Genetic Diversity of Transcriptional Activator-Like Effector Genes in Chinese Isolates of *Xanthomonas oryzae* pv. *oryzicola*

Zhi-Yuan Ji, Muhammad Zakria, Li-Fang Zou, Li Xiong, Zheng Li, Guang-Hai Ji, and Gong-You Chen

First, second, third, fourth, fifth, and seventh authors: School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, 200240, China; and sixth author: Key Laboratory for Plant Pathology of Yunnan Province, College of Plant Protection, Yunnan Agricultural University, Kunming, 650201, China.

Accepted for publication 7 January 2014.

ABSTRACT

Ji, Z.-Y., Zakria, M., Zou, L.-F., Xiong, L., Li, Z., Ji, G.-H., and Chen, G.-Y. 2014. Genetic diversity of transcriptional activator-like effector genes in Chinese isolates of *Xanthomonas oryzae* pv. *oryzicola*. *Phytopathology* 104:672-682.

Xanthomonas oryzae pv. *oryzicola* causes bacterial leaf streak (BLS), a devastating disease of rice in Asia countries. *X. oryzae* pv. *oryzicola* utilizes repertoires of transcriptional activator-like effectors (TALEs) to manipulate host resistance or susceptibility; thus, TALEs can determine the outcome of BLS. In this report, we studied genetic diversity in putative *tale* genes of 65 *X. oryzae* pv. *oryzicola* strains that originated from nine provinces of southern China. Genomic DNAs from the 65 strains were digested with *Bam*HI and hybridized with an internal fragment of *avrXa3*, a *tale* gene originating from the related pathogen, *X. oryzae* pv. *oryzae*, which causes bacterial leaf blight (BLB). Southern blot analysis indicated that the strains contained a variable number (9 to 22) of *avrXa3*-hybridizing fragments (e.g., putative *tale* genes). Based on the number and size of hybridizing bands, strains were classified into 14

genotypes (designated 1 to 14), and genotypes 3 and 10 represented 29.23 and 24.64% of the total, respectively. A high molecular weight *Bam*HI fragment (HMWB; ≈6.0 kb) was present in 12 of the 14 genotypes, and sequence analysis of the HMWB revealed the presence of a C-terminally truncated *tale*, an insertion element related to *IS1403*, and genes encoding phosphoglycerate mutase and endonuclease V. Primers were developed from the 6.0-kb HMWB fragment and showed potential in genotyping *X. oryzae* pv. *oryzicola* strains by polymerase chain reaction. Virulence of *X. oryzae* pv. *oryzicola* strains was assessed on 23 rice cultivars containing different resistance genes for BLB. The *X. oryzae* pv. *oryzicola* strains could be grouped into 14 pathotypes (I to XIV), and the grouping of strains was almost identical to the categories determined by genotypic analysis. In general, strains containing higher numbers of putative *tale* genes were more virulent on rice than strains containing fewer *tales*. The results also indicate that there are no gene-for-gene relationships between the tested rice lines and *X. oryzae* pv. *oryzicola* strains. To our knowledge, this is the first description of genetic diversity of *X. oryzae* pv. *oryzicola* strains based on *tale* gene analysis.

Bacterial leaf streak (BLS) caused by *Xanthomonas oryzae* pv. *oryzicola* is a major threat to rice production in Asia, Africa, and Australia (12,13,28,30,33). The disease is especially serious in Asia, where hybrid rice is widely grown and causes yield losses up to 40 to 60% in severe epidemics (16,17,30,52). The *X. oryzae* pv. *oryzicola*–rice interaction is a model system for studying how bacterial pathogens cause disease in rice (30,52) and also provides important clues regarding the pathogenicity of *X. oryzae* pv. *oryzae*, which causes bacterial leaf blight (BLB) in rice. A classical gene-for-gene model for resistance exists for the *X. oryzae* pv. *oryzae*–rice pathosystem, and the most cost-effective and environmentally sound approach to combating BLB is the use of resistant rice varieties that interact with cognate avirulence (*avr*) genes in *X. oryzae* pv. *oryzae* (2,14,19,28,39,43). However, with the exception of *Rxo1*, a resistance (*R*) gene of maize origin that interacts with *X. oryzae* pv. *oryzicola* *AvrRxo1* (38,43,50), rice varieties with *R* genes specific to *X. oryzae* pv. *oryzicola* are not available. Thus, studies to discover additional gene-for-gene interactions between *X. oryzae* pv. *oryzicola* and rice need to be undertaken.

Molecular fingerprinting techniques, including restriction fragment length polymorphism (RFLP); amplified fragment length

polymorphism (AFLP); and BOX-conserved repetitive elements, enterobacterial repetitive intergenic consensus (ERIC), and repetitive sequence-based (Rep)-polymerase chain reaction (PCR), have been used to investigate genetic diversity in phytopathogenic bacteria, including *X. oryzae* pvs. *oryzae* and *oryzicola* (1,13,18,22,34,35). However, polymorphisms identified using these approaches were not associated with bacterial virulence in host plants. In the last decade, comparative analysis of *X. oryzae* genomic sequences has revealed that many pathogenicity- and virulence-related genes are highly conserved between *X. oryzae* pvs. *oryzae* and *oryzicola* (5,15,37). However, a notable exception is the variation in the number of transcriptional activator-like effector (*tale*) genes among the two pathovars (15,40). For example, *X. oryzae* pv. *oryzicola* strains BLS256 and BLS303 contain ≈28 and 33 *tale* genes (6), which exceed the numbers identified in *X. oryzae* pv. *oryzae* strains MAFF311018 (31), KACC10331 (23), and PXO99^A (37). Previously, we tracked *tale* gene variations in different strains of *X. oryzae* pv. *oryzae* (44) and in Chinese isolates of *X. citri* subsp. *citri*, the citrus canker pathogen (48). It seems likely that the polymorphisms in *tale* genes may correlate with bacterial virulence in host plants. This prompts the following questions: do larger numbers of *tale* genes in *X. oryzae* pv. *oryzicola* strains result in a higher level of virulence? What is the distribution of *tale* genes in *X. oryzae* pv. *oryzicola* strains within rice-growing regions?

TALEs are a structurally and functionally distinct class of proteins present in *Xanthomonas* spp. that are secreted via the type III secretion system (T3SS) (3,47). TALEs are delivered into plant nuclei and subsequently bind and activate upstream se-

Corresponding author: G.-Y. Chen; E-mail address: gyouchen@sjtu.edu.cn

* The e-Xtra logo stands for “electronic extra” and indicates that the online version contains two supplemental figures.

<http://dx.doi.org/10.1094/PHYTO-08-13-0232-R>
© 2014 The American Phytopathological Society

quences (UPT-box) present in *R* or susceptibility (*S*) genes (3,21,41,45). TALEs share several common features, including an N-terminal region required for secretion via the T3SS, a C-terminus containing nuclear localization signals (NLS), and an acidic activation domain (AD) typical of transcription factors. TALEs generally contain a variable central domain of tandem, nearly identical 33- to 34-amino-acid (aa) repeats that confer DNA-binding specificity (4,11,26,29). Residues at the 12th and 13th positions are hypervariable and are known as repeat-variable diresidues (RVDs). The RVDs bind to specific, single nucleotides and, consequently, determine disease susceptibility or resistance. For example, PthXo1, a TALE in *X. oryzae* pv. *oryzae* strain PXO99^A, binds to the UPT-box of the sucrose transporter gene *OsSWEET11* (*Os8N3*). PthXo1 binding activates expression of *OsSWEET11* and facilitates nutrient acquisition by the pathogen, thus resulting in susceptibility to BLB (9,45). Similarly, AvrXa27 binds to the UPT-box of *Xa27* and activates its expression, thus leading to BLB resistance (14). However, similar interactions between TALEs and genes encoding resistance or susceptibility have not been identified for the *X. oryzae* pv. *oryzicola*–rice pathosystem. Thus, the survey of *X. oryzae* pv. *oryzicola* *tale* gene diversity conducted below will ultimately provide further insight into this host–pathogen interaction.

The objectives of this study were to evaluate the distribution of *tale* genes in *X. oryzae* pv. *oryzicola* strains collected in China and to investigate potential gene-for-gene interactions between *X. oryzae* pv. *oryzicola* and rice. This was undertaken by collecting *X. oryzae* pv. *oryzicola* isolates from nine rice-growing provinces, determining the number and size of *tale* genes by hybridization analysis, and assessing virulence in rice lines containing different *R* genes for BLB.

MATERIALS AND METHODS

Isolation of bacterial strains. Rice leaves showing typical BLS symptoms in the field were collected and sent to us by local county technicians in local protection stations from major rice-growing provinces of southern China from 2008 to 2009 (Table 1). One of five leaves in each sample was surface sterilized and crushed in 1.5-ml microcentrifuge tubes containing 1 ml of sterile distilled H₂O (dH₂O) using a plastic pestle (Axygen, Hanzhou, China). Each bacterial suspension was serially diluted, plated to nutrient agar (NA) and NA containing 5% skimmed milk (51) plates, and incubated at 28°C for 4 days. A single yellow, polysaccharide-rich bacterial representative of those showing extra-cellular protease haloes surrounding colonies isolated from each sample was selected, purified, and identified using *X. oryzae* pv. *oryzicola*-specific primers XoocF/XoocR (49) and *hrpE3F*/*hrpE3R* (10) by colony PCR amplification. *X. oryzae* pv. *oryzicola* isolates were individually infiltrated into tobacco leaves (*Nicotiana benthamiana*) for hypersensitive response (HR) detection using needleless syringes and inoculated into rice line IR24 (susceptible to *X. oryzae* pv. *oryzicola*) using the leaf-needling method (52). Strains causing typical HR in tobacco and BLS symptoms in rice IR24 were maintained at –80°C in nutrient broth containing 30% glycerol for further investigation. In total, 65 *X. oryzae* pv. *oryzicola* strains were collected from different rice samples for which detailed genetic backgrounds were known by the local technicians.

