Preliminary Analysis of the Population Structure of *Xanthomonas oryzae* pv. *oryzae* in China

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中国水稻白叶枯病菌群体结构的初步分析

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摘 要:用两个 DNA 探针,pJEL101 和 pBSavrXa10,测定了 78 个来自 18 个地区的水稻白叶枯病菌系的 RFLP 型,其中主要来自华北、东北和少数来自华中、华南,以分析其群体结构和遗传多样性。各菌系的基因组 DNA 分别用限制性内切酶 *Eco*R Ⅰ和 *Bam*H Ⅰ酶切探针 pJEL101 或 pBSavrXa10。通过分子杂交,分别鉴定出 16 种 RFLP 型。病原型 1、2 和 6 的多数来自华北和东北菌系表现 14 种 RFLP 型。它们之间多数只有一带之差,或有 3～4 个带位不同。然而,它们与来自广东和云南的菌系相比,差别较大,有 7～8 条带位不同。根据参试菌系的 RFLP 带型分簇(Cluster),如以彼此之间的带位相似率达 85%为界,可分为 6 簇,多数病原型 1、2 和 6 的菌系为簇 1,少数几个菌系,LN43、GD1358 和 YN5 分别为簇 4、5 和 6。参试的菌系群体遗传变异为 0.77(pJEL101)和 0.83(pBSavrXa10)。也分析了亚群体的各病原型内的遗传变异,初步分析表明中国的水稻白叶枯病菌系的遗传变异多种多样。但尚需测试更多的来自华南、华中和西南地区的菌系,方能较系统地分析全国菌系的群体结构及其遗传关系。

关键词:水稻白叶枯病;遗传多样性;群体结构;RFLP 型;菌系

Abstract: Seventy eight strains of *Xanthomonas oryzae* pv. *oryzae* collected mostly from North, Northeast and a few from Center and South China have been assessed the RFLP types for analyzing the population structure and genetic diversity by using two probes, pJEL101, and pBSavrXa10. Genomic DNA from each strain of *X. o.* pv. *oryzae* was digested to completion by enzymes *Eco*RI or *Bam*HI for blots probed with pJEL101 or pBSavrXa10. Sixteen RFLP types were identified respectively. Fourteen RFLP types were found in strains of pathotypes 1, 2 and 6, which were from North and Northeast China (using pJEL101). Among them differed only at one or 3–4 banding positions. In comparison between those with RFLP types of strains from Guangdong and Yunnan provinces were distinct from one another at 7 or 8 positions varied. Cluster analysis based on RFLP banding showed six groupings at 85% similarity. The majority of strains from pathotypes 1, 2 and 6 were grouped in clusters 1, A few strains, LN43, GD1358 and YN5 were grouped in clusters 4,5 and 6, respectively. The genetic diversities of the population of strains tested was 0.77 (for pJEL101) and 0.83 (for pBSavrXa10) respectively. Genetic diversities for each pathotypes tested as a subpopulation has been also calculated. The preliminary analysis here showed that the genetic diversity of *X. o.* pv. *oryzae* in China was various. It will be necessary to assess more strains collected from South, Center and Southwest China for analyzing the population constructure and genetic relationship of *X. o.* pv. *oryzae* in China systematically.

Key words: bacterial blight; genetic diversity; population structure; RFLP type; strains

In previous studies, *Xanthomonas oryzae* pv. *oryzae* (*X. o.* pv. *oryzae*) population in China have been classified seven pathotypes on the pathogenicity patterns on a set of Chinese differential cultivars[1]. The host–pathogen response rice–*Xoo* system was, however, still not clear–cut. The pathotype groupings provided little

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Note:The project was cooperated with Leach, at Kansas State University, USA; Rebecca, and Mew at IRRI in 1989 and 1993.

insight into the genetic structure of the bacterial population. Recently, restriction fragment length polymorphism (RFLP) analysis, which exploits the abundant variation in the DNA sequence, has been used to generate a large number of markers for the measurement of genetic diversity in population[2,4]. In order to better understand the population structure of *X. o.* pv. *oryzae*, two probes were used to measure the genetic diversity of *X. o.* pv. *oryzae* populations and relate the diversity to previous characterization of the pathogen in China.

