Nucleic Acids Research, 2005, Vol. 33, No. 2 ***577–586***
doi:10.1093/nar/gki206

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

Byoung-Moo Lee*, Young-Jin Park, Dong-Suk Park, Hee-Wan Kang[1], Jeong-Gu Kim, Eun-Sung Song, In-Cheol Park, Ung-Han Yoon, Jang-Ho Hahn, Bon-Sung Koo, Gil-Bok Lee, Hyungtae Kim[2], Hyun-Seok Park[2], Kyong-Oh Yoon[2], Jeong-Hyun Kim[2], Chol-hee Jung[2], Nae-Hyung Koh[2], Jeong-Sun Seo[2,3] and Seung-Joo Go

National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Korea, [1]Graduate School of Bio and Information Technology, Hankyong National University, Ansung 456-749, Korea, [2]Macrogen Inc., World Meridian Venture Center 10F, #60-24, Gasan-dong, Geumcheon-gu, Seoul 153-023, Korea and [3]College of Medicine, Seoul National University, Chongno-gu 151-742, Korea

Received September 30, 2004; Revised December 8, 2004; Accepted January 5, 2005 DDBJ/EMBL/GenBank accession no. AE013598

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 4637 open reading frames (ORFs) of which 3340 (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 production, and genes encoding extracellular plant cell wall-degrading enzymes. 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 γ-subdivision 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 pathogenesis (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

*To whom correspondence should be addressed. Tel: +82 31 299 1751; Fax: +82 31 299 1798; Email: lbmoo@rda.go.kr

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

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

The nucleotide sequence of a pathogen's genome is an important step to understanding the mechanisms of pathogenesis and the processes that limit the host range of the strain. The nucleotide sequence of the genomes of several phytopathogenic bacteria, such as *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Xylella fastidiosa* and two *Xanthomonas* species, have been recently determined (14–18). Among bacteria classified in the genus *Xanthomonas*, the whole-genome sequences of *X.axonopodis* pv. *citri* (*Xac*; the causal bacterium of citrus canker) and *X.campestris* pv. *campestris* (*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 *Xanthomonas* species with known genomes (it is a monocotyledon rather than a dicotyledon), it is likely that the *Xoo* genome will include distinct genes that are critical to interactions with rice.

Here, we report the nucleotide sequence and genome structure of *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 *avr* genes, including *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

Xanthomonas oryzae pv. *oryzae* str. KACC10331 (KXO85), a representative Korean race 1 strain that is virulent to rice carrying the *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 SmaI/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 clones constructed using 40 kb genome fragments in the pEpiFOS™-5 vector (Epicentre technologies, USA) and 2895 BAC clones with 112 kb genome fragments generated in the pIndigoBAC-5 vector (Epicentre 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 *Xoo* genome and direct sequencing of PCR products. Assembly was confirmed by comparing PacI, PmeI and SwaI restriction maps to computational predictions.

Gene annotation

ORFs were identified using Glimmer 2.0 (http://www.tigr.org/software/glimmer/) (21) or GeneMark (http://opal.biology.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 *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 *Xoo* genome was 63.7%, which is slightly lower than that of the *Xac* (64.7%), *Xcc* (65.0%) and *R.solanacearum* (67.0%) genomes, but is higher than that of the genomes of other phytopathogenic bacteria, such as *X.fastidiosa* (52.6%), *A.tumefaciens* (58–60%) and *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 *dnaA* boxes, was identified between the deduced gene for the 50S ribosomal protein L34 and the predicted *gyrB* locus expressing *dnaA*, *dnaN* and *recF6*. Two separate sets of 23S–5S and 16S ribosomal RNA (rRNA) genes, each consisting of two operons,

