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Review

Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice

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Abstract

Xanthomonas oryzae pv. *oryzae* is the causal agent of rice bacterial blight disease. Numerous genes critical for virulence have been identified. This article reviews current knowledge on the molecular mechanisms of *X. oryzae* pv. *oryzae* virulence.

Keywords: Virulence; Avirulence; Protein secretion systems; Extracellular polysaccharide; Extracellular enzymes

1. Introduction

Bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* has become a major rice disease in tropical Asian countries in last three decades because of the extensive deployment of more nitrogen-responsive modern rice varieties. There have also been reports on the occurrence of the disease in Australia, Africa, and Latin America as well as in North America [1,2]. Bacterial blight can cause severe yield loss of up to 50% depending on the rice growth stage, the geographic location and seasonal conditions [3].

Rice bacterial blight is a vascular disease. *X. oryzae* pv. *oryzae* cells enter the epitheme, the tissue connecting the water pores (hydathodes) with the xylem, where they multiply and move to the xylem vessels. Once in the vascular system, *X. oryzae* pv. *oryzae* continues to grow until the xylem vessels are clogged with bacterial cells and extracellular polysaccharide (EPS or xanthan). Rice plants become wilted if infection occurs during the seedling or early tillering stage. When infection occurs in later stages, lesions of leaf blight enlarge in length and width and turn gradually from grayish green to chlorotic [1]. During the infection, *X. oryzae* pv. *oryzae* employs diverse tools to overcome the host innate defense system resulting in blight disease.

Since bacterial blight is a major rice disease from an economic point of view, numerous studies have been carried out on disease diagnosis, pathogen isolation, epidemiology and disease management. The last decade has seen a dramatic advancement in our understanding of the molecular basis of the rice/*X. oryzae* pv. *oryzae* interaction, with the cloning of the two rice resistance (*R*) genes, *Xa1* and *Xa21*[4,5], the bacterial avirulence (*avr*) genes, *avrXa5*, *avrXa7* and *avrXa10*[6], the hypersensitive response and pathogenicity (*hrp*) genes [7] and many other virulence-related genes [8–14]. Studies on the pathogen population structure using molecular genotyping methods have provided information on pathogen evolution. Studies on diseases caused by *X. campestris* species have facilitated research on rice bacterial blight, since both *Xanthomonas* species share common virulence mechanisms. Here we give a brief review on the current knowledge of the molecular mechanisms of *X. oryzae* pv. *oryzae* virulence.

2. Compatible and incompatible interactions between *X. oryzae* pv. *oryzae* and rice

The outcome of plant-pathogen interactions depends on the genes carried by the host and the pathogen. In many cases, the host-encoded resistance gene product recognizes the presence of the pathogen-encoded *avr* gene product. This interaction triggers a rapid induced defense response, called an incompatible interaction. In the incompatible reaction, the pathogen is restricted to the primary infection site. The *avr* genes alone are not sufficient to specify incompatibility but require the presence of *hrp* genes (that will be discussed later

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in this review). If the pathogen lacks the appropriate *avr* gene or the host plants lack the *R* gene, a compatible interaction is established. In this case, the pathogen is able to overcome innate host defenses, and cause disease on rice through the use of extracellular enzymes, extracellular polysaccharides and other factors.

More than 20 rice genes conferring resistance to *X. oryzae* pv. *oryzae* have been identified genetically, while only two, *Xa1* and *Xa21*, have been cloned. *Xa1* is a pathogen-inducible gene encoding a typical R protein that contains a nucleotide-binding sequence and a leucine-rich repeat domain (LRR). It confers resistance to *X. oryzae* pv. *oryzae* Japanese race 1 and is predicted to be intracellular [4]. In contrast, *Xa21* is a constitutively expressed gene encoding a protein with a presumed extracellular LRR domain and a cytoplasmic kinase domain. It is a unique type of *R* gene and controls resistance to *X. oryzae* pv. *oryzae* Philippine race 6 (PR6) [5]. The resistance conferred by *Xa21* is developmentally regulated, indicating that there are other gene(s) epistatically regulating the defense pathway [15]. The corresponding *avr* genes for *Xa1* and *Xa21* have not been isolated from *X. oryzae* pv. *oryzae* races which are avirulent on *Xa1* and *Xa21* rice plants. Based on the structural differences of the *R* genes it is expected that *avrXa1* and *avrXa21* will also be distinct. Three *avr* genes, *avrXa5*, *avrXa7* and *avrXa10*, have been isolated from *X. oryzae* pv. *oryzae* Philippine race 2 [6]. However, their corresponding rice *R* genes have not been cloned yet. Only when the two partners are available, will it be possible to scrutinize their roles during the incompatible interaction.

