MOLECULAR PLANT PATHOLOGY (2006) 7(5), 303–324 DOI: 10.1111/J.1364-3703.2006.00344.X

Pathogen profile

Xanthomonas oryzae pathovars: model pathogens of a model crop

DAVID O. NIÑO-LIU[1], PAMELA C. RONALD[2] AND ADAM J. BOGDANOVE[1]*

[1]*Department of Plant Pathology, Iowa State University, Ames, IA 50011, USA*
[2]*Department of Plant Pathology, University of California, Davis, CA 95616, USA*

SUMMARY

Xanthomonas oryzae pv. oryzae and *Xanthomonas oryzae* pv. oryzicola cause bacterial blight and bacterial leaf streak of rice (*Oryza sativa*), which constrain production of this staple crop in much of Asia and parts of Africa. Tremendous progress has been made in characterizing the diseases and breeding for resistance. *X. oryzae* pv. oryzae causes bacterial blight by invading the vascular tissue, while *X. oryzae* pv. oryzicola causes bacterial leaf streak by colonizing the parenchyma. In rice there are 29 major genes for resistance to bacterial blight, but so far only a few quantitative resistance loci for bacterial leaf streak. Over 30 races of *X. oryzae* pv. oryzae have been reported. Both pathogens exhibit genetic variation among isolates. Mechanisms of pathogenesis and resistance have begun to be elucidated. Members of the AvrBs3/PthA family of transcription activator-like effectors play a major role in the virulence of *X. oryzae* pv. oryzae and possibly *X. oryzae* pv. oryzicola. Cloning of six rice resistance genes for bacterial blight and one from maize effective against bacterial leaf streak has uncovered a diversity of structure and function, some shared by genes involved in defence in animals. This article reviews research that spans a century. It also presents a perspective on challenges for sustainable control, and opportunities that interactions of *X. oryzae* pathovars with rice present as models for understanding fundamental aspects of bacterial pathogenesis of plants and plant disease resistance, as well as other aspects of plant and microbial biology, with implications also for animal innate immunity.

INTRODUCTION

Xanthomonas oryzae pv. oryzae (Xoo) and *Xanthomonas oryzae* pv. oryzicola (Xoc) cause bacterial blight (BB) and bacterial leaf streak (BLS) of rice (*Oryza sativa*), respectively. BB is one of the most serious diseases of rice, and BLS is emerging in importance. Rice is a worldwide staple as well as a model for cereal biology (Bennetzen and Ma, 2003; Ronald and Leung, 2002; Shimamoto and Kyozuka, 2002). Xoo and Xoc are important both from the standpoint of food security and as models for understanding fundamental aspects of bacterial interactions with plants. Despite the close relatedness of Xoo and Xoc (Ochiai and Kaku, 1999; Vauterin *et al.*, 1995), they infect their host in distinct ways. Genetic resistance to BB and BLS is also distinct. Both pathogens exhibit significant diversity among isolates. There is remarkable structural and functional diversity also among genes for resistance to these pathogens. This article presents a detailed review of these and other findings garnered from applied and fundamental studies of these pathogens and the diseases they cause that span nearly a century. It also presents a perspective on challenges for improved control of BB and BLS, and opportunities that the interactions of Xoo and Xoc with rice present for advances in understanding not only bacterial pathogenesis of plants and plant disease resistance, but also other aspects of plant and microbial biology, and innate immunity in animals.

* *Correspondence*: Tel.: +1 515 294 3421; Fax: +1 515 294 9420; E-mail: ajbog@iastate.edu

XANTHOMONAS ORYZAE PV. ORYZAE AND *X. ORYZAE* PV. ORYZICOLA

Discovery and classification

BB was first characterized in the Fukuoka Prefecture of Japan in 1884 (Fig. 1). It was originally believed to be caused by acidic soil (Tagami and Mizukami, 1962, as cited by Ou, 1972). In 1909, masses of bacteria were isolated from the (acidic) turbid dewdrops of infected rice leaves, and the disease was reproduced by inoculating healthy leaves with these dewdrops. Shortly thereafter its aetiology as a bacterial disease was established, and the causal agent was isolated and classified as *Bacillus oryzae* (Bokura, 1911, as cited by Mizukami and Wakimoto, 1969). The bacterium was renamed *Pseudomonas oryzae* and later *Xanthomonas oryzae* (Ishiyama, 1922). In 1978, it was reclassified as *X. campestris* pv. oryzae (Dye, 1978). BLS was first discovered in the Philippines in 1918 (Fig. 1) and named bacterial stripe (Reinking, 1918, as cited by Ou, 1972) although it was erroneously referred to as bacterial blight for several years. In a study in southern China in 1957, the

Fig. 1 Geographical distribution and symptoms of bacterial blight (BB) and bacterial leaf streak (BLS) of rice. (a) World map. Regions where BB and/or BLS are prevalent are highlighted, although bacterial BB has been reported also in South America. BB was discovered in the Fukuoka Prefecture of Japan (white star) in 1884 and BLS in the Philippines (yellow star) in 1918. BB is distributed in both temperate and tropical regions, but BLS is prevalent primarily in tropical regions. (b) Rice plot severely affected by BB. (c) Rice plants at the ripening phase showing BB symptoms. (d) Advanced BB. (e) Close-up of BB symptoms. (f) Pale-yellow leaf syndrome caused by the BB pathogen. (g) Seedling blight or 'kresek' syndrome caused by the BB pathogen. (h) BLS. (i) Close-up of BLS symptoms; droplets of bacterial exudate create a stippled appearance. Photos b and c courtesy of C. Vera Cruz. Photo d courtesy of V. Verdier. Photos e, f, g and i courtesy of T. Mew, reprinted from the *Compendium of Rice Diseases*, 1992, American Phytopathological Society, St. Paul, MN. Photo h courtesy of H. Kaku.

