The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice

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ABSTRACT

The nucleotide sequence was determined for the genome of *Xanthomonas oryzae* pathovar *oryzae* (Xoo) KACC10331, a bacterium that causes bacterial blight in rice (*Oryza sativa* L.). The genome is comprised of a single, 4,941,439 bp, circular chromosome that is G + C rich (63.7%). The genome includes 4,637 open reading frames (ORFs) of which 3,340 (72.0%) could be assigned putative function. Orthologs for 80% of the predicted Xoo genes were found in the previously reported *X. axonopodis* pv. *citri* (Xac) and *X. campestris* pv. *campestris* (Xcc) genomes, but 245 genes apparently specific to Xoo were identified. Xoo genes likely to be associated with pathogenesis include eight with similarity to *Xanthomonas* avirulence (avr) genes, a set of hypersensitive reaction and pathogenicity (hrp) genes, genes for exopolysaccharide synthesis functions as a virulence determinant (6). The presence of these genes provides insights into the interactions of this pathogen with its gramineous host.

INTRODUCTION

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is affiliated with the *X. campestris* sub-division of the Proteobacteria and is the causal agent of bacterial blight (BB) on rice (*Oryza sativa* L.). BB disease is a major rice disease in tropical Asian countries where high-yielding rice cultivars are often highly susceptible to the disease. BB is a vascular disease resulting in tannish-gray to white lesions along the leaf veins. In severely infested fields, the disease can cause yield losses as high as 50% (1).

In the last decade, our understanding of the molecular basis of interactions between the rice and *X. oryzae* pv. *oryzae* has been advanced by elucidation of the functional roles of genes associated with pathogenesis. The representative gene groups include effector or avirulence genes (avr), hypersensitive response and pathogenicity (hrp) genes, genes associated with production of extracellular polysaccharides or cell wall degradation. In phytopathogenic bacteria, the type III protein secretion system (TTSS) encoded by *hrp* genes plays a central role in eliciting defense responses, such as the rapid cell death response called the hypersensitive reaction (HR), on non-host or resistant host plants and pathogenesis on susceptible hosts (2). Some Hrp proteins form a pilus that has been proposed to function as conduit that directly translocates effector proteins such as avirulence factors into plants (3).

In addition to the TTSS, the type II secretion system may play a role in secretion of other *Xoo* virulence factors, such as extracellular enzymes like xylanase (4,5), and like other *Xanthomonas* species, the *gum* gene cluster involved in exopolysaccharide synthesis functions as a virulence determinant (6).

Control of BB traditionally involves the introduction of host resistance genes that mediate strain-specific initiation of defense responses due to *gene-for-gene* interactions of the
resistance gene product with the product of the pathogen\textit{avr} or effector genes (7,8). However, introduction of individual plant resistance genes frequently results in a change in the pathogenic diversity of \textit{X. oryzae pv. oryzae} populations, and new races of the pathogen emerge that are able to overcome the deployed resistance (8). Although several \textit{avr} genes from \textit{Xoo} have been characterized (9), the complete set of \textit{avr} genes encoded in the \textit{Xoo} genome are unknown. Information on these additional pathogen \textit{avr} genes may be useful to predict the stability of their corresponding disease resistance genes (10). So far only two avirulence genes, \textit{avrXa10} and \textit{avrXa7}, have been cloned and sequenced from \textit{Xoo} (9). Although several resistance genes, including \textit{Xa1}, \textit{Xa5} and \textit{Xa21}, have been cloned from rice (11–13), the genes corresponding to the characterized \textit{Xoo} \textit{avr} genes (\textit{avrXa10} and \textit{avrXa7}) have not been cloned.

The nucleotide sequence of a pathogen’s genome is an important step to understanding the mechanisms of pathogenicity and the processes that limit the host range of the strain. The nucleotide sequence of the genomes of several phytopathogenic bacteria, such as \textit{Agrobacterium tumefaciens}, \textit{Pseudomonas syringae}, \textit{Ralstonia solanacearum}, \textit{Xylella fastidiosa} and two \textit{Xanthomonas} species, have been recently determined (14–18). Among bacteria classified in the genus \textit{Xanthomonas}, the whole-genome sequences of \textit{X.anaxonopodis pv. citri} (\textit{Xac}; the causal bacterium of citrus canker) and \textit{X.campestris pv. campestris} (\textit{Xcc}; the causal bacterium of cabbage black rot) have been reported (16). Several candidate genes related to pathogenicity, such as the set of translocated effectors produced by a strain, as well as genes related to general biological processes have been deduced from these genome sequences. Because rice is taxonomically so distinct from the hosts for the other \textit{Xanthomonas} species with known genomes (it is a monocotyledon rather than a dicotyledon), it is likely that the \textit{Xoo} genome will include distinct genes that are critical to interactions with rice.

Here, we report the nucleotide sequence and genome structure of \textit{Xoo} str. KACC10331 isolated from diseased rice in Korea. This isolate was selected because it represents an important race in Korea (race 1), and because it contains several \textit{avr} genes, including \textit{avrXa21} (19). Because of its importance as a pathogen, our analysis of the genome sequence focused on genes associated with pathogenicity genes.