3. Avirulence determinants

The isolated *X. oryzae* pv. *oryzae avr* genes, *avrXa5*, *avrXa7* and *avrXa10*, are members of the gene family from *Xanthomonas* that are typified by the first cloned member of the family, *avrBs3*, from *X. campestris* pv. *vesicatoria*[16]. The striking feature of the *avr* gene family is the presence of a repeat sequence in the central domain of the encoded protein with each repeat of 34 amino acids. The number of repeats in individual members varies, but the amino acids in each repeat are conserved, with the exception of amino acids at positions 12 and 13, which are referred to as variable regions. The arrangements of the variable regions of all characterized family members appear to be a critical feature for the race specificity of the proteins. AvrXa7 and AvrXa10 have been well studied. They contain 25.5 and 15.5 copies of repeats, respectively. Replacement of the repeat region of AvrXa10 with that of AvrXa7 and AvrBs3 resulted in specificity change. The chimeric AvrXa10 with the AvrXa7 repeat domain conferred specificity for the resistance gene *Xa7* and lost specificity to *Xa10*. The chimeric AvrXa10 with the AvrBs3 repeat domain conferred on *X. oryzae* pv. *oryzae* an ability to trigger a hypersensitive response (HR) on pepper plants, a non-host to *X. oryzae* pv. *oryzae* that normally does not give an HR. Moreover, this chimeric *avr* gene conferred an avirulent phenotype to *X. campestris* pv. *vesicatoria* on pepper plants containing the *Bs3* gene [17].

AvrXa7 and AvrXa10, as with other AvrBs3 family members, have an additional structural feature. The carboxyl terminus contains a domain that is structurally similar to the acidic activation domain of many eukaryotic transcription factors, in addition to three nuclear localization signal (NLS) sequences. Removal of the C-terminal 38 codons containing the putative activation domain or replacement of three hydrophobic amino acid residues in the C-terminal domain was concomitant with the loss of avirulence activity in rice and transcriptional activation in a yeast assay system. The lost transcription activation function and avirulence activity can be restored by the activation domain from the herpesvirus protein VP16, indicating that the transcriptional activation domain is required for AvrXa7 and AvrXa10 function [18,19]. Additional experiments demonstrated that AvrXa7 binds double-stranded DNA with a preference for dA/dT-rich sequences. Furthermore, mutations in the three NLS sequences also impaired the avirulence activity and nuclear localization in yeast cells, while addition of the sequence of the NLS motif from SV40 T-antigen restored both activities [20]. These results suggest that recognition of AvrXa7 and AvrXa10 takes place in the host nucleus and requires the transcriptional activation domain.

The molecular mechanism underlying AvrXa21 activity has recently been studied. In a screen of a Tn*5* transposon insertional mutant library of *X. oryzae* pv. *oryzae* PR6, two *X. oryzae* pv. *oryzae* genes, *raxP* and *raxQ*, were found to be required for AvrXa21 activity. *raxP* and *raxQ*,which reside in a genomic cluster of sulfur assimilation genes, encode an ATP sulfurylase and adenosine-5'-phosphosulfate (APS) kinase. These enzymes function together to produce activated forms of sulfate, APS and 3'-phosphoadenosine-5'-phosphosulfate (PAPS). PR6 strains carrying disruptions in either gene, PR6γ*raxP* or PR6γ*raxQ*, are unable to produce APS and PAPS and are virulent on *Xa21*-containing rice lines. RaxP and RaxQ are similar to the bacterial symbiont *Sinorhizobium meliloti* host specificity proteins, NodP and NodQ [8]. In *S. meliloti*, PAPS produced by NodP and NodQ is used by *nodH*- or *noeE*-encoded sulfotransferases for Nod factor sulfation [21]. An *X. oryzae* pv. *oryzae* gene, *raxST*, similar to *nodH* and *noeE*, has also been isolated recently (da Silva et al., unpublished data). These results demonstrate that sulfation plays a critical role in determining AvrXa21 activity and reveal a commonality between symbiotic and phytopathogenic bacteria in controlling host specificity. It is not yet known whether sulfation takes place on the AvrXa21 effector or if it is involved in the regulation of the AvrXa21 effector synthesis, nor has the AvrXa21 effector yet been identified. Since sulfated molecules act as specific ligands or receptors in many organisms, it is plausible that the AvrXa21 effector, either a carbohydrate or a protein, is sulfated, and interacts specifically with the Xa21 extracellular domain.