disease was again characterized as distinct from bacterial blight and called bacterial leaf streak. The causal agent was distinguished from that of bacterial blight and given the name *Xanthomonas oryzicola* (Fang *et al.* 1957, as cited by Ou, 1972). The pathogen was later renamed *X. translucens* f. sp. *oryzae* (Goto, 1964, as cited by Ou, 1972). It has also been referred to as *X. translucens* f. sp. *oryzicola* and *X. campestris* pv. *oryzicola* (Ou, 1985). In 1990 both pathogens were elevated to their current status as a new species and named *Xanthomonas oryzae* pv. oryzae and *X. oryzae* pv. oryzicola (Goto, 1992; Swings *et al.*, 1990). The species resides within the family Xanthomonadaceae in the Gammaproteobacteria.

Morphology and physiology

X. oryzae is a rod-shaped, round-ended, Gram-negative species. Individual cells vary in length from approximately 0.7 μm to 2.0 μm and in width from 0.4 μm to 0.7 μm. Cells are motile by means of a single polar flagellum. Colonies on solid media containing glucose are round, convex, mucoid and yellow in colour due to the production of the pigment xanthomonadin, characteristic of the genus (Bradbury, 1984). *X. oryzae* cells produce copious capsular extracellular polysaccharide (EPS). This EPS is important in the formation of droplets or strands of bacterial exudate from infected leaves, providing protection from dessication and aiding in wind- and rain-borne dispersal (Ou, 1972; Swings *et al.*, 1990) (Fig. 2).

X. oryzae is obligately aerobic and does not form spores. Optimal temperature for growth is between 25 and 30 °C. Like the genus as a whole, *X. oryzae* is catalase-positive, unable to reduce nitrate and a weak producer of acids from carbohydrates (Bradbury, 1984). Pathovars oryzae and oryzicola can be differentiated by (a) acetoin production (Xoo–, Xoc+), (b) growth on

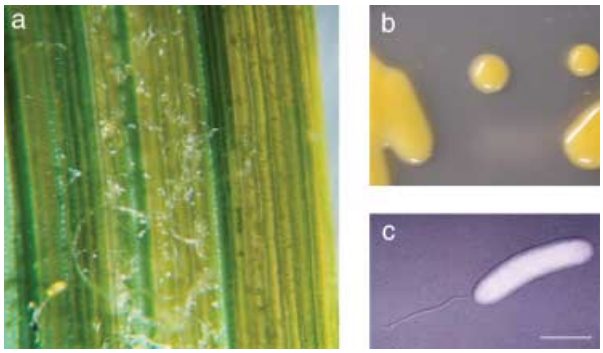


Fig. 2 Morphology of *Xanthomonas oryzae*. (a) Strands and condensed droplets of ooze consisting of *X. oryzae* pv. oryzicola cells coated in extracellular polysaccharide exuded on to the surface of an infected rice leaf. (b) Colonies of *X. oryzae*. pv. oryzae on glucose yeast extract agar. (c) Scanning electron micrograph of a single *X. oryzae* pv. oryzae cell (bar, 1.0 μm; courtesy of K. Tsuchiya).

L-alanine as sole carbon source (Xoo–, Xoc+), (c) growth on 0.2% vitamin-free casamino acids (Xoo–, Xoc+) and (d) resistance to 0.001% $Cu(NO_3)_2$ (Xoo+, Xoc–) (Gossele *et al.*, 1985).

