-spaced bands in a ladder-like pattern with this primer. The other polymorphic RAPD primers also generated useful information (Figures 2-7). For example, OPA-03, as in Figure 2, amplified at least 2-3 intense bands for each isolate, in addition to faint bands. The isolates IXoII (lane 8) of

Table 1. List of *Xanthomonas oryzae* pv. *oryzae* isolates used in the present investigation

Strain/isolate	Race/group	Geographical origin	Reference/source
IXoIa	Ia	Hyderabad	A. P. K. Reddy
IXo3863	Ia	Chinsurah	S. S. Gnanamanickam
IXo10	Ia	-	S. S. Gnanamanickam
IXo2	Ia	-	S. S. Gnanamanickam
IXoIb	Ib	Hyderabad	A. P. K. Reddy
IXo3813	Ib	Pantanagar	S. S. Gnanamanickam
IXo3841	Ib	Gurdaspur	S. S. Gnanamanickam
IXo15	Ib	-	S. S. Gnanamanickam
IXoII	II	Hyderabad	A. P. K. Reddy
IXo3856	II	Maruteru	S. S. Gnanamanickam
IXo3858	II	Hyderabad	S. S. Gnanamanickam
IXo14	II	-	S. S. Gnanamanickam
IXo9	ND*	Cuttack	R. N. Misra
IXoCI	ND	Ludhiana	Sukhwinder Singh
IXoCII	ND	Ludhiana	Sukhwinder Singh
IXoCIII	ND	Ludhiana	Sukhwinder Singh
PXo35	I	Philippines	S. S. Gnanamanickam
PXo86	II	Philippines	S. S. Gnanamanickam

*ND, Race not determined.

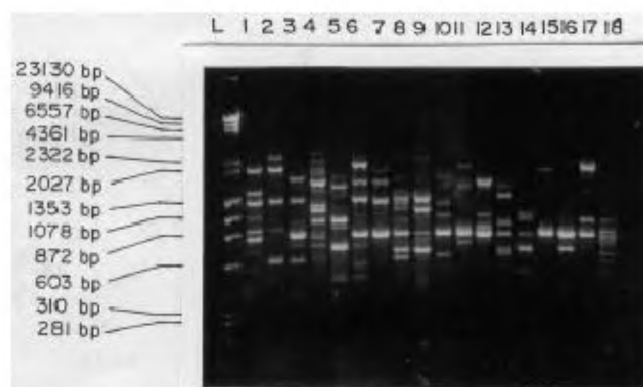


Figure 1. RAPD profile of *X.o. pv. oryzae* DNA using primer OPK-12. Lanes 1-18 contain amplification products obtained using DNA of isolates IXoCI (1), IXoCII (2), IXoCIII (3), PXo86 (4), PXo35 (5), IXoIa (6), IXoIb (7), IXoII (8), IXo2 (9), IXo9 (10), IXo10 (11), IXo14 (12), IXo3856 (13), IXo3858 (14), IXo3863 (15), IXo3841 (16), IXo15 (17) and IXo3813 (18). Lane L contains λ /HindIII and ϕ 174/HaeIII marker.



Figure 2. RAPD profile of *X.o. pv. oryzae* DNA using primer OPA-03. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains λ /HindIII.

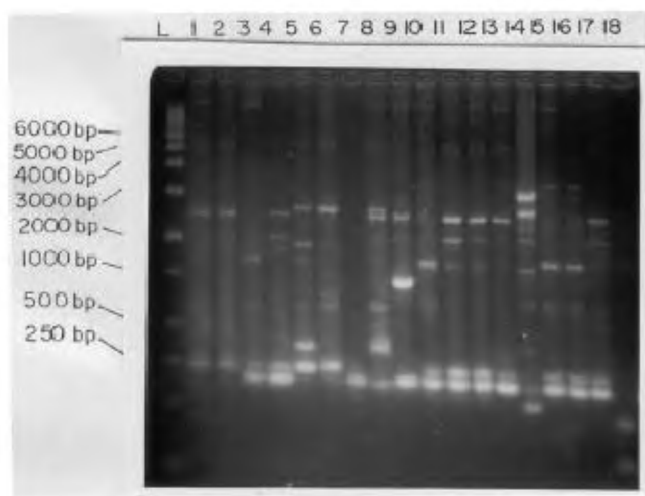


Figure 3. RAPD profile of *X.o. pv. oryzae* DNA using primer OPA-04. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains 1 kb ladder.

