Comparative AFLP Profiles among Strains of Korean Races of *Xanthomonas oryzae* pv. oryzae.

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ABSTRACT

We used an amplified fragment length polymorphism (AFLP) analysis, a novel PCR-based technique, to differentiate *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) of Korean races. The 6 strains of *Xoo* K1, K2, K3 races were tested with 81 AFLP primer combinations to identify the best selective primers. The primer combinations were selected according to their reproducibility, number of polymorphic bands and polymorphism detected among *Xoo* strains. 18 strains of *Xoo* K1, K2 and K3 races were analyzed with the selected combinations of primer set. Some primer combinations (*Eco* R I +1 / *Mse* I +1) could differentiate *Xoo* of Korean races that were not distinguished by other fingerprinting analysis. Thus AFLP fingerprinting permitted very fine discrimination among different races.

Keywords **:** *Xanthomonas oryzae* pv. *oryzae*, Race, AFLP

INTRODUCTION

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive diseases of rice in Asia. The use of resistance varieties has been an effective and economical control method against BLB (Adhikari *et al.*, 1995). However, The varietal control of the BLB have not always successful, because of diversities of the pathogen. The 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 has been considered to be urgent. In Korea, a differential systems distinct from that of IRRI was established. According to the systems in korea, strains of *Xoo* has been classified into races K1 to K5 on the basis of their pathogenicity to the differential varieties, but K4 and K5 has not been found since 1986. The genetic diversity of *Xoo* was mainly characterized by RFLP analysis, using the repetitive element (pJEL101) as the RFLP probe (Horita, *et al.* 1995; Kaku *et al.*, 1996; Leach *et al.*, 1995). However, RFLP results by pJEL101 were not classified Korean races. Amplified restriction fragment length polymorphism (AFLP) is a new and high resolution fingerprinting method for bacterial species. (Jassen, et al., 1996; Lin *et al.*, 1996). AFLP generates fingerprints from DNA of both eukaryotic and

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prokaryotic origin without any prior knowledge of the sequence (Vos *et al.*, 1995).

In this study, korean races of *Xoo* were compared with AFLP profiles to set up the standard for genetic diversity analysis. It was also studied here whether the strains of each races could be classified each other using the results obtained by AFLP methods.

MATERIALS AND METHODS

The strains of *Xoo* used in this study were eighteen local field isolates collected in regions of Chonbuk and Chonnam province in Korea. The occurrences disease has been recorded frequently in Korea from 2001 to 2002. The sources and available information concerning the strains are presented in Table 1. All strains have been verified their races by pathogenicity test. For the genomic DNA extraction, the bacteria were grown overnight on a rotary shaker in liquid YPG medium. The bacterial genomic DNA was extracted using genomic DNA extract kit (Promega). Eighteen of *Xoo* strains were analyzed using commercial AFLP Microbial Fingerprinting kit (Applied Biosystems). Genomic DNA (500 *ng* per each sample) was digested 5 U of *Eco* R I and 5 U of Mse I, and ligated by T_4 DNA ligase (5 U μl^{-1}, NEB) for overnight at 4.0℃. Digested (D) and ligated (L) DNA were diluted (D) and the resulting DLD DNA was then stored at 4℃ until used. DLD DNA (4.0 μl) was added to 20.0 μl mixture containing 1.0 μl *Eco* R I-primer (1.0 μM), 1.0 μl *Mse* I-primer (5.0 μM), 15 μl AFLP core amplification mix. PCR reaction was performed with the touch-down PCR thermal profile, with the initial annealing temperature of 65℃ and subsequent reduction by 1℃ per cycle to 56 ℃ . Amplified fragments were separated by electrophoresis on 6% polyacrylamide gels and

Table 1. The strains of *Xanthomonas oryzae* pv. *oryzae* used in this study.

Strain	Race	Location	Year
HB01013	K1	Chonnam	2001
HB01014	K2	Chonnam	2001
HB01015	K3	Chonnam	2001
HB0205	K3	Chonbuk	2002
HB0207	K1	Chonbuk	2002
HB0208	K2	Chonnam	2002
HB0209	K2	Chonnam	2002
HB0210	K1	Chonbuk	2002
HB0217	K3	Chonnam	2002
HB0219	K3	Chonnam	2002
HB0221	K3	Chonbuk	2002
HB0222	K1	Chonbuk	2002
HB0223	K3	Chonbuk	2002
HB0224	K3	Chonnam	2002
HB0229	K2	Chonnam	2002
HB0230	K1	Chonnam	2002
HB0232	K2	Chonnam	2002
HB0234	K1	Chonbuk	2002

Table 2. Number of polymorphic bands obtained by AFLP analysis.