1 Materials and Methods

1.1 Bacterial strains and DNA probes

Seventy eight strains were used in this study in RFLP analysis. Most of the strains were collected from North and Northeast China, a few from South and Southwest China. The data of collection, source cultivar, and pathotype grouping for the *X. o.* pv. *oryzae* strains used are shown in Table 1. All stock cultures were kept as lyophilized cultures. The strains were revived in fresh slants of PBS medium for DNA isolation.

Two DNA probes were used in this study. One is pJEL101, a pUC18 plasmid containing a 2.4 kb *Eco*R I − *Hin*d III fragment derived from Philippine race 2 strain PXO86 of *X. o.* pv. *oryzae*. The insert of pJEL101 contains a high-copy-number repetitive DNA element present in DNA from over 100 *X. o.* pv. *oryzae* strains tested. Other one is plasmid pBSavrXa10, containing a 3.1 kb *Bam*H I fragment internal to the avrXa10 gene of *X. o.* pv. *oryzae*[3].

1.2 DNA analysis

Genomic DNA from strains of *X. o.* pv. *oryzae* was isolated as described by Leach *et al.*[4], and digested with *Eco*R I or *Bam*H I for blots probed with pJEL101 or pBSavrXa10. DNA fragments were electrophoresed in 0.7% agarose gel (20 by 21.5 cm) in TBE buffer. A 1-kb ladder was included in gel as a size standard. Southern transfer onto a nylon membrane was done according to instructions of the manufacturer. The entire pJEL101 and pBSavrXa10 plasmids were labeled with ^{32}P by using a nick translation kit. Hybridization and washing conditions were high stringency. Autoradiography was done at −80 ℃ with Cronex film with Cronex Lightning-Plus intensifying screens. DNA from strains of same geographic origin analyzed in the same gel primarily. Distinct RFLP types were analyzed in a single blot to confirm that each RFLP type was unique.

1.3 Data analysis

To determine the genetic relationships among RFLP types, the presence or absence of bands at 20 (for using pJEL101, based on appearing a difference in banding) or 19 (for using pBSavrXa10, appeared 19 total markers) different marker positions was converted into binary data; i. e., the presence or absence of a band was coded as 1 or 0 respectively[4].

Cluster method, UPGMA[5] was used to infer phylogenetic relationships among strains of *X. o.* pv. *oryzae* based on RFLP data. Clusters that separated at the 85% similarity level were grouped and given designations, with cluster 1 being the cluster whose strains were most similar, cluster 2 being the cluster whose units were the second most similar, and so on. Genetic diversity (H) was calculated according to the following equation[4].

$H=[n/(n-1)]\times(1-X_i^2)$, $i=1$, where X_i is the frequence of the i^{th} haplotype in the population, and n is the number of strains examined.

2 Results

2.1 RFLP analysis

Seventy eight strains of *X. o.* pv. *oryzae* for DNA polymorphisms were examined with two DNA probes pJEL101 and pBSavrXa10, sixteen RFLP types were identified respectively (Fig.1, Fig.2). Most of strains tested within pathotypes 1, 2 and 6 were grouped in RFLP types 1 to 4 (Table 2), among them differed only at one banding position. The banding patterns among some RFLP types were distinct from one another at several banding positions, i. e., 7 or 8 positions vary between RFLP 11 and 1, 15, 6, respectively(Fig. 3). Similarly, using pBSavrXa10, 7, 8 and 11 positions vary between RFLP type 11 and 10, 13 and 14, respectively (Fig. 2).