In addition to their role in avirulence activity, some *avr* genes, such as *avrBs2*, *pthA*, *avrA*, *avrE*, *avrBm*, *avrRPM1* and *avrPto*, have been found to function in aggressiveness or disease symptom expression [22]. It has been demonstrated that transposon-insertional inactivation of the *X. oryzae* pv. *oryzae avr* genes, *avrXa7* and *avrXa5*, and four homologous genes, *aB3.5*, *aB3.6*, *aB4.3* and *aB4.5*, caused shorter lesions on rice line IR24, which contains no resistance genes relevant to the wild-type bacterial strain. However, such a dual function has not been found for *avrXa10*[23]. These results may help explain the retention of *avr* genes in general, and more specifically, the presence of many copies of the genes in *X. oryzae* pv. *oryzae*. The deployment of new rice varieties to control the pathogen may select for new versions of the gene family that avoid recognition for resistance but retain function for aggressiveness. Recently, it has also been found that the transcription activation domain and the NLS sequences in AvrXa7 are required for the virulence activity, suggesting that these *avr* gene products are virulence factors that enter the host nucleus and directly affect host gene transcription (20).

Adaptation of an avirulent bacterial race to host varieties containing a single dominant *R* gene often results from the loss of function of the corresponding *avr* gene. However, the fitness penalty associated with loss of the *avr* gene may prevent disease epidemics. This idea was evaluated with near-isogenic rice lines (NIL) containing single *R* genes (*Xa7*, *Xa10* and *Xa4*) at two field sites. It was found that bacterial blight disease severity was high in all 3 years on the *Xa10* and *Xa4* NILs but not on the *Xa7* NIL. Only a few *X. oryzae* pv. *oryzae* strains were virulent on the *Xa7* NIL, and these strains displayed a reduction in aggressiveness. Laboratory analysis showed that loss of avirulence and reduced aggressiveness was associated with mutations at the 3' terminus of the *avirXa7* allele. These results suggest that *Xa7* is a durable *R* gene due to a fitness penalty in *X. oryzae* pv. *oryzae* strains carrying alterations in *avrXa7*[24].

4. Hypersensitive response and pathogenicity (*hrp*) genes

The interaction of many Gram-negative plant and animal pathogenic bacteria with their hosts depends on a conserved type III protein secretion system (TTSS). In phytopathogenic bacteria, the TTSS is encoded by *hrp* genes for eliciting HR on non-host or resistant host plants and for pathogenesis on susceptible hosts. The *hrp* genes were first identified in the bean pathogen, *Pseudomonas syringae* pv. *phaseolicola*[25]. Since then, *hrp* genes have been isolated and characterized from a variety of plant pathogenic bacteria, including *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas*. At least nine *hrp* genes are conserved in both plant and animal bacterial pathogens and are believed to encode the core components of the TTSS (referred to as *hrc* for *hrp* conserved). An additional set of non-conserved proteins, encoded in the *hrp* gene clusters, is essential for secretion and/or translocation. Among the non-conserved proteins are secreted proteins such as the subunits of the *Hrp* pilus. The *Hrp* pilus has been shown to be essential for type III secretion in vitro and is supposed to function as a conduit for translocation of bacterial proteins, such as virulence and avirulence factors, into plant cells.

Knowledge concerning *hrp* genes in *Xanthomonas* rises mostly form *X. campestris* species. The 23-kb *hrp* gene cluster from *X. campestris* pv. *vesicatoria*, the causal agent of bacterial spot on pepper and tomato, is by far the best characterized among the xanthomonad *hrp* genes [26]. The *hrp* cluster is located on the chromosome and contains six operons, designated *hrpA* to *hrpF*. Most of these operons encode proteins for components of TTSS, which are conserved in other bacteria. *HrpF* has been found to be a putative type III translocon protein required for pathogenicity [27]. Expression of the six operons is induced in the plant and is regulated by the products of the regulatory genes *hrpX* and *hrpG*. *HrpG* belongs to the OmpR family of two-component response regulators and activates the expression of *hrpA* and *hrpX*. *hrpX* encodes a protein belonging to the AraC family of positive transcriptional activators. It controls the expression of operons *hrpB* to *hrpF* as well as the *avrXv3* and a number of putative virulence factors [28,29].

