AvrXa3: A novel member of *avrBs3* gene family from *Xanthomonas oryzae* pv. *oryzae* has a dual function*

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Abstract Two positive clones pUAV45 and pUAV47 were identified from the cDNA library of Jxo III, a race 3 strain of *Xanthomonas oryzae* pv. *oryzae* Dye (*Xoo*) in Japanese system, using Tn5 based technology. pUAV45 clone contained a 25.4 kb *Xoo* genomic DNA insert. Southern blot analysis with *avrXal0* as the probe showed that DNA insert in pUAV45 shares homology with *avrX10*. Furthermore within the *Xoo* insert, a smaller 5.7 kb *Kpn*I fragment (pUAV5k) was identified through hybridization with *avrXa10*. The transformation of pUAV45 and pUAV5k into the strain Pxo99 (race 6 in Philippine system) led to the decrease of Pxo99 pathogenicity on rice cultivar Wase Aikoku 3 (*Xa3*) and the increase of the pathogen pathogenicity on Cas209 (*Xal0*). The result of sequence analysis showed that there is a 2598 bp open reading frame (ORF) within the 5.7 kb *Kpn*1 fragment (pUAV5k). The ORF shared high identity (97%) with *avrXal0*. The deduced sequence of the ORF contained 8.5 tandem repeat units of 34-amino-acids, one leucine zipper (LZ), three nuclear localization signal (NLS) motifs, and an acidic activation transcriptional domain (AAD) at C-terminus. We named this ORF *avrXa3* and it is classified as a new member of *avrBs3* (*avr/pth*) family with the dual-function determined by alternations of avirulence and aggressiveness on rice cultivars carrying different '*R*' genes.

Keywords: rice, *Xanthomonas oryzae* pv. *oryzae*, *avrXa3*, *avrBs3* (*avr/pth*) family, functional domains.

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye (Swings et al. 1990), *Xoo*, is a major rice disease worldwide. It occurs in Australia, Africa, Latin America, North America, and is particularly destructive in Asia[1~3]. As a model of "gene-for-gene" disease, the incompatible interaction occurs when a plant resistant gene (*R*) is matched by a corresponding avirulence gene (*A*) of the pathogen[4]. Many bacterial *avr* genes have been identified from plant pathogenic bacteria[6~8]. Canteros and Bonas first found the close structural similarities between two *avr* genes from *Xanthomonas. campestris* pv. *vesicatoria* (Doidge) Dye[9]. Further studies by Gabriel and Leach indicated that the same characteristics existed in the *avr* genes from other Xanthomonads (*Xanthomonas campestris* pv. *mulvacearum*, *X. auxonopodis* pv. *citri* and *X. oryzae* pv *oryzae*.) and they called them *avr/pth* family[10, 11]. Because *avrBs3* is the first reported member in this family, Bonas considered that it should be named *avrBs3* gene family[5]. The structure of the gene family is characterized by 3 kb in length, identical 3′ and 5′ terminals, more than 12 of the 102-bp tandem repeat in the central region of each *avrBs3*-homologue. All deduced sequences of AvrBs3-like proteins from *Xanthomonas* share 90%~97% identity with each other and have one leucine zipper (LZ), three nuclear localization signals (NLS), and an acidic transcriptional activation domain (AAD) at C-terminal. However, different *avrBs3* homologues may contain different numbers of 102-bp repeats, and within these repeats the sequences share 91%~100% identity with each other. The differences among them are located in a two-codon region referred to as the variable region (the 12th and 13th sites of 34-amino-acids). The number of 34 amino acids repeats and variable regions control virulence specificity[12]. Other structures might be also required for the interactions between pathogen and host as well as the inductions of host defense responses[13, 14].

In general it is difficult to clone pathogenicity (*pth*) genes from *Xoo* due to the strictly restricted modification system within *Xoo*[15]. Thus in most cases isolations and characterizations of genes related

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to pathogenicity from *Xoo* depend on the homology among extrinsic genes from other phytobacteria[16]. Hopkins et al. used *avrBs3* of *Xcv* as a probe to hybridize against the genomic library of Pxo86 (*Xoo*) and identified two *avr* genes, *avrXa7* and *avrXa10*. When they were transferred into a virulent strain, Pxo99, the interaction phenotype between the trans-conjugants and rice cultivars carrying the resistance gene *Xa7* or *Xa10* was altered. Further research demonstrated that both *avr* genes were homologous to *avrBs3*.