**MATERIALS AND METHODS**

**Bacterial strain, library construction, sequencing and assembly**

\textit{Xanthomonas oryzae pv. oryzae} str. KACC10331 (KX085), a representative Korean race 1 strain that is virulent to rice carrying the \textit{Xa21} resistance gene, was used in this study. The genome sequence was determined through the whole-genome shotgun approach (20). The nucleotide sequence of the inserts carried by 49 087 clones with 1–2 kb inserts (8.6-fold genome coverage) and 14 783 clones with 8–10 kb inserts (2.4-fold genome coverage) in pUC18 Smal/BAP vector (Invitrogen, USA) were determined from both ends using BigDye™ terminator (Applied Biosystems, USA) and an ABI3700 automated sequencer. In addition to the above sequences, nucleotide sequences were obtained from both ends of 3025 inserts carried by fosmid constructs using 40 kb genome fragments in the pEpiFOS™-5 vector (Epiconcept technologies, USA) and 2895 BAC clones with 112 kb genome fragments generated in the pIndigoBAC-5 vector (Epiconcept technologies, USA). The inserts in these libraries covered 98% of the genome and the sequences from both ends of fosmid and BAC clones were used to confirm the orientation and integrity of the sequence contigs to validate the final sequence assembly. The reported sequence (GenBank accession no. AE013598) was assembled from 70 689 115 bp of accumulated nucleotide sequence using Phred/Phrap/Consed software package (http://genome. washington.edu). The scaffolds were created using mate information between contig groups. Gap closures between scaffolds or contigs were accomplished by primer-walking on BAC, cosmid or plasmid templates spanning \textit{Xoo} genome and direct sequencing of PCR products. Assembly was confirmed by comparing PacI, PmeI and ScaI restriction maps to computational predictions.

**Gene annotation**

ORFs were identified using Glimmer 2.0 (http://www.tigr.org/software/glimmer/) (21) or GeneMark (http://opalbiology. gatech.edu/GeneMark/) (22). In a few cases, open reading frames (ORFs) were identified by similarities detected using BLAST. Annotation was completed using BLAST and tRNAscan-SE (23) in reflection of the functional categories for clusters of orthologous groups (COGs). Annotation of transporter proteins was assisted from the KEGG databases (http://www.genome.jp/kegg/kegg2.html) (24).

**Database submission**

The sequence and annotation of the genome were submitted to the GenBank database with the accession no. AE013598.

**RESULTS AND DISCUSSION**

**General features**

The basic features of the \textit{X. oryzae pv. oryzae} str. KACC10331 genome are reported in Figure 1 and Table 1. The assembled sequence was consistent with a single, 4 941 439 bp, circular chromosome. No autonomous plasmids were apparent. The average G + C content of \textit{Xoo} genome was 63.7%, which is slightly lower than that of the \textit{Xac} (64.7%), \textit{Xcc} (65.0%) and \textit{R. solanacearum} (67.0%) genomes, but is higher than that of the genomes of other phytopathogenic bacteria, such as \textit{X. fastidiosa} (52.6%), \textit{A. tumefaciens} (58–60%) and \textit{P. syringae} (58.4%). Most of the genome was coding sequence, and contained 4637 ORFs predicted to encode polypeptides. Tentative functional assignments could be made for 3340 (72.0%) of the proposed genes based on their inclusion in known COGs (or sequence similarity). The remaining 1297 genes (27.9%) were predicted to express hypothetical proteins of unknown function. An origin of replication, consisting of \textit{dnaA} boxes, was identified between the deduced gene for the 50S ribosomal protein L34 and the predicted \textit{gyrB} locus expressing \textit{dnaA}, \textit{dnaN} and \textit{recF} genes. Two separate sets of 23S–5S and 16S ribosomal RNA (rRNA) genes, each consisting of two operons,
were also identified. Genes encoding tRNAs that recognize 54 codons were also found.

Comparative genomics

The alignment of the three organisms shown in Figure 2 suggests that many rearrangement events (reverse match; red) have been occurred between Xoo and Xac. Many of these events are located around the putative origin of replication. In alignments between Xoo and Xcc, only a few forward matches (blue) were observed. This is also evident in closer comparisons (DNA:DNA similarities); the entire length of the Xoo genome is non-co-linear and matched diagonally with the genomes of Xac and Xcc (Figure 3). The alignment between Xac and Xcc were previously shown to contain only three major rearrangement events; one of these was an inversion around the putative terminus of replication and the other two were inversions with translocations symmetrically located with respect to the putative origin of replication (16).

To find genes specific to the Xoo genome, the entire genome sequence was compared to the reported genome sequences of X. axonopodis pv. citri (AE008923) and X. campestris pv. campestris (AE008922). Xoo genome contains 245 species-specific genes (known: 95, unknown: 45, hypothetical: 105) that are not present in either the Xac or Xcc genomes. Although 95 genes appear to encode functional proteins, most (150) were of unknown function. Putative functions of representative Xoo genome species-specific genes were in restriction–modification (RM), a TonB-dependent siderophore receptor, toxin production (MlrB, Rtx), a TTSS effector and phage-related proteins. In addition, the rax genes of Xoo are species specific, and are involved in type I secretion and sulfation required to elicit the rice-resistant protein Xa21 (25).
Figure 2. Nucleotide alignments of Xoo (x-axis) versus Xac (y-axis), left; and Xoo (x-axis) versus Xcc (y-axis), right. Each point in the plot corresponds to an MUM of >25 bp.

Figure 3. Linear genomic comparisons of X.oryzae pv. oryzae with X.axonopodis pv. citri and X.campestris pv. campestris. Top, Xac; middle, Xoo; bottom, Xcc. The colored ticks represent the reading frames from top to bottom; +1 frame, +2 frame, +3 frame, a whole forward frame, a whole reverse frame, –1 frame, –2 frame and –3 frame. The red lines in between the genomes represent DNA:DNA similarities (BLASTN matches) between the two DNA sequences.
Mobile elements