race II and IXo2 (lane 9) of race Ia indicated an identical profile with OPA-03, while isolate IXo3858 (lane 14) revealed presence of many intense bands. OPA-04 (Figure 3) showed presence of intense and unique bands for a few isolates, namely IXo3858 (lane 14) and IXo2 (lane 9). OPA-10 (Figure 4) generated an intense band of approximately 2 kb present in all the isolates, except for IXo 3856 (lane 13), where it appeared to be slightly faint compared to other isolates, accompanied by several faint bands. Although the molecular weight of this fragment of 2 kb is similar in all the isolates, the DNA sequence homology still needs to be confirmed. Nevertheless such intense and common amplification product might be pathovar-specific and cloning and sequencing of such products might be useful in generating diagnostic markers. With primer OPA-11 (Figure 5), a band of ~2.5 kb was present in most of the isolates, except IXoII (lane 8) and IXo2 (lane 9). Some isolates had a

distinctly similar pattern, except for 1 or 2 band variations. OPK-07 (Figure 6) showed the presence of a range of 4 to 11 distinct bands in all the 18 isolates. OPK-17 (Figure 7) showed the presence of intense and distinct bands for each isolate.

In another approach using PCR, variable length sequences between copies of the repetitive element



Figure 4. RAPD profile of *X.o. pv. oryzae* DNA using primer OPA-10. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains 1 kb ladder.



Figure 5. RAPD profile of *X.o. pv. oryzae* DNA using primer OPA-11. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains λ /HindIII.



Figure 6. RAPD profile of *X.o. pv. oryzae* DNA using primer OPK-07. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains λ /HindIII and ϕ 174/HaeIII marker.

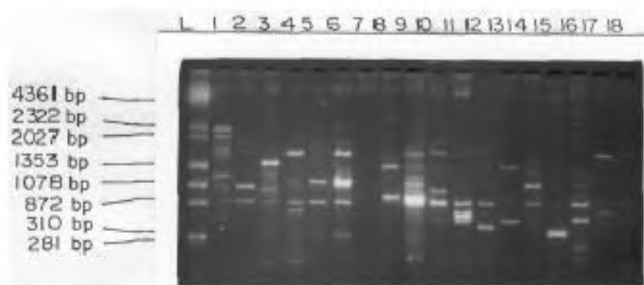


Figure 7. RAPD profile of *X.o. pv. oryzae* DNA using primer OPA-17. Lanes 1–18 contain amplification products obtained using DNA of same isolates as indicated in Figure 1. Lane L contains λ /HindIII and ϕ 174/HaeIII marker.