Races of *Xanthomonas oryzae* pv. *oryzae* have been monitored by pathogenicity on differential rice cultivars. In general Japanese plant pathologists divided *Xoo* into 5 races, while 6 races by International Rice Research Institute (IRRI) in Philippines and 7 pathotypes in China[17]. The races of plant pathogens may vary depending on the differential cultivars used. At present most japonica rice cultivars used in China have *Xa3* gene and most indica rice culivars have *Xa4*[18]. Therefore studies on *avrXa3* and *avrXa4* not only have profound impacts on theoretical studies but also are helpful for the understanding of the variations within *Xoo* races and exploring the properties of *avr* genes of this bacterium. Disease resistance of rice cultivars (Wase Aikoku 3 and IRBB3) carrying *Xa3* is triggered by the strain JXO Ⅲ (race 3) in Japanese system. Our laboratory has screened the *avr* mutants of JXO Ⅲ by Tn5 mutagenesis and identified 49 positive clones using the probe derived from the flanking DNA sequence of Tn5 to hybridize the JXO Ⅲ genomic library in the previous research[1)]. In this paper we report 2 clones pUAV45 and pUAV47 containing *avr* genes screened from the 49 positive clones and identification of a novel *avrXa3* gene belonging to *avrBs3* family.

1 Materials and methods

1.1 Bacterial strains, plasmids and culture media

Sources and characteristics of the bacterial strains and plasmids used in this study are shown in Table 1. *Xoo* strains were grown on NA medium at 28 ℃. *Escherichia coli* strains were cultured on Luria-Bertani (LB) medium at 37 ℃. The concentrations of antibiotics are 100 μg/mL for rifampicin (Rif), 20 μg/mL for kanamycin (Km), 25 μg/mL for spectinomycin (Sp), 50 μg/mL for ampicilin (Ap) and 20 μg/mL for cephalexin (Cp) added in the media respectively.

Table 1. Bacterial strains and plasmids used in this study

Strains	Relevant characteristics	Source or reference
Bacterial strains		
Xoo		
JXO Ⅲ	Japanese system, Race 3, Riff resistant, incompatible on Wise Aikoku 3 (*Xa3*) and compatible on Cas209(Xa3)	This laboratory
PXO99	Philippine system, race 6, riff resistant, compatible on Wise Aikoku 3 and Cas209	International Rice Research Institute, the Philippines
E. coli.		
DH5α	F *recA*, Φ80d *lacZ*, ΔM12	Bethesda Research Laboratories
S17-1	294 *recA*, Tp^r, Sp^r	Kansas State University
Plasmids		
pUFV034	Cosmid, Mob^+, $LacZ^+$, *IncW*, Nms, Km^r,	Florida University
pUAV45	JXOⅢ genomic library, 25.4kb insert in pUFR034, Kmr	This study
pUAV5K	From pUAV45, 5.8 kb *Kpn*I insert in pUFR034 Km^r	This study
pUAV5E3	From pUAV45, 4.3 kb *Eco*RI insert in pUFR034, Km^r	This study
pUAV5E4	From pUAV45, 3.6 kb *Eco*RI insert in pUFR034, Km^r	This study
pUAV5B	From pUAV45, 2.8 kb *Bam*HI insert in pUFR034, Km^r	This study
pBS3.1A	*avrXa10* clone in pBluescriptKS vector, Ap^r	Kansas State University

1) Huang, Y.C. The cloning of a virulence gene avrXa3 of *Xanthomonas oryzae* from JxoⅢ, a strain of race 3 in Japanese system. Ph. D Dissertation. Nanjing Agricultural University, 1999.

1.2 Plant materials and pathogenicity assay

Rice cultivar Wase Aikoku 3 carries *Xa3* gene and Cas209 carries *Xa10* gene of bacterial leaf blight resistance. Rice was planted in pots (diameter ≥30 cm) and grown in growth chambers at 28 ~ 32 ℃ in day light. Plants were inoculated by leaf-cutting method at booting or flowering stage. Symptoms (phenotypes and lesion lengths) were assessed 14 days after the inoculation. The disease severity (%) was expressed by the lesion length divided by the full length of the inoculated leaf. Rice cell suspension was prepared as described[19]. Of the 1 mL *Xoo* bacterial suspension (OD_{620}=1) was added to every 50 mL of rice cell suspension, then the rice cell suspension was shacked in darkness at 28 ℃. After inoculation, the cell suspensions were sampled every 6 hours and stained with Evans blue and the rate of cell death was recorded under the microscope.