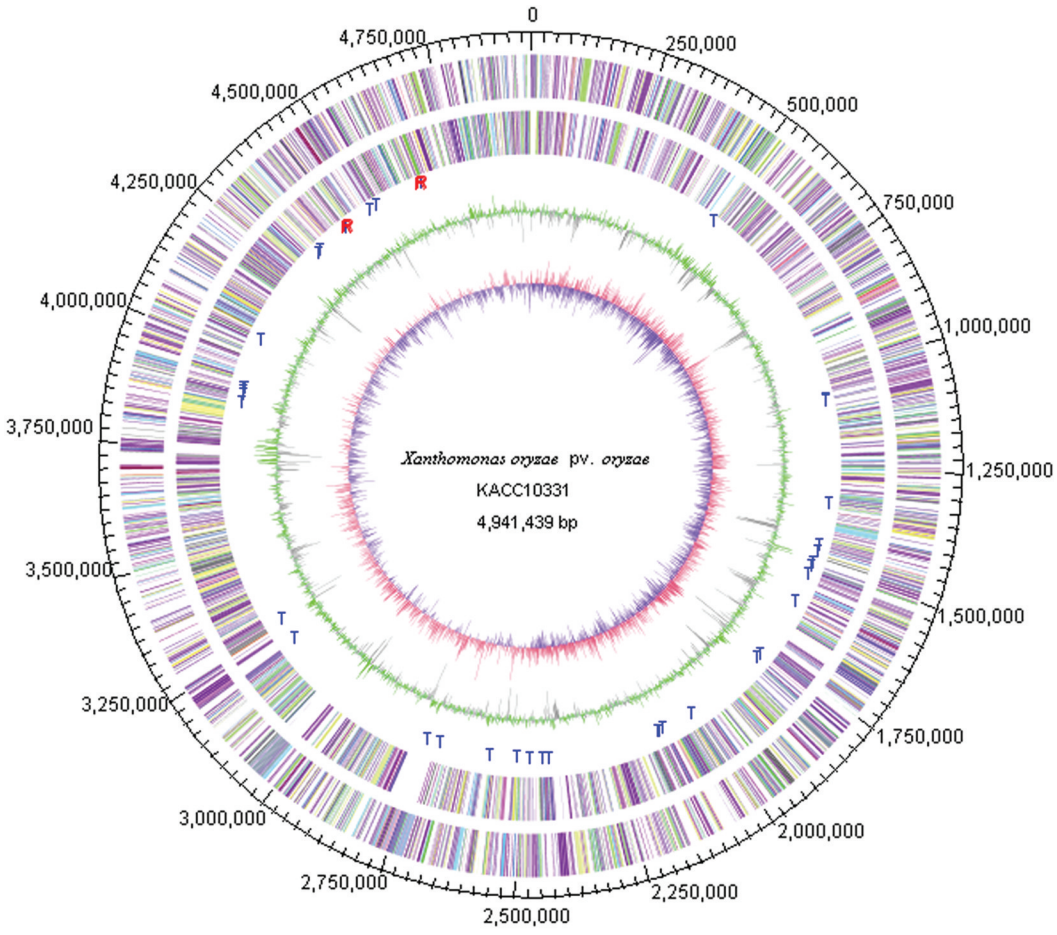

Figure 1. Circular genome map of *X.oryzae* pv. *oryzae* str. KACC10331. Overall structure of the *X.oryzae* pv. *oryzae* genome. The putative origin of replication is at 0 kb. The outer scale indicates the coordinates (in base pair). Red symbols (character R) are positions of rRNA and blue symbols (character T) are tRNAs. The distribution of genes is shown on the first two rings within the scale. The next circle (green) shows G + C content and central circle (blue/red) shows GC-skew value. The window size of G + C content and GC-skew are 1000 nt.

Table 1. General features of the *Xanthomonas oryzae* pv. *oryzae* genome

Length (bp)	4 941 439
G + C content (%)	63.7
Protein coding genes	
With function assigned	3340
Conserved hypothetical	1151
Hypothetical	146
Total	4637
Transfer RNA	54
Ribosomal RNA operons	2
Plasmids	0
Insertion sequence element (IS)	207

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).