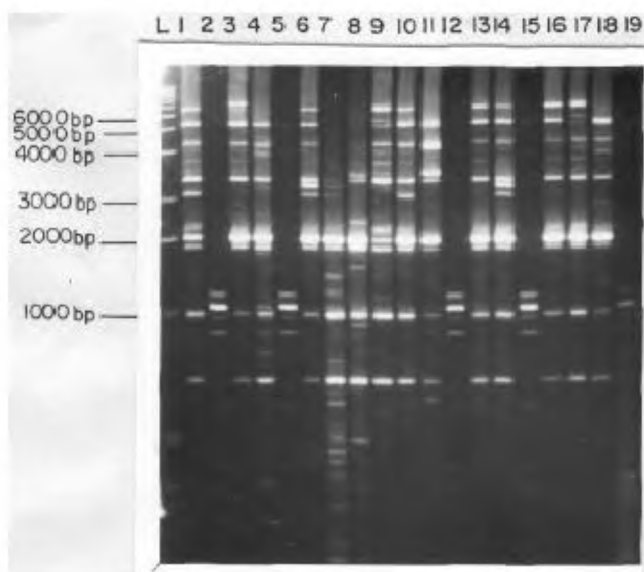


Figure 8. *IS1112*-based PCR profile of *X.o. pv. oryzae* DNA using primer pJEL1 and pJEL2. Lanes 1–19 contain amplification products obtained using DNA of isolates IXo15 (1), PXo86 (2), IXoCII (3), IXoCI (4), IXoIb (5), IXo14 (6), IXo10 (7), IXo9 (8), IXo3841 (9), IXoCIII (10), IXoII (11), IXo2 (12), IXo3863 (13), IXo3813 (14), IXoIa (15), PXo35 (16), IXo3844 (17) not included in present analysis, IXo3858 (18) and IXo3856 (19). Lane L contains 1 kb ladder.

IS1112 were amplified using two outwardly-directed primers (PJEL1 and PJEL2) specific to the ends of the element as described by George *et al.*²³. Reaction mixtures for PCR amplification were prepared in a final volume of 24 μ l overlaid with 24 μ l of mineral oil. The reaction mix contained 1X final concentration of Taq polymerase buffer, 185 μ M each of dATP, dCTP, dGTP and dTTP, 50 pmol each of primer PJEL1 and PJEL2, 10% of dimethylsulphoxide, 31 mM of Tris (pH 9.5), 10 ng of genomic DNA and 2 U Taq polymerase. PCR amplification was performed with a Perkin Elmer Cetus Thermocycler using the following cycles: 1 initial stage at 94°C for 1 min, 30 cycles of denaturation at 94°C for 10 s, annealing at 62°C for 1 min and extension at 65°C for 10 min, followed by a final extension at 65°C for 15 min and a final soak at 4°C. The amplified products

were loaded on a 0.5% agarose/0.75% SynergelTM (equivalent of 2% agarose) in 0.5X TBE buffer, stained with ethidium bromide and photographed on a UV transilluminator. All the amplification reactions were repeated more than thrice for each isolate and only reproducible band patterns were used for scoring.

Figure 8 demonstrates the amplification profile of 16 Indian isolates along with two isolates from Philippines of *X.o. pv. oryzae* using pJEL1 and pJEL2 primers. All the isolates yield multiple DNA amplification products in the range of approximately 10 to 0.2 kb. The number of scorable bands obtained using pJEL1 and pJEL2 primers ranged from 6 to 18 bands per isolate. Many intense bands of 3 kb and above were present in most of the isolates, whereas bands of approximately 2 kb and 1.6 kb were present in 13 isolates, except for isolates PXo86, IXoIb, IXo2, IXoIa and IXo3856 (lanes 2, 5, 12, 15 and 19). Similarly only few bands below 3 kb are present uniformly in all isolates, except for isolate IXo10 (lane 7), where a ladder-like pattern of low intensity bands in addition to intense bands is observed.

A similarity index X_D was calculated for all pairwise comparisons expressing the probability that a fragment in one genotype was also found in another²⁷. Different fingerprint parameters were studied for in-depth analysis of each primer; these are represented in Table 2. The average number of bands detected by the RAPD-PCR approach was as high as 7.4 and the level of band sharing between isolates was as low as 0.21, indicating high level of polymorphism. The primer OPA-04 generated highly polymorphic bands with the least probability of identical match of 1.19×10^{-4} . Although OPK-7 had the highest number of average bands per lane, many of them were monomorphic thus increasing the average similarity index value. The fingerprint parameters using PJEL1 and PJEL2 primer indicate the average number of bands as approximately 12 per isolate; however, most of the bands are monomorphic. The number of polymorphic bands between pairs was the lowest (3.72) with these two primers. The average similarity index value and the value of probability of identical match were considerably higher than those generally obtained with RAPD markers.