BACTERIAL LEAF BLIGHT AND BACTERIAL LEAF STREAK

Distribution and impact

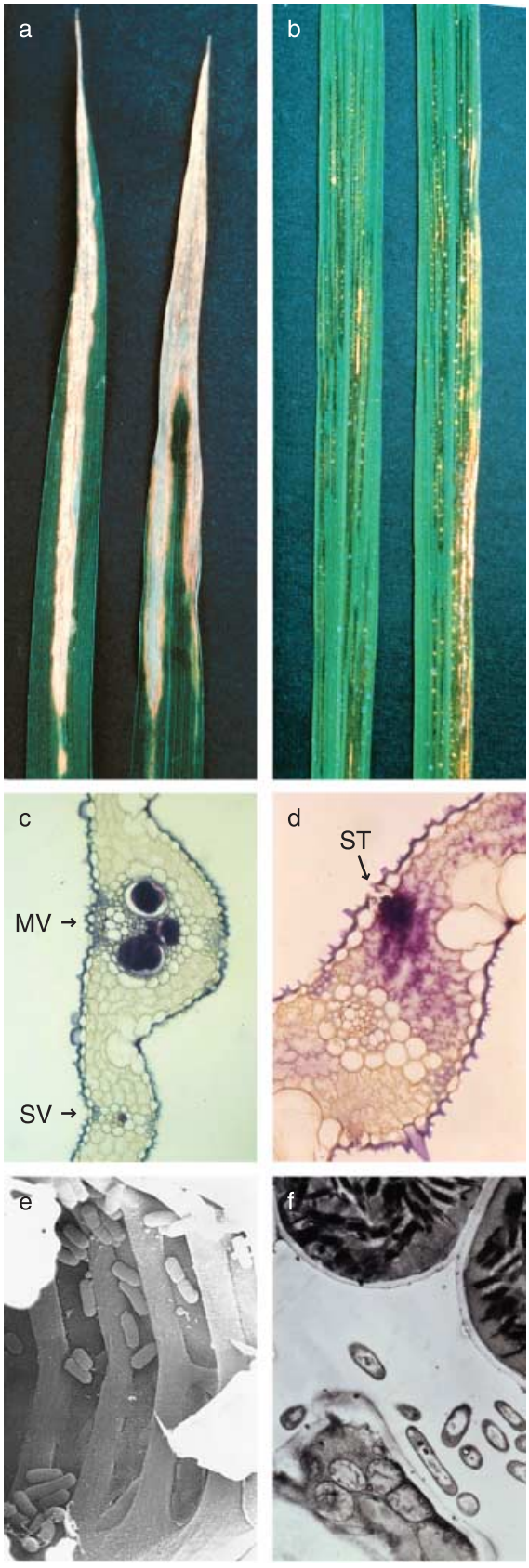
BB and BLS are endemic to much of Asia and parts of West Africa. BB is prevalent in both tropical and temperate areas, and has also been reported in Australia, Latin America and the Caribbean (Mew *et al.*, 1993). By contrast, BLS is restricted largely to tropical and subtropical Asia, including southern China, Thailand, Malaysia, India, Viet Nam, the Philippines and Indonesia, but it also affects rice-growing regions of northern Australia (Awoderu *et al.*, 1991; Moffett and Croft, 1983; Ou, 1985; Sigee, 1993; Singh *et al.*, 1980) and recently has become a significant problem in parts of West Africa (J. Notteghem, personal communication). In the United States, although an apparent mild outbreak of BB was reported in the late 1980s (Jones *et al.*, 1989), it was later determined that the bacterium associated with the disease was not Xoo (Ryba-White *et al.*, 1995). Quarantines for Xoo and Xoc are in place in the United States and other rice-growing countries where the diseases are not endemic, but also in places where they are present, to prevent the introduction of new virulent strains. Xoc is listed in the 2002 Agricultural Bioterrorism Protection Act of the USA as a potential bioterrorism agent, necessitating strict biosecurity measures (to limit access to pathogen stock cultures in research laboratories) in addition to standard biosafety measures (to prevent release of the pathogen into the environment). Implementation of biosecurity measures is an unfortunate necessity that somewhat restricts US research on this important pathogen.

Damage due to BB increased significantly following the widespread cultivation of high-yielding and nitrogen-responsive dwarf hybrid varieties of rice in the 1960s. Prior to the more recent incorporation of resistant varieties and implementation of strict quarantine measures in Japan, BB damage there was reported to range from 20 to 30% and as high as 50% (Ou, 1972). In tropical countries BB is even more destructive. Reports from the Philippines, Indonesia and India estimate that losses due to the kresek syndrome of BB, which affects recently transplanted seedlings, have reached 60–75%, depending on weather, location and rice variety (Ou, 1985; Reddy *et al.*, 1979). In addition to reducing yield, BB may also affect grain quality by interfering with maturation (Goto, 1992; Ou, 1985).

Although documentation does not exist for many areas in which BLS is present, available reports suggest that yield losses due to this disease typically range from 0 to 20% depending on the rice variety and climatic conditions (Ou, 1985). Without strong wind and rain, secondary spread of BLS is limited and the effect of the disease dwindles rapidly as the growth of new leaves compensates for damage to infected leaves (Ou, 1985). Under conditions favourable for spread, however, BLS may affect entire fields and cause damage comparable with BB, e.g. reductions in grain weight of up to 32% (Ou, 1985). Overall, however, BLS is less economically important than BB. Nevertheless, BLS is increasing in significance in parts of Asia where hybrid rice varieties are grown, such as China, as these varieties can be particularly susceptible to the pathogen (Xie *et al.*, 1990) (Z. Qi and Q. Zhang, personal communication).

Pathogen modes of infection, symptoms, and signs

Xoo enters the rice leaf typically through hydathodes at the leaf tip and leaf margin (Ou, 1985). Cells on the leaf surface may become suspended in guttation fluid as it exudes at night and enter the plant by swimming, or passively as the fluid is withdrawn into the leaf in the morning (Curtis, 1943). Bacteria multiply in the intercellular spaces of the underlying epitheme, then enter and spread into the plant through the xylem (Noda and Kaku, 1999) (Fig. 3). Xoo may also gain access to the xylem through wounds or openings caused by emerging roots at the base of the leaf sheath (Ou, 1985). Within the xylem, Xoo presumably interacts with xylem parenchyma cells (Hilaire *et al.*, 2001) (Fig. 3). The pathogen moves vertically through the leaf through primary veins but also progresses laterally through commissural veins. Within a few days bacterial cells and EPS fill the xylem vessels and ooze out from hydathodes, forming beads or strands of exudate on the leaf surface, a characteristic sign of the disease and a source of secondary inoculum (Mew *et al.*, 1993). Xoc, by contrast, penetrates the leaf mainly through stomata, multiplies in the substomatal cavity and then colonizes the intercellular spaces of the parenchyma (Ou, 1985) (Fig. 3). Like Xoo,