DNA extraction and Southern blot analysis. Total genomic DNAs from 65 *X. oryzae* pv. *oryzicola* strains were extracted using the AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen). DNA samples (3 µg) were digested with *Bam*HI at 37°C for 4 h, separated in 1.2% agarose gels, and then transferred to Hybond N⁺ nylon membranes (Millipore, Billerica, MA). The probe was a digoxigenin-labeled 1,368-bp internal *Sph*I fragment of *avrXa3* (GenBank accession number AY129298.1) originating from *X. oryzae* pv. *oryzae* (44). This 1,368-bp fragment contains

8.5 tandem repeats of a 102-bp sequence and part of the leucine zipper (LZ) coding region (44). This internal fragment of *avrXa3* was used as a probe to detect the number and size of *tale* genes in each *X. oryzae* pv. *oryzicola* strain. DNA labeling, hybridization, and detection were performed as recommended by the manufacturer (Roche, Sweden); all hybridization experiments were repeated twice.

Analysis of virulence. Because *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae* are close pathovars in rice (30), we assumed that *R* genes for BLB in different rice lines against *X. oryzae* pv. *oryzae* containing corresponding *tale* genes may occasionally match a *tale* gene contained in these 65 *X. oryzae* pv. *oryzicola* strains. To investigate whether there are rice lines resistant to *X. oryzae* pv. *oryzicola* infection, 23 rice lines containing different *R* genes for BLB (41) were used for virulence assessment of the collected *X. oryzae* pv. *oryzicola* strains (Table 1). Rice plants were maintained in a greenhouse at Shanghai Jiao Tong University with a photoperiod of 14 h of light (30°C) and 10 h of darkness (25°C), while tobacco plants were grown in the greenhouse with a photoperiod of 14 h of light (24°C) and 10 h of darkness (18°C). *X. oryzae* pv. *oryzicola* strains were grown overnight on NA plates at 28°C, washed twice, and resuspended in dH₂O to an optical density at 600 nm = 0.6 prior to inoculation. Leaves of 4-week-old rice seedlings were infiltrated with bacterial suspensions using a needleless syringe (51), and each leaf was infiltrated with the same strain in three different spots. Five secondary leaves were inoculated with each strain. Lesion lengths were measured 6 days after inoculation. Virulence levels were evaluated according to lesion size as follows: level 0, lesions ≤0.1 cm; level 1, lesions 0.1 to 0.5 cm; level 2, 0.5 to 1.5 cm; level 3, 1.5 to 2.0 cm; level 4, 2.0 to 2.5 cm; level 5, 2.5 to 3.0 cm; level 6, 3.0 to 3.5 cm; level 7, 3.5 to 4.0 cm; level 8, 4.0 to 4.5 cm; level 9, 4.5 to 5.0 cm; and level 10, lesions >5.0 cm in length. Based on the virulence assessment, unweighted pair-group arithmetic method (UPGMA) cluster analysis (18) of simple matching coefficients (a similarity value that can be applied to measure qualitative, multistate parameters) was used to quantify *X. oryzae* pv. *oryzicola* virulence in different rice lines. All virulence assays were performed in three independent experiments.

Data analysis. The number and size of *tale* genes were converted to binary form, where 1 indicates the presence of a band and 0 indicates the absence of the corresponding band. An intensely hybridizing band was considered to contain more than one *tale* gene in the analysis. Bands separated by >2 mm were considered different. Genetic diversity was measured by the percentage of variable bands and the Shannon diversity index (25). Jaccard similarity coefficients for pairs of *X. oryzae* pv. *oryzicola* strains were calculated using NTSYS (v. 2.1; Exeter Software, Setauket, NY) using SIMQUAL with the Dice coefficient; results were subjected to cluster analysis using the UPGMA (18).

The diversity of virulent pathotypes was measured by a simple matching coefficient similarity matrix, based on a matrix of virulence levels generated by the SIMQUAL routine in NTSYS (18). Cluster analysis was performed using UPGMA with the arithmetic mean method in the SAHN routine of NTSYS (18). To estimate the strength of the groups generated by cluster analysis, bootstrap analysis was performed on 1,000 replications using Winboot software (http://books.irri.org/DPS14_content.pdf).

Isolation and sequencing of large *tale*-containing *Bam*HI fragments. The fine structure of putative *tale*-containing 6.0-kb high molecular weight bands (HMWBs) was investigated in *X. oryzae* pv. *oryzicola* strains RS105 and JSB2-24. Genomic DNA from these two strains was digested with *Bam*HI, and 6.0- to 8.0-kb fragments were purified from 1.0% agarose gels. The purified HMWBs were then ligated into *Bam*HI-digested vector pBluescript II SK(–) for sequencing (Invitrogen, Shanghai, China). The full-length HMWB sequences from RS105 and

TABLE 1. Lesion lengths of the tested *Xanthomonas oryzae* pv. *oryzicola* strains in 23 different rice varieties

Strains ^a	Lesion length (cm) for rice lines and genes shown ^b											
	Asominor	BJ1	Cas209	DV85	IR24	IR26	IRBB1	IRBB2	IRBB3	IRBB4	IRBB5	IRBB7
	<i>Xa17</i>	<i>xa5</i> , <i>xa13</i>	<i>Xa10</i>	<i>Xa7</i>	<i>Xa18</i>	<i>Xa4</i>	<i>Xa1</i>	<i>Xa2</i>	<i>Xa3</i>	<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>
SCB04-1	1.44	0.92	1.48	0.42	1.04	1.76	0.9	1.84	0.78	0.78	1.34	0.86
YNB1-1	1.4	0.74	1.94	0.34	1.42	1.28	0.38	2.34	0.48	2.18	1.32	2.18
YNB9-3	1.38	1.66	2.74	0.82	2.98	2.38	2.84	2.3	1.82	1.36	1.78	2.2
SCB9-02	1.48	1.78	2.86	0.68	2.56	2.14	2.86	2.26	1.34	1.34	1.58	2.28
ZJB9-2	1.24	1.94	2.88	0.64	2.64	2.32	2.82	2.36	1.28	1.34	2.2	2.28
HANB9-1	1.38	1.96	2.48	0.84	2.86	2.44	2.66	2.2	1.72	1.38	1.8	1.74
JSB2-24	1.3	1.82	2.92	0.82	2.52	1.9	2.98	2.08	1.34	1.44	1.8	2.1
GDB3-143	1.48	1.42	2.74	0.8	2.5	2.44	2.52	2.36	1.46	1.26	2.26	2.28
HNB16-97	1.54	1.64	2.4	0.76	2.94	2.24	2.86	2.7	1.36	1.3	2.24	2.32
HNB16-100	1.48	1.78	2.9	0.58	2.76	2.5	2.68	2.18	1.3	1.32	1.78	1.72
AHB4-204	1.36	1.6	2.54	0.68	2.66	2.48	2.42	2.24	1.32	1.8	1.8	1.82
HNB16-46	1.48	1.88	2.56	0.66	2.68	2.16	2.74	2.76	1.34	1.46	2.24	2.2
AHB4-147	1.64	1.62	2.54	0.64	2.6	2.28	2.82	2.2	1.44	1.66	1.74	1.78
AHB4-205	1.12	1.34	2.64	0.54	2.78	2.66	2.08	2.4	1.84	1.4	2.18	2.22
AHB4-118	1.28	1.44	2.84	0.76	2.8	2.88	2.42	2.22	1.56	1.78	2.38	1.8
AHB3-7	1.38	1.82	2.9	0.54	2.76	2.48	2.64	2.14	1.24	1.56	1.86	1.8
AHB2-131	1.54	2.26	2.64	1.28	2.72	2.74	2.36	2.36	1.8	1.8	2.28	2.16
GDB8-47	1.58	1.52	2.58	0.94	2.98	2.72	2.06	2.78	1.34	1.78	2.42	2.32
ZJB01-25	1.38	1.36	2.96	1.32	2.5	2.68	2.24	2.2	1.3	1.78	1.82	1.8
HNB12-26	1.58	1.62	2.78	0.84	2.68	2.12	2.38	2.28	1.78	1.54	1.76	1.82
SCB9-5	1.42	1.74	2.68	0.68	2.66	2.42	2.48	2.24	1.4	1.62	1.72	2.22
HNB3-17	3.26	1.42	2.6	2.62	3.7	1.5	1.44	1.92	2.36	0.8	1.84	1.24
YNB01-3	3.42	1.44	2.9	2.68	2.16	1.64	1.66	1.62	1.8	0.64	1.82	1.78
JSB5-2	4.66	1.36	2.06	1.48	2.32	3.52	1.88	3.14	1.34	2.2	3.54	2.74
SCB04-2	1.24	0.6	1.42	1.76	3.02	3.12	3.36	2.24	1.74	2.4	2.7	1.78
YNB63	1.4	0.82	1.46	1.82	3.28	3.84	3.4	2.3	1.84	2.4	2.76	1.64
GXB9-31	1.42	0.72	1.38	1.68	3.48	3.12	3.04	1.92	1.66	2.34	2.7	1.84
SCB9-24	1.44	0.7	1.64	1.7	3.38	3.22	3.46	2.16	2.24	2.16	2.78	1.84
HANB9-23	1.58	0.66	1.46	1.76	3.1	3.06	3.74	2.24	1.78	1.82	2.28	1.66
SCB9-18	1.46	0.56	1.48	1.88	3.5	3.5	3.82	2.4	2.36	2.18	2.78	1.66
HANB8-2	1.54	0.64	1.48	1.74	2.58	3.24	3.96	2.16	1.72	2.44	2.26	1.72
RS85	1.2	0.86	1.68	1.52	3.24	3.38	3.84	2.2	2.14	1.7	2.84	1.86
ZJB9-46	2.46	0.74	1.74	1.9	2.72	3.38	2.62	3.56	3.54	1.8	2.76	2.22
YNB9-41	2.38	0.66	1.72	1.66	2.82	3.48	2.92	3.16	2.74	1.96	2.56	2.28
SCB9-39	2.34	0.8	1.72	1.6	2.62	3.42	3.02	3.64	3.64	1.76	2.7	2.3
YNB21	1.34	0.58	2.4	1.76	2.42	2.08	2.68	1.32	1.72	1.74	1.36	2.28
GDB3-12	1.46	0.62	2.42	1.62	2.4	2.16	2.86	1.22	1.62	1.56	1.48	2.42
GXB3-4	1.36	0.72	2.34	1.82	2.04	2.64	2.82	1.42	1.7	1.68	1.34	2.4
GXB3-23	1.38	0.8	2.46	1.48	2.44	2.8	2.7	1.3	2.18	1.82	1.76	2.2
GXB3-8	1.3	0.82	2.84	1.82	2.14	2.32	2.6	1.28	2.28	2.12	1.38	2.34
GDB3-092	1.42	0.8	2.48	1.84	2.08	2.86	2.74	1.8	2.38	2.24	1.8	2.48
GDB3-087	1.44	0.86	2.36	1.44	2.26	2.3	3.16	1.3	1.78	2.26	1.38	2.3
JSB3-120	1.26	0.96	2.9	1.14	2.12	2.02	2.62	1.3	2.32	1.78	1.36	2.4
JSB3-102	1.28	1.26	2.28	1.24	2.46	2.5	2.78	1.74	2.46	2.22	1.78	2.3
YNB9-53	1.22	0.78	2.4	1.44	2.08	2.24	3.28	1.26	2.28	1.68	1.38	2.22
HANB9-48	1.48	0.58	2.36	1.36	2.1	2.36	2.84	1.38	1.74	1.84	1.42	2.26
HANB9-35	1.28	0.64	2.28	1.26	2.38	2.42	3.12	1.92	1.82	1.66	1.74	2.36
GXB9-32	1.36	0.86	2.26	1.22	2.42	2.24	2.74	1.28	1.54	1.64	1.32	2.18
GXB1-6	1.28	0.76	2.76	1.34	2.24	2.18	2.66	1.78	1.64	1.8	1.42	1.72
JSB3-28	1.68	0.98	2.36	1.3	2.32	2.22	2.86	1.32	1.56	1.68	1.38	2.24
AHB1-58	1.26	0.7	2.52	1.44	2.16	2.44	3.02	1.32	1.66	1.88	1.74	2.3
RS105	2.42	1.52	2.58	2.56	2.66	3.08	2.94	1.28	1.44	0.68	0.84	1.8
ZJB01-19	1.84	1.44	1.84	1.52	2.16	4.46	3.44	3.68	1.72	1.8	1.32	1.42
ZJB01-12	1.78	1.32	1.88	1.68	2.12	4.12	3.08	3.7	1.64	1.44	1.34	1.32
YNB9-44	1.92	1.44	1.94	1.66	2.46	4.36	3.16	3.54	1.72	1.68	1.18	1.24
ZJB9-38	1.98	1.32	1.94	1.18	2.36	4.34	3.22	3.76	1.48	1.84	1.86	1.46
SCB9-25	1.74	1.48	1.88	1.82	2.26	4.24	2.84	3.3	1.78	1.78	1.38	1.3
RS119	2.86	1.66	2.8	2.9	2.78	2.38	2.92	2.36	2.3	1.78	1.7	1.8
HANB10-10	2.96	2.06	2.8	2.76	2.6	2.24	3.3	2.24	1.8	1.32	1.7	1.52
HNB1-19	2.68	2.12	2.94	2.4	2.56	2.24	2.88	2.38	2.18	1.78	2.26	1.78
HANB01-12	2.9	2.16	2.84	1.68	2.84	2.36	2.46	2.24	2.28	1.36	2.36	1.66
HNB07-3	3.96	2.26	1.46	2.16	2.56	2.38	2.42	2.82	1.38	2.36	1.46	1.66
GDB3-4	1.26	0.96	1.76	1.26	1.74	3.1	1.82	2.78	2.2	2.28	3.14	1.26
ZJB9-12	2.26	1.74	1.84	3.12	3.52	3.64	1.74	1.74	2.78	2.78	1.36	2.62
JSB1-39	2.46	1.88	1.86	3.44	3.88	3.88	1.72	1.8	2.8	2.26	1.3	2.52
Average ^d	1.77	1.26	2.30	1.46	2.59	2.72	2.70	2.21	1.82	1.74	1.91	1.97