The RAPD and *IS1112* gel profiles were also analysed critically for presence of a unique fragment for each isolate. The molecular weight of these fragments was calculated using SEQUAID program²⁸. Table 3 gives information on such unique fragments obtained in 8 specific isolates using specific primers. The isolate IXo3858 is found to generate more unique fragments compared to other isolates, indicating that it might contain more diverse sequence compared to other isolates in the present study. The identification of unique fragments for each isolate will help in rapid identification of the isolate and also can be further utilized to design a diagnostic marker which is isolate-specific. Such mark-

Table 2. Analysis of RAPD-PCR and *IS1112*-based PCR DNA fingerprint patterns

Marker	Fingerprint parameter			
	Average number of bands ($N \pm SD$)	Average similarity index ($X_D \pm SD$)	Average number of polymorphic bands between pairs	Probability of identical match by chance (X_D) ^N
RAPD markers				
OPA-03	6.31 \pm 2.00	0.44 \pm 0.26	7.06	5.62 $\times 10^{-3}$
OPA-04	5.79 \pm 2.65	0.21 \pm 0.26	9.14	1.19 $\times 10^{-4}$
OPA-10	6.84 \pm 2.58	0.43 \pm 0.23	7.79	3.11 $\times 10^{-3}$
OPA-11	5.68 \pm 2.03	0.58 \pm 0.23	4.77	4.53 $\times 10^{-2}$
OPK-07	7.47 \pm 2.52	0.59 \pm 0.49	6.12	2.03 $\times 10^{-2}$
OPK-12	6.26 \pm 2.92	0.38 \pm 0.49	7.76	2.64 $\times 10^{-3}$
OPK-17	4.73 \pm 2.07	0.28 \pm 0.45	6.81	2.38 $\times 10^{-3}$
<i>IS1112</i> -based primers				
pJEL1 and pJEL2	11.95 \pm 3.72	0.73 \pm 0.15	3.72	2.32 $\times 10^{-2}$

Similarity index was calculated as

$$X_D = \frac{2N_{AB}}{(N_A + N_B)},$$

where N_{AB} is number of bands present in both lanes; N_A is the total number of bands in lane A; and N_B is the total number of bands in lane B.

Table 3. Unique fragments for specific isolates

Primer	Molecular weight of unique fragments* (bp)	Isolate
OPA-03	2020	IXo3858
OPA-04	4400	IXo3858
OPA-04	1950	IXo2
OPA-10	1460	IXoIb
OPA-11	1460	IXo3858
OPK-07	530	IXoCIII
OPK-12	2630	IXoCII
OPK-17	420	IXo3858
pJEL1 and pJEL2	3700	IXo3858
pJEL1 and pJEL2	1830	IXo9
pJEL1 and pJEL2	1450	IXo10
pJEL1 and pJEL2	960	IXo3841

*Molecular weights calculated by using computer software SEQAID²¹.

ers can be utilized for tracking the pathogenic isolates and also to study their fitness in field.

Virulence typing of *X.o. pv. oryzae* is done by using differential rice cultivars², which gives an estimate of pathogenicity of these isolates. However, this approach is time consuming, laborious and shows changes in disease resistance with changes in rice cultivars, plant age and environmental conditions. The racial classification is based on the interaction of an *avr* gene in the pathogen and *Xa* gene in the host. In rice, more than twenty-one *Xa* genes are identified which makes the assessment of the overall genetic similarity among the Indian iso-

lates rather difficult. In previous studies, *X.o. pv. oryzae* population in India has been shown to consist of three races/groups, Ia, Ib and II, on the basis of pathogenicity patterns on five indica varieties²⁹. According to another report, out of 86 *X.o. pv. oryzae* Indian isolates, 70 have been classified into either Ia, Ib or II, while 5 belong to new races 3 and 4, and the remaining 11 less-virulent strains have not been assigned any race³⁰. Biological pathotyping can assess only a few genetic loci and which in turn may be subject to selection, thus making it difficult to infer the genetic diversity and evolution of the pathogen.