Table 1. *X. o.* pv. *oryzae* **strains from China used in RFLP analysis**

Strain	Pathotype[1]	RFLP type[2]			Year of collection	Geographic origin (grouping)	Varieties
		pBSavrXa10	pJEL101	Combined			
HLJ72	1	2	2	1	1985	Echeng, Heilongjiang	Chengjian 6
HB20	1	2	2	1	1984	Chengde, Hebei	Jigeng 60
HB21	1	2	2	1	1984	Fengning, Hebei	Muzhan 2
NX39	1	2	2	1	1984	Yongning, Ningxia	Jingyin 35
NX40	1	2	2	1	1985	Wuzhong Ningxia	Xinghuozhan
LN51	1	2	2	1	1985	Donggou Liaoning	7530-4-3
LN52	1	2	2	1	1985	Donggou, Liaoning	Zhongdan 1
LN55	1	2	2	1	1985	Donggou, Liaoning	Jingyue 1
LN56	1	2	2	1	1985	Liaozhong, Liaoning	Liaogeng 5
BJ7	1	3	1	2	1985	Daxing, Beijing	78-10
HB30	1	3	1	2	1985	Zhuoxian, Hebei	Zhonghua 8
LN45	1	3	1	2	1985	Liaozhong, Liaoning	Liaogeng 5
HLJ69	1	3	1	2	1985	Wuchang, Heilongjiang	Jigeng 60
HLJ68	1	3	1	2	1984	Wuchang, Heilongjiang	Jigeng 60
LN44	1	3	2	3	1984	Kaiyuan, Liaoning	Liaogeng 5
BJ3	1	4	4	4	1984	Shunyi, Beijing	Nongken 5
JL62	1	4	4	4	1984	Ji'an, Jilin	Nuodao
HB25	1	14	2	5	1984	Baigezhuang, Hebei	Zhonghua 8
HB24	1	6	6	6	1984	Tangxian, Hebei	Qianjunbang
HB23	1	6	6	6	1984	Tangxian, Hebei	720
HB32	1	6	1	7	1984	Henan	Zhengeng 107
BJ2	1	1	2	8	1984	Fangshan, Beijing	Yuefu
BJ1	1	1	1	9	1984	Fangshan, Beijing	Zhonghua 8
SC38	1	1	1	9	1977	Sichuan	–
BJ11	1	9	9	10	1984	Daxing, Beijing	Qiuyou 20
BJ12	1	9	9	10	1985	Haidian, Beijing	Jingdao 2
BJ74	1	9	14	11	1986	Shuangqiao, Beijing	Zhongzuo 180
KS6-6	2	1	1	9	–	Jiangsu	–
BJ4	2	1	1	9	1984	Haidian, Beijing	Yueshi
BJ9	2	1	1	9	1985	Daxing, Beijing	Zhonghua 9
BJ10	2	1	1	9	1985	Daxing, Beijing	78-10
HB14	2	1	1	9	1984	Tanghai, Hebei	Jingyue / C57
HB17	2	1	1	9	1984	Fengrun, Hebei	7608
HB22	2	1	1	9	1984	Tangxian, Hebei	720
HB26	2	1	1	9	1984	Baigezhuang, Hebei	Zhonghua 8
HB27	2	1	1	9	1984	Tanghai, Hebei	Kenfeng 2
HB29	2	1	1	9	1984	Funing, Hebei	Xingshi
HLJ71	2	1	1	9	1985	Baoshan, Heilongjiang	Jingyin 127
HUN1	2	1	1	9	–	Hunan	–
SD38	2	1	5	12	1984	Linyi, Shandong	Jingyi 119
HN31	2	1	5	12	1984	Kaifeng, Henan	Zhonghua 8
SX73	2	1	6	13	1985	Nanzhen, Shaanxi	Zhenshan 97
BJ5	2	1	16	14	1984	Haidian, Beijing	Zhongxi 8419
HB18	2	1	8	15	1984	Fengrun, Hebei	Zhonghua 9
HB19	2	1	8	15	1984	Fengrun, Hebei	Kengfeng 5

Table 1-continued

Strain	Pathotype[1]	RFLP type[2]			Year of collection	Geographic origin (grouping)	Varieties
		pBSavrXa10	pJEL101	Combined			
HN33	2	1	7	16	1984	Kaifeng, Henan	Xindao 68-11
JL64	2	1	2	8	1985	Zhangjiadian, Jilin	126
JL65	2	1	2	8	1985	Gudianzi, Jilin	126
JL67	2	1	2	8	1985	Yongji, Jilin	Jingyin 127
LN48	2	2	2	1	1985	Donggou, Liaoning	Zhonghua 9
NX42	2	6	1	7	1985	Wuzhong, Ningxia	Ninggeng
HN34	2	6	1	7	1984	Kaifeng, Henan	Zhengeng 107
JL63	2	7	1	17	1984	Ji'an, Jilin	Jigeng 60
HN35	2	7	1	17	1984	Kaifeng, Henan	Zhengeng 107
JL63	2	7	2	18	1984	Ji'an, Jilin	Zhongzuo 75
BJ8	2	8	1	19	1984	Daxing, Beijing	8-10
TJ13	2	8	1	19	1984	Jixian, Tianjin	Kenxi 2
KS1-21	2	13	13	20	-	Jiangsu	-
HN36	3	6	1	7	1985	Xinxiang, Henan	Zhengeng 107
HN37	3	7	5	21	1985	Xinxiang, Henan	68-11
LN57	4	1	5	12	1985	Liaozhong, Liaoning	Liaogeng 5
ZHE173	4	5	2	22	-	Zhejiang	-
FJ1	4	5	2	22	-	Fujian	-
ZHE18	4	5	2	22	-	Zhejiang	-
ZHE35	4	5	2	22	-	Zhejiang	-
JL76	4	16	16	23	1985	Tonghua, Jilin	Tongxi 22
GD1358	5	11	11	24	1982	Xinyi, Guangdong	Zhi 20-5
HB15	6	2	2	1	1984	Zunhua, Hebei	Jingyue 1 / C 57
HB16	6	2	1	25	1984	Zunhua, Hebei	Jingyue 1
LN47	6	2	2	1	1985	Xinmin, Liaoning	292
LN53	6	2	2	1	1985	Donggou, Liaoning	Zhongdan 2
LN49	6	4	3	26	1985	Kaiyuan, Liaoning	Zhongzuo 59
LN50	6	4	3	26	1985	Kaiyuan, Liaoning	Tegeng 1
LN54	6	4	3	26	1985	Donggou, Liaoning	Jingyue 1
LN43	6	10	10	27	1984	Liaoning	Liaogeng 5
JS49-6	7	1	1	9	-	Changsha, Hunan	-
Jiangling 691	?	12	12	28	1981	Hubei	IR8
YN5	?	15	15	29	-	Yunnan	-