Strains	Primer combination	No. of polymorphic bands/ total bands(average)
HB01013[1)] vs. HB01014	*Eco R* I+ 0 / *Mse I* + 2	2/35
	Eco R I+ 1 / *Mse I* + 1	4/56
	Eco R I+ 2 / *Mse I* + 0	2/44
HB01013 vs. HB01015	*Eco R* I+ 0 / *Mse I* + 2	10/35
	Eco R I+ 1 / *Mse I* + 1	12/56
	Eco R I+ 2 / *Mse I* + 0	7/44
HB01014 vs. HB01015	*Eco R* I+ 0 / *Mse I* + 2	5/35
	Eco R I+ 1 / *Mse I* + 1	9/56
	Eco R I+ 2 / *Mse I* + 0	5/44

[1)]Representative strains of each races: HB01013 (K1), HB01014(K2), HB01015(K3).

fingerprint patterns were visualized as described by Vos *et al.* (1995). To identify the best selective primers, tests were conducted on six representative strains of K1, K2, and K3 races with 81 primer combinations. The entire sets of eighteen strains was then analyzed, using selected combinations of primers. The reproducibility of AFLP was assessed by comparing the fingerprinting obtained from duplicate assays of six strains each other based on all primer combinations.

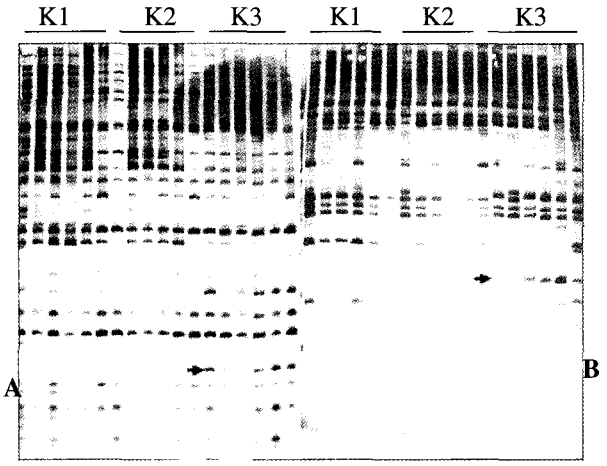

Fig 1. Section of AFLP fingerprint of *Xoo* Korean races. Arrows indicate polymorphic bands to differentiate K3 among *Xoo* K1, K2, and K3 races. The same 18 strains (each 6 isolates of each races) were used for each primer combinations. Primer combinations of A and B are each different *Eco* R I +1/*Mse* I +1 sequence.

RESULTS AND DISCUSSION

AFLP markers were assessed for their usefulness in characterizing molecular diversity among *Xoo* strains. The representatives (HB01013, HB01014, HB01015, HB0205, HB0207, and HB02017) of K1, K2, and K3 races of *Xoo* strains were analyzed with all 81 primer combinations. Among all the primer combinations tested, some primer combinations that showed similar pattern within strains of same race were selected. Some primer combinations were selected according to their reproducibility, polymorphic band and polymorphism detected among *Xoo* races, and All *Xoo* strains were analyzed with selected primer combinations. The number of polymorphic fragments for each primer combinations varied from 2 to 12 with an average of 6.2 polymorphic fragments per primer combination (Table 2).

With *Eco* R I +2 / *Mse* I +0, or *Eco* R I +0 / *Mse* I +2 primer pairs, the each representative strains of race K1, K2 and K3, showed a similar AFLP pattern. When *Eco* R I +1 / *Mse* I +1 primer pair was used as primer combinations, strains HB01013 and HB01014, representatives of race K1 and K2 respectively, showed

a similar AFLP pattern. However, precise comparison revealed that there was difference between the patterns of the two strains. The strain HB01015, representative of race K3 was also distinguishable by each specific AFLP pattern though many common bands were observed. The most numerous bands were obtained *Eco* R I+1 / *Mse* I +1 primer pairs.

When *Eco* R I +0 / *Mse* I +2 was used as primer pairs, the pattern was less complex than that observed in *Eco* R I +1 / *Mse* I +1, and each tested strain could be differentiated clearly and easily by the pattern. With *Eco* R I +1 / Mse I +1 primer combinations, the strain of K3 race showed a very unique polymorphic band (Fig. 1). The strains of race K3 showed the similar AFLP patterns. However, this strains were differentiated by specific band. In contrast, the strains of race K1 and K2 showed a similar AFLP pattern not only in *Eco* R I +1 / *Mse* I+1, but also in other primer combinations, respectively. However, distinctive difference among the pattern of strains of K1 and K2 race were revealed with careful comparison.

Based on the results obtained in this paper, it can be considered to distinguish the strains of Korean races of *Xoo* by AFLP patterns. When the strains belong to same races were analyzed with sixteen primer combinations, profiles of AFLP patterns were always the same. The results obtained here showed that the genetic diversity among the strains of *Xoo* could be detected using the *Eco* R I +1 / *Mse* I +1 primer combinations so that AFLP may be applicable to race differentiation. Some combinations permitted to differentiate *Xoo* strains that were not distinguished by RFLP analyses. Further comparison using many strains is necessary to prove that the AFLP analysis can be used to differentiate the Korean races of *Xoo*. AFLP analyses allowed establishing the phylogenetic relationships among *Xoo* strains. Further application of the AFLP technique to the study of *Xoo* will be conducted.

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