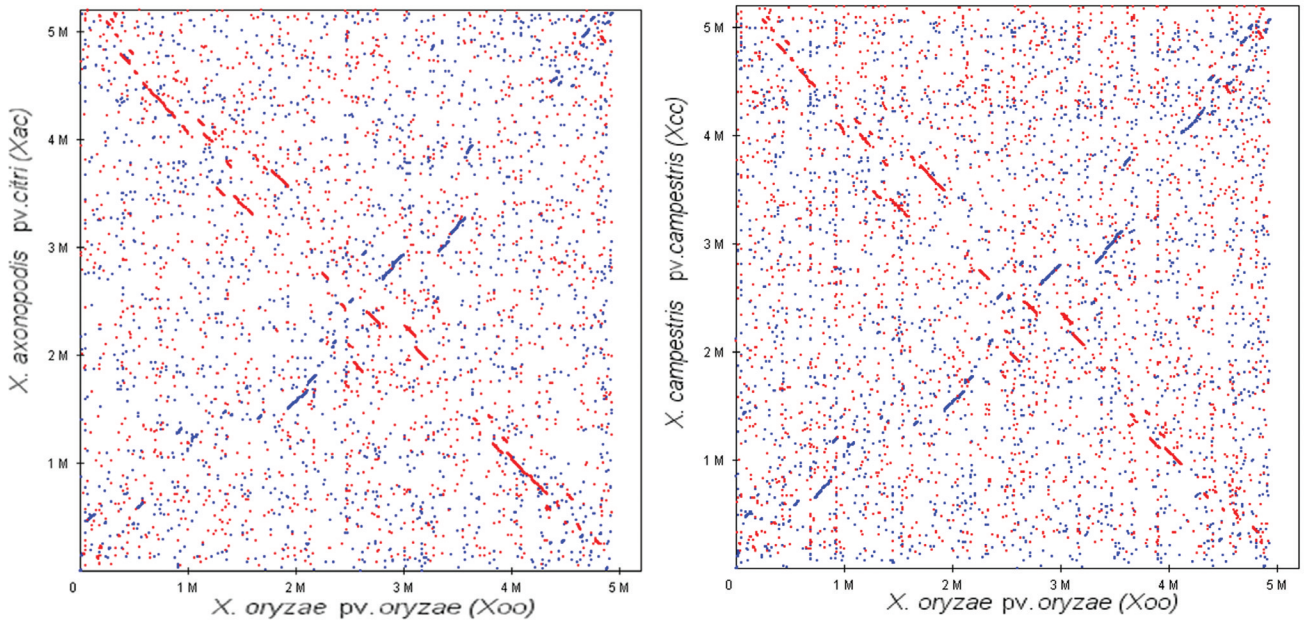

Figure 2. Nucleotide alignments of *Xoo* (*x*-axis) versus *Xac* (*y*-axis), left; and *Xoo* (*x*-axis) versus *Xcc* (*y*-axis), right. Each point in the plot corresponds to an MUM of ⩾25 bp.

