Comparative RFLP Profiles among Representative Strains of Japanese Races of *Xanthomonas oryzae* pv. *oryzae* Using the Repetitive DNA Probe pJEL101

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Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in Asia. Use of varietal resistance has been an effective and economical control method against the disease. However, the results are not always successful, because of the existence of many races of the pathogen. Therefore, information on the race distribution is required to select rice cultivars resistant to the disease. Race differentiation is laborious and time-consuming work due to growing differential varieties, inoculation and scoring. Therefore, development of new methods for the purpose has been expected.

Restriction fragment length polymorphism (RFLP) analysis has been used to classify bacterial genera, species, and pathovars³⁾. Leach *et al.*⁵⁾ detected highly repetitive DNA sequences in the genome of *X. o.* pv. *oryzae*. They indicated that one sequence designated as pJEL101 can be used as a probe for pathovar and strain identification as well as for population analysis^{5,6)}.

In Japan, a differential system distinct from that of IRRI was established. According to the system, strains of *X. o.* pv. *oryzae* from Japan and Indonesia have been classified into races I to VII on the basis of their pathogenicity to the differential varieties^{1,8,9,11)}. The representative strains of Japanese races of *X. o.* pv. *oryzae* have been used as reference strains not only in rice breeding program but in various aspects of the research in other countries as well as in Japan^{1,5,9,11)}.

In this study, therefore, the representative strains of Japanese races of *X. o.* pv. *oryzae* were compared by RFLP using the probe pJEL101 to set up the standard for genetic diversity analysis. It was also studied here whether the strains representative of each race are

distinguishable each other and from Indonesian strains by RFLP using the same probe towards the application to race differentiation.

The strains of *X. o.* pv. *oryzae* used in this study were T7174 (race I) ; T7147 (race II) ; T7133 (race III) ; H75373 and Xo-7435 (race IV) ; H75304 and Xo-7306 (race V) and H8584 (race VII). Of these strains, Xo-7435 and Xo-7306 were obtained in Indonesia by Yamamoto *et al.*¹¹⁾, and have been also used as representatives of races IV and V, respectively. Source and available information concerning the representative strains is presented in Table 1. In addition to the representatives of the races, certain strains from races I, II and III were analyzed for RFLP pattern to compare the genetic diversity within a race.

The bacterial strains were grown in liquid medium containing 10 g of peptone, 5 g of sucrose and 1 g of L-glutamic acid per liter for 16 hr at 28°C on a rotary shaker. The bacterial cells were harvested and washed with STE buffer. Genomic DNA was extracted by the modified procedure of Wilson⁹⁾. The bacterial DNA (approximately 1 µg) was digested to completion with *Eco*RI, *Bam*HI, *Cla*I or *Hind*III at 37°C for 6 hr. The DNA fragments were separated by electrophoresis in 0.8% agarose gels immersed in Tris-borate buffer. Transfer of the DNA fragments from agarose gel onto nylon membranes, and Southern hybridization were done as described by the manufacturer of the Hybond-N membrane (Amersham Japan Co. Ltd.). The probe pJEL101⁵⁾, a pUC18 plasmid containing a 2.4 kb *Eco*RI-*Hind*III fragment derived from PXO86 of Philippine race 2, was labeled with photobiotin⁷⁾. Blots were prehybridized at 42°C for 2 hr in a solution composed of 5× SSPE, 5× Denhardt's solution, 0.5% SDS, 50% formamide

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Table 1. Representative strains of each race of *Xanthomonas oryzae* pv. *oryzae* in Japanese differential system

Strain	Race	Location	Year	Reference
T7174	I	Kyoto Prefecture, Japan	1971	Ezuka <i>et al.</i> ¹⁾
T7147	II	Gifu Prefecture, Japan	1971	Ezuka <i>et al.</i> ¹⁾
T7133	III	Mie Prefecture, Japan	1971	Ezuka <i>et al.</i> ¹⁾
H75373	IV	Okinawa Prefecture, Japan	1975	Horino <i>et al.</i> ⁴⁾
H75304	V	Miyazaki Prefecture, Japan	1975	Horino <i>et al.</i> ⁴⁾
H8584	VII	Miyazaki Prefecture, Japan	1982	Noda and Ohuchi ⁸⁾
Xo-7435	IV	Bali, Indonesia	1974	Yamamoto <i>et al.</i> ¹¹⁾
Xo-7306	V	Bali, Indonesia	1973	Yamamoto <i>et al.</i> ¹¹⁾

Fig. 1. Southern blot analysis of total DNA from the representatives of each race of *Xanthomonas oryzae* pv. *oryzae* from Japan and Indonesia. The probe was photobiotin-labeled pJEL101. Genomic DNA was digested with *Eco*RI (A), *Hind*III (B) and *Cla*I (C). Lane 1, T7174 from Japan (race I); lane 2, T7147 from Japan (race II); lane 3, T7133 from Japan (race III); lanes 4 and 5, H75373 and Xo-7435, from Japan and Indonesia, respectively (race IV); lanes 6 and 7, H75304 and Xo-7306, from Japan and Indonesia, respectively (race V), and lane 8, H8584 from Japan (race VII).

and 1 mg/ml denatured salmon sperm DNA. For hybridization, denatured labeled probe was added directly to the prehybridization solution, and blots were incubated at 42°C for 16–18 hr. Detection of the blots were carried out using BLUGENE™ non-radioactive nucleic acid detection kit (BRL, Gaithersburg, Maryland).