Five insertion sequences (IS; IS1112 = TNX8, IS1113 = TNX1, IS1114, TNX6 and TNX7) had been previously identified in another strain of X. oryzae pv. oryzae (26–30) and 109 and 108 transposable elements were identified in the genomes of Xac and Xcc, respectively (16). Interestingly, the Xoo genome contained more than twice the number of transposable elements as either the Xac or Xcc genomes. A total of 271 out of 478 protein coding sequences (CDS) in the identified IS elements of the Xoo genome showed significant similarity to transposases, indicating that these have played an important evolutionary role in horizontal gene transfer and also in internal rearrangement of the genome. In the Xoo genome, a total of 207 genes were associated with mobile genetic elements. Included in this total were the genes for transposases located within IS and transposons as well as 37 apparent prophage-related genes. The Xoo IS elements could be classified into six known IS families: IS3, IS4, IS5, IS30, INSYC and IS630 (31,32). The IS5 family was the most abundant in the Xoo genome with 117 copies detected out of a total of 207 identified IS elements. In Xcc, the IS5 family is highly represented, with 16 copies of IS1478 (33), whereas in Xac the IS3 family is more abundant, with 21 copies of a member not previously described in Xanthomonas (ISXac3) (16). Many of these IS elements were located near strain-specific genes where altered codon usage and distinct G + C content suggests that these adjacent genes may have been acquired through horizontal transfer. Genes encoding for virulence/avirulence determinants in another plant pathogenic bacteria, P. syringae, have been previously reported to be associated with mobile genetic elements (34).

Bacteriophage can also mediate evolution and horizontal gene transfer of virulence factors and other new traits (35). A large population of bacteriophage has been found to be specifically present in Xoo strains (36). A prophage-related gene cluster (27 kb) encoding tail proteins, integrase, capsid, lytic enzyme and replication proteins suggestive of an intact prophage, was detected at about 1.7 Mb in the Xoo genome. Surprisingly, the cluster was very similar to the XccP1 phage in the Xcc genome; however, Xoo lacks orf8, which is predicted to encode a phage-related tail fiber protein and five hypothetical proteins between the int and orf37 genes, which were included in Xcc genome. Thus, the total length of the prophage gene cluster in the Xoo genome is less than that found in the Xcc genome. A strong amino acid identity (74–97%) of the clustered prophage genes was observed between Xoo and Xcc. Xac lacked most of the tail genes, but a strong amino acid identity (77–96%) of prophage remnants was also observed between Xac and Xoo.

Metabolic characteristics and RM systems

The three Xanthomonas pathogens with known genomes have numerous and diversified pathways for intermediary, small molecule and DNA metabolism. In Xcc, but not Xac, genes that function in the assimilation and conversion of nitrate and nitrite into ammonium (nasTACDEF and cysG) were identified. The Xoo genome contained only nasT (3 copies) and nasF (2 copies), suggesting that Xoo, like Xac, does not have this activity. An ABC-type oligopeptide transport system (oppA, oppB and oppC) was identified in the Xoo genome that could facilitate the entry of small oligopeptide products. These observations suggest that Xoo has different nitrate assimilation and oligopeptide transport capabilities than either Xac or Xcc.

Many bacteria can sense their population density using any of several cell-to-cell communication systems to alter expression of specific genes when the population reaches a threshold density. This phenomenon is known as quorum sensing (37). Phytopathogenic bacteria, such as Azotobacter, Erwinia carotovora and R. solanacearum, have quorum sensing mechanisms similar to that of the LuxR/LuxI system from Vibrio fisheri, and utilize acyl-homoserine lactones (AHLs) to regulate several virulence genes. Although the basic mechanism of AHL-mediated quorum sensing is generally well understood in vitro, the dynamics of signal sensing and regulation in nature are more difficult to define, and new levels of complexity are now surfacing. For example, different bacteria produce different AHLs, and a given species may produce more than one AHL. The acyl side chains of known AHL molecules vary in length (4–18 carbons), can contain double bonds, or are frequently substituted with a carbonyl or hydroxyl group at the C3 position (38,39). In addition, quorum sensing regulation may be quite strain specific, with different strains making substantially different sets of AHLs, or no detectable AHLs at all (40,41). In the Xoo genome, genes for acetylation, O-acetyltransfer, and dehydrogenation of homoserine were identified, but genes exhibiting sequence similarity to LuxR/LuxI were not obvious.

Two DNA RM systems have been reported previously in Xoo (42–44) that affected the efficiency of transposon mutagenesis and transformation. Two type II RM systems were identified in the Xoo genome, which corresponded to XorI and XorII. In addition, three type I DNA RM systems were present.

Extracellular polysaccharides, lipopolysaccharide and surface-borne features

A characteristic of Xoo that is similar to other Xanthomonas species is the ability to form mucoid colonies when cultured on media supplemented with glucose. This phenotype results from the production of copious amounts of the extracellular polysaccharide (EPS), known as xanthan gum, which is formed by the activity of the gum operon products (45). The EPS is a repeating pentamer composed of two subunits (inner and outer membrane antigen) and one of glucuronic acid, and contains certain modifications like acetylation (46). EPS can play a critical role in facilitating adhesion of bacteria to the host surface during initial stages of plant–pathogen interactions and disease development (47). A transposon insertion in the gumG homolog of Xoo causes loss of EPS production as well as virulence in rice. Reversal of the gumG mutation of Xoo restored the EPS production and virulence (6). A gum operon (16 kb) was identified in the Xoo genome that consisted of 13 genes, gumBDEFGHIJKLMN, which was similar to the gum operon of Xcc except for the existence of gumN in the Xoo genome.

Three distinct genes, wsoD (O-antigen acetylase), oma (outer membrane antigen) and rbyC (which functions in O-antigen biosynthesis) were found in three separate regions in the Xoo genomes. However, genes for O-antigen synthesis in Xcc genome are organized as a single cluster containing...
many more genes (48). The first region contains genes coding for transferases, epimerases, translocases and deduced sugar transport proteins whereas the second region contains the xanAB and rmlDABC genes involved in nucleotide-sugar and dTDP-t-rhamnose biosynthesis (49). O-antigens of Xoo lack significant sequence similarity to counterparts in Xcc and Xac genomes. These observations are consistent with the lipopolysaccharide (LPS) O-antigen being pathovar specific. Much of the traditional interest in LPS molecules originates in their complex interaction with host defenses and their contribution to virulence in pathogenic bacteria. O-antigens form hydrophilic surface layers that may function in host-range and pathogenicity by acting as a barrier against plant toxins (50,51).