^a Strains are abbreviated by the letters indicating their places isolated. AH = Anhui, GD = Guangdong, GX = Guangxi, HAN = Hainan, HN = Hunan, JS = Jiangsu, SC = Sichuan, YN = Yunnan, ZJ = Zhejiang, and RS = rice streak.

^b Bacterial leaf streak (BLS) lesion length (cm). Resistance genes against bacterial leaf blight contained in near-isogenic lines are referred from Verdier et al. (42).

^c Mean of bacterial leaf streak (BLS) lesion length caused by one strain in 23 rice lines.

^d Mean of BLS lesion length caused by 65 strains in one rice line.

TABLE 1. (Continued from preceding page)

Lesion length (cm) for rice lines and genes shown ^b											
IRBB8	IRBB10	IRBB11	IRBB11(T)	IRBB13	IRBB14	IRBB21	Java14	Tetep	TN1	Wase Aikoku	Average ^c
<i>Xa8</i>	<i>Xa10</i>	<i>Xa11</i>	<i>Xa11</i>	<i>xa13</i>	<i>Xa14</i>	<i>Xa21</i>	<i>Xa1, Xa3</i>	<i>Xa2</i>	<i>Xa14</i>	<i>Xa3</i>	
1.22	0.78	0.88	0.44	1.3	0.82	1.36	0.9	0.98	0.92	1.48	1.09
1.76	1.38	1.46	0.48	0.4	0.78	0.98	0.88	0.48	0.94	1.62	1.18
3.74	1.9	2.38	1.86	1.36	1.46	1.82	2.26	1.78	1.24	2.24	2.01
3.38	1.72	1.84	1.94	1.74	1.36	2.12	2.3	1.7	1.36	2.26	1.95
3.7	1.56	1.56	1.86	1.44	1.44	2.24	2.88	1.62	1.42	2.14	1.99
3.54	1.92	1.84	2.36	1.76	1.38	1.86	2.9	1.76	1.44	2.34	2.02
3.58	1.78	2.3	1.78	1.4	1.34	1.68	2.84	1.88	1.46	2.12	1.96
3.7	2	1.68	2.2	1.7	1.48	2.22	2.44	1.64	1.36	2.34	2.01
3.56	2.22	1.94	1.74	1.8	1.8	2.2	2.62	1.9	1.26	2.12	2.06
3.58	2.38	2.4	2.2	1.92	1.44	1.78	2.88	1.86	1.38	2.18	2.04
3.56	1.74	2.46	1.82	1.4	1.64	1.72	2.7	1.84	1.36	2.06	1.96
3.66	2.3	2.14	1.54	1.76	1.3	1.74	2.6	1.76	1.34	2.44	2.03
3.68	1.72	2.38	1.8	1.84	1.4	2.24	2.74	1.8	1.48	1.74	1.99
3.4	2.08	2.36	1.86	1.54	1.48	1.84	2.74	1.84	1.3	2.28	2.00
3.36	1.92	2.2	2.24	1.76	1.8	1.76	2.22	1.84	1.36	2.32	2.04
3.36	1.64	1.78	1.9	1.76	1.4	1.7	2.24	2.38	1.36	1.84	1.93
3.42	1.8	2.48	2.22	2.3	1.84	1.8	2.74	2.44	1.34	2.02	2.19
3.7	1.52	1.78	2.36	2.28	1.72	2.3	2.52	2.34	1.84	2.26	2.16
3.4	2.2	1.62	1.84	1.8	1.94	1.82	2.56	2.42	1.3	2.06	2.01
3.56	2.34	1.76	1.74	1.8	1.84	1.82	2.3	2.08	1.32	2.22	2.00
3.62	1.8	2.36	1.82	1.82	1.88	1.74	2.78	2.36	1.46	2.44	2.06
1.46	2.26	1.92	1.78	3.24	2.6	1.58	2.86	3.32	2.1	1.6	2.15
1.42	1.72	1.82	2.78	3.32	2.72	3.24	2.78	3.44	2.46	1.88	2.22
1.76	3.44	3.12	2.36	1.8	5.02	0.86	1.46	2.28	1.28	2.3	2.43
2.68	2.48	4.28	2.14	3.7	3.32	3.36	2.84	2.46	1.88	2.36	2.47
2.76	2.38	4.06	2.42	3.26	2.98	3.24	2.62	2.48	1.6	2.22	2.48
2.6	2.12	4.44	2.76	2.66	3.36	3.16	2.64	2.34	1.86	2.24	2.41
3.3	2.2	4.44	2.24	3.72	2.74	2.76	2.7	2.3	1.56	2.22	2.47
2.74	2.26	4.26	2.28	3.86	3.3	3.32	2.64	2.38	1.96	2.34	2.46
3.32	2.18	4.4	2.26	3.76	3.4	3.32	2.76	2.36	1.86	2.26	2.59
3.24	2.08	4.22	2.5	3.52	3.2	3.4	2.24	2.26	1.72	2.5	2.45
3.18	2.24	4.06	2.7	3.88	3.48	3.26	2.48	2.48	1.52	2.4	2.53
2.3	2.34	3.88	2.28	3.24	2.86	3.22	3.18	1.28	2.3	1.32	2.51
2.26	2.42	4.38	2.32	3.4	3.34	2.8	3.32	1.42	2.24	1.6	2.51
2.32	2.18	4.46	2.4	3.16	3.24	3.18	3.26	1.42	2.32	1.44	2.56
3.6	1.64	1.86	3.28	1.84	1.72	1.38	2.24	2.84	1.9	1.62	1.98
3.76	1.54	2.1	3.28	1.82	1.92	1.38	2.22	2.84	2.34	1.84	2.04
3.62	1.76	2.44	2.9	1.4	2.3	1.86	2.3	2.54	2.44	1.76	2.07
3.64	2.48	1.84	2.3	1.78	1.86	1.84	2.78	3.26	2.44	1.54	2.13
3.82	2.4	1.84	3.3	1.34	1.54	1.8	2.26	3.12	2.08	1.26	2.09
3.64	2	2.24	3.28	1.8	2.36	1.3	2.24	2.26	2.36	1.48	2.17
3.56	2.12	2.44	2.8	1.3	1.8	1.8	2.04	2.76	2.28	1.84	2.07
3.72	1.96	2.42	2.18	1.54	1.78	1.3	2.38	2.62	2.36	1.42	1.99
3.68	2.18	2.36	3.28	1.7	2.36	1.72	2.68	2.64	2.2	1.8	2.21
3.38	2.24	1.98	3.2	4.42	1.88	1.38	2.14	2.44	2.36	1.34	2.13
3.74	1.86	1.94	3.36	1.9	1.92	1.78	2.28	2.64	2.44	1.62	2.05
3.68	2.24	1.94	2.82	1.84	1.64	1.42	2.4	2.24	2.62	1.78	2.07
3.42	2.16	1.96	2.84	1.88	2.34	1.44	2.08	2.72	2.76	1.62	2.01
3.76	2.24	2	3.44	1.34	1.58	1.4	2.48	2.9	2.68	1.58	2.04
3.52	2.44	2.02	3.38	1.68	1.54	1.26	2.74	2.8	2.44	1.66	2.06
3.58	1.8	1.74	1.84	1.8	1.86	1.78	2.2	2.82	2.3	1.8	2.00
2.88	1.5	0.36	0.46	0.78	0.44	1.82	2.68	2.88	2.66	1.88	1.83
2.34	3.66	1.74	1.78	1.72	2.72	1.84	2.26	2.3	1.3	1.58	2.17
2.34	3.6	2.36	1.48	1.8	2.52	1.26	2.4	2.16	1.72	1.82	2.13
2.4	3.46	1.96	1.72	1.32	2.72	1.76	2.34	2.38	1.24	1.96	2.15
2.32	3.86	2.4	1.6	1.88	2.8	1.36	2.38	2.4	1.22	1.74	2.20
1.88	3.22	1.6	1.68	1.86	2.28	1.64	2.34	2.48	1.46	1.94	2.09
1.86	1.98	1.76	1.76	3.3	2.28	1.82	3.28	2.84	2.36	0.8	2.27
1.84	1.5	1.5	1.74	2.82	2.28	1.78	2.86	2.88	2.34	0.58	2.15
2.24	1.48	1.94	1.84	3.18	2.72	1.78	3.22	2.74	2.38	0.98	2.29
1.76	2.4	1.78	1.34	3.32	2.86	2.22	3.36	2.62	2.76	0.82	2.28
1.86	1.86	1.92	1.8	3.34	2.26	1.8	2.74	4.02	1.66	2.36	2.28
3.8	1.64	3.72	1.78	2.74	1.44	2.24	2.56	4.38	0.74	1.96	2.20
1.96	2.04	2.88	1.74	2.84	2.66	1.78	2.68	3.2	2.94	1.84	2.42
1.84	2.2	2.56	1.7	2.8	2.7	1.48	2.6	3.36	2.6	1.58	2.40
3.00	2.13	2.41	2.17	2.22	2.16	1.98	2.52	2.38	1.85	1.86	...