1.3 Genetic manipulations

Plasmid extraction, transformation, electrophoresis, enzymatic ligation and sub-cloning were conducted as methods described[20]. Dig random labeling kit was used for the probe synthesis. Probe labeling and Southern blot was performed as recommended by the manufacturer (Roche Co., Ltd.). Restriction map was constructed using double-enzyme digestion method. Bi-parental mating method[15] was used for the conjugation between *E. coli* and the appropriate *Xoo* strains used.

1.4 Sequence analysis

DNA fragments were sequenced by TaKaRa Biotechnology (Dalian) Co., Ltd. Sequence homology was analyzed with Blast (www.ncbi.nih.nlm.org.) and the sequence alignment was conducted by BioEdit program.

2 Results

2.1 Function of the cosmid clone pUAV45

The strain JXO III was mutated using Tn5 mediated mutagenesis (S17-1/pZJ25::Tn5) and the conjugant was screened on PSA-Rif-Km plate. The conjugation efficiency was achieved at very low level (about 0.2×10^{-7}). A total of 6000 conjugants were obtained. Pathogenicity assay of the mutants from JXO III on rice cultivars Wase Aikoku 3 (*Xa3*) and IR26 (*Xa4*) showed that only mutant 3201 significantly altered the phenotype from incompatible to compatible on Wase Aikoku 3 with the average of lesion length increased from 0.36±0.29 cm to 5.38±0.89 cm. This result suggested that 3201 is an *avrXa3* mutant. Genomic DNA of 3201 was digested with *Bam*H1 and then ligated into pUC19. The recombinant clones were grown on LB+Ap+Km plate. The probe plasmid harbored Km resistance gene and the flanking DNA sequence of *avr* gene targeted. The probe was hybridized against the JXOIII genomic DNA library by colony *in situ* blot. Totally 49 positive clones were selected but all 49 clones failed to be transformed into the mutant 3201 or JXOV, a strain of race 5, using bi-parental mating method. However, when PXO99, a strain of race 6 in Philippine system, was used as recipient, 9 out of 49 clones were successfully transformed. Four rice cultivars: Wase Aikoku 3 (*Xa3*), IR26 (*Xa4*), Java14 (*Xa1*) and Cas209 (*Xa10*), were inoculated with 9 conjugants and 2 conjugants, PXO99/pUAV45 and PXO99/pUAV47 were found to have altered pathogenicity on Wase Aikoku 3 with significantly shorter lesions. Especially for PXO99/pUAV45, its phenotype in interaction with Wase Aikoku 3 was obviously incompatible as compared to others (Table 2).

Table 2. The pathogenicity of conjugants PXO99/pUAV45 and PXO99/pUAV47 on rice cultivars

Bacterial strains	Pathogenicity (%) a)			
	Wase Aikoku3/*Xa3*	IR26/*Xa4*	Java14/*Xa1*	Cas209/*Xa10*
PXO99	94.1±8.6A	62.3±24.7A	74.4±33.3A	67.4±16.2A
PXO99/pUAV45	39.2±20.4B	82.9±10.4A	87.3±6.7A	86.9±6.1B
PXO99/pUAV47	54.3±14.5BC	66.5±27.9A	66.5±27.9A	87.1±11.7B
PXO99/pUFR034	85.9±10.3C	68.9±17.9A	68.9±17.9A	93.4±10.6B

a) Disease severity (%)=(lesion length/leaf length)×100
Different letters represent significant differences ($p \geqslant 0.01$)

2.2 Homologous analysis of pUAV45 with *avrXal0*

The DNA fragments from genomic library of JXO Ⅲ in pUAV45 were 25.4 kb in length. The restriction map was shown in Fig. 1 (a), and (c). Southern blot analysis showed that all of the 5.7 kb *Kpn*I fragment, 3.6 kb *Eco*RI fragment and 2.8 kb *Bam*HI fragment hybridized with the probe of *avrXal0* and further sequence analysis indicated that they were on an overlapping position of the DNA fragment inserted in pUAV45 (Fig. 1 (b) and (c)).