Xoc may also gain access through wounds, but it remains restricted to the apoplast of the mesophyll tissue and does not invade the xylem (Ou, 1985). Xoc also exudes from natural openings in the leaf in chains or strands, or under moist conditions as small beads of ooze. Yellow exudate on the leaf surface is a typical sign of BLS, and as is the case for Xoo, it may fall into irrigation water or be dispersed by wind, rain, insects or other means, and contribute to spread of the disease (Mew *et al.*, 1993; Nyvall, 1999).

BB and BLS symptoms are easily differentiated in the early stages of disease and reflect the different modes of infection by each pathogen. Foliar symptoms of BB usually become evident at the tillering stage as small, green water-soaked spots at the tips and margins of fully developed leaves. The spots expand along the veins, merge, and become chlorotic and then necrotic, forming opaque, white to grey coloured lesions that typically extend from the leaf tip down along the leaf veins and margins (Fig. 3). BLS symptoms, by contrast, begin with small, water-soaked lesions anywhere along the leaf between the veins. Veins act as barriers as infected areas expand and coalesce lengthwise, resulting in the symptom for which the disease is named. Streaks are translucent and typically yellow (Fig. 3). At later stages infected leaves turn greyish white and die. When infection results from entry through breaks in the leaf as might occur due to high wind, symptoms may extend across the leaf break and expand lengthwise killing most or all of the leaf. As both diseases progress their symptoms become less differentiable. Leaf blades may wilt and roll as they dry up and die. Leaves with BLS may also turn white or grey from the growth of opportunistic or saprophytic fungi, and thus resemble BB. Depending on the growth conditions or the degree of resistance of the cultivars, BB and BLS may be confused with each other or with unrelated physiological disorders of the plant. Xoo and Xoc often occur in rice fields simultaneously, and individual leaves may show symptoms of both diseases (Goto, 1992; Mew *et al.*, 1993) (R. Sonti, personal communication).

Fig. 3 Host tissue specificity of *Xanthomonas oryzae* pathovars. (a) Rice leaves with bacterial blight lesions extending along the veins from the leaf tip and margins, resulting from invasion of the xylem via hydathodes by *X. oryzae* pv. oryzae. (b) Rice leaves with leaf streak symptoms caused by colonization of the interveinal tissue via stomata by *X. oryzae* pv. oryzicola. (c) Cross-section of a rice leaf infected with *X. oryzae* pv. oryzae, stained to show the location of bacterial cells, apparent as dark blue to purple colour within the xylem vessels of the midvein (MV) and a secondary vein (SV). (d) Cross-section of a rice leaf infected with *X. oryzae* pv. oryzicola stained as in (c), showing bacterial colonization of the substomatal chamber and the mesophyll parenchyma. ST, stoma. (e) Scanning electron micrograph of *X. oryzae* pv. oryzae cells in a xylem vessel of a rice leaf. (f) Transmission electron micrograph of *X. oryzae* pv. oryzicola cells in the intercellular spaces of the mesophyll parenchyma of a rice leaf. Photos a and b courtesy of T. Mew, reprinted from the *Compendium of Rice Diseases*, 1992, American Phytopathological Society, St. Paul, MN. Photos c, d and f courtesy of H. Kaku. Photo e courtesy of J. Leach.

In the tropics, and particularly on susceptible cultivars of *O. sativa* ssp. *indica*, Xoo causes two disease syndromes with symptoms distinct from typical bacterial blight: kresek and pale-yellow leaf (Nyvall, 1999). Kresek is a seedling blight that occurs shortly after transplant from nurseries to the field. The common practice of cutting leaf tips before transplanting plays an important role in the development of the syndrome. Cut leaves serve as an infection court for the pathogen, and after a few days, water-soaked spots develop just beneath the cut tips. In addition, broken roots resulting from pulling seedlings off the seedbed serve as entry points for bacteria present in flood-irrigated fields. Bacteria spread through the vascular system to the growing point of the plant, infecting the base of other leaves, and killing entire plants in 2–3 weeks. Plants that survive kresek suffer arrested tiller growth, a stunted appearance and an overall yellowish-green colour (Goto, 1992; Nyvall, 1999; Ou, 1985). Pale-yellow leaf is observed in older plants and is sometimes considered a secondary effect of seedling leaf blight and wilt. Whereas older leaves appear green and healthy, younger leaves are uniformly pale yellow or whitish, and tillers do not grow fully (Mew *et al.*, 1993; Ou, 1985).

Sources of primary inoculum, dissemination and survival

Outbreaks of both BB and BLS are more likely to occur during the monsoon season of the south-east Asian and Indian oceans (from June to September) than at other times of the year (Mew *et al.*, 1993). Wind and rain disseminate bacteria from infected rice plants and other hosts, as well as contaminated rice stubble from previous crop seasons—the most important sources of primary inoculum. Severe epidemics often occur following typhoons, the fierce winds, wind-blown rain and hail of which both wound rice plants and disperse bacteria. Bacteria may also be disseminated in irrigation water (Nyvall, 1999), as well as by humans, insects and birds (Nyvall, 1999; Ou, 1985).