JSB2-24 were recorded in GenBank as accession numbers KF447887 and KF460460, respectively.

Long PCR for detection of HMWBs in *X. oryzae* pv. *oryzicola* strains. Primers HMWBF (5'-GGAGTTGAGAGGTC

CGCCGTTACAGTTGGACACAGGCC-3') and HMWBR (5'-GCGGCTGCAACGCGCCGCGTGTGCGCTGAATGGAAGC-3') were synthesized to amplify HMWBs in representative strains of each genotype. PCR reactions were carried out in a 25- μ l reaction mixture containing 30 ng of genomic DNA, 0.2 μ M each primer, 0.1 mM each dNTP, and 2 U of *Ex-Taq* DNA polymerase with GC buffer II (TaKaRa, Dalian, China). The PCR conditions were as follows: 94°C for 3 min; 35 cycles at 94°C for 20 s, 58°C for 20 s, and 72°C for 3 min; followed 72°C for 5 min. PCR was performed using a Mastercycler pro *S* (Eppendorf International). PCR products were separated by electrophoresis on 1.0% agarose gels, purified using the AxyPrep PCR clean-up kit (Axygen), and then ligated into the pMD18-T vector (TaKaRa) for sequencing (Invitrogen).

RESULTS

Isolation of *X. oryzae* pv. *oryzicola* strains. Diseased rice leaves showing BLS symptoms were collected from rice fields in nine provinces of southern China and processed for bacterial strain isolation. *X. oryzae* pv. *oryzicola* strains, showing extracellular protease activity on NA-milk plates (not all data shown) (Supplemental Figure 1), were initially identified by colony PCR using the *X. oryzae* pv. *oryzicola*-specific primers XooC/F/XooC/R (49) and *hrpE3F/hrpE3R* (10) (not all data shown) and were then confirmed to induce HR in tobacco (data not shown) and produce BLS symptoms in the susceptible rice line IR24 (Table 1). In total, 65 pathogenic *X. oryzae* pv. *oryzicola* strains were isolated from the following provinces (number of strains isolated in parentheses): Anhui ($n = 7$), Guangdong ($n = 6$), Guangxi ($n = 6$), Hainan ($n = 7$), Hunan ($n = 7$), Jiangsu ($n = 9$), Sichuan ($n = 8$), Yunnan ($n = 8$), and Zhejiang ($n = 7$) (Table 1). Virulence was assessed in 23 rice lines containing different BLB *R* genes (41), and the tested strains caused typical BLS symptoms in all the tested rice lines (Table 1). Strains SCB04-1 and YNB1-1, which were originally isolated from Sichuan and Yunnan, respectively, showed reduced virulence compared with the other 63 strains (Table 1), indicating a potential genetic variation in virulence.

***X. oryzae* pv. *oryzicola* strains are classified into 14 genotypes.** Southern blot hybridizations were performed to determine the number and the size of *tale* genes in the 65 *X. oryzae* pv. *oryzicola* strains. For these experiments, the central *SphI* fragment of *avrXa3* (44) was hybridized to *Bam*HI-digested genomic DNAs. The majority of *Bam*HI fragments hybridizing to the probe were 2.5 to 4.8 kb, which indicates the presence of 9 to 33 repeat units of the 102-bp sequence that is located in the conserved, central region of *tale* genes. Southern blot analysis indicated that the 65 strains could be subdivided into 28 unique *tale* haplotypes, which were designated A* to W (Fig. 1). Each strain generally contained 9 to 22 hybridizing bands of 2.5 to 8.0 kb in size (Table 2). The most notable, visible difference among strains was the occurrence of high molecular weight *Bam*HI fragments (HMWBs) (Fig. 1, see bands designated A to D, near 6.0 kb). Strains SCB04-1 and YBN1-1 were the most unusual in terms of hybridization patterns and exhibited several unique bands (see bands marked with asterisk; Fig. 1B; Table 2). Strain SCB04-1 contained only nine *Bam*HI fragments with homology to the *avrXa3* probe (Fig. 1B). Based on the number and size of hybridizing fragments, the tested strains were classified into 14 different genotypes designated 1 to 14 by UPGMA cluster analysis (Fig. 2A; Table 2).

Our results indicated that strains collected from Yunnan, Jiangsu, and Sichuan provinces are more diverse than those collected from other regions (Tables 2 and 3). Genotypes 3 and 10 were the most common and represented 29.23 and 24.64% of the 65 strains, respectively, while genotypes 1, 2, 5, 7, 11, and 12 were rare and consisted of a single strain (Table 3). Strains SCB04-1 and YNB1-1, which showed unusual hybridization patterns (Fig. 1), were assigned to genotypes 1 and 2, respectively.

Fig. 1. Southern blot analysis of potential transcriptional activator-like effector (*tale*) genes in 65 strains of *Xanthomonas oryzae* pv. *oryzicola*. Genomic DNA of the strains was digested with *Bam*HI and hybridized with an internal *Sph*I fragment of *avrXa3* (44). Molecular weight markers (bp) are shown in the left lane. Hybridizing bands of various sizes are marked with different letters (A*, A to W), and bands marked with an asterisk were unique to a single strain. Strains were named using a letter designation by province of origin, followed by the letter B and a number. Province abbreviations are as follows: AH, Anhui; GD, Guangdong; GX, Guangxi; HAN, Hainan; HN, Hunan; JS, Jiangsu; SC, Sichuan; YN, Yunnan; and ZJ, Zhejiang. Strains designated with RS represent *X. oryzae* pv. *oryzicola* strains isolated from Jiangsu province prior to 2008. Southern hybridization experiments were repeated twice, with similar results.