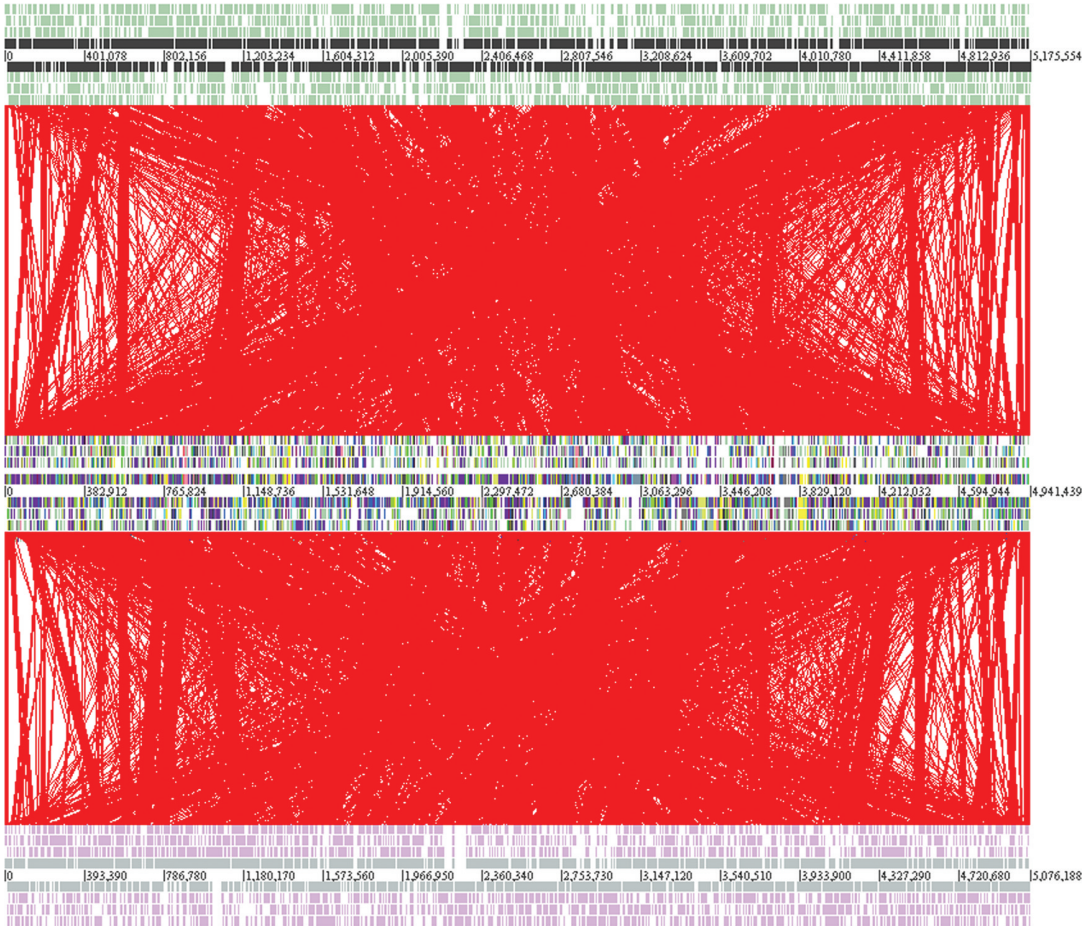

Figure 3. Linear genomic comparisons of *X.oryzae* pv. *oryzae* with *X.axonopodis* pv. *citri* and *X.campestris* pv. *campestris*. Top, *Xac*; middle, *Xoo*; bottom, *Xcc*. The colored ticks represent the reading frames from top to bottom; +1 frame, +2 frame, +3 frame, a whole forward frame, a whole 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, ISNCY 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 *orf*8, which is predicted to encode a phage-related tail fiber protein and five hypothetical proteins between the *int* and *orf*37 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 *A.tumefaciens*, *Erwinia carotovora* and *R.solanacearum,* have quorum sensing mechanisms similar to that of the *LuxR*/*LuxI* system from *Vibrio fischeri*, 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 *Xor*I and *Xor*II. 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 of glucose, two subunits of mannose 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, *gumBCDEFGHIJKLMN,* which was similar to the *gum* operon of *Xcc* except for the existence of *gumN* in the *Xoo* genome.

Three distinct genes, *wxo*D (O-antigen acetylase), *oma* (outer membrane antigen) and *rbf*C (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-L-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 glycine-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 phenylacetic 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 found 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 extracellular enzymes and is required for virulence of many phytopathogens to their host plants (59). Cellualse, 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 lyase), four xylanases, six xylosidases and one 1,4-β-cellobiosidase. *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 (*xpsEFGHIJKLMN* 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* (66). 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 (*rpfABFCGDIE*) 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 effect 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