2.3 Functional analysis of pUAV45 and its sub-clones

Based on the results of restriction mapping and Southern blot analysis, we recovered 4.3 kb and 3.6 kb *Eco*RI fragments, 5.7 kb *Kpn*I fragment and 2.8 kb *Bam*HI fragment from the agarose gel and ligated them into the vector pUFR034. The recombined plasmids were named pUAV5E3, pPUAV5E4, pUAV5K and pUAV5B respectively. All sub-clones were transformed into PXO99. When the conjugants were inoculated on rice cultivars Wase Aikoku 3 with leaf-cutting method, Pxo99/pUAV45 and Pxo99/pUAV5k showed decreased pathogenicity with the disease incidence changing from 44.31% to 35.67% and 33.68% on Wase Aikoku 3 respectively. Introduction of pUAV5E3 into PXO00 also led to the decrease in the pathogencity of the trans-conjugants. On the contrary, the transformations of sub-clones into PXO99 all led to the increase of its pathogenicity on rice cultivar Cas209 (*Xal0*) (Table 3).

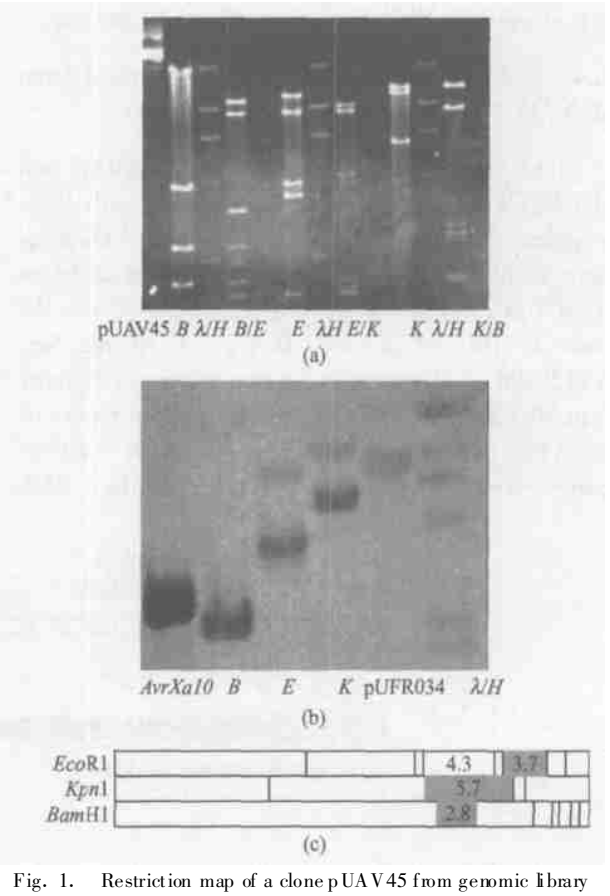

Fig. 1. Restriction map of a clone pUAV45 from genomic library and detection for homologous fragments with *avrXal0*. (a) Restriction map of pUAV45; (b) hybridization of pUAV45 restricted fragments with the probe of *avrXal0* by Southern blot analysis; (c) relative positions of homologous sequences of *avrXal0* in pUAV45; *E*= *Eco*R1, *K*= *Kpn*1, *B*= *Bam*H1, *B*/*E* and *K*/*E* are fragments from digestion of two enzymes. λ*H* is a DNA marker.

Table 3. Changes of pathogenicity of a Xoo strain Pxo99 transferred by sub-clones derived from pUAV45

Bacterial strains	Pathogenicity (%) [a]			
	Wase Aikoku 3/ *Xa3*		Cas209/ *Xal0*	
PXO99	44.31±13.63	ABCD	51.72±15.29	ABCD
PXO99/ pUFR034	43.54±12.72	ABCD	55.05±7.74	ABCD
PXO99/ pUFR45	35.67±10.33	CDE	61.46±11.16	A
PXO99/ pUFR5K	33.68±12.16	DE	59.92±12.81	ABC
PXO99/ pUFR5E3	39.02±9.44	BCDE	58.61±11.03	ABC
PXO99/ pUFR5E4	45.90±14.00	A	42.93±6.46	ABCD
PXO99/ pUFR5B	46.23±15.17	ABC	—	—

a) Disease severity (%) = (lesion length/ whole leaf length)× 100%
Different letters show significant differences ($p \geqslant 0.01$)

In the cell suspension of Wase Aikoku 3, compatible wild type strain Pxo99 caused the cell death at the rate of (50.48±2.12)% 18 hours after inoculation. When the conjugants (Pxo99/pUAV45, Pxo99/pUAV5K, Pxo99/pUAV5E3) and rice cell suspensions were co-cultured, the cell death rate was only (18.25±1.00)%, (24.29±1.84)% and (24.35±1.86)% respectively. The reactions on rice cell suspension were coincident with that on plant.