[1]Pathotype was determined by inoculation to the rice differential cultivars. IR26, Java14, Tetep, Nanjing 15, Jingang 30.

[2]RFLP type determined from Southern blot analysis of *Eco*R I -digested total DNA and *Bam*H I digested total DNA from strains of *X. o.* pv. *oryzae* with pJEL101 and pBSavrXa10 as probes respectively.

To determine the genetic relationships among strains of pathotypes, the banding patterns were analyzed by using cluster method, UPGMA. All strains were similar to each other at a level of 85% similarity, six clusters of strains were observed in sixteen RFLP types using pJEL101 probe (Fig. 4). Pathotypes represented in the six clusters were as follows: cluster 1, pathotypes 1, 2 and 6; cluster 2, pathotypes 3 and 4; cluster 3, pathotype 1; cluster 4, 5 and 6, pathotypes 6, 5 and unknown (strain YN5), respectively. Most of strains used were contained within one cluster: pathotype 1 (26 of 28 strains

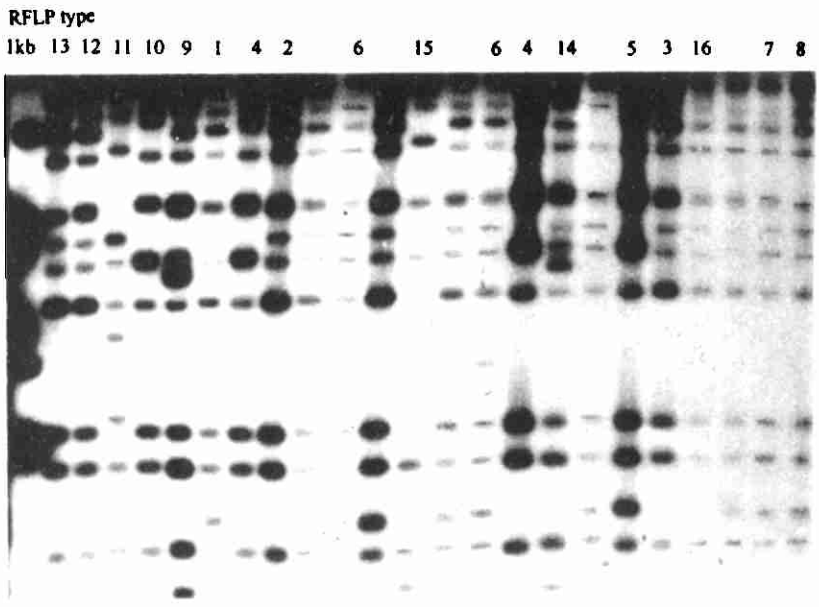

Fig. 1. Southern blot analysis of genomic DNA of strains of *X. o.* pv. *oryzae* in China showed 16 RFLP types (The probe used was ^{32}P–labeled pJEL101)

tested) pathotype 2 (29 of 31 strains), and pathotype 6 (7 of 8 strains) in cluster 1 (Table 2).