The Xoo genome also contains genes for type IV fimbriae and for several glycan-rich outer membrane proteins that are associated with host colonization and adhesion in many pathogenic bacteria (52). For example, xadA encodes an outer membrane protein implicated in virulence that is coordinately regulated with other pathogenicity determinants by hrpG (53). Two alleles of xadA were identified in the Xoo genome, similar to the Xac genome. Only one allele is present in the Xcc genome. The fibrillin genes of Xoo are different from those of the Xac and Xcc genomes.

### Potential pathogenicity and virulence determinants

RTX toxins are important virulence factors for a variety of human and animal pathogens (54), and have been found in several plant pathogenic bacteria, including X.fastidiosa, Rhizobium leguminosarum and E.carotovora (14,55,56). The genes for two apparent RTX toxins, rtxA and rtxC, were identified in the Xoo genome but were not detected in the Xcc or Xac genomes. Xoo has been reported to produce several toxins, including phynelactic acid (PAA), trans-3-methylthio-acrylic acid (MTAA) and 3-methylthio-propionic acid, that can cause wilting and chlorosis (57). Thus, the RTX toxin genes in the Xoo genome may also be virulence factors.

Motility in several different plant pathogenic species is important for virulence (58). The genomic sequence of Xoo, like Xac and Xcc, includes genes required for flagellar biosynthesis and chemotaxis. Unlike those from Xac or Xcc, the Xoo genes for chemotaxis receptors and flagella biogenesis are organized into two clusters spread over 62 kb, and only two copies of the methyl-accepting chemotaxis protein gene (mcp) are present.

Many plant pathogenic bacteria secrete a variety of plant cell wall degrading enzymes, such as cellulases, xylanases, pectinases and proteases. The general secretory pathway (GSP), referred to as type II secretion system, secretes the pectinases and proteases. The general secretory pathway (GSP), referred to as type II secretion system, secretes the extracellular enzymes and is required for virulence of many phytopathogens to their host plants (59). Cellulase, protease and pectate lyase from Xanthomonas species have been suggested to play crucial roles in virulence and in bacterial nutrition (5,60–62). The Xoo genome contains genes for various extracellular enzymes, including the genes for seven types of cellulases, six different proteases, a polygalacturonase, pectin degrading enzymes (one pectin esterase, two pectate lyases), four xylanases, six xylosidas and one β-celluliosidase. Xoo has more genes involved in degradation of pectin, cellulose and xylanase than either Xcc or Xac. Xylanase and protease have been shown to play a role in Xoo pathogenesis (4,5). This is logical since bacterial blight is a vascular disease and because Xoo multiplies and spreads in the xylem vessel where xylan is abundant (63). Thus, xylanase may function to degrade the xylan and produce energy Xoo cells to multiply in the xylem vessel. Xylanase genes have not yet been identified in Xcc and Xac genomes, suggesting xylanase production can be regarded as characteristic factor in Xoo pathogenesis.

Secretion of the aforementioned extracellular enzymes usually involves the GSP encoded by the xps gene cluster (64,65). Homologs of the Xps system (xpsEFHIJKLNM and xpsD) were identified in the Xoo genome and showed >79% amino acid identity to their counterparts of other Xanthomonas strains. Null mutations in these genes block secretion of degradative enzymes from bacterial cells, causing a substantial loss of virulence (5). Similarly, a Xoo GSP mutant that was not able to secrete xylanase showed reduced pathogenicity on rice plant (5).

The synthesis of extracellular cell wall degrading enzymes and exopolysaccharides are transcriptionally regulated by the products of rpf (regulation of pathogenicity factor) genes (66). This is a complex regulatory system, and also involves a small diffusible molecule called DSF (diffusible signal factor) (67). The expression levels of proteases and endoglucanases were reduced, e.g. when the rpfE gene was inactivated in Xcc (68). In the case of Xoo, the rpfC gene effects EPS production and virulence on rice (68). An rpf cluster was identified in the Xoo genome that had a unique organization (rpfABFGCDIE) relative to its counterparts in the Xac and Xcc genomes. The Xoo genome lacked an rpfH, which is homologous to the transmembrane sensor domain of rpfC and may stabilize rpfC in the cell membrane of X.campestris (69). In the Xoo genome, four copies of rpfI genes that are involved in the regulation of extracellular enzyme and EPS synthesis were identified. In the case of Xcc, a transposon insertion in rpfI (orf4) did not affect polygalacturonate lyase production, but led to reduced levels of protease and endoglucanase. These alterations in the levels of extracellular enzymes did not affect the pathogenicity of Xcc (66).

### Hypersensitive reaction and pathogenicity (hrp) and avirulence (avr) genes

Virulence and regulatory genes required for bacterial pathogenicity are commonly found in pathogenicity islands (PAIs) that encode for a type III protein secretion system assembled from hrp gene products (70–72). A hrp gene cluster was identified in the Xoo genome that included 26 genes inclusive of hpa2 and hrpF (Figure 4). The Xoo hrp PAI (31.3 kb) was larger than its counterparts of Xac (25.6 kb) and Xcc (23.1 kb) due to the presence of four transposase genes (about 6 kb) located between hpaB and hrpF genes. Otherwise, the clusters were very similar. Strong amino acid identity was observed between several orthologous hrp genes of Xoo and Xac: hpaF (74%), hpaP (76%), hrpD5 (79%), hpaA (82%). In contrast, hrpF (68%), hpa1 (65%), hrpB5 (66%) and hrpB7 (65%) in these bacteria exhibited relatively low similarity. It is interesting to note that the products of hrpF and hpa1 are predicted to be exposed or secreted components of the type III secretion system, and this feature could contribute to their diversity due
to distinct selective pressures in the different hosts. A homolog to hrpW, a proposed pectate lyase, was not readily apparent in the Xoo genome but, as mentioned earlier, several candidate pectate lyase genes were identified that could function similarly to hrpW. One of these pectate lyase genes was tentatively designated hrpW based on sequence similarity. The hrpW of many other pathogens indeed has HR-eliciting activity, but it does not have measurable pectate lyase activity (72). Erwinia amylovora also produces hrpW (72). Furthermore, overexpression of hrpW in E. amylovora can complement the hrpN mutation, which drastically reduces the ability of E. amylovora to cause HR or disease and suggests that hrpN and hrpW are functionally redundant (72).