All the results suggested that the sub-clones pUAV5E3 and pUAV5K should carry the entire or

part of *avrXa3* DNA sequence with dual functions.

2.4 Sequence analysis of sub-clones derived from pUAV45

The DNA fragments inserted in pUAV5K and pUAV5E3 were sequenced and analyzed with Blast program. The results showed that the 5.8 kb fragment in pUAV5K harbored an open reading frame (ORF) of 2598 bp and the structure of ORF was the same as that of *avrBs3* family (GenBank No. AY129298). The identity of two sequences is more than 96.6% except the numbers of 102 bp repeat units (Fig. 2). Shine-Delgarno −35 and −10 sequences were found on the −81st site and the −56th site upstream of the ORF. They shared 96.6% identity to the corresponding sequence of *avrBs3*. The ribosome-binding site (rbs) was on the −14th site. At both ends of ORF, there were the characteristic restricted sites of *avrBs3* gene family, *Bam*HI, *Pst*I and *Sst*I. In the central region the numbers of 102 bp repeat was 8.5 and within 34 amino acids encoded by the 102 bp repeat there are 5 leucine residues. One leucine zipper (LZ, 1732 ~ 1917 bp), three nuclear localization signal (NLS, 2140 ~ 24154 bp, 2275 ~ 2289 bp, 2383 ~ 2589 bp) motifs, one acidic transcriptional activation domain (AAD, 2467 ~ 2589 bp) exist at the C-terminus of the fragment (Fig. 2. (a), (b)).

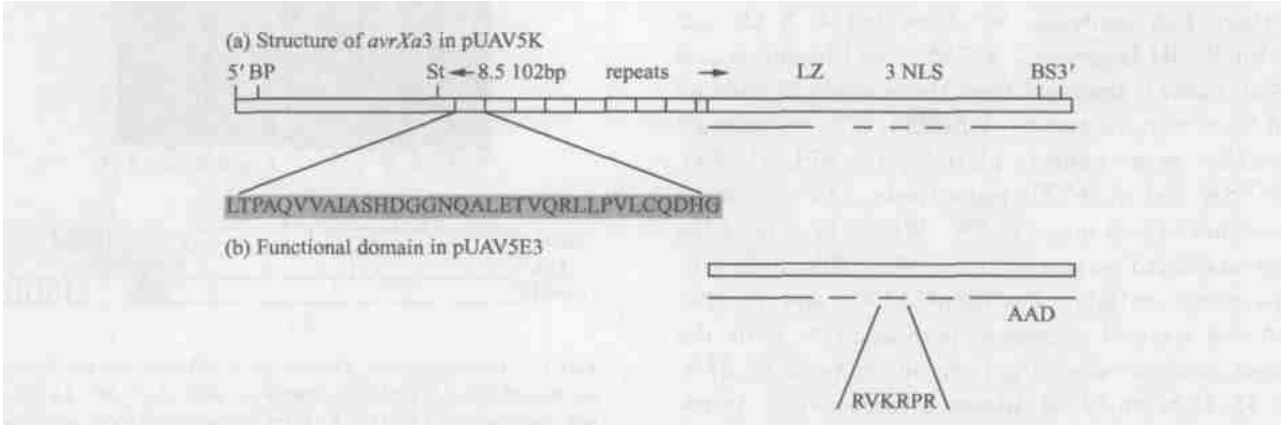

Fig. 2. Schematic map of domains in plasmids: pUAV5K and pUAV5E3. pUAV5K contains a complete *avrXa3* and another contains partial sequence. (a) Organization of a complete *avrXa3* in pUAV5K showing the102 repeat domain in central region followed by a putative LZ, 3 NLSs and a AAD at 3′; (b) a partial structure of *avrXa3* in pUAV5E3 containing one NLS and one AAD only.