Other hosts of Xoo include several species of wild rice (*O. sativa*, *O. rufipogon*, *O. australiensis*) and a number of gramineous weeds (*Leersia oryzoides* and *Zizania latifolia* in temperate regions and *Leptochloa* spp. and *Cyperus* spp. in the tropics). Virtually all species of wild rice can serve as hosts for Xoc (Moffett and Croft, 1983), but other alternative hosts have not been identified. In a study conducted at the International Rice Research Institute (IRRI) in the late 1960s, none of several gramineous weeds and crop species inoculated with Xoc developed disease (Ou, 1985).

In temperate regions, Xoo can survive the winter in the rhizosphere of weeds of the genera *Leersia* and *Zizania* as well as in the base of the stem and the roots of rice stubble (Mizukami and Wakimoto, 1969). In addition, in temperate regions, Xoo can survive in the soil for 1–3 months depending on the soil moisture and acidity, though this is not considered an important source of inoculum (Ou, 1985). Xoo can overwinter in piled straw as well; this source of inoculum may acquire importance in areas where little or no weedy hosts occur (Ou, 1985). In the tropics, high temperature, humidity and an abundance of host plants typically allow Xoc, and Xoo, to persist throughout the year (Ou, 1985). Both pathovars can be isolated easily from seed of infected plants (Sakthivel *et al.*, 2001; Xie and Mew, 1998). Nevertheless, controversy exists over how long bacteria can survive in stored grain and whether seed-borne transmission is important.

Control

Control measures for BB include cultural practices, chemical and biological control, disease forecasting, and, most importantly, host genetic resistance, typically major gene resistance. Few studies have been conducted on control methods for BLS, although many of the same measures used for BB can be expected to be effective against BLS. As is the case for BB, in practice, host genetic resistance is the most important control measure for BLS, although it is so far limited to quantitative resistance (Gnanamanickam *et al.*, 1999; Sheng *et al.*, 2005; Tang *et al.*, 2000).

Cultural practices useful for BB control vary depending on the location and disease incidence records. At the nursery stage, methods include seed disinfection (see below), proper nursery drainage, and removal of diseased plants, weeds and debris. Prior to transplanting, fields may be disinfected by burning rice straw left from the previous season. Weeds are removed from canals and ridges in order to reduce natural habitats for the pathogen and its dispersal through irrigation water. At the paddy field stage, judicious fertilization and proper plant spacing are the most recommended cultural methods of control (Goto, 1992; Mizukami and Wakimoto, 1969). Fertilization must avoid an excess of nitrogen as it stimulates rapid vegetative growth of the plant, which favours disease development. Application of fertilizers rich in potassium and phosphorus, as well as application of agrochemicals at the maximum tillering to booting stages or after a typhoon or a severe flood are common practices (Goto, 1992; Ho and Lim, 1979; Mizukami and Wakimoto, 1969).

Chemical control of BB in rice fields began in the 1950s with the preventative application of Bordeaux mixture (hydrated lime and copper sulfate) and the testing of several antibiotics, mercuric and copper compounds. Laboratory tests determined that streptomycin derivatives and mercuric compounds were most effective, but they were found to damage rice grains when sprayed at the heading stage in the field (Mizukami and Wakimoto, 1969). In the 1960s, different kinds of agrochemicals were developed from repeated field trials and made available on a large commercial scale, mostly in Japan. They were based on L-chloramphenicol, nickel-dimethyldithiocarbamate, dithianon and fentiazon. Most were unreliable, however, owing to variability

in sensitivity among the pathogen population (Gnanamanickam *et al.*, 1999; Mizukami and Wakimoto, 1969; Ou, 1973). Although seed transmission of the disease is an uncertain source of primary inoculum, disinfection of rice seeds with mercuric compounds, antibiotic solutions or hot water is practised in several countries in tropical Asia. In temperate regions, chemical control of BB in nurseries and paddy fields includes the application of probenazole to the paddy water before and after transplanting the seedlings, in order to inhibit bacterial multiplication and prevent or retard the disease. Other chemicals such as tecloftalam, phenazine oxide and nickel dimethyldithiocarbamate are sprayed directly on plants (Goto, 1992; Mizukami and Wakimoto, 1969). However, chemical control of BB in the tropical monsoon climate of Asia is impractical, and no truly effective bactericide is commercially available for disease control (Lee *et al.*, 2003; Ou, 1973).