The expression of hrp and several effector genes in other Xanthomonas strains is regulated by the transcriptional activator, hrpX (73). Expression of hrpX, in turn, is regulated by hrpG, a response regulator of the OmpR subclass of two-component signal transduction systems (74). Homologs to hrpX and hrpG were present at similar locations in the Xoo, Xac and Xcc genomes. Genes regulated by hrpX in other Xanthomonas strains usually include a plant-inducible-promoter (PIP) box (TTCG\(_{15}\)-TTCG) in their promoters (75). Fourteen copies of a similar sequence, TTCG\(_{16}\)-TTCGn, were identified in the Xoo genome (Table 2). Four of these apparent PIP boxes were located in predicted promoter regions of the hrp gene cluster. Another was associated with the promoter of an avr gene and one was near a PopC-like leucine-rich protein. The remaining eight were dispersed elsewhere in the genome, and were associated with a peptidase, an iron receptor protein, ribonucleotide-diphosphate reductase and three hypothetical proteins.

Pathogenicity trials using characterized isogenic lines of rice (IRBB1, 3, 4, 5, 7, 8, 10, 13, 14, 21) suggested that Xoo str. KACC10331 harbors at least nine avr genes corresponding to Xa1, 3, 4, 5, 7, 8, 10, 13, 14 (Unpublished data). Eight homologs of known avr genes were identified and scattered in the Xoo chromosome. Four homologs of the avrBs3/\(pNhA\) family of avirulence genes were identified as well as individual homologs of avrBs3 and avrBs2. Two homologs of popC, an avr-like effector gene originally characterized from \(R\. solanacearum\), were also identified (Table 3). These genes all exhibited higher sequence similarity to their counterparts of Xac than to those of Xcc. Although we did identify the avrXa7 gene, surprisingly, no genes identical to avrXa10 (9,76) were found in the genome. This is consistent with the observations of this strain’s virulence to rice lines IRBB5 and IRBB10 that serve as indicator varieties for bacteria expressing avrXa5 and avrXa10, and avirulence to rice line IRBB7, which is the indicator for bacteria expressing avrXa7 (unpublished data).

The avrBs2 from \(X\. campestris\) pv. \(vesicatoria\) is highly conserved in strains of \(X\. campestris\) and was previously reported in the Xoo genome (77). AvrBs2 is a TTSS translocated effector that acts as a virulence factor in susceptible hosts but elicits defense responses in resistant hosts (78,79).

**Table 2.** The proposed hrpX regulon in *Xanthomonas oryzae* pv. *oryzae*

| PIP position | Distance (bp) | Gene ID | Gene product |
|--------------|--------------|---------|--------------|
| hrp gene cluster | | | |
| 77095 | 144 | XOO0082 | hrpC |
| 80817 | 1995 | XOO0085 | hrpU |
| 80734 | 83 | XOO0086 | hrpB1 |
| 89672 | 137 | XOO0095 | hpa1 |
| 89740 | 125 | XOO0096 | hpa2 |
| Extended Hrp conserved regulon | | | |
| 4661123 | 62 | XOO4391 | Xanthomonas conserved hypothetical |
| 3186454 | 542 | XOO2979 | Conserved hypothetical |
| 3070661 | 205 | XOO2861 | \(\beta\)-ketoadipate enol-lactone hydrolase |
| 2856630 | 1972 | XOO2699 | Polygalacturonase |
| 3352034 | 245 | XOO3122 | Conserved hypothetical |
| 4251305 | 2058 | XOO3959 | Endopolygalacturonase |
| 4611239 | 927 | XOO4332 | 2-K-3-DdG permease |
| 115257 | 10270 | XOO0111 | Conserved hypothetical with GGDEF domain |
| 2098427 | 275 | XOO1992 | Iron receptor |
| 1533310 | 4182 | XOO1487 | Cysteine protease |
| 494176 | 148 | XOO0475 | Ribonucleotide-diphosphate reductase |
| 460543 | 6414 | XOO0459 | 3-oxoacyl-[ACP] reductase |
The Xoo AvrBs2 homolog like the X.campestris gene, exhibited regions with similarity to enzymes that synthesize or hydrolyze phosphodiester bonds (78,79). X.campestris strains harboring avrBs2 genes with mutations in these regions overcame resistance to the corresponding resistance gene Bs2, suggesting the enzyme activity might be critical to avirulence function (79).

All three of the Xoo, Xac and Xcc genomes contained genes coding for PopC-like leucine-rich-repeat (LRR) proteins. LRR motifs are commonly involved in protein–protein interactions and are found in the three major classes of plant-resistance genes (80) and in the PopC protein of R. solanacearum (81). Xoo PopC consisted of a 677-amino acid protein that carries 10 tandem LRRs. Many other bacteria-pathogenic plants and animal encode for a YopJ homolog, a cysteine protease necessary for virulence (82). Similar to Xac, Xoo lacks a recognizable YopJ homolog.