The results showed that the representative strains of Japanese races I, II, III, IV, V and VII were distinguished by the RFLP patterns, irrespective of the restriction enzyme used. The RFLP patterns of *Eco*RI-, *Hind*III-, and *Cla*I-digests of total DNA of the representative strains are shown in Fig. 1A, 1B and 1C, respectively.

The probe pJEL101 hybridized extensively to digested total genomic DNA from the strains used and numerous bands were detected. The number of the bands was also dependent on the restriction enzyme used.

When *Eco*RI, most commonly used restriction enzyme, was used as a cutter, T7174 and T7147, repre-

sentatives of races I and II, respectively, showed a similar RFLP pattern. However, precise comparison revealed that there was difference between the patterns of the two strains. Other strains were also distinguishable by each specific RFLP pattern, though many common bands were observed (Fig. 1A). The most numerous bands were obtained in the *Cla*I-digested total DNA (Fig. 1C). When *Hind*III was used as restriction enzyme, the hybridization was less complex than that observed in DNA digested with *Eco*RI, and each representative strain could be differentiated clearly and easily by the pattern (Fig. 1B).

Among the strains, H8584, representatives of race VII, showed a very unique pattern, irrespective of the restriction enzymes used. In contrast, T7174 and T7147, representatives of races I and II, respectively, showed a similar RFLP pattern not only in *Eco*RI digests as mentioned above, but also in other two enzymes digests. However, precise comparison revealed that there was

Fig. 2. Southern blot analysis of certain strains belonging to races I (A and B), II and III of *X. o. pv. oryzae* together with representatives of Japanese races IV, V and VII. The probe was photobiotin-labeled pJEL101, and genomic DNA was digested *Bam*HI.

difference between the patterns of the two strains. The strains T7133 and H75373, representative of races III and IV, also revealed the similar RFLP patterns. However, the two strains were differentiated by a specific band or bands. Other strains were also distinguishable by each specific RFLP pattern, though many common bands were observed.

Between Japanese and Indonesian strains, race IV strains H75373 and Xo-7435 from Japan and Indonesia, respectively, showed a different pattern, while the same pattern was observed between the race V strains H75304 and Xo-7306 from Japan and Indonesia, respectively.

Based on the results obtained here, it appears possible to distinguish the representative strains of Japanese races of *X. o. pv. oryzae* by RFLP patterns. However, when the strains belonging to the same race were analyzed with the same method, RFLP patterns were not always the same.

Figure 2 shows the comparative RFLP patterns of *Bam*HI-digests from certain strains belonging to race I, II and III, respectively, together with those of the representative strains of other Japanese races. Among the strains belonging to the same race, all of them did not show the same RFLP patterns, and were grouped into subgroups on the basis of their patterns. However, overlapping of the RFLP patterns among the races was

not observed except a small number of strains between races I and II.

Ogawa⁹⁾ tested the pathogenicity of certain strains of *X. o. pv. oryzae*, and he demonstrated that race I and III strains were grouped into two categories A and B on the basis of their pathogenicity.

In race I, race IA strain T7174 and H9156 showed a different pattern from other strains belonging to race IB. A similar result was obtained between T7133 (IIIA) and Q6803 (IIIB). Thus, the difference in pathogenicity may coincide with the RFLP patterns.

Further comparison using many strains is necessary to prove that the RFLP analysis can be used to differentiate races of *X. o. pv. oryzae*, though RFLP may be applicable to race differentiation, if the probe is modified or new probe related pathogenicity is developed.

The results obtained here showed that the genetic diversity among the Japanese strains of *X. o. pv. oryzae* can be detected using the probe pJEL101.

Assessment of the genetic diversity of Japanese strains as well as those from South East Asia, *i.e.* Thailand, Malaysia, India, Nepal, Bangladesh, Myanmar including Indonesia and the Philippines is underway.

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和 文 摘 要

加来久敏・平八重一之・落合弘和・兼松聡子・野田孝人・土屋健一・日比忠明：日本産およびインドネシア産イネ白葉枯病菌各レース代表菌株の RFLP 解析

本邦産イネ白葉枯病菌の遺伝的多様性解析を目的として、さらに遺伝子解析によるレース判別の可能性を探るため、イネ白葉枯病菌各レースの代表菌株について RFLP 解析を行った。イネ白葉枯病菌由来の高頻度反復配列を含む pJEL101 をプローブとして、各代表菌株の全 DNA を各種制限酵素で切断した断片についてサザンブロット解析を行った結果、いずれの制限酵素を用いても各代表菌株は固有の RFLP パターンを示し、相互の重複は認められなかった。また、同じレースに属する日本産とインドネシア産菌株は互いに相同な場合と異なる場合とがみられた。さらに、一部の品種に対する病原性の差異からレース IB および IIIB に分類された菌株について検討した結果、それらは各々レース IA および IIIA とは異なる RFLP パターンを示した。しかしながら、同一レースに属する複数の菌株を供試して同様な解析を行った場合、RFLP パターンによりさらに細分類が可能であり、またレース I および II の一部の菌株間でパターンの重複が認められた。以上の結果から、pJEL101 をプローブとして RFLP パターンによる遺伝的多様性の解析、さらに各レース代表菌株の識別が可能であることが明らかとなった。しかしながら、本法をレース判別に適用するためにはプローブの改良あるいは新プローブの開発が必要であると考えられた。

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