Sequencing data of the 4.3 kb insert in pUAV5E3 showed that there was only a 124-amino acid ORF containing three NLS and one AAD. The NLS and AAD had 81% identity with the corresponding conserved domains of *avrBs3* (Fig. 2 (b)).

There are 8.5 repeats of 34-amino acid in deduced sequence encoded by 102 bp in pUAV5K, which is 7, 17, 10 repeats less than those in *avrXa10*, *avrXa7* and *avrBs3* respectively (Fig. 3 (b)). The 12th and 13th sites of the 33-amino acid repeats are different among *avrXa3*, *avrXa10*, *avrXa7* and *avrBs3*. Furthermore the 5th repeat in *avrXa3* is only 99 bp in length.

AAD domain identified in *avrBs3* (*avr*/*pth*) family has similarity to eukaryotic AAD at the last 38 codons (date not listed). The AAD domain is characterized by the presence of acidic and hydrophobic residues within the domain. The AAD domain of *avrXa3* is identical to that of *avrXa10*. However compared to *avrBs3*, it has 8 more codons and 3 different amino acids.

3 Discussion

More than 40 *avr* genes from plant pathogenic bacteria have been cloned and sequenced on the basis of "gene for gene" theory. There is no or very little information about the relationships among most of *avr* gene homologues except for the members within *avrBs3* and *avrRxv*/*YopJ* families[2]. Previous researches have suggested that the members of *avrBs3* family are more than 3 kb in length and contain more than 12 tandem repeats of 102 bp in the central regions. However *avrXa3* identified in this research only has 2598 bp and 8.5 repeats of 34-amino aicd in the central region. *AvrXa7* and *avrXa10* derived from a Philippine strain Pxo86 has 25.5 and 15.5 34-amino acid repeats respectively. All the members in *avrBs3* family have different numbers of 34-amino acid repeats. Deletion of this units and the domain swaps