Due to the importance of the TTSS to pathogenicity, analysis of the system in different species will provide insight into their adaptation to their respective host plants. A homologue of *hrpX*, *hrpXo*, was the first *hrp* gene identified from *X. oryzae* pv. *oryzae*. A mutation in *hrpXo* results in the loss of pathogenicity on rice and the loss of HR on non-hosts such as *Datura stramonium* and radishes. The pathogenicity and the ability to trigger the HR is restored by complementing the mutant with the heterologous *hrpXc* gene from *X. campestris* pv. *campestris*. Conversely, *hrpXo* can also complement non-pathogenic mutants of *X. campestris* pv. *campestris* and *X. campestris* pv. *armoraciae*. This result indicates that *hrpXo* and *hrpXc* are functionally equivalent [30]. PCR amplification of *hrpXo* in 16 *X. campestris* pathovars and 12 *X. oryzae* pv. *oryzae* strains indicates that the *hrpX* gene is highly conserved in *Xanthomonas* and suggests that the functional equivalence may extend into other pathovars of *X. campestris*[30,31]. By using a portion of the *X. campestris* pv. *vesicatoria hrp* region as a probe, an *X. oryzae* pv. *oryzae hrp* region has also been isolated [7]. The 12.2-kb DNA fragment covers the region corresponding to *hrpA*, *hrpB*, *hrpC* and *hrpD* of *X. campestris* pv. *vesicatoria*. Tn*5* insertions in the *hrp* region abolished the ability to elicit disease on rice and an HR on non-host tomato. Complementation of the Tn*5* mutants with the wild-type *hrp* region restored the ability to cause disease on rice and to elicit HR on tomato, indicating that *hrp* gene-encoded TTSS is also required for the pathogenicity and HR of *X. oryzae* pv. *oryzae*[7].

Detailed analysis of the 12.2-kb clone of *X. oryzae* pv. *oryzae* revealed two genes, *hpa1* and *hpa2*, upstream of the

hrp region [7]. An *X. oryzae* pv. *oryzae* strain with a deletion covering *hpa1* and *hpa2* has reduced pathogenicity and elicited a weak HR on non-host and resistance host plants. Single mutation analysis showed that loss of *hpa1* function is the principal cause of the reduction in pathogenicity. The putative product of *hpa1* is similar to harpin proteins from *P. syringae* pathovars and *Erwinia* species and the harpin-like protein PopA from *Ralstonia solanacearum*[32–35]. Harpins are a group of proteins that share regions of high glycine content and are secreted by TTSS. Some harpins, such as the products of *hrpW*, *popA*, *hrpZ* and *hrpN*, elicit HR. *HrpN* is also required for pathogenicity. Sequence similarity with *hrpW* from *P. syringae* pv. *tomato* in the DNAs from *Xanthomonas* species has been reported [36]. Recently, an orthologue of *hpa1* from *X. campestris* pv. *vesicatoria*, *xopA*, has been identified. Its product is secreted by *hrp*-dependent TTSS and is required for growth in plants and full avirulence of the pathogen [37]. These results imply that *X. oryzae* pv. *oryzae* Hpa1 may also be secreted by *hrp*-dependent TTSS.

Compelling evidence from studies on avirulence proteins of bacterial pathogens points to the possibility that *hrp* gene-encoded TTSSs are responsible for translocation of avirulence proteins into the host plant cells. For example, AvrBs3 is secreted into conditioned culture medium through TTSS [38]. Moreover, expression of *avrBs3* in pepper plant cell resulted in the induction of an *R* gene-specific HR [39]. Similarly to *Yersinia* outer proteins, a type III translocation signal has been proposed to be located within the first 58 codons of AvrBs2, an avirulence protein from *X. campestris* pv. *vesicatoria* to pepper plants [40]. Evidence for translocation of *X. oryzae* pv. *oryzae avr* gene products into rice cells through TTSS is very limited. Similarly to AvrBs3, AvrXa10 protein purified from *Escherichia coli* or *X. oryzae* pv. *oryzae*, when infiltrated into rice leaves, did not elicit HR in rice carrying the *Xa10* resistance gene [41]. These results do not provide direct evidence that *X. oryzae* pv. *oryzae avr* proteins are translocated through a TTSS. Nonetheless, because AvrXa5, AvrXa7 and AvrXa10 are members of the AvrBs3 family, it is reasonable to assume that these *X. oryzae* pv. *oryzae avr* gene products are also translocated into rice cells through TTSS.