CONCLUSION

Many researchers have tried to elucidate the mechanisms of Xoo virulence and host resistance at a molecular level and, as a result, a large number of Xoo genes associated with pathogenesis have been isolated and characterized. Nevertheless, many aspects of virulence and avirulence mechanisms of Xoo are still not understood. In this study, we presented the whole-genome sequence of Xoo and used that sequence to identify genes that might be involved in virulence and that may be specific to the pathovar oryzae.

Xoo, the bacterial blight pathogen on rice, is the third Xanthomonas species whose whole-genomic sequence has been completely defined. Comparative genomics between Xoo and the other two Xanthomonas genomes (Xcc and Xac) showed high homology of more than 80% in genes associated with virulence determinants, suggesting analogous functions in pathogenesis. The Xoo genome contained approximately twice as many transposable elements as the genomes of Xcc and Xac. Transposable elements are potential agents of large-scale genome reorganization by virtue of their ability to induce chromosomal rearrangements such as deletions, duplications, inversions and reciprocal translocations. We also identified 245 genes in the Xoo genome that were not found in the genomes of Xcc or Xac. Some of these genes may be responsible for the certain types of pathogenicity and host specificity profiles of Xoo. Host specificity, for example, may result from combining different subsets of genes found in each genome, such as genes encoding avr effector proteins, components of secretion systems (hrp elements of the type III secretion system), regulatory elements (rpf, regulation of pathogenesis factor), type IV fimbriae and surface components (LPS O-antigen operons). These findings in the sequence information of Xoo genome provide a basis for experimental approaches to better understand mechanisms by which the pathogen invades and induces disease or resistance in its host plant.

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REFERENCES

1. Ezuka,A. and Kaku,H. (2000) A historical review of bacterial blight of rice. Bull. Natl. Inst. Agrobiol. Resour. (Japan), 15, 53–54.
2. Lindgren,P.B. (1997) The role of hrp genes during plant-bacterial interactions. Annu. Rev. Phytopathol., 35, 129–152.
3. Rossier,O., Van den Ackerveken,G. and Bonas,U. (2000) HrpB2 and HrpF from Xanthomonas are type III-secreted proteins and essential for pathogenicity and recognition by the host plant. Mol. Microbiology, 38, 828–838.
4. Xu,G.W. and Gonzalez,C.F. (1989) Evaluation of TN4431-induced protease mutants of Xanthomonas campestris pv. oryzae for growth in plants and pathogenicity. *Phytopathology*, 79, 1210–1215.

5. Ray,S.K., Rajeshwari,R. and Sonit,R.V. (2000) Mutants of Xanthomonas oryzae deficient in general secretory pathway are virulent deficient and unable to secrete xylanase. *Mol. Plant Microbe Interact.*, 13, 594–401.

6. Dharmapuri,S. and Sonit,R.V. (1999) A transposon insertion in gmuG homologue of Xanthomonas oryzae pv. oryzae causes loss of extracellular polysaccharide production and virulence. *FEMS. Microbiol. Lett.*, 179, 53–59.

7. Keen,N.T. (1990) Gene-for-gene complementarity in plant–pathogen interactions. *Annu. Rev. Genet.*, 24, 447–463.

8. Leach,J.E. and White,F.F. (1996) Bacterial avirulence genes. *Annu. Rev. Phytopathol.*, 34, 153–179.

9. Hopkins,C.M., White,F.F., Choi,S.-H., Guo,A. and Leach,J.E. (1992) Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.*, 30, 187–224.

10. Blair,M.W., Garris,A.J., Iyer,A.S., Chapman,B., Kresovich,S. and McCouch,S.R. (2003) High resolution genetic mapping and candidate gene identification at the Xa5 locus for bacterial blight resistance in rice (*Oryza sativa L.*). *Theor. Appl. Genet.*, 107, 62–73.

11. Yoshimura,S., Umehara,Y., Kurata,N., Nagamura,Y., Sasaki,T., Minobe,Y. and Iwata,N. (1996) Characterization of candidate clones of Xa-1, the bacterial blight resistance gene in rice, isolated by map-based cloning. *Theor. Appl. Genet.*, 93, 117–122.

12. Wang,G.L., Song,W.Y., Ruan,D.L., Sideris,S. and Ronald,P.C. (1996) The cloned gene, Xa21, confers resistance to multiple Xanthomonas oryzae pv. oryzae isolates in transgenic rice plants. *Plant. Mol. Microbe Interact.*, 9, 850–855.

13. Simpson,A.J., Reinach,F.C., Arruda,P., Abreu,F.A., Acencio,M., Leach,J.E., Vera Cruz,C.M., Bai,J. and Leung,H. (2001) Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.*, 39, 187–224.

14. Rajeshwari,R. and Sonti,R.V. (2000) Mutants of *Xanthomonas campestris pv. oryzae* isolate 955-2955 cause loss of extracellular polysaccharide production and virulence. *FEMS. Microbiol. Lett.*, 193, 271–279.

15. Neson,R.J., Baraoidan,M.R., Vera Cruz,C.M., Yap,I.V., Leach,J.E. (1998) Distribution of Xa21 in a collection of rice varieties. *Mol. Plant Microbe Interact.*, 11, 1126–1132.

16. Wang,G.L., Song,W.Y., Ruan,D.L., Sideris,S. and Ronald,P.C. (1996) The cloned gene, Xa21, confers resistance to multiple Xanthomonas oryzae pv. oryzae isolates in transgenic rice plants. *Plant. Mol. Microbe Interact.*, 9, 850–855.

17. Keen,N.T. (1990) Gene-for-gene complementarity in plant–pathogen interactions. *Annu. Rev. Genet.*, 24, 447–463.

18. Leach,J.E. (1994) Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.*, 60, 3275–3283.

19. Leach,J.E. (1994) Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.*, 60, 3275–3283.

20. Wilson,R.K. and Mardis,E. (1997) Shotgun sequencing. In Birren,B., Nusbaum,C., Clark,A., Davis,R.W., Glodek,A., et al. (2001) *The genomic sequence of rice*. ASM Press, Washington, DC, pp. 211–230.