Biological control is an environmentally friendly and cost-effective alternative to chemical control. Bacterial antagonists of Xoo have received particular attention as biocontrol candidates, largely because of their rapid growth, easy handling and effective colonization of the rhizosphere (Vasudevan *et al.*, 2002). In India, about 40 bacterial isolates antagonistic to Xoo were identified through plate and field assays. Among those antagonists native strains of the rice-associated rhizobacteria *Pseudomonas fluorescens* and *P. putida* strain V14i (also used in biocontrol of the rice sheath blight pathogen *Rhizoctonia solani*) significantly suppressed BB severity when sprayed on leaves (Sivamani *et al.*, 1987). For both agents, there was a significant correlation between endophytic survival in rice tissues and the extent of disease suppression (Gnanamanickam *et al.*, 1999; Johri *et al.*, 2003). Similarly, different species of *Bacillus* have been employed as seed treatment before sowing, root dips prior to transplanting and foliar sprays in the fields. In at least one study, BB was suppressed by almost 60%, and plant height and grain yield increased by two-fold (Vasudevan *et al.*, 2002). Although the mechanisms of BB suppression are not known, a recent investigation of biocontrol of the rice sheath blight disease has suggested that a rice systemic resistance response to the agents may be involved, as has been observed in other systems (Vasudevan *et al.*, 2002). Despite promising results such as these, biological agents have not seen widespread use in the control of BB.

Forecasting of BB and BLS is difficult because epidemics are dependent on the rice cultivars and cultural practices in use, in addition to environmental and geographical conditions. Methods used for forecasting may include scouting for early disease development and tracking climatic conditions (Mizukami and Wakimoto, 1969). In temperate locations, monitoring of bacteriophage strains specific for Xoo has been used in forecasting since the 1960s. Under particular agroenvironmental conditions, an increment of bacteriophage population in irrigation water and paddy fields early in the planting season correlates well with an increase in bacterial populations and is used to predict BB outbreaks. However, the bacteriophage forecasting system is not practised extensively in tropical Asia, because rice cultivation is mostly rainfed, limiting the use of phage detection in paddy fields (Murty and Devadath, 1982; Wakimoto and Mew, 1979). In general also, disease forecasting has been of limited utility because chemical control is unavailable or impractical.

Breeding and deployment of resistant cultivars carrying major resistance (*R*) genes has been the most effective approach to controlling BB. To date, 29 *R* genes to BB have been identified (see Table 1 for details and references), mostly from *O. sativa* ssp. *indica* cultivars, but some also from *japonica* varieties, and from related wild species including *O. longistaminata*, *O. rufipogon*, *O. minuta* and *O. officinalis* (Brar and Khush, 1997; Lee *et al.*, 2003). In addition, several resistance genes or alleles have been produced by mutating cultivated rice lines, e.g. by treatment with *N*-methyl-N-nitrosourea or thermal neutron irradiation, or by somaclonal mutagenesis (Gao *et al.*, 2001; Lee *et al.*, 2003; Nakai *et al.*, 1988). Some *R* genes are effective only in adult plants (e.g. *Xa21*) whereas most do not seem to be developmentally regulated (e.g. *Xa23, Xa26*). Curiously, *Xa3* is typically effective only in adult plants, but against at least one race it is effective at all stages of growth. Some genes condition resistance to a wide spectrum of Xoo races (e.g. *Xa21, Xa23*), whereas others are effective against only one or a few races that may be limited to a particular geographical location (e.g. *Xa1*). Most *R* genes to BB are dominant, but some are recessive (e.g. *xa5, xa13*), and some display semidominance (e.g. *Xa27*). Among the handful of genes that have been cloned there is remarkable structural diversity (see below).

Most *R* genes to BB have been introgressed into the background of the susceptible *indica* cultivar IR24 to develop a set of near isogenic lines (NILs), and some have been pyramided, either through classical breeding and marker-assisted selection or through genetic engineering, to develop new plant types and NILs (Narayanan *et al.*, 2002; Sanchez *et al.*, 2000; Singh *et al.*, 2001). Pyramid lines have displayed higher levels and/or wider spectra of resistance to BB than the parental NILs with single *R* genes, suggesting synergism and complementation among *R* genes (Adhikari *et al.*, 1999a; Huang *et al.*, 1997; Narayanan *et al.*, 2002). With pyramid lines, it is possible to conduct quantitative analysis on the effect of each gene and their interactions, but most importantly, to maximize the performance and durability of genetic resistance.

Resistance of rice to specific Xoo races is governed by both major *R* genes with a qualitative effect that condition complete resistance (CR) and polygenes with a quantitative effect (quantitative trait loci, QTL) that condition partial resistance (PR) (Koch and Parlevliet, 1991b; Li *et al.*, 2006). A recent study of the epistatic effects between R genes and QTL for resistance in rice revealed a complex genetic network in which the interactions between alleles at the rice *R* loci and alleles at the corresponding

Table 1 Genes conferring resistance to the bacterial blight pathogen *Xanthomonas oryzae* pv. oryzae.