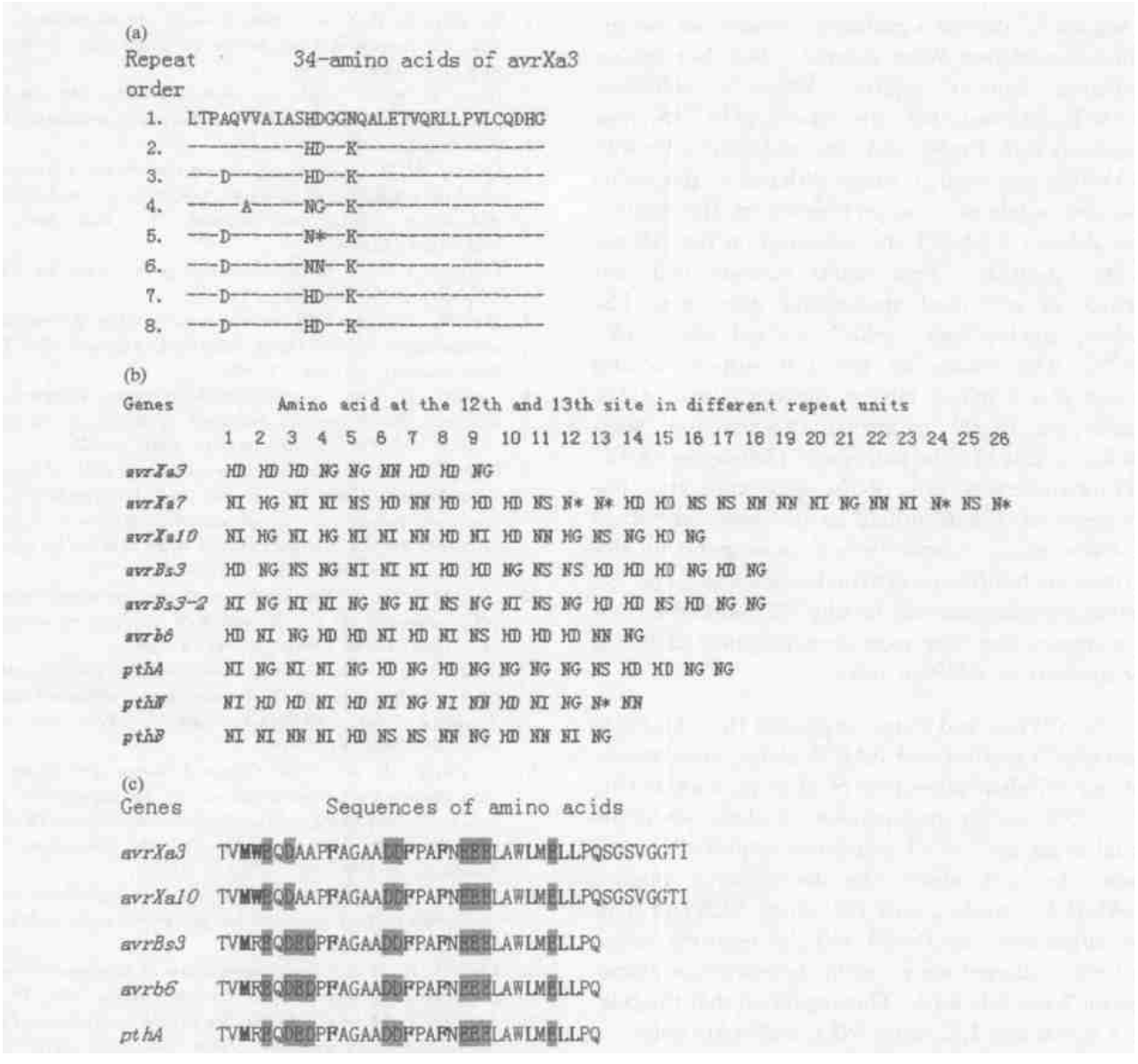

Fig. 3. Deduced amino acid sequence of the *avrXa3* shows domain of repeat units and comparison of the 12th and 13th amino acids in the 34-amino acid variable region. (a) The 12th and 13th amino acids in the 34-amino acid variable region of *avrXa3* repeat unit; (b) comparison of repeat members of *avrBs3* family and their variable regions, * show deleted amino acid residues; (c) deduced acidic transcriptional activation domain of *avrXa3* in some other members of *avrBs3* family, gray shadow indicates acidic amino acid; boldface types indicate hydrophobic groups.

between different *avrBs3* homologues provided evidence that the number of the repeats affected both avirulence and virulence specificity[14, 16, 23]. The variable amino acid region is also related to the race specificity[14]. For the above reasons, we identified the *avrXa3* as a new member of *avrBs3* family.

In general, determination of *avr* gene's function relies on mobilization of *avr* genes into the corresponding *aver* gene mutants or other compatible strains and then assessment of the pathogenicity of altered strains on the rice cultivars carrying corresponding *R* genes. Pxo99, a race6 strain in Philippine system, has compatible phenotypes on rice cultivars carrying *Xa3*, *Xa4*, *Xa7*, *Xa8* and *Xa10*. Leach reported that when *avrXa7* and *avrXa10*, cloned from Pxo86 (a race 2 strain in Philippine system), were transferred into Pxo99, the conjugants altered the interaction phenotypes on cultivars carrying *Xa7* and *Xa10* with dominant depression of aggressiveness of the bacteria[16].

In this study we identified two *avr* gene mutants (3201, 3202) from JXOIII and their

pathogenicity showed significant increase on the incompatible cultivar Wase Ailoku 3 but decrease on compatible cultivar cas209. When a sub-clone pUAV5K, derived from the cosmid pUAV45, was introduced into Pxo99 and the conjugant (Pxo99/pUAV5K) was used to infect differential rice cultivars, the symptom was suppressed on the cultivar Wase Aikoku 3 (*Xa3*) and enhanced on the cultivar cas209 (*Xa10*). These results demonstrated that *avrXa3* is a typical dual-acting *avr* gene like *avrBs3*, *avrPto* and *avrXa7* but not like *avrXa10*[14]. The results on rice cell suspension also showed that *avrXa3* altered the phenotype of the transformed Pxo99 (Pxo99/pUAV5K) on Wase Aikoku 3, just like the pathogenic phenotypes of JXOIII on suspension cells of the same cultivars. The *avr* genes of *Xoo* identified so far seem only affect the lesion size in complementary experiments on rice cultivars with different genetic background. The hypersensitive response was usually not induced on rice by corresponding *Xoo* races or conjugants harboring *avr* genes from different races.