2.2 Genetic diversity

Genetic diversity values were calculated for each pathotype based on RFLP data derived with probes pJEL101 and pBSavrXa10 (Table 2). Approximative values, 0.77 and 0.83 were estimated

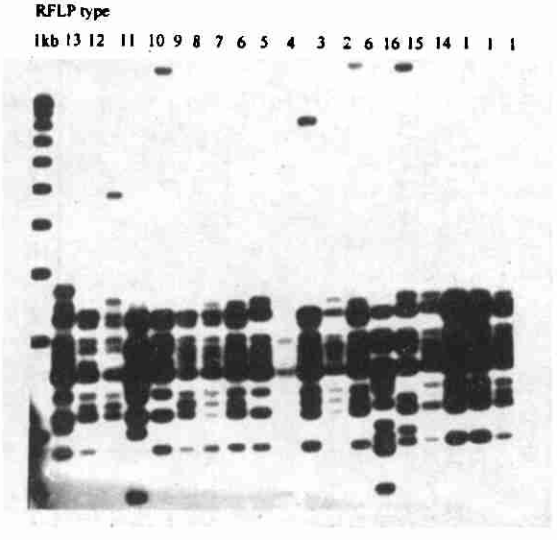

Fig. 2. Southern blot analysis of genomic DNA of strains of *X. o.* pv. *oryzae* in China showed 16 RFLP types (The probe used was ^{32}P–labeled pBSavrXa10)

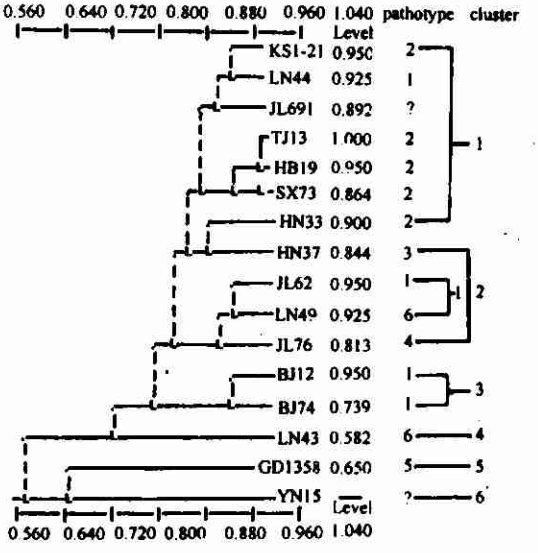

Fig. 3. Dendrogram displaying the relationships among the 16 RFLP types for strains of *X. o.* pv. *oryzae* after southern blot analysis using ^{32}P– labeled pJEL101 as a probe. Similarity coefficients were grouped by using the unweighted pair group method with arithmatic means

Table 2. Frequencies of RFLP types of *X. o.* pv. *o.ryzae* according to pathotype grouping and the estimated genetic diversity of each group (pJEL101 and pBSavrXa10)

RFLP type	Type strain	Total population		Frequency of RFLP type in each pathotype grouping													
				1		2		3		4		5		6		7	
		NS	NP	NS	%[1)]	NS	%[1)]	NS	%[1)]	NS	%[1)]	NS	%[1)]	NS	%[1)]	NS	%[1)]
pJEL101																	
1	JL13	29	37.2	8	28.6	18	58.0	1	33.3					1	12.5	1	100
2	LN44	24	30.1	12	42.9	5	16.1			4	66.6			3	37.5		
3	LN49	3	3.8											3	37.5		
4	JL62	2	2.6	2	7.1												
5	HN37	4	5.1			2	6.5	1	33.3	1	16.7						
6	HB23	3	3.8	2	7.1	1	3.2										
7	HN33	1	1.3			1	3.2										
8	HB19	2	2.6			2	6.5										
9	BJ12	2	1.3	2	7.1												
10	LN43	1	1.3											1	12.5		
11	GD1358	1	1.3									1	100				
12	JL691	1	1.3					1	33.3								
13	KS1-21	1	1.3			1	3.2										
14	BJ74	1	1.3	1	3.6												
15	YN15	1	1.3			1	3.2										
16	JL76	2	2.6	1	3.6					1	16.7						
Total no. of strains		78		28		31		3		6		1		8		1	
Genetic diversity		0.77		0.74		0.65		1.0		0.67		0		0.79		0	
pBSavrXa10																	
1	HB17	27	34.6	3	11.1	22	68.8							1	11.1	1	100
2	HLJ72	14	17.9	9	33.3	1	3.1							4	44.4		
3	LN44	6	7.7	6	22.2												
4	JL62	5	6.4	2	7.4									3	33.3		
5	ZHE173	4	5.1							4	80.0						
6	HB23	6	7.7	3	11.1	2	6.3	1	33.3								
7	HN37	4	5.1			3	9.4	1	33.3								
8	TJ13	2	2.6			2	6.3										
9	BJ12	3	3.8	3	11.1												
10	LN43	1	1.3											1	11.1		
11	GD1358	1	1.3									1	100				
12	JL691	1	1.3					1	33.3								
13	KS1-21	1	1.3			1	3.1										
14	HB25	1	1.3	1	3.7												
15	YN15	1	1.3			1	3.1										
16	JL76	1	1.3							1	20.0						
Total no. of strains		78		27		32		3		5		1		9		1	
Genetic diversity		0.83		0.83		0.52		1.0		0.4		0		0.75		0	