Gene	Chromosome*	Inheritance†	Source‡	Features	References
Xa1	4	D	Kogyoku	Cloned, NBS-LRR type; expression induced by wounding and bacterial inoculation; resistance to Japanese race I; ineffective against all Philippine races.	(Yoshimura *et al.*, 1998)
Xa2	4	D	Tetep	Linked to *Xa1*; possibly receptor-like kinase type; resistant to Japanese race II; susceptible to all Philippine races.	(He *et al.*, 2006; Oryzabase, 2006)
Xa3	11	D	Wase Aikoku 3	Other names: *Xa4b*, *Xa6*, *Xa9*, *Xaw*; resistance to Philippine races 1, 2, 4, 5 and all Japanese races at booting (adult) stage only; resistance to Philippine race 3 at all growth stages.	(Kaku and Ogawa, 2000; Ogawa *et al.*, 1986; Oryzabase, 2006; Qi and Mew, 1985; Sun *et al.*, 2004)
Xa4	11	D	TKM 6	Linked to *Xa26*; resistance to Philippine races 1, 4, 5, 7, 8, and 10; most widely used before defeat in 1970s	(Wang *et al.*, 2001)
xa5	5	r	Aus Boro lines (e.g. DZ192)	Cloned; encodes TFIIA gamma subunit; resistance to Philippine races 1, 2, 3, 5, 7, 8, 9, and 10.	(Iyer and McCouch, 2004)
Xa7	6	D	DV85	Resistance to Philippine race 1 at booting stage and to Philippine races 2, 3, 5, 7, 8, and 10 at all growth stages.	(Lee and Khush, 2000; Porter *et al.*, 2003)
xa8	7	r	PI231129	Resistance to Philippine races 5 and 8.	(Sidhu *et al.*, 1978; Singh *et al.*, 2002)
Xa10	11	D	Cas 209	Linked to *Xa4*; resistance to Philippine races 2, 5, and 7.	(Oryzabase, 2006; Yoshimura *et al.*, 1983)
Xa11	ND	D	IR8, IR944	Resistance to Japanese races IB, II, IIIA and V; ineffective against all Philippine races.	(Mew, 1987; Oryzabase, 2006)
Xa12	4	D	Kogyoku	Also known as *Xakg*; resistance to Japanese race V.	(Mew, 1987; Oryzabase, 2006)
xa13	8	r	BJ1 (Aus Boro)	Cloned; homologous with the *Medicago truncatula MtN3* gene; role in pollen development; resistance to Philippine race 6.	(Chu *et al.*, 2006)
Xa14	4	D	TN1	Resistance to Philippine races 5 and 8.	(Oryzabase, 2006)
xa15	ND	r	M41, a Harebare mutant line	Created by thermal neutron irradiation of seed; wide spectrum of resistance to Japanese races.	(Gnanamanickam *et al.*, 1999; Nakai *et al.*, 1988)
Xa16	ND	D	Tetep	Resistance to Japanese isolates H8581 and H8584.	(Oryzabase, 2006)
Xa17	ND	D	Asominori	Resistance to Japanese isolate H8513.	(Oryzabase, 2006)
Xa18	ND	D	IR24, Toyonishiki	Resistance to Burmese isolates BM8417 and BM8429; ineffective against all Philippine races.	(Liu *et al.*, 2004; Oryzabase, 2006)
xa19	ND	r	XM5	Created by mutagenesis (*N*-methyl-N-nitrosourea); resistance to Philippine races 1, 2, 3, 4, 5, and 6.	(Lee *et al.*, 2003; Oryzabase, 2006)
xa20	ND	r	XM6	Created by mutagenesis (*N*-methyl-N-nitrosourea); resistance to Philippine races 1, 2, 3, 4, 5, and 6.	(Lee *et al.*, 2003; Oryzabase, 2006)
Xa21	11	D	*O. longistaminata*	Cloned; receptor-like kinase type; developmentally regulated; resistance only at adult stage to Philippine races 1, 2, 3, 4, 5, 6, 7, 8 and 9.	(Song *et al.*, 1995)
Xa22	11	D	Zhachanglong	Linked to *Xa26*; broad spectrum resistance.	(Oryzabase, 2006; Sun *et al.*, 2004; Wang *et al.*, 2003)

Table 1 *continued.*

Gene	Chromosome*	Inheritance†	Source‡	Features	References
Xa23	11	D	*O. rufipogon*	Strong resistance at all growth stages to all Philippine races and most Japanese and Chinese races.	(Zhang *et al.*, 1998, 2001)
xa24	ND	r	DV86, DV85, Aus 295	Resistance to Philippine race 6.	(Khush and Angeles, 1999; Lee *et al.*, 2000)
Xa25(a)	4	D	HX-3, a somaclonal mutant of Minghui 63	Resistance to Philippine races 1, 3, and 4, and to several Chinese races.	(Gao *et al.*, 2001, 2005)
Xa25(b)	12	D	Minghui 63	Resistance to Philippine race 9.	(Chen *et al.*, 2002)
Xa26	11	D	Minghui 63	Cloned; receptor-like kinase; linked to *Xa4* and *Xa3*; broad-spectrum resistance to Philippine and Chinese races; affected quantitatively and qualitatively by genetic background of the host cultivar.	(Sun *et al.*, 2004; Yang *et al.*, 2003)
Xa27	6	SD	*O. minuta*	Cloned; no informative sequence similarities; resistance to Philippine races 2 and 5; involves induction by the pathogen; developmentally regulated or a dosage effect in the cv. CO39 genetic background.	(Gu *et al.*, 2004, 2005; Lee *et al.*, 2003)
xa28	ND	r	Lota Sail	Resistance to Philippine races 2 and 5.	(Lee *et al.*, 2003)
Xa29(t)	1	D	*O. officinalis*	*Xa29* is a tentative (t) designation; not fully characterized.	(Tan *et al.*, 2004)

*ND, not determined.

†D, dominant; r, recessive; SD, semidominant.