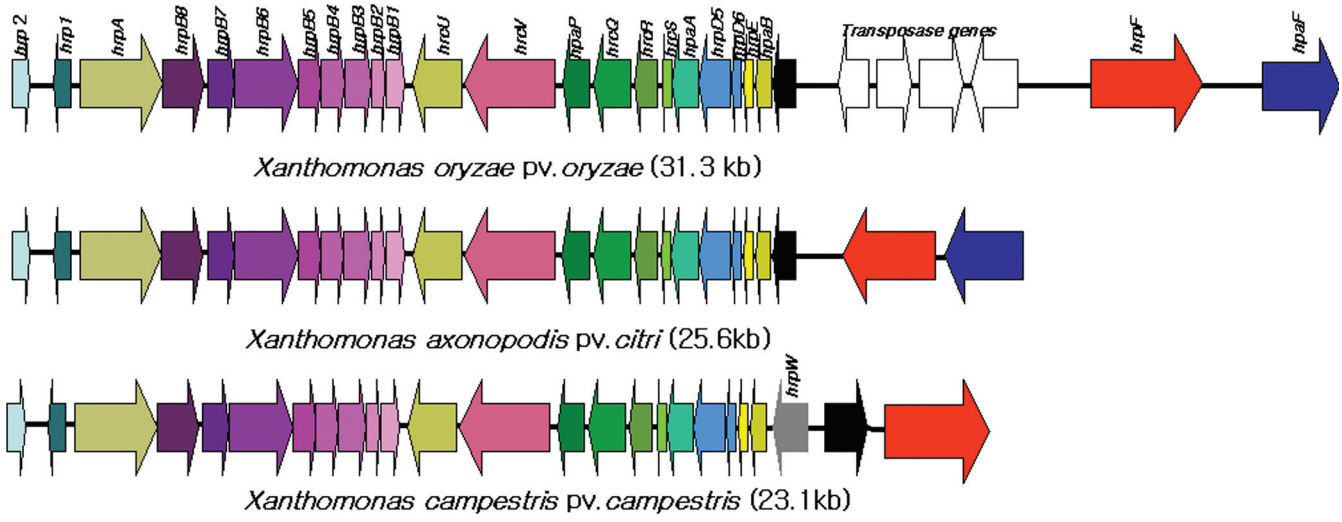

Figure 4. Comparisons of the *hrp* gene cluster of the three *Xanthomonas* species.

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 ($TTCGN_{15}TTCG$) in their promoters (75). Fourteen copies of a similar sequence, $TTCGN_{16}TTCGn$, 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*/*pthA* 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 *avrBs*2 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	*hrcQ*
80817	1995	XOO0085	*hrcU*
80734	83	XOO0086	*hrpB1*
89672	137	XOO0095	*hpa1*
89740	125	XOO0096	*hpa2*
Extended *Hrp* conserved regulon			
4666123	62	XOO4391	*Xanthomonas* conserved hypothetical
3186454	542	XOO2979	Conserved hypothetical
3070661	205	XOO2861	β-ketoadipate enol-lactone hydrolase
2856630	1972	XOO2699	Polygalacturonase
3352034	245	XOO3122	Conserved hypothetical
4231305	2058	XOO3959	Endopolygalacturonase
4611239	927	XOO4332	2-K-3-DdG permease
115257	10 270	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