Assignment of *X. oryzae* pv. *oryzicola* strains to 14 pathotypes. The 65 *X. oryzae* pv. *oryzicola* strains were inoculated into 23 rice lines containing different *R* genes for BLB and assessed for virulence. All 65 *X. oryzae* pv. *oryzicola* strains caused typical water-soaked BLS lesions in the 23 rice lines when evaluated 6 days post inoculation, indicating that rice lines with BLB resistance are susceptible to the *X. oryzae* pv. *oryzicola* strains. BLS lesion lengths were 0.34 to 5.02 cm (Table 1). Strains SCB04-1 (containing 9 putative *tale* genes, genotype 1) and YNB1-1 (16 putative *tale* genes, genotype 2) showed attenuated virulence in rice line DV85, with mean lesion lengths of 0.42 and 0.34 cm, respectively (Table 1). In contrast, genotype 8, containing 22 putative *tale* genes (Fig. 2; Table 2), displayed hypervirulence in the rice lines, with a mean lesion length of 2.53 cm (Table 4). These results suggest a potential correlation between *X. oryzae* pv. *oryzicola* virulence and the number of putative *tale* genes in a particular strain. In contrast, rice lines BJ1 (containing *xa5* and *xa13*) and DV85 (*xa5*, *Xa7*, *xa13*, and *Xa24*) (32,41) were somewhat less susceptible to the *X. oryzae* pv. *oryzicola* strains than the other rice lines (Tables 1 and 4) and exhibited BLS mean lesion lengths of 1.27 and 1.48 cm, respectively (Table 1). Conversely, rice lines IRBB8 (*Xa8*), IR26 (*Xa4*), IR24 (*Xa18*), and Java 14 (*Xa1*, *Xa3*) (32,41) were more susceptible to the *X. oryzae* pv. *oryzicola* strains and developed mean lesion lengths of 3.03, 2.74, 2.60, and 2.55 cm, respectively (Table 1). These results suggest that BLS resistance and *X. oryzae* pv. *oryzicola* virulence is quantitative and controlled by multiple genes in the host (rice) and pathogen (*X. oryzae* pv. *oryzicola*).

To determine whether the acquisition of multiple *tale* genes in *X. oryzae* pv. *oryzicola* is correlated with virulence, we sought to cluster pathotypes of the 65 *X. oryzae* pv. *oryzicola* strains using the SIMQUAL subroutine in NTSYS (18). When a 50% matching coefficient was applied, the 65 strains were discriminated into 14 pathotypes (designated I to XIV) based on virulence in the 23 rice lines (Fig. 2B). Interestingly, most of the strains clustered by genotype were assigned to the same pathotype (Fig. 2), although there were several exceptions. For example, strains HANB10-10 (genotype 13) and GXB1-6 (genotype 10) clustered in pathotype III, strains RS85 (genotype 6) and GDB8-47 (genotype 3) were

assigned to pathotype X, and JSB3-102 (genotype 10) was classified as pathotype VI (Fig. 2).

Analysis of HMWBs and PCR analysis. With the exception of SCB04-1 and YNB1-1, *X. oryzae* pv. *oryzicola* strains contained a large, ~6.0-kb *Bam*HI fragment that hybridized with the *avrXa3* probe (Figs. 1 and 3A). Based on the common structure of mature *tale* genes (Fig. 3B), this 6.0-kb *Bam*HI fragment (HMWB) could contain up to 35 repeats of the 102-bp conserved sequence. This prompted us to investigate the fine structure of the 6.0-kb HMWB and determine whether this locus could be used in genotyping *X. oryzae* pv. *oryzicola* strains by PCR. To address these objectives, we isolated the 6.3- and 6.5-kb HMWBs from strains JSB2-24 and RS105, respectively (Fig. 3A, black arrows), and cloned these fragments into pBluescript. The sequenced HMWBs were designated HMWB-a (TAL2-24) and HMWB-b (TAL105); these are depicted in Figure 3B and were deposited as GenBank accession numbers KF447887 and KF460460, respectively. Sequence analysis showed that these loci were similar in structure. Both loci contained a truncated *tale* lacking the C-terminal region that encodes LZ, NLS, and AD (Fig. 3B). Furthermore, both loci encoded *IS1403*, phosphoglycerate mutase (*pgm*), and endonuclease V (*enaV*). The two loci differed in the number of repeat units of the 102-bp conserved sequence; HMWB-a (*tal2-24*) and HMWB-b (*tal105*) contained 19.5 and 21.5 repeat units, respectively (Fig. 3B). Interestingly, this locus was also identified in the genome (AAQN01000001) of *X. oryzae* pv. *oryzicola* strain BLS256 (5); the annotated region (*tal2h*) encodes a C-terminally truncated *tale* and *XOC_1555*, *XOC_1554* and *XOC_1553* (Fig. 3B). The latter three genes share 98% nucleotide identity to *IS1403* (24), *pgm*, and *enaV* (data not shown). In *tal2h*, the number of repeat units of the 102-bp sequence is 20.5. These data suggest that the HMWB loci are conserved but vary in the number of repeat units. It should be mentioned that HMWB homologues were not detected in the genomes of *X. oryzae* pv. *oryzae* strains MAFF311018, KACC10331, and PXO99^A (BLAST analysis; data not shown). Multiple sequence alignment using mature TALEs, including AvrXa3 (accession number AY129298.1), AvrXa10 (AAA92974.1) (Fig. 3B), and AvrXa27 (AAV54168.1) revealed the absence of a 58-aa sequence at the N-terminus of TAL2-24, TAL105, and TAL2h, respectively

TABLE 2. Transcriptional activator-like effector (*tale*) genotypes of *Xanthomonas oryzae* pv. *oryzicola* strains collected from different rice-growing regions of south China from 2008 to 2009

Genotypes	<i>tale</i> fragments absent ^a	Strains ^b	Number of strains (%) ^c	Number of putative <i>tale</i> genes ^d
1	A*ABCDEFGHIJKMNSTUVW	SCB04-1	1 (1.54)	9
2	A*ABCDGLOPRSUVW	YNB1-1	1 (1.54)	16
3	A*ABCNPVW	YNB9-3, SCB9-02, ZJB9-2, HANB9-1, JSB2-24, GDB3-143, HNB16-97, HNB16-100, AHB4-204, HNB16-46, AHB4-147, AHB4-205, AHB4-118, AHB3-7, AHB2-131, GDB8-47, ZJB01-25, HNB12-26, SCB9-5	19 (29.23)	20
4	ABCNPVW	HNB3-17, YNB01-3	2 (3.08)	20
5	A*ABCHNPVW	JSB5-2	1 (1.54)	19
6	A*BCNPVW	SCB04-2, YNB63, GXB9-31, SCB9-24, HANB9-23, SCB9-18, HANB8-2, RS85	8 (12.31)	20
7	A*ABNPVW	GDB3-4	1 (1.54)	21
8	A*ACDW	ZJB9-46, YNB9-41, SCB9-39	3 (4.62)	22
9	A*ABCVW	ZJB9-12, JSB1-39	2 (3.08)	22
10	A*ACDNUVW	YNB21, GDB3-12, GXB3-4, GXB3-23, GXB3-8, GDB3-092, GDB3-087, JSB3-120, JSB3-102, YNB9-53, HANB9-48, HANB9-35, GXB9-32, GXB1-6, JSB3-28, AHB1-58	16 (24.64)	20
11	A*ACDFNUVW	RS105	1 (1.54)	18
12	A*ACDNPVW	ZJB01-19, ZJB01-12, YNB9-44, ZJB9-38, SCB9-25	5 (7.69)	21
13	A*ACDNPVW	RS119, HANB10-10, HNB1-19, HANB01-12	4 (6.15)	20
14	A*ABDNUV	HNB07-3	1 (1.54)	20

^a The *tale* fragments absent in the tested strains. Letters (A*, A–W) represent *tale* fragments or bands of the tested isolates hybridized by Southern blot with the central *Sph*I fragment of *avrXa3* as the probe.

^b Strains are abbreviated by letters indicating their place of isolation: AH = Anhui, GD = Guangdong, GX = Guangxi, HAN = Hainan, HN = Hunan, JS = Jiangsu, SC = Sichuan, YN = Yunnan, and ZJ = Zhejiang; RS = rice streak.

^c Values in parentheses indicate percentage of a genotype of the total strains.

^d Number of possible *tale* genes are estimated based on the number and size of the hybridized bands by Southern blot.

(Supplemental Figure 2); whether this deletion is significant in pathogen virulence or host specificity awaits further investigation. To evaluate whether the HMWBs could be used for detection of *X. oryzae* pv. *oryzicola* by PCR, we designed primers HMWBF and HMWBR. HMWBF maps upstream of the first repeat unit in *tal2-24*, *tal105*, and *tal2h'*, and primer HMWBR is located at the stop codon of *IS1403* (Fig. 3B, panel b, horizontal arrows). The primers were then used in PCR with representative strains of 14 genotypes. PCR products were amplified from 12 of the genotypes, and the size of PCR products (Fig. 3C) was consistent

with the calculation based on HMWBF locus structures (Fig. 3B) detected by Southern blot analysis (Fig. 3A), implying that these primers can be used to genotype *X. oryzae* pv. *oryzicola* strains isolated from rice-growing regions. The PCR products shown in Figure 3C were also sequenced, and subsequent analysis indicated that PCR product length was dependent on the number of repeat units in the C-terminally truncated portion of the HMWBs (data not shown). It is also important to note that PCR products were not detected using strains SCB04-1 and YNB1-1 (Fig. 3C), which is consistent with the absence of HMWB loci in these two strains.

Fig. 2. Classification of 65 *Xanthomonas oryzae* pv. *oryzicola* strains into distinct genotypes and pathotypes. **A**, Dendrogram showing relatedness of *X. oryzae* pv. *oryzicola* strains based on Southern blot analysis and the presence or absence of hybridizing *Bam*HI fragments. The dendrogram was obtained by cluster analysis using the unweighted pair group method (UPGMA) in SIMQUAL with the Dice coefficient of NTSYS (18,25). Clusters were delineated at an arbitrary level of similarity equal to 0.97. **B**, Dendrogram based on virulence assessment of *X. oryzae* pv. *oryzicola* strains inoculated to 23 different rice lines containing different resistance genes for bacterial leaf blight. The dendrogram was constructed by cluster analysis using UPGMA with the arithmetic mean method in the SAHN routine of NTSYS (18). Pathotypes were designated I to XIV and clustered at an arbitrary level of similarity equal to 0.5.