Similarity indices calculated on the basis of PCR band profiles from both the approaches, (RAPD-PCR and *IS1112*-based PCR) were averaged to obtain a similarity matrix. The purpose of averaging the similarity matrices obtained by all the markers was to maximize the number of loci. Moreover, if cluster analysis was performed using each primer data, some of the isolates would cluster similarly, while others would not. Therefore to get an overall view about the genetic diversity, it is essential to average the similarity matrices obtained by maximum number of markers. The similarity matrix was further utilized for cluster analysis using TAXAN 4.0 (ref. 31). The cluster analysis of *X.o. pv. oryzae* isolates provides a new insight in their genetic relationship (Figure 9). At a cutoff value of 0.57 similarity, 16 isolates are grouped in five clusters and 2 isolates are loosely grouped with them. This similarity level obtained by PCR-based approaches appears to be similar

to our earlier observation²⁵ with hybridization-based probes like (TG)₁₀, pV47, pBS101 repeat element and *avr Xa-10*. However, the clustering of isolates is different in the present investigation. Our hybridization-based approach²⁵ could group the two isolates from Philippines together, whereas in the present investigation the isolates from Philippines are grouped into separate clusters. PXo86 groups with IXo1a and IXo1b in cluster III and PXo35 clusters with IXo14 and IXo3856 in cluster IV. The isolates which group together in hybridization as well as PCR-based approach are IXoCI and IXoCII in cluster I, IXoII and IXo2 in cluster II, IXo10 and IXo14.

As depicted in Figure 9, cluster I includes IXoCI, IXoCII, IXo15 and IXo3813, the latter two belong to race Ib. IXoII and IXo3658 (race II) and IXo2 (race Ia) group together in cluster II. Cluster III includes PXo86, isolate from Philippines belonging to race II and IXo1a and IXo1b, Indian isolates of race Ia and Ib, respectively. IXoCIII and PXo35 group together in cluster IV. Cluster V includes IXo10 and IXo3863 of race Ia, IXo3841 of race Ib and IXo14 of race II. The isolates IXo9 and IXo3856 (race II) are loosely grouped with cluster I and V, respectively. These isolates can be representatives of a new race and need to be characterized by using more rice differentials. Earlier studies by Nelson *et al.*³² have shown that the isolates belonging to a different race, but clustering together on molecular analysis when analysed using more *R* genes showed

presence of new races. The loosely-grouped isolates can also be good candidates for analysing new sources of resistance.

In our molecular pathotyping work, we have used selected isolates collected from different parts of the country and most of them have been characterized earlier using rice differentials. The analysis of such selected isolates may serve as a platform study for refined characterization of new isolates whose racial classification has not been determined. The characterization of isolates at a molecular level may further help in delineating isolates belonging to the same race or group, in studying the pathogenic diversity across the country and monitoring isolates at field level. Handling of a large number of isolates for biological pathotyping is practically difficult and hence alternatively a small group of representative isolates clustering together on molecular basis can be used for analysing the population structure existing in the country, as well as looking for more resistant lines in the host. With the use of arbitrary primers and *IS1112*-based primers, we have shown a new range of molecular genetic markers which can be efficiently used in strain or isolate identification and in assessing genetic variability as well as population structure in *X.o. pv. oryzae* isolates.

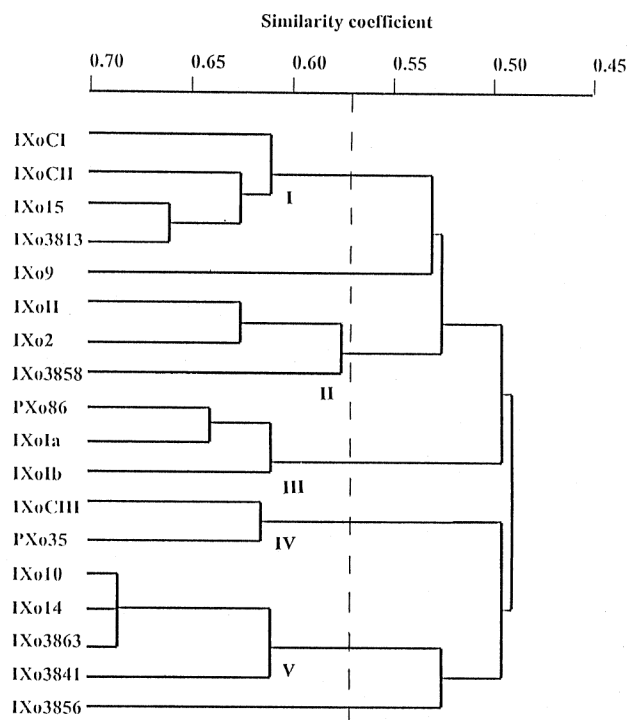


Figure 9. Dendrogram of 18 isolates based on combined similarity indices of the RAPD-PCR gel profile and *IS1112*-based PCR profile.