Table 3. Putative effector/avirulence genes of *Xoo*, *Xac* and *Xcc*

Gene ID	Name	Family	*Xoo*/*Xac*/*Xcc*	PIP box	Location
XOO0168/XAC0076/XCC0052	*avrBs2*	*avrBs2*	Y/Y/Y	Y/Y/Y	C/C/C
XAC0286/XCC1629	*avrXccE1*/*avrXacE1*	*avrPphE*	N/Y/Y	-/Y/Y	-/C/C
XAC3224	*avrXacE2*	*avrPphE*	N/Y/N	-/N/-	-/C/-
XACb0011	*avrXacE3*	*avrPphE*	N/Y/N	-/Y/-	-/P/-
XOO2131/XACa0022	*pthA1*	*avrBs3*	Y/Y/N	N/N/-	C/P/-
XOO3013/XACa0039	*pthA2*	*avrBs3*	Y/Y/N	N/N/-	C/P/-
XOO3015/XACb0015	*pthA3*	*avrBs3*	Y/Y/N	N/N/-	C/P/-
XOO2275/XACb0065	*pthA4*	*avrBs3*	Y/Y/N	N/N/-	C/P/-
XCC2100	*avrBs1*	*avrBs1*	N/N/Y	-/-/N	-/-/C
XCC2099	*avrBs1.1*	*avrBs1*	N/N/Y	-/-/N	-/-/C
XCC2109	*avrXccC*	*avrC*	N/N/Y	-/-/Y	-/-/C
XCC3731	*avrXccB*	*yopJ*	N/N/Y	-/-/Y	-/-/C
XCC4229	*avrXccA1*	*avrXca*	N/N/Y	-/-/N	-/-/C
XCC2396	*avrXccA2*	*avrXca*	N/N/Y	-/-/N	-/-/C
XOO1762/XAC3090/XCC4186	Leucine-rich protein	*popC*	Y/Y/Y	Y/N/Y	C/C/C
XOO0065/XAC0393	*hpaF*	*popC*	Y/Y/N	Y/N/-	C/C/-
XCC2565	Leucine-rich protein	*popC*	N/N/Y	-/-/Y	-/-/C
XOO1239,XOO4256/XAC0571	Conserved hypothetical protein	*avrBs3*	Y/Y/N	N,N/N/-	C/C/-
XOO4255	*avrXa7*	*avrBs3*	Y/N/N	N/-/-	C/-/-

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 pathogenecity 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.

ACKNOWLEDGEMENTS

We thank Dr S. H. Choi for kindly providing the strain of *Xoo*, and Prof. J. E. Leach at Colorado State University and Prof. S. W. Hutcheson at University of Maryland for stimulating discussions and proofreading of the manuscript. Project funding was from the BioGreen21 Foundation under Rural Development Administration, Korea. Funding to pay the Open Access publication charges for this article was provided by National Institute of Agricultural Biotechnology.