TABLE 3. Genotypes of *Xanthomonas oryzae* pv. *oryzicola* strains collected from different provinces of south China

Province	Genotypes ^a														Strains ^b
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Anhui	...	...	6	...	...	...	...	...	...	1	...	...	...	...	7
Jiangsu	...	...	1	...	1	1	...	...	1	3	1	...	1	...	9
Hunan	...	...	4	1	...	...	...	...	...	...	...	...	1	1	7
Hainan	...	...	1	...	...	2	...	...	...	2	...	...	2	...	7
Guangdong	...	...	2	...	...	...	1	...	...	3	...	...	...	...	6
Guangxi	...	...	...	...	...	1	...	...	...	5	...	...	...	...	6
Yunnan	...	1	1	1	...	1	...	1	...	2	...	1	...	...	8
Sichuan	1	...	2	...	...	3	...	1	...	...	...	1	...	...	8
Zhejiang	...	...	2	...	...	...	...	1	1	...	...	3	...	...	7
Total	1	1	19	2	1	8	1	3	2	16	1	5	4	1	65

^a Number of strains belonging to one genotype.

^b Total strains collected in this province.

TABLE 4. Virulence comparison of 14 genotypes of *Xanthomonas oryzae* pv. *oryzicola* in rice lines containing different resistance genes against bacterial leaf blight

Rice line	Lesion length (cm) for genotypes ^a														Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Asominor	1.44	1.40	1.42	3.34 ^S	4.66 ^S	1.41	1.26	2.39	2.36	1.36	2.42	1.95	2.85	3.96 ^S	2.37
BJ1	0.92	0.74	1.69	1.43	1.36	0.70	0.96	0.73	1.81	0.80	1.52	1.42	2.00	2.26	1.34
Cas209	1.48	1.94	2.71	2.75	2.06	1.50	1.76	1.73	1.85	2.46	2.58	2.01	2.85	1.46	2.13
DV85 ^b	0.42 ^R	0.34 ^R	0.78	2.65	1.48	1.73	1.26	1.72	3.28	1.47	2.56	1.74	2.44	2.16	1.82
IR24	1.74	1.42	2.71	2.93	2.32	3.20 ^S	1.74	2.72	3.70 ^S	2.25	1.66	2.17	2.70	2.56	2.47
IR26	1.76	1.28	2.42	1.57	3.52 ^S	3.31 ^S	3.10 ^S	3.43 ^S	3.76 ^S	2.36	3.08 ^S	4.10 ^S	2.31	2.38	2.82
BB1	0.90	0.38 ^R	2.57	1.55	1.88	3.58 ^S	1.82	2.85	1.73	2.84	2.94	3.11 ^S	2.89	2.42	2.35
BB2	1.84	2.34	2.33	1.77	3.14 ^S	2.20	2.78	3.45 ^S	1.77	1.43	1.28	3.21 ^S	2.31	2.82	2.37
BB3	0.78	0.48 ^R	1.47	2.08	1.34	1.94	2.20	3.31 ^S	2.79	1.92	1.44	1.63	2.14	1.38	1.86
BB4	0.78	2.18	1.52	0.72	2.20	2.18	2.28	1.84	2.52	1.85	0.68	1.54	1.56	2.36	1.80
BB5	1.34	1.32	1.98	1.83	3.54 ^S	2.64	3.14 ^S	2.67	1.33	1.50	0.84	1.32	2.01	1.46	1.97
BB7	0.86	2.18	2.05	1.51	2.74	1.75	1.26	2.27	2.57	2.28	1.80	1.42	1.69	1.66	1.94
BB8	1.22	1.76	3.55	1.44	1.76	2.98	3.80 ^S	2.29	1.90	3.63 ^S	2.88	2.36	1.93	1.86	2.47
BB10	0.78	1.38	1.92	1.99	3.44 ^S	2.24	1.64	2.31	2.12	2.07	1.50	3.22 ^S	1.84	1.86	2.12
BB11	0.88	1.46	2.07	1.87	3.12 ^S	4.27 ^S	3.72 ^S	4.24 ^S	2.72	2.07	0.36 ^R	1.74	1.75	1.92	2.41
BB11(T)	0.44 ^R	0.48 ^R	1.95	2.28	2.36	2.41	1.78	2.33	1.72	2.97	0.46 ^R	1.45	1.67	1.8	1.82
BB13	1.30	0.40 ^R	1.75	3.28 ^S	1.80	3.55 ^S	2.74	3.27 ^S	2.82	1.84	0.78	1.56	3.16 ^S	3.34 ^S	2.33
BB14	0.82	0.78	1.58	2.66	5.02	3.22 ^S	1.44	3.15 ^S	2.68	1.90	0.44	2.25	2.54	2.26	2.30
BB21	1.36	0.98	1.92	2.41	0.86	3.23 ^S	2.24	3.07 ^S	1.63	1.55	1.82	1.61	1.90	1.8	1.92
Java14	0.90	0.88	2.59	2.82	1.46	2.62	2.56	3.25 ^S	2.64	2.34	2.68	2.40	3.18 ^S	2.74	2.47
Tetep	0.98	0.48 ^R	1.96	3.38 ^S	2.28	2.38	4.38 ^S	1.37	3.28 ^S	2.72	2.88	2.43	2.77	4.02 ^S	2.64
TN1	0.92	0.94	1.39	2.28	1.28	1.75	0.74	2.29	2.77	2.38	2.66	1.60	2.46	1.66	1.86
Wase Aikoku	1.48	1.62	2.18	1.74	2.3	2.32	1.96	1.45	1.71	1.62	1.88	1.82	0.80	2.36	1.83
Average	1.09	1.18	2.02	2.19	2.43	2.48	2.20	2.53	2.41	2.07	1.79	2.09	2.25	2.28	...

^a Lesion lengths in mean caused by each genotype. R = resistant to the tested genotypes if lesion length is <0.5 cm and S = more susceptible to the tested genotypes if lesion length is >3.0 cm.

Fig. 3. Analysis of a high molecular weight band (HMWB) in the 14 genotypes of *Xanthomonas oryzae* pv. *oryzicola*. **A**, Southern blot analysis of strains representing the 14 genotypes of *X. oryzae* pv. *oryzicola*. Genomic DNAs of the strains were digested with *Bam*HI and hybridized with an internal *Sph*I fragment of *avrXa3* (44). Bands demarcated with black arrows were isolated and sequenced. **B**, Fine-structure maps of a known transcriptional activator-like effector (*tale*) gene product and HMWBs identified in the present study. **(a)** Fine structure of the mature TALE encoded by *avrXa10*. T3S, type III secretion signals at the N-terminus of a mature TALE; LZ, leucine zipper; NLS, nuclear localization signal; AD, acidic transcriptional activation domain; B, *Bam*HI; P, *Pst*I; S, *Sph*I; 58, amino acid sequence of 58 residues that is present in *AvrXa10*. **(b)** Functional map of the HMWB locus in strain JSB2-24 (designated TAL2-24). This locus is predicted to contain an immature *tale* with a C-terminal truncation, insertion element *IS1403*, phosphoglycerate mutase (*pgm*), and endonuclease V (*enaV*). The number of repeat units in TAL2-24 is 19.5. **(c)** Functional map of the HMWB locus in strain RS105 (designated TAL105). This locus contains all the components present in TAL2-24 but contains 21.5 repeat units in the central region. **(d)** Functional map of the HMWB locus in strain BLS256 (accession CP003057.1). This locus contains an immature *tale* (designated TAL2h'), *XOC_1555*, *XOC_1554*, and *XOC_1553*. The latter three genes show 98% nucleotide identity to *IS1403*, *pgm*, and *enaV* in strains JSB2-24 and RS105. TAL2h' contains 20.5 repeat units. **C**, Detection of polymorphisms in the HMWB loci by polymerase chain reaction (PCR) amplification. Positions of the specific primers used in strain genotyping are shown by the horizontal arrows in B (b). PCR products were separated by electrophoresis in a 1% agarose gel. PCR was performed twice with similar results, and products were sequenced. NC represents that water was used as the template (negative control). Molecular size markers (bp) are indicated to the left.

DISCUSSION

In this study, we evaluated genetic diversity within putative *tale* genes in *X. oryzae* pv. *oryzicola* strains isolated from rice-growing provinces in southern China. When an internal fragment of *avrXa3* was used as a probe (44), 65 *X. oryzae* pv. *oryzicola* strains (Table 1) were clustered into 14 genotypes containing 9 to 22 potential *tale* genes (Table 2; Figs. 1 and 3). The majority of strains were assigned to genotypes 3 and 10 (Tables 2 and 3). There was an obvious absence of the “gene-for-gene” resistance paradigm when these 65 strains were inoculated to 23 rice lines harboring different *R* genes for BLB (32,41). The 65 strains were classified into 14 pathotypes (I to XIV) by UPGMA cluster analysis, and there was a strong correlation between pathotype and genotype groupings (Fig. 2). Furthermore, the acquisition of greater numbers of *tale* genes was associated with increased virulence (Tables 1, 2, and 4), supporting the contention that *tale* genes are involved in pathogenicity. Furthermore, we demonstrate the potential use of the HMWB loci in genotyping *X. oryzae* pv. *oryzicola* strains by PCR (Fig. 3C), and we propose that molecular typing using *tale* genes may be a more useful approach for *X. oryzae* pv. *oryzicola* than random amplified polymorphic DNA; AFLP; RFLP; and BOX-, ERIC-, and Rep-PCR (1,13,18,22, 34,35) for *X. oryzae* pv. *oryzicola*.