21. Whitehead,N.A., Wernegreen,B., Slater,H., Simpson,N.L. and Salmond,G.P.C. (2001) Characterization of insertion of IS476 and two newly identified insertion elements in the chromosome. *J. Mol. Biol.*, 196, 445–455.

22. Krylov,V.N. (2003) Role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria. *Genetika*, 39, 595–620.

23. Ezuka,A. and Kaku,H. (2000) A historical review of bacterial blight of rice. *Bull. Natl. Inst. Agrobiol. Resour., Japan*, 15, 61–74.

24. Tong,B., Bauer,W.D. and Coplin,D. (2003) Quorum sensing in plant pathogenic bacteria. *Annu. Rev. Phytopathol.*, 41, 455–482.

25. Faqua,C. and Eberhard,A. (1999) Signal generation in autoinduction systems: synthesis of acylated homoserine lactones by Lux-type proteins. In Dunny,G.M. and Winans,S.C. (eds), *Cell–Cell Signaling in Bacteria*. ASM Press, Washington, DC, pp. 211–230.

26. Whitehead,N.A., Barnard,A.M.L., Slater,H., Simpson,N.L. and Salmond,G.P.C. (2001) Quorum-sensing in gram-negative bacteria. *FEMS Microbiol. Rev.*, 25, 365–404.

27. Cha,C., Gao,P., Chen,Y.C., Shaw,P.D. and Farrand,S.K. (1998) Production of acyl-homoserine lactone quorum sensing signals by gram-negative plant-associated bacteria. *Plant Mol. Microbe Interact.*, 11, 1119–1219.

28. Elami,O., Delome,S., Lemanceau,P., Stewart,G., Laue,B., Glickmann,E., Oger,P.M. and Dessaux,Y. (2001) A historical review of bacterial blight of rice. *Bull. Natl. Inst. Agrobiol. Resour., Japan*, 15, 61–74.

29. Von Bodman,S.B., Bauer,W.D. and Coplin,D. (2003) Quorum sensing in plant pathogenic bacteria. *Annu. Rev. Phytopathol.*, 41, 455–482.
47. Vojnov,A.A., Zarregarieua,A., Dow,J.M., Daniels,M.J. and Dankert,M.A.(1998) Evidence for a role in the mumB and mumC gene products in the formation of xanthan from its pentasaccharide repeating unit by Xanthomonas campestris. Microbiology, 144, 1487–1493.

48. Vorhö lter,F.J., Niehaus,K. and Puhler,A. (2001) Lipopolysaccharide biosynthesis in Xanthomonas campestris pv. campestris: a cluster of 15 genes is involved in the biosynthesis of the LPS O-antigen and the LPS core. Mol. Genet. Genomics., 266, 79–95.

49. Koplin,R., Wang,G., Hotte,B., Priefer,U.B. and Puhler,A. (1993) A 3.9-kb DNA region of Xanthomonas campestris pv. campestris that is necessary for lipopolysaccharide production encodes a set of enzymes involved in the synthesis of TDP-rhamnose. J. Bacteriol., 175, 7786–7792.

50. Dow,J.M., Osbourn,A.E., Wilson,T.J. and Daniels,M.J. (1995) A locus determining pathogenicity of Xanthomonas campestris is involved in lipopolysaccharide biosynthesis. Mol. Plant Microbe Interact., 8, 678–777.

51. Whitfield,C. (1995) Biosynthesis of lipopolysaccharide O antigens. Trends Microbiol., 3, 178–185.

52. Cao,H., Baldini,R.L. and Rahme,L.G. (2001) Common mechanisms of xanthan biosynthesis in Xanthomonas. Mol. Genet. Genomics., 266, 655–671.

53. Noel,L., Thieme,F., Nennstiel,D. and Bonas,U. (2001) cDNA-AFLP analysis unravels a genome-wide hprG-regulon in the plant pathogen Xanthomonas campestris pv. vesicatoria. Mol. Microbiol., 41, 1271–1281.

54. Welch,R., Forestier,A., Lobo,C., Pellett,A., Thomas,S.W. and Rowe,G. (1992) The synthesis and function of the Escherichia coli hemolysins and related RTX exotoxins. FEMS Microbiol. Immunol., 105, 29–36.

55. Orenskij,I., Twelker,S. and Hynes,M.F. (1999) Cloning and characterization of a Rhizobium leguminosarum gene encoding a bacteriocin with similarities to RTX toxins. Appl. Environ. Microbiol., 65, 2833–2840.

56. Kuhnert,P., Heyberger-Meyer,B., Burnens,A.P., Nicolet,J. and Frey,J. (1997) Detection of RTX toxin genes in gram-negative bacteria with a set of specific probes. Appl. Environ. Microbiol., 63, 2258–2265.

57. Noda,T., Saito,Z., Iwasaki,S. and Ohuchi,A. (1989) Isolation and structural elucidation of phytotoxic substances produced by Xanthomonas campestris pv. oryzae. Bull. Hokurika Natl Agric. Exp. Stn., 105, 105–129.

58. Rudolf,K. (1993) Xanthomonas. Chapman & Hall, London, UK.

59. Stathopoulos,C., Hendrixson,D.R., Thanassi,D.G., Hultgren,S.J., St Geme,J.W. III and Curtiss,R. (2000) Secretion of virulence determinants by the general secretory pathway in gram-negative pathogens: an evolving story. Microbes Infect., 2, 1061–1072.

60. Kamoun,S. and Kado,C.I. (1990) A plant-inducible gene of Xanthomonas campestris pv. campestris encodes an exocellular component required for growth in the host and hypersensitivity on nonhosts. J. Bacteriol., 172, 5165–5172.