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 Sonti,R.V. (2000) Mutants of *Xanthomonas oryzae* deficient in general secretory pathway are virulent deficient and unable to secrete xylase. *Mol. Plant Microbe Interact.*, **13**, 394–401.
6. Dharmapuri,S. and Sonti,R.V. (1999) A transposon insertion in *gumG* 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) Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant Microbe Interact.*, **5**, 451–459.
10. 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.
11. 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.
12. 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.
13. 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 plants. *Mol. Plant Microbe Interact.*, **9**, 850–855.
14. Simpson,A.J., Reinach,F.C., Arruda,P., Abreu,F.A., Acencio,M., Alvarenga,R., Alves,L.M., Araya,J.E., Baia,G.S., Baptista,C.S., Barros,M.H. *et al.* (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature*, **406**, 151–157.
15. Buell,C.R., Joardar,V., Lindeberg,M., Selengut,J., Paulsen,I.T., Gwinn,M.L., Dodson,R.J. *et al.* (2003) The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl Acad. Sci.*, **100**, 10181–10186.
16. da Silva,A.C.R., Ferro,J.A., Reinach,F.C., Farah,C.S., Furlan,L.R., Quaggio,R.B., Monteiro-Vitorello,C.B., Van Sluys,M.A., Almeida,N.F., Jr, Alves,L.M.C. *et al.* (2002) Comparison of genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*, **417**, 459–463.
17. Salanoubat,M., Genin,S., Artiguenave,F., Gouzy,J., Mangenot,S., Arlat,M., Billault,A., Brottier,P., Camus,J.C., Cattolico,L. *et al.* (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, **415**, 497–502.
18. Wood,D.W., Setubal,J.C., Kaul,R., Monks,D.E., Kitajima,J.P., Okura,V.K., Zhou,Y., Chen,L., Wood,G.E., Almeida,N.F., Jr *et al.* (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science*, **294**, 2317–2323.
19. Lee,S.W., Choi,S.H., Han,S.S., Lee,D.G. and Lee,B.Y. (1999) Distribution of *Xanthomonas oryzae* pv. *oryzae* strains virulent to *Xa21* in Korea. *Phytopathology*, **89**, 928–933.
20. Wilson,R.K. and Mardis,E. (1997) Shotgun sequencing. In Birren,B., Green,E.D., Klapholz,S., Myers,R.M. and Roskams,J. (eds), *Genome Anlysis: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. I, pp. 397–454.
21. Delcher,A.L., Harmon,D., Kasif,S., White,O. and Salzberg,S.L. (1999) Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.*, **27**, 4636–4641.
22. Borodovsky,M. and McIninch,J. (1993) GeneMark: parallel gene recognition for both DNA strands. *Computers Chemistry*, **17**, 123–133.
23. Lowe,T.M. and Eddy,S.R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.*, **25**, 955–964.
24. Ogata,H., Goto,S., Sato,K., Fujibuchi,W., Bono,H. and Kanehisa,M. (1999) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.*, **27**, 29–34.
25. da Silva,F.G., Shen,Y., Dardick,C., Burdman,S., Yadav,R.C., de Leon,A.L. and Ronald,P.C. (2004) Bacterial genes involved in type I secretion and sulfation are required to elicit the rice *Xa21*-mediated innate immune response. *Mol. Plant Microbe Interact.*, **17**, 593–601.
26. Neson,R.J., Baraoidan,M.R., Vera Cruz,C.M., Yap,I.V., Leach,J.E., Mew,T.W. and Leung,H. (1994) Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.*, **60**, 3275–3283.
27. Zhu,W., Magbanua,M.M. and White,F.F. (2000) Identification of two novel *hrp*-associated genes in the *hrp* gene cluster of *Xanthomonas oryzae* pv. *oryzae*. *J. Bacteriol.*, **182**, 1844–1853.
28. Goel,A.K., Rajagopal,L., Nagesh,N. and Sonti,R.V. (2002) Genetic locus encoding functions involved in biosynthesis and outer membrane localization of xanthomonadin in *Xanthomonas oryzae* pv. *oryzae*. *J. Bacteriol.*, **184**, 3539–3548.
29. Rajeshwari,R. and Sonti,R.V. (2000) Stationary-phase variation due to transposition of novel insertion elements in *Xanthomonas oryzae* pv. *oryzae*. *J. Bacteriol.*, **182**, 4797–4802.
30. Leach,J.E., White,F.F., Rhoads,M.L. and Leung,H. (1990) A repetitive DNA sequence differentiates *Xanthomonas campestris* pv. *oryzae* from other pathovars of *X.campestris*. *Mol. Plant Microbe Interact.*, **3**, 238–246.
31. Birkenbihl,R.P. and Vielmetter,W. (1989) Complete maps of IS1, IS2, IS3, IS4, IS5, IS30 and IS150 locations in *Escherichia coli* K12. *Mol. Gen. Genet.*, **220**, 147–153.
32. Matsutani,S., Ohtsubo,H., Maeda,Y. and Ohtsubo,E. (1987) Isolation and characterization of IS elements repeated in the bacterial chromosome. *J. Mol. Biol.*, **196**, 445–455.
33. Chen,J.H., Hsieh,Y.Y., Hsiau,S.L., Lo,T.C. and Shau,C.C. (1999) Characterization of insertion of IS476 and tow newly identified insertion sequences, IS1478 and IS 1479, in *Xanthomonas campestris* pathovar. *campestris*. *J. Bacteriol.*, **181**, 1220–1228.
34. Kim,J.F., Charkowski,A.O., Alfano,J.R., Collmer,A. and Beer,S.V. (1998) Sequences related to transposable elements and bacteriophages flank avirulence genes of *Pseudomonas syringae*. *Mol. Plant Microbe Interact.*, **11**, 1247–1251.
35. Krylov,V.N. (2003) Role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria. *Genetika*, **39**, 595–620.
36. Ezuka,A. and Kaku,H. (2000) A historical review of bacterial blight of rice. *Bull. Natl. Inst. Agrobiol. Resour., Japan*, **15**, 61–74.
37. Von Bodman,S.B., Bauer,W.D. and Coplin,D. (2003) Quorum sensing in plant pathogenic bacteria. *Annu. Rev. Phytopathol.*, **41**, 455–482.
38. Fuqua,C. and Eberhard,A. (1999) Signal generation in autoinduction systems: synthesis of acyllated homoserine lactones by LuxI-type proteins. In Dunny,G.M. and Winans,S.C. (eds), *Cell–Cell Signaling in Bacteria*. ASM Press, Washinton, DC, pp. 211–230.
39. Whitehead,N.A., Barnard,A.M.L., Slater,H., Simpson,N.J.L. and Salmond,G.P.C. (2001) Quorum-sensing in gram-negative bacteria. *FEMS Microbiol. Rev.*, **25**, 365–404.
40. Cha,C., Gao,P., Chen,Y.C., Shaw,P.D. and Farrand,S.K. (1988) Production of acyl-homoserine lactone quorum sensing signals by gram-negative plant-associated bacteria. *Mol. Plant Microbe Interact.*, **11**, 1119–1129.
41. Elasri,M., Delome,S., Lemanceau,P., Stewart,G., Laue,B., Glickmann,E., Oger,P.M and Dessaux,Y. (2001) Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soil borne *Pseudomonas* spp. *Appl. Environ. Microbiol.*, **67**, 1198–1209.
42. Choi,S.H. and Leach,J.E. (1994) Identification of the XorII methyltransferase gene and a vsr-homolog from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Gen. Genet.*, **244**, 383–390.
43. Wang,R.Y.H., Shedlarski,J.G., Farber,M.B., Kuebbing,D. and Ehrlich,M. (1980) Two sequence specific endonucleases from *Xanthomonas oryzae*. Characterization and unusual properties. *Biochim. Biophys. Acta*, **606**, 371–385.
44. Choi,S.H., Vera Cruz,C.M. and Leach,J.E. (1998) Distribution of *Xanthomonas oryzae* pv. *oryzae* DNA modification systems in Asia. *Appl. Environ. Microbiol.*, **64**, 1663–1668.
45. Katzen,F., Becker,A., Zorreguieta,A., Puhler,A. and Ielpi,L. (1996) Promoter analysis of the *Xanthomonas campestris* pv. *campestris gum* operon directing biosynthesis of the xanthan polysaccharide. *J. Bacteriol.*, **178**, 4313–4318.
46. Coplin,D.L. and Cook,D. (1990) Molecular genetics of extracelluar polysaccharide biosynthesis in vascular phytopathogenic bacteria. *Mol. Plant Microbe Interact.*, **3**, 271–279.