- Swings, J., Van den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W. and Kersters, K., *Int. J. Syst. Bacteriol.*, 1990, **40**, 309–311.
- Mew, T. W., *Annu. Rev. Phytopathol.*, 1987, **25**, 359–382.
- Browning, J. A. and Frey, K. J., *Annu. Rev. Phytopathol.*, 1969, **7**, 355–382.
- Burdon, J. J. and Jarosz, A. M., in *Plant Population Genetics, Breeding and Genetic Resources* (eds Brown, A. H. D. *et al.*), Sinauer Associates Inc, Sunderland, Mass, 1990, pp. 215–228.
- Leonard, K. J. and Czochor, R. J., *Annu. Rev. Phytopathol.*, 1980, **18**, 237–238.
- Marshall, D. R., *Ann. NY Acad. Sci.*, 1977, **287**, 1–20.
- Mew, T. W., Bridge, J., Hibino, H., Bonman, J. M. and Merca, S. D., in *Rice Seed Health*, Intl. Rice Res. Inst., Philippines, 1988, pp. 101–115.
- Saiki, R. K., Gelfand, D. H., Stoffels, S., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A., *Science*, 1988, **239**, 487–491.
- Welsh, J. and McClelland, M., *Nucleic Acids Res.*, 1990, **18**, 7213–7218.
- Berg, D. E., Akopyants, N. S. and Kersulyte, D., *Methods Mol. Cell. Biol.*, 1994, **5**, 13–24.
- Williams, J. G. K., Kubelik, A. R., Lival, K. J., Rafalski, J. A. and Tingey, S. V., *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
- Permaul, K., Pillay, D. and Pillay, B., *Lett. Appl. Microbiol.*, 1996, **23**, 307–311.
- Pooler, M. R., Ritche, D. F. and Hartung, J. S., *Appl. Environ. Microbiol.*, 1996, **62**, 3121–3127.
- Yao, J. D. C., Comly, J. M. and Krajden, M. J., *J. Clin. Microbiol.*, 1995, **33**, 2195–2198.
- Manulis, S., Valinsky, L., Lichter, A. and Gabriel, D. W., *Appl. Environ. Microbiol.*, 1994, **60**, 4094–4099.
- Hamelin, R. C., Ouellette, G. B. and Bernier, L., *Appl. Environ. Microbiol.*, 1993, **59**, 1725–1755.

17. Duncan, S., Barton, J. E. and O'Berin, P., *Mycol. Res.*, 1993, **97**, 1075–1082.
18. Liew, E. C. Y. and Irwin, J. A. G., *Mycol. Res.*, 1994, **98**, 1284–1290.
19. Wyss, P. and Bonfante, P., *Mycol. Res.*, 1993, **97**, 1351–1357.
20. Leach, J. E., White, F. M., Rhoads, M. L. and Leung, H., *Mol. Plant-Microb. Interact.*, 1990, **3**, 238–246.
21. Leach, J. E., Rhoads, M. L., VeraCruz, C. M., White, F. F., Mew, T. W. and Leung, H., *Appl. Environ. Microbiol.*, 1992, **58**, 2188–2195.
22. Adhikari, T. B., VeraCruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W. and Leach, J. E., *Appl. Environ. Microbiol.*, 1995, **61**, 966–971.
23. George, M. L. C., Leach, J. E. and Nelson, R. J., *Int. Rice Res. Inst. Newsl.*, 1995, 30–31.
24. Vera Cruz, C. M., Ardales, D. Z., Skinner, J. T., Nelson, R. J., Louws, F. J., Mew, T. W. and Leach, J. E., *Phytopathology*, 1996, **86**, 1352–1359.
25. Rajebhosale, M. D., Chowdhari, V. K., Ramakrishna, W., Tamhankar, S., Gnanamanickam, S. S., Gupta, V. S. and Ranjekar, P. K., *Theor. Appl. Genet.*, 1997, **95**, 103–111.
26. Chen, J., Lamikanra, O., Chang, C. J. and Hopkins, D. L., *Appl. Environ. Microbiol.*, 1995, **61**, 1688–1690.
27. Wetton, J. H., Carter Parkin, D. T. and Walters, D., *Nature*, 1987, **327**, 147–149.
28. Rhoads, D. D. and Roufa, D. J., SEQAID II, Kansas State Univ., Mol. Genet. Lab., Manhattan, 1989.
29. Reddy, A. P. K., in Proceedings of the International Workshop on Bacterial Blight of Rice, IRRI, 1989, pp. 79–88.
30. Rehman, F. U., Gnanamanickam, S. S., Adhikari, T. B. and Alvarez, A. M., *Int. Rice Res. Newsl.*, 1993, **18**, 33–34.
31. Swartz, D., *TAXAN 4.0 Users Manual*, Information Resource Group, Maryland Biotechnology Institute, University of Maryland, College Park, MD, USA.
32. Nelson, R. J., Baradian, M. R., Vera Cruz, C. M., Yap, I. M., Leach, J. E., Mew, T. and Leung, H., *Appl. Environ. Microbiol.*, 1994, **60**, 3275–3283.