61. Dow,J.M., Davies,H.A. and Daniels,M.J. (1998) A metalloprotease from Xanthomonas campestris that specifically degrades proline/hydroxyproline-rich glycoproteins of the plant extracellular matrix. Mol. Plant Microbe Interact., 11, 1085–1093.

62. Dow,J.M., Clarke,B.R., Milligan,D.E., Tang,J.L. and Daniels,M.J. (1990) Extracellular proteases from Xanthomonas campestris pv. campestris, the black rot pathogen. Appl. Environ. Microbiol., 56, 2994–2998.

63. Ezuka,A. and Kaku,H. (2000) A historical review of bacterial blight of rice. Bull. Natl. Inst. Agrobiol. Resour. Japan, 15, 148–149.

64. Dums,F., Dow,J.M. and Daniels,M.J. (1991) Structural characterization of the bacterial phytopathogen Xanthomonas campestris pathovar campestris pv. campestris: relatedness to secretion systems of other gram-negative bacteria. Mol. Gen. Genet., 229, 357–364.

65. Hu,N.T., Hung,M.N., Chou,S.J., Tang,F., Chiang,D.C., Huang,H.Y. and Wu,C.Y. (1992) Cloning and characterization of a gene required for the secretion of extracellular enzymes across the outer membrane by Xanthomonas campestris pv. campestris. J. Bacteriol., 174, 2679–2687.

66. Dow,J.M., Feng,J.X., Barber,C.E., Tang,J.L. and Daniels,M.J. (2000) Novel genes involved in the regulation of pathogenicity factor production within the rpf gene cluster of Xanthomonas campestris. Microbiology, 146, 885–891.

67. Barber,C.E., Tang,J.L., Feng,J.X., Pan,M.Q., Wilson,T.J., Slater,H., Dow,J.M., Williams,P. and Daniels,M.J. (1997) A novel regulatory system required for pathogenicity of Xanthomonas campestris is mediated by a small diffusible signal molecule. Mol. Microbiol., 24, 555–566.

68. Tang,J.L., Feng,J.X., Li,Q.Q., Wen,H.X., Zhou,D.L., Wilson,T.J., Dow,J.M., Ma,Q.S. and Daniels,M.J. (1996) Cloning and characterization of the rpfC gene of Xanthomonas oryzae pv. oryzae: involvement in exopolysaccharide production and virulence to rice. Mol. Plant Microbe Interact., 9, 664–666.

69. Slater,H., Alvarez-Morales,A., Barber,C.E., Daniels,M.J. and Dow,J.M. (2000) A two-component system involving an HD-GYP domain protein links cell–cell signaling to pathogenicity gene expression in Xanthomonas campestris. Mol. Microbiol., 38, 986–1003.

70. Pugsley,A.P. (1993) The complete general secretory pathway in gram-negative bacteria. Microbiol. Rev., 57, 30–108.

71. Lindgren,P.B. (1997) The role of hpr genes during plant–bacterial interactions. Annu. Rev. Phytopathol., 35, 129–152.

72. He,S.Y. (1998) Type III protein secretion systems in plant and animal pathogenic bacteria. Annu. Rev. Phytopathol., 36, 363–392.

73. Dow,J.M. and Daniels,M.J. (1994) Pathogenicity determinants and global regulation of pathogenicity of Xanthomonas campestris pv. campestris. Curr. Top. Microbiol. Immunol., 192, 29–41.

74. Wengelnik,K., Vanden Ackerveken,G. and Bonas,U. (1996) HrpG, a key hrp regulatory protein of Xanthomonas campestris pv. vesicatoria is homologous to two-component response regulators. Mol. Plant Microbe Interact., 9, 704–712.

75. Fenselau,S. and Bonas,U. (1995) Sequence and expression analysis of the hrpB pathogenicity operon of Xanthomonas campestris pv. vesicatoria which encodes eight proteins with similarity to components of the Hrp, Ysc, Spa, and Fli secretion systems. Mol. Plant Microbe Interact., 8, 845–854.

76. Bai,J., Choi,S.H., Ponciano,G., Leung,H. and Leach,J.E. (2000) Xanthomonas oryzae pv. oryzae avirulence genes contribute differently and specifically to pathogen aggressiveness. Mol. Plant Microbe Interact., 13, 1322–1329.

77. Mazzola,M., Leach,J.E., Nelson,R. and White,P.F. (1994) Analysis of the interaction between Xanthomonas oryzae pv. oryzae and the rice cultivars IR24 and IRBB21. Phytopathology, 84, 392–397.

78. Swords,K.M., Dahlbeck,D., Kearney,B., Roy,M. and Staskawicz,B. (1996) Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in Xanthomonas campestris pv. vesicatoria avrBs2 protein secretion systems. Mol. Plant Microbe Interact., 9, 466–469.

79. Mudgett,M.B., Chesnokova,O., Dahlbeck,D., Clark,E.T., Rossier,O., Bonas,U. and Staskawicz,B. (2000) Molecular signals required for type III secretion and translocation of the Xanthomonas campestris AvrBs2 protein to pepper plants. Proc. Natl Acad. Sci. USA, 97, 13324–13329.

80. Young,N.D. (2000) The genetic architecture of resistance. Curr. Opin. Plant Biol., 3, 285–290.

81. Guenon,E., Timmers,A.C., Boucher,C. and Arlat,M. (2000) Two novel proteins, PopB, which has functional nuclear localization signals, and PopC, which has a large leucine-rich repeat domain, are secreted through the hpr-secretion apparatus ofRalstonia solanacearum. Mol. Microbiol., 36, 261–277.

82. Lahaye,T. and Bonas,U. (2001) Molecular secrets of bacterial type III effector proteins. Trends Plant Sci., 6, 479–485.