In the related pathogen, *X. oryzae* pv. *oryzae*, TALEs with substantial effects on virulence were shown to activate sucrose transporter genes of the *OsSWEET* family to promote disease development (8,42). However, *X. oryzae* pv. *oryzicola* TALEs are not known to target the *OsSWEET* family in rice. It is likely that *tale* genes in *X. oryzae* pv. *oryzicola* also have specific targets in rice, because the *tale* gene products in other *Xanthomonas* spp. are known to localize to plant nuclei (2,4,9,11,14,19,26,29,36,42). Thus, it is tempting to speculate that *X. oryzae* pv. *oryzicola* uses TALEs to promote BLS susceptibility in rice, perhaps using mechanisms that are quite different from *X. oryzae* pv. *oryzae*. Several pieces of evidence support this hypothesis: (i) *X. oryzae* pv. *oryzicola* has the ability to suppress *avrXa10-Xa10*-mediated resistance (27); (ii) *Xa27* resistance can be triggered by *X. oryzae* pv. *oryzicola* when this pathogen harbors *avrXa27* from *X. oryzae* pv. *oryzae* (39); (iii) BLB is a vascular disease caused by *X. oryzae* pv. *oryzae* whereas BLS occurs in the leaf parenchyma, implying very different infection arsenals for the two pathogens; and (iv) *X. oryzae* pv. *oryzicola* TALEs lack an 8-aa peptide (SGSVGGTI) that is present at the C-terminus of *X. oryzae* pv. *oryzae* TALEs (7). The final observation may alter the functionality of TALEs, an observation that merits further study.

It has been hypothesized that the evolution of TALEs may occur as the pathogen adapts to the selective pressure conferred by host *R* genes (3,30,38). The bacterial leaf blight pathogen, *X. oryzae* pv. *oryzae*, uses TALEs such as PthXo1, PthXo2, and PthXo3 (2,46) to promote virulence, and it seems likely that *X. oryzae* pv. *oryzicola* also uses *tale* gene products as virulence factors. It is relevant that the 65 *X. oryzae* pv. *oryzicola* strains differed in the number of hybridizing bands (e.g., potential *tale* genes) and varied in virulence. For example, strain JSB5-2 containing band F and lacking band H was more virulent in rice lines Asominor and IRBB14 than strain RS105, which contains band H but not F (Fig. 1; Table 1). Conversely, RS105 displayed stronger virulence in rice lines DV85 and IRBB21 than JSB5-2 (Table 1). Thus, the putative *tale* gene encoded by band F may function as a virulence determinant for JSB5-2 in Asominor and IRBB14, whereas band H gene may be important in the virulence of RS105 in rice lines DV85 and IRBB21. Although it is tempting to correlate the presence of a particular band with virulence, such an association is highly speculative. Thus, it would be quite useful to isolate or engineer an *X. oryzae* pv. *oryzicola* strain that lacks TALEs and use this for reintroducing *tale* genes, an approach that

could be used to systematically dissect the contribution of individual *tale* genes in virulence (6,42).

An important outcome of the present study was the identification of the HMWB loci in 63 of the 65 *X. oryzae* pv. *oryzicola* strains. Sequence analysis indicated that the HMWB loci contained a C-terminally truncated *tale*, and encoded IS1403 (*XOC_1555*), *pgm* (*XOC_1554*), and *enaV* (*XOC_1553*) (Fig. 3B); the latter three loci are conspicuously absent from the genomes of *X. oryzae* pv. *oryzae* strains KACC10331 (23), MAFF311018 (31), and PXO99^A (37). It is also noteworthy that the C-terminally truncated TALEs lack a 58-aa peptide that generally maps at the N terminus of mature TALEs. This raises an interesting question: did the mature *tale* genes in *X. oryzae* pv. *oryzae* strains such as *AvrXa3*, *AvrXa10*, and *AvrXa27* perhaps evolve from the HMWB loci described in this study? Furthermore, some of the virulence properties might be present in the HMWB loci, which possibly represent immature, ancestral *tale* genes. In support of this speculation, we previously isolated an immature *tale* from an *X. oryzae* pv. *oryzae* strain which reduced *X. oryzae* pv. *oryzae* virulence in rice (20), suggesting that C-terminus-truncated *tale* genes still play roles in bacterial virulence in rice. In preliminary studies, we discovered that derivatives of *tal3a* and *tal3b* from *X. oryzae* pv. *oryzae* containing truncations in the C-terminus end still function in virulence (unpublished data). These truncated derivatives, like the HMWB loci described above, lack the 58-aa peptide and the NLS and AD domains. Although the mechanistic basis is unclear, these “immature” *tale* gene products may be transported via the T3SS into the host cytoplasm, or perhaps they bind a mature TALE for localization to the host nucleus. More detailed studies of *tale* gene function in *X. oryzae* pv. *oryzicola* will help elucidate the role of these factors in modulating the outcome of the host–pathogen interaction.

ACKNOWLEDGMENTS

This work was supported by the State Key Basic Research and Development Project of China (20112CB114003), the Natural Science Foundation of China (31230059), and the Special Fund for Agro-Scientific Research in the Public Interest of China (201303015). Z.-Y. Ji and M. Zakria contributed equally to this work. Z.-Y. Ji performed experiments; G.-Y. Chen and L.-F. Zou designed experiments; G.-H. Ji collected strains; and Z.-Y. Ji, G.-Y. Chen, and M. Zakria wrote the article.

LITERATURE CITED

- Adhikari, T. B., Cruz, C., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and Leach, J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Appl. Environ. Microbiol.* 61:966-971.
- Antony, G., Zhou, J., Huang, S., Li, T., Liu, B., White, F., Yang, B. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os11N3*. *Plant Cell* 22:3864-3876.
- Boch, J., and Bonas, U. 2010. *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. *Annu. Rev. Phytopathol.* 48:419-436.
- Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., and Bonas, U. 2009. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 326:1509-1512.
- Bogdanove, A. J., Koebnik, R., Lu, H., Furutani, A., Angiuoli, S. V., Patil, P. B., Van Sluys, M. A., Ryan, R. P., Meyer, D. F., Han, S. W., Aparna, G., Rajaram, M., Delcher, A. L., Phillippy, A. M., Puiu, D., Schatz, M. C., Shumway, M., Sommer, D. D., Trapnell, C., Benahmed, F., Dimitrov, G., Madupu, R., Radune, D., Sullivan, S., Jha, G., Ishihara, H., Lee, S. W., Pandey, A., Sharma, V., Sriariyanun, M., Szurek, B., Vera-Cruz, C. M., Dorman, K. S., Ronald, P. C., Verdier, V., Dow, J. M., Sonti, R. V., Tsuge, S., Brendel, V. P., Rabinowicz, P. D., Leach, J. E., White, F. F., and Salzberg, S. L. 2011. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic *Xanthomonas* spp. *J. Bacteriol.* 193:5450-5464.
- Cao, Y., Zou, L., Zhao, W., Ji, Z., Zou, H., and Chen, G., 2011. Establishment of knockout mutagenesis in *avrBs3/pthA* family genes of *Xanthomonas oryzae* pv. *oryzicola*. *J. Zhejiang Univ. Agric. Life Sci.* 37:40-48.
- Chen, G. Y., Zou, L. F., Wu, X. M., Li, Y. R., and Wang, J. S. 2005.