ACKNOWLEDGEMENTS. We thank Dr A. P. K. Reddy, and Dr R. N. Misra for providing *X.o. pv. oryzae* isolates. M.D.R. is thankful to the Council of Scientific and Industrial Research, New Delhi for award of Senior Research Fellowship. This work was supported in part by the Rockefeller Foundation, USA. M.S. acknowledges the financial assistance and research facilities extended under Asian Rice Biotechnology Network.

Received 16 May 2000; revised accepted 19 January 2001

Application of PCR fingerprinting techniques for identification and discrimination of *Salmonella* isolates

Anjali Tikoo[†], A. K. Tripathi*, S. C. Verma*, N. Agrawal* and Gopal Nath^{†,***}

[†]Department of Microbiology, Institute of Medical Sciences and

*School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi 221 001, India

Different PCR-based DNA fingerprinting techniques were evaluated for identification and discrimination of bacterial strains. Fifteen strains of *Salmonella enterica* subspecies *enterica* serotypes Typhi (10), Paratyphi A (1) and Typhimurium (3) collected over a period of 15 years from stool, blood, and urine samples and river Ganga were tested. All the strains were analysed by restriction analysis of the amplified 16s rDNA (ARDRA), random amplified polymorphic DNA (RAPD) and BOX-PCR methods. In ARDRA, strains belonging to the same species were identified by identical fingerprints; RAPD on the other hand, divided *Salmonella* into 9 different groups. Two ciprofloxacin (CIP)-resistant strains showed similar PCR fingerprints which were different from the rest of the 12 strains. In BOX-PCR, all the strains of *Salmonella* showed 6 different groups which showed the presence of a common band. Serotype Typhimurium could be placed in the same group by BOX-PCR. CIP-resistant strains also produced one characteristic extra band by BOX-PCR. It was observed that RAPD had higher discriminatory power than BOX-PCR and was a simple and rapid technique for use in epidemiological studies of isolates belonging to *S. enterica*.

THE genus *Salmonella* consists of bacilli that are primarily parasites of the intestine of a large number of vertebrate species, including man. It causes enteric fever, gastroenteritis, septicaemia with or without focal suppuration and also carrier state in human beings. Epidemiological studies are done for the prevention and treatment of infections. The ability to distinguish isolates of *Salmonella* may be very important to trace the source of outbreak/s. The relationship, not only among the isolates from patients and environments, but also amongst the multiple isolations from the same patient may be interesting. The methods that have been used for deciphering the relatedness among the isolates are biotyping, antibiogram, plasmid typing, phage typing, serotyping, ribotyping and PCR fingerprinting. Some of the above techniques have low discriminatory potential, whereas others demand considerable amount of expertise, time and equipment.

***For correspondence. e-mail: gnath@banaras.ernet.in