Note: NS = No. of strains, NP = No. of pathotypes, JL691 = Jiangling 691. [1)]Representing the percentage of pathotype.

respectively. Genetic diversity calculated for each pathotype treated as a subpopulation (H_R) was the highest for pathotype 6 (0.79) and 1 (0.84) and the lowest for pathotypes 5 and 7 (both 0) using probes pJEL101 and pBSavrXa10, respectively (Table 2).

On the basis of the occurrence of RFLP types, most of the pathotypes were heterogeneous groups, pathotype 1 and 2, with seven and eight different types in 28 and 31 strains tested (using pJEL101) with both seven types in 27 and 31 strains tested (using pBSavrXa10). The other pathotypes showed hetrogeneous groups also, even though in a few strains treated , i. e., pathotypes 3, 4 and 6, with two, three and four types in 2, 3 and 6 strains tested. The other two pathotypes, 5 and 7, with one each different type in one strain (Table 2).

3 Discussion

In *X. o.* pv. *oryzae* in China, we observe much greater variations in molecular phenotypes than in pathogenic patterns as Leach *et al.* reported[4]. Although avirulence loci and the genomic distribution of the repetitive element are not functionally related, our observation would suggest that the molecular phenotypes as revealed by pJEL101 or pBSavrXa10 hybridization patterns, change at a higher rate than avirulence loci. In general, the avirulence loci must take account that measurement of diversity at avirulence loci is limited by the number of rice differential cultivars available for difining race. Alternatively, the repetitive element may play an important role in race differentiation. Rabecca *et al.* based on DNA and pathotypic analysis, together with information on the spatial and temporal distribution of the pathogen types from their studies, a general picture of *X. o.* pv. *oryzae* evolution in the Philippines is presented[5].

The relationship between pathotype and putative phylogeny was assessed with two probes that yielded relatively robust phylogenies. Similar groups of strains were inferred from the RFLP data obtained. Strains of pathotypes 1, 2 and 6 formed a large heterogeneous cluster based on the two probes. A single strain, LN43 of pathotype 6, strain, GD1358 of pathotype 5, and a strain YN5, of unknown pathotype appeared to be only remotely related to the other strains based on the both probes.

Based on the analysis above, it appeared that some pathotypes consisted of multiple lineages, and some lineages consisted of multiple pathotypes. Many strains with very different RFLP patterns proved to be good candidates for a new race group and were useful in screening new sources of resistance [6]. The molecular technique will be a very available tool for explanation the host–pathogen response in rice–*Xoo* system in China and reconnoitring new races.

To determine whether the inferred population structure would depend on the particular probe utilized, we compared the results of RFLP analysis using two different element probes. Similar estimates of diversity were obtained for some of the pathotypes which were assessed a few strains between the two probes used. Somewhat variation of diversity however, was appeared for estimating pathotypes 1 and 2 between the two probes. The genetic diversities estimated based on pJEL101 and pBSavrXa10 were 0.74, 0.83 for pathotype 1, and 0.65, 0.52 for pathotype 2, respectively. The preliminary results suggested that the both DNA probes can be used for characterizing the population structure of *X. o.* pv. *oryzae* in China.

Although the dendrogram constructed on the basis of RFLP banding patterns provides new insight into the evolutionary relationship among those strains predominantly from North and Northeast China and a few ones from other regions of China only. Therefore, an intensive analysis of the pathogen population structure and genetic diversities including the background regarding the time, location of collection and host origins of strains will be very necessary.

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