47. Vojnov,A.A., Zorreguieta,A., Dow,J.M., Daniels,M.J. and Dankert,M.A. (1998) Evidence for a role for the *gumB* and *gumC* gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*. *Microbiology*, **144**, 1487–1493.
48. Vorhölter,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 dTDP-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**, 768–777.
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 for pathogens of plants and animals. *Annu. Rev. Phytopathol*., **39**, 259–284.
53. Noel,L., Thieme,F., Nennstiel,D. and Bonas,U. (2001) cDNA-AFLP analysis unravels a genome-wide *hrpG*-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. Oresnik,I.J., 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. Hokuriku Natl Agric. Exp. Stn*., **30**, 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.,III (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*: relatedness to secretion systems of other gram negative bacteria. *Mol. Gen. Genet*., **229**, 357–364.
65. Hu,N.T., Hung,M.N., Chiou,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 cross 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**, 50–108.
71. Lindgren,P.B. (1997) The role of *hrp* genes during plant–bacterial interactions. *Annu. Rev. Phytopathol*., **35**, 129–152.
72. He,S.Y. (1998) Type β 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., Van den 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,F.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.J. (1996) Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria avrBs2*. *J. Bacteriol*., **178**, 4661–4669.
79. Mudgett,M.B., Chesnokova,O., Dahlbeck,D., Clark,E.T., Rossier,O., Bonas,U. and Staskawicz,B.J. (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. Gueneron,M., 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 *hrp*-secretion apparatus of *Ralstonia 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.