5. Other virulence determinants

Bacterial flagella are important virulence factors for pathogenesis in animals and plants [42,43]. For example, it has been found that a flagella-minus mutant strain of *Agrobacterium tumefaciens* had reduced virulence on sunflower [44]. *X. oryzae* pv. *oryzae* is a motile bacterium that bears a single flagellum. The flagellum is induced under limited nutrition conditions [45]. Studies demonstrated that *X. oryzae* pv. *oryzae* cells move towards a synthetic medium and towards exudates of susceptible rice plants, whereas no chemotaxis towards exudates of resistant plants was observed [42]. This suggests that chemotaxis may facilitate reaching the infection site before the bacteria penetrate into rice leaf tissue. Upon entrance into plant tissue, flagella may no longer be instrumental to virulence. In *X. campestris*, switch from non-swarming wild-type to swarming type in swarm plate is concomitant with loss of xanthan production and virulence. It was also observed that *X. campestris* pv. *campestris* is non-motile and non-flagellated in the xylem fluid of infected cabbage leaves [46]. Recently, an *X. oryzae* pv. *oryzae* flagellar operon region has been isolated, which contains four ORFs. One of the ORFs, *flhF*, encodes a putative GTP-binding protein. Mutation in *flhF* resulted in weak chemotaxis. However, inoculation of the mutant on rice leaves with a scissors-clipping method did not show reduced virulence, suggesting that chemotaxis is not required for virulence once the bacterial cells enter rice leaves [45]. Inoculation experiments with *X. oryzae* pv. *oryzae* strains carrying deletions in the flagella structural genes are needed to determine the role of chemotaxis in *X. oryzae* pv. *oryzae* virulence. Recently, it has been shown that the flagella of *Yersinia* species, an animal pathogen, also function as a secretion system to secrete virulence-associated phospholipase [47]. It will be interesting to see whether flagella of phytopathogenic bacteria also play a role in secretion of virulence factors.

Extracellular enzymes have long been considered to play a role in virulence of phytobacterial pathogens. Numerous studies have been done on extracellular enzymes of cellulytic, pectolytic, proteolytic, lipolytic and amylolytic functions from *X. campestris* species. However, the role of a specific enzyme during pathogenesis has not yet been elucidated, and in most cases, the results were circumstantial and not conclusive [48]. Likewise, extracellular enzymes have also been considered as candidates for virulence factors of *X. oryzae* pv. *oryzae* although evidence is still limited. A protease-deficient mutant of *X. oryzae* pv. *oryzae* has been shown to have reduced pathogenicity and multiplication in plants [9]. Despite the ambiguity in the exact role of the extracellular enzymes, it is clear that the general secretory pathway (GSP, also referred to as type II secretion system), which secretes extracellular enzymes, is required for bacterial virulence on host plants. *X. campestris* GSP mutants are virulence deficient and accumulate enzymes in the periplasm. Recently, a GSP mutant has been generated from *X. oryzae* pv. *oryzae*. This mutant is also deficient in virulence on rice plants and is unable to secrete xylanase [10]. Because *X. oryzae* pv. *oryzae* mainly grows in xylem vessels, where xylan is abundant, it is reasonable to hypothesize that xylanase may function as an *X. oryzae* pv. *oryzae* virulence factor. However, studies on xylanase from other bacterial pathogens, such as *Erwinia chrysanthemi*, have shown that xylanase-deficient mutants are still virulence proficient [49]. It is possible that other protein(s) secreted by the GSP may play an important role in virulence.

Like other *Xanthomonas* species, a striking feature of *X. oryzae* pv. *oryzae* is that it produces copious amounts of

EPS when cultured in medium supplemented with sucrose. Spontaneous loss of virulence associated with reduction of EPS production has been observed in long-term storage and culture [11]. Therefore, EPS is regarded as a virulence determinant. The virulence role of EPS has been attributed to several features. EPS is highly hydrated, so that it provides protection against desiccation and hydrophobic bacteriostatic compounds in the air-filled plant intercellular space. The adhesion quality may facilitate pathogen attachment to plant cell surfaces. Partially purified EPS preparations have been found to induce rice leaf wilting. This may be due to cell membrane leakage caused by EPS [50,51].