- avr/pth13* gene of *Xanthomonas oryzae* pv. *oryzicola*, a novel virulence member of *avrBs3/PthA* family, strengthening virulence of *Xanthomonas oryzae* pv. *oryzae* on rice. *Chin. J. Rice Sci.* 19:291-296.
8. Chen, L. Q., Hou, B. H., Lalonde, Takanaga, H., Hartung, M. L., Qu, X. Q., Guo, W. J., Kim, J. G., Underwood, W., Chaudhuri, B., Chermak, D., Antony, G., White, F. F., Somerville, S. C., Mudgett, M. B., and Frommer, W. B. 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* 468:527-532.
9. Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., Li, X., Fu, B., Li, Z., Bennetzen, J. L., Zhang, Q., and Wang, S. 2006. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* 20:1250-1255.
10. Cui, Y. P., Zou, L. F., Zou, H. S., Li, Y. R., Zakria, M., and Chen, G. Y. 2013. HrpE3 is a type III effector protein required for full virulence of *Xanthomonas oryzae* pv. *oryzicola* in rice. *Mol. Plant Pathol.* 14:678-692.
11. Deng, D., Yan, C. Y., Pan, X. J., Mahfouz, M., Wang, J. W., Zhu, J. K., Shi, Y. G., and Yan, N. 2012. Structural basis for sequence-specific recognition of DNA by TAL effectors. *Science* 335:720-723.
12. Fang, C. T., Ren, H. C., Chen, T. Y., Zhu, Y. K., Fan, H. C., and Wu, S. C. 1957. A comparison of the rice bacterial leaf blight organism with the bacterial leaf streak organisms of rice and *Leersia hexandra* Swartz. *Acta Phytopathol. Sin.* 3:99-124.
13. Gonzalez, C., Szurek, B., Manceau, C., Mathieu, T., Sere, Y., and Verdier, V. 2007. Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. *Mol. Plant-Microbe Interact.* 20:534-546.
14. Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G. L., White, F. F., and Yin, Z. 2005. *R* gene expression induced by a type-III effector triggers disease resistance in rice. *Nature* 435:1122-1125.
15. Hajri, A., Brin, C., Zhao, S., David, P., Feng, J. X., Koebnik, R., Szurek, B., Verdier, V., Boureau, T., and Poussier, S. 2012. Multilocus sequence analysis and type III effector repertoire mining provide new insights into the evolutionary history and virulence of *Xanthomonas oryzae*. *Mol. Plant Pathol.* 13:288-302.
16. Han, Q. D., Chen, Z. W., Deng, Y., Lan, T., Guan, H. Z., Duan, Y. L., Zhou, Y. C., Lin, M. C., and Wu, W. R. 2008. Fine mapping of qBLSr5a, a QTL controlling resistance to bacterial leaf streak in rice. *Acta Agron. Sin.* 34:587-590.
17. He, W. A., Huang, D. H., Cen, Z. L., Zhang, Y. X., Ma, Z. F., Liu, C., Chen, Y. Z., Lu, S. N., Liu, K. Y., and Li, R. B. 2010. Research progress on rice resistance to bacterial leaf streak. *J. Plant Genet. Resour.* 11:116-119.
18. Hu, J., Zhang, Y., Qian, W., and He, C. 2007. Avirulence gene and insertion element-based RFLP as well as RAPD markers reveal high levels of genomic polymorphism in the rice pathogen *Xanthomonas oryzae* pv. *oryzae*. *Syst. Appl. Microbiol.* 30:587-600.
19. Hummel, A. W., Doyle, E. L., and Bogdanove, A. J. 2012. Addition of TAL effector binding sites to a pathogen strain-specific rice bacterial blight resistance gene makes it effective against additional strains and against bacterial leaf streak. *New Phytol.* 195:883-893.
20. Ji, Z. Y., Yang, J., Wang, Y. P., Zhou, D., Li, Y. R., Zou, L. F., and Chen, G. Y. 2009. Identification of a specific avirulence gene from a Chinese race C8 of *Xanthomonas oryzae* pv. *oryzae*. *Chin. J. Rice Sci.* 23:463-469.
21. Kay, S., Hahn, S., Marois, E., Hause, G., and Bonas, U. 2007. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science* 318:648-651.
22. Leach, J. E., Rhoads, M. L., Vera Cruz, C. M., White, F. F., Mew, T. W., and Leung, H. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. *Appl. Environ. Microbiol.* 58:2188-2195.
23. Lee, B. M., Park, Y. J., Park, D. S., Kang, H. W., Kim, J. G., Song, E. S., Park, I. C., Yoon, U. H., Hahn, J. H., Koo, B. S., Lee, G. B., Kim, H., Park, H. S., Yoon, K. O., Kim, J. H., Jung, C. H., Koh, N. H., Seo, J. S., and Go, S. J. 2005. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res.* 33:577-586.
24. Lee, Y. A., and Chiu, S. P. 1998. IS1403 and IS1404: analysis and distribution of two new insertion sequences in *Xanthomonas campestris*. *Bot. Bull. Acad. Sin.* 39:231-239.
25. Lewontin, R. C. 1972. The apportionment of human diversity. *Evol. Biol.* 6:381-398.
26. Mak, A. N., Bradley, P., Cernadas, R. A., Bogdanove, A. J., Stoddard, B. L. 2012. The crystal structure of TAL effector PthXo1 bound to its DNA target. *Science* 335:716-719.
27. Makino, S., Sugio, A., White, F. F., and Bogdanove, A. J. 2006. Inhibition of resistance gene-mediated defense in rice by *Xanthomonas oryzae* pv. *oryzicola*. *Mol. Plant-Microbe Interact.* 19:240-249.
28. Mew, T. W., Alvarez, A. M., Leach, J. E., and Swings, J. 1993. Focus on bacterial blight of rice. *Plant Dis.* 77:5-12.
29. Moscou, M. J., and Bogdanove, A. J. 2009. A simple cipher governs DNA recognition by TAL effectors. *Science* 326:1501.
30. Niño-Liu, D. O., Ronald, P. C., and Bogdanove, A. J. 2006. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Mol. Plant Pathol.* 7:303-324.
31. Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A., and Kaku, H. 2005. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *Jpn. Agric. Res. Q.* 39:275-287.
32. Ogawa, T., 1993. Methods and strategy for monitoring race distribution and identification of resistance genes to bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*) in rice. *Jpn. Agric. Res. Q.* 27:71-80.
33. Ou, S. H. 1984. Exploring tropical rice diseases: a reminiscence. *Annu. Rev. Phytopathol.* 22:1-11.
34. Rademaker, J. L., Hoste, B., Louws, F. J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P., and de Bruijn, F. J. 2000. Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. *Int. J. Syst. Evol. Microbiol.* 2:665-677.
35. Raymundo, A. K., Briones, A. M., Jr., Ardales, E. Y., Perez, M. T., Fernandez, L. C., Leach, J. F., Mew, T. W., Ynalvez, M. A., McLaren, C. G., and Nelson, R. J. 1999. Analysis of DNA polymorphism and virulence in Philippine strains of *Xanthomonas oryzae* pv. *oryzicola*. *Plant Dis.* 83:434-440.
36. Romer, P., Recht, S., Strauß, T., Elsaesser, J., Schornack, S., Boch, J., Wang, S., Lahaye, T., 2010. Promoter elements of rice susceptibility genes are bound and activated by specific TAL effectors from the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae*. *New Phytol.* 187:1048-1057.
37. Salzberg, S. L., Sommer, D. D., Schatz, M. C., Phillippy, A. M., Rabinowicz, P. D., Tsuge, S., Furutani, A., Ochiai, H., Delcher, A. L., Kelley, D., Madupu, R., Puiu, D., Radune, D., Shumway, M., Trapnell, C., Aparna, G., Jha, G., Pandey, A., Patil, P. B., Ishihara, H., Meyer, D. F., Szurek, B., Verdier, V., Koebnik, R., Dow, J. M., Ryan, R. P., Hirata, H., Tsuyumu, S., Won Lee, S., Seo, Y. S., Sriariyanum, M., Ronald, P. C., Sonti, R. V., Van Sluys, M. A., Leach, J. E., White, F. F., and Bogdanove, A. J. 2008. Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. *BMC Genomics* 9:204.
38. Tang, D., Wu, W., Li, W., Lu, H., and Worland, A. J. 2000. Mapping of QTLs conferring resistance to bacterial leaf streak in rice. *Theor. Appl. Genet.* 101:286-291.
39. Tian, D., and Yin, Z., 2009. Constitutive heterologous expression of *avrXa27* in rice containing the *R* gene *Xa27* confers enhanced resistance to compatible *Xanthomonas oryzae* strains. *Mol. Plant Pathol.* 10:29-39.
40. Triplett, L. R., Hamilton, J. P., Buell, C. R., Tisserat, N. A., Verdier, V., Zink, F., and Leach, J. E. 2011. Genomic analysis of *Xanthomonas oryzae* isolates from rice grown in the United States reveals substantial divergence from known *X. oryzae* pathovars. *Appl. Environ. Microbiol.* 77:3930-3937.
41. Verdier, V., Cruz, C. V., and Leach, J. E. 2012. Controlling rice bacterial blight in Africa: needs and prospects. *J. Biotechnol.* 159:320-328.
42. Verdier, V., Triplett, L. R., Hummel, A. W., Corral, R., Cernadas, R. A., Schmidt, C. L., Bogdanove, A. J., and Leach, J. E. 2012. Transcription activator-like (TAL) effectors targeting OsSWEET genes enhance virulence on diverse rice (*Oryza sativa*) varieties when expressed individually in a TAL effector-deficient strain of *Xanthomonas oryzae*. *New Phytol.* 196:1197-1207.
43. White, F. F., and Yang, B. 2009. Host and pathogen factors controlling the rice-*Xanthomonas oryzae* interaction. *Plant Physiol.* 150:1677-1686.
44. Wu, X. M., Li, Y. R., Zou, L. F., and Chen, G. Y. 2007. Gene-for-gene relationships between rice and diverse *avrBs3/pthA* avirulence genes in *Xanthomonas oryzae* pv. *oryzae*. *Plant Pathol.* 56:26-34.
45. Yang, B., Sugio, A., and White, F. F. 2005. Avoidance of host recognition by alterations in the repetitive and C-terminal regions of AvrXa7, a type III effector of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant-Microbe Interact.* 18:142-149.
46. Yang, B., Sugio, A., and White, F. F. 2006. *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *Proc. Natl. Acad. Sci. USA* 103:10503-10508.
47. Yang, B., and White, F. F. 2004. Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol. Plant-Microbe Interact.* 17:1192-1200.
48. Ye, G., Hong, N., Zou, L., Zou, H., Zakria, M., Wang, G., and Chen, G. 2013. *tale*-based genetic diversity of Chinese isolates of the citrus canker pathogen *Xanthomonas citri* subsp. *citri*. *Plant Dis.* doi:10.1094/pdis-12-12-1201-RE
49. Zhang, H., Jiang, Y. H., Hu, B. S., Liu, F. Q., and Xu, Z. G. 2008. Specific detection of *Xanthomonas oryzae* pv. *oryzicola* by PCR techniques. *Acta Phytopathol. Sin.* 38:1-5.
50. Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J., and Hulbert, S. 2005. A maize resistance gene functions against bacterial streak disease in rice.

51. Zou, H. S., Yuan, L., Guo, W., Li, Y. R., Che, Y. Z., Zou, L. F., and Chen, G. Y. 2011. Construction of a Tn5-tagged mutant library of *Xanthomonas oryzae* pv. *oryzicola* as an invaluable resource for functional genomics. *Curr. Microbiol.* 62:908-916.
52. Zou, L. F., Wang, X. P., Xiang, Y., Zhang, B., Li, Y. R., Xiao, Y. L., Wang, J. S., Walmsley, A. R., and Chen, G. Y. 2006. Elucidation of the *hrp* clusters of *Xanthomonas oryzae* pv. *oryzicola* that control the hypersensitive response in nonhost tobacco and pathogenicity in susceptible host rice. *Appl. Environ. Microbiol.* 72:6212-6224.