Both White and Bonas suggested that the NLSs (generally 3 motifs) and AAD domains were responsible for avirulent phenotype of plant pathogenic bacteria. Deletion or mutagenesis of these structures would cause the loss of avirulence conferred by *avr* genes. In this study the recombinant plasmid pUAV5E3 containing only LZ, three NLS and AAD was introduced into Pxo99 and the resulting conjugant was conferred the recipient the avirulence phenotype on Wase Aikoku 3. This suggested that the fragment containing LZ, three NLS and AAD plays the same role as a whole *avrXa3*. But it is still not clear whether the other DNA sequences in pUAV5E3 will affect the interactions between *Xoo* and its host. It will be interesting to determine the functions of the residues upstream of these domains and their effects on the specificity of the pathogen on different rice cultivars.

References

1 Ou, S. H. Rice Diseases. Commonwealth Mycological Institute Aberystwyth, England, 1985, 70~74.

2 Ryba-white, M. et al. Comparison of *Xanthomonas oryzae* pv. *oryzae* strains from Africa, North America, and Asia by RFLP analysis. Intl. Rice Research Notes, 1995, 20: 25.

3 Swings, J. et al. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. Nov., rev. Int. J. Syst. Bacteriol., 1990, 40: 309.

4 Ellingboe, A. H. Genetic Interactions in hosts and pathogens. In: Molecular Aspects of Pathogenicity and Resistance. Minnesota: APS Press, 1996, 33~46.

5 Bonus, U. et al. Genetic and characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet., 1989, 218: 127.

6 Gabriel, D. W. et al. Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum* with specific resistance genes in cotton. Proc. Natl. Acad. Sci. USA, 1986, 83: 6415.

7 Leach, J. E. et al. Bacterial avirulence genes. Annu. Rev. Phytopathol., 1996, 34: 153.

8 Zhu, W. et al. *AvrXa21* contains an acidic transcriptional activation domain in the functionally conserved C terminus. Mol. Plant Microbe Interact., 1998, 11: 824.

9 Canteros, B. et al. A gene from *Xanthomonas campestris* pv. *vesictoria* that determines avirulence in tomato is related to *avrBs3*. Mol. Plant-Microbe Interact. 1991, 4: 628.

10 Kamdar, H. V. et al. Restoration of pathogen city of avirulent *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *campestris* pathovars by reciprocal complementation with *hrpXo* and *hrpXc* genes and identification of *HrpX* function by sequence analysis. J. Bacteriol., 1993, 175: 2017.

11 Swarup, S. et al. An *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on non-hosts. Mol. Plant-Microbe Interact., 1992, 5: 204.

12 Yang, B. et al. The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. Proc. Natl. Acad. Sci. USA, 2000, 97: 9807.

13 Gabriel, D. W. et al. Role of nuclear localizing signal sequences in three diseases phenotypes determined by *Xanthomonas avr/pth* gene family. In: Biology of Plant-Microbe Interactions. The International Society for Molecular Plant-Microbe Interactions, Minnesoda, USA, 1996, 197~202.

14 Shen, Y. et al. Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice. Microbes and Infection, 2002, 4: 1361.

15 Choi, S. H. et al. Genetic manipulation of *Xanthomonas oryzae* pv. *oryzae*. International Rice Research Institute, 1994, 19: 31.

16 Hopkins, C. M. et al. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. Mol. Plant Microbe Interaction, 1992, 5(6): 451.

17 Wang J. S. et al. Phytobacteriology (in Chinese). Beijing: Chinese Agricultural Publishing House, 2001.

18 Zhang, Q. Progress on rice bacteria leaf blight resistance and genetic research in China. In: Progress of Genetic Research on Disease Resistance of Key Crops in China (in Chinese). Nanjing: Jiangsu Science and Technology Publishing House, 1990, 1~13.

19 Physiology Teaching and Research Section. Laboratory Guide for Plant Physiology, Huadong General University. Beijing: People Education Publishing House, 1982.

20 Sambrook, J. et al. Molecular Cloning: A Laboratory Manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.

21 Thomas L. et al. Molecular secrets of bacterial type III effecter proteins. Trends in Plant Science, 2001, 6(10): 497.

22 White, F. F. et al. Prospects for understanding avirulence gene function. Current Opinion in Plant Biology, 2000, 3: 291.

23 Yang, Y. N. et al. Host specific symptoms and increased release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from leaves are determined by the 102 bp tandem repeats of *pthA* and *avrb6*, respectively. Mol. Plant Microbe Interact., 1994, 7: 345.