So far, several gene loci involved in EPS synthesis have been identified from *X. oryzae* pv. *oryzae*. One clone was shown to be able to complement a transposon-inactivated *gum* gene, *gumGxo*, a homologue of the *X. campestris* pv. *campestris gumG* gene which has been shown to be involved in the acetylation of xanthan [12]. An *X. oryzae* pv. *oryzae* strain carrying mutation in *gumGxo* does not produce EPS and is virulence deficient. The complementing clone contains a cluster of *gum* synthesis genes which are homologous to the *gum* gene cluster containing *gum A* through *gumM* from *X. campestris* pv. *campestris*. The *X. oryzae* pv. *oryzae* gene *gumM* has also been verified to be required for EPS production. Mutants of *X. oryzae* pv. *oryzae gumM* recovered from stationary-phase culture are also deficient for virulence and EPS production [11]. Recently by Tn*5* mutagenesis, a novel genomic locus which is not located in the *gum* cluster has been identified to be required for EPS and virulence in *X. oryzae* pv. *oryzae*. It has been found that two of the transposon insertions are in genes that encode a putative sugar nucleotide epimerase and a putative glycosyltransferase. Interestingly, the DNA region containing these genes has a low G + C content (51.7%), deviating from the typical G + C content of *X. oryzae* pv. *oryzae* (approximately 65%), suggesting a horizontal transfer. Such a locus has not been reported in other *Xanthomonas* species [13].

In *X. oryzae* pv. *oryzae*, EPS synthesis has been found to be controlled by the *rpfC* (regulation of pathogenicity factor) gene that is part of a two-component system. Strains carrying a mutation in *rpfC* have greatly reduced EPS production and virulence but still attain maximum population levels in rice plants [52]. These results suggest that EPS is a virulence determinant in *X. oryzae* pv. *oryzae*.

Yellow xanthomonadins are membrane-bound pigments produced by members of the *Xanthomonas* genus. It is not known whether these pigments are related to bacterial pathogenicity. A study with naturally occurring white strain and pigment-minus mutants of *X. campestris* pv. *campestris* showed that the pigments do not play a decisive role during pathogenesis. However, an *X. oryzae* pv. *oryzae* mutant deficient in xanthomonadin production was isolated and was found to be deficient for virulence on rice, as well as auxotrophic for aromatic acids (Aro^-). A genetic study showed that an ORF encoding a homologue of *E. coli* shikimate dehydrogenase is responsible for the xanthomonadin production and virulence. It can complement *E. coli aroE*$^-$ mutant. Shikimate dehydrogenase activity was found in wild-type strain but not in the mutant [14].

Molecular genotyping has helped to study pathogen populations and has revealed some interesting phenomena. In a DNA fingerprinting study of Indian isolates of *X. oryzae* pv. *oryzae*, it has been found that the IxoII isolates have comparatively more 'A' and 'C' methylation in the GATC and CCGG sequences, respectively, than the IxoIa and IxoIb isolates. As IxoII is reported to be more pathogenic than IxoIa and IxoIb, the increased 'A' and 'C' methylation at various loci in IxoII isolates may be related to their pathogenicity [53]. The mechanism is not yet clear.

6. Conclusions

As bacterial blight is an important rice disease, numerous studies have been carried out to explore the mechanisms of the pathogen virulence and host resistance. A significant number of *X. oryzae* pv. *oryzae* genes have been found to be involved in virulence. These include genes required for EPS production; genes for synthesis and regulation of other virulence and avirulence factors; and genes encoding components of secretion systems for delivery of extracellular enzymes and virulence and avirulence factors into plant cells. It is now clear that *X. oryzae* pv. *oryzae* virulence is very complex and not yet completely characterized. Efforts are therefore needed to identify other genes required for virulence and to elucidate the concerted functions of all the virulence determinants. To this end, genomic studies will play a significant role. When the complete genomic sequences of *X. oryzae* pv. *oryzae* and related *Xanthomonas* species become available, comparative genomic analysis is expected to accelerate the identification of all secreted effectors and other secretion systems required for virulence. In addition, the sequence information will provide a basis for rational experimental dissection of the functional regulation of the virulence determinants that should lead to full elucidation of *X. oryzae* pv. *oryzae* virulence on rice.

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