‡*Oryza sativa* cultivar (roman type), or *Oryza* species (italics).

avirulence loci in Xoo lead to CR, and interactions between rice QTL for resistance and corresponding aggressiveness loci in Xoo lead to PR. Race specificity of the QTLs during PR and strong genetic overlap between CR and PR suggested that PR is essentially a 'weaker' CR (Li *et al*., 2006).

The distinction between CR and PR may be masked by the fact that some QTLs for BB may in fact be 'defeated' dominant *R* genes, or, in a sense, *R* genes that have lost their qualitative nature and adopted new, intermediate phenotypes (Koch and Parlevliet, 1991a; Li *et al*., 1999). An example is *Xa4*, a single dominant gene for resistance to BB widely used in Asian rice breeding programmes. *Xa4* conferred durable resistance in cultivars IR20 and IR64, among others developed at IRRI, before being overcome by the emergence of two new Chinese races in the early 1970s (Mew *et al*., 1992). The breakdown of *Xa4*-mediated resistance was manifested by significant changes in the qualitative action of *Xa4* (i.e. loss of dominance) and by a quantitative reduction of ~50% in the magnitude of the effect of the *Xa4* gene (Li *et al*., 1999). However, the defeated *Xa4* can still act as a recessive QTL and show quantitative complementation when pyramided with other resistance genes in elite cultivar breeding. This complementation results partially from residual effects of the defeated *Xa4* against the new virulent Xoo races and partially from its epistatic/additive effects with undefeated *R* genes (Li *et al*., 2001; Narayanan *et al*., 2002).

Notably, despite some effort (Das, 1977; Rao *et al*., 1972), no major genes for resistance to BLS have been identified in rice. In contrast to resistance to BB, available genetic resistance to BLS in rice is quantitative (Tang *et al*., 2000). Breeding efforts have identified several QTLs conditioning resistance to BLS in *indica* rice. Such QTLs combined were able to explain almost 85% of the genetic variation, a promising prospect for the breeding of resistant elite rice cultivars (Sheng *et al*., 2005; Tang *et al*., 2000). Also of promise, a gene from maize that mediates defence responses to each of several tested Xoc strains was recently cloned and shown to provide resistance to BLS as a transgene in rice (Zhao *et al*., 2004b, 2005) (see below).

DIVERSITY AMONG STRAINS OF *X. ORYZAE* PATHOVARS

BB is characterized by a high degree of race–cultivar specificity. There are over 30 reported races of isolates from several countries (e.g. Adhikari *et al*., 1999b; Mew, 1987; Noda *et al*., 1996, 2001). A set of races identified in the Philippines using five differential rice cultivars (Mew, 1987) has been used widely for identifying and classifying resistance to BB in other cultivars (Lee *et al*., 2003; Ogawa *et al*., 1991) (Table 1). It has been noted, however, that screening for resistance to pathogen populations specific to particular geographical locations and tailoring regional breeding programmes accordingly are important (Mew, 1987).

Xoo also has a high degree of genetic diversity among different isolates, based on RFLP and pathotype analyses of more than 300 strains from different parts of Asia, using a repetitive insertion sequence (IS) element as the RFLP probe (Adhikari *et al*., 1995). In this study, isolates formed five clusters, each with more than one pathotype. Some correlation of clusters with geographical distribution and specific pathotypes (races) was observed, indicating that tailoring breeding programmes for specific regions is indeed a tenable approach to control, although there was also evidence of movement of strains among regions.

Xoc has not been studied as broadly, but analysis of strains isolated at different times and different locations in the Philippines showed a level of diversity similar to Xoo (Raymundo *et al*., 1999). Clusters of isolates were not robust, and there was no correlation between clusters and the reactions of a diverse set of rice varieties. A wide distribution of lineages over time and location was observed, suggesting movement and long-term survival. The lack of major gene resistance for Xoc may be a factor in the persistence of diverse genotypes.

PHYSIOLOGY AND MOLECULAR BIOLOGY OF BB AND BLS

In the past 15 years, research has shed light on the physiology of rice resistance to BB, and on the bacterial and host determinants of incompatibility, i.e. avirulence (*avr*) and *R* genes (Flor, 1971). More recently, mechanisms involved in pathogenesis have also begun to be unravelled. Considerably less has been elucidated regarding the physiology and molecular biology of BLS, but recent work is providing clues concerning both mechanisms of pathogenesis and the nature of host resistance to this disease. A graphical depiction of some of the molecular interactions between *X. oryzae* pathovars and rice is given in Fig. 4.

Physiology of rice resistance to bacterial blight

In the incompatible interaction between a race 2 strain of Xoo and plants carrying the *Xa10* gene, host cationic and anionic peroxidases accumulated in the lumen of xylem vessels (Reimers *et al*., 1992). The cationic peroxidase PO-C1 was shown to be secreted from xylem parenchyma cells into the lumen (Young *et al*., 1995), and its accumulation coincided with thickening of secondary cell walls. This thickening reduced the area of the pit membranes, through which the pathogen can come into contact with xylem parenchyma cells (Hilaire *et al*., 2001) (see Fig. 3e). Avirulent bacterial cells infiltrated into the parenchyma of rice leaves induced the accumulation of a lignin-like substance (Reimers and Leach, 1991). These physiological responses took place also in the compatible interaction with a virulent strain, but later and to a lesser extent (Hilaire *et al*., 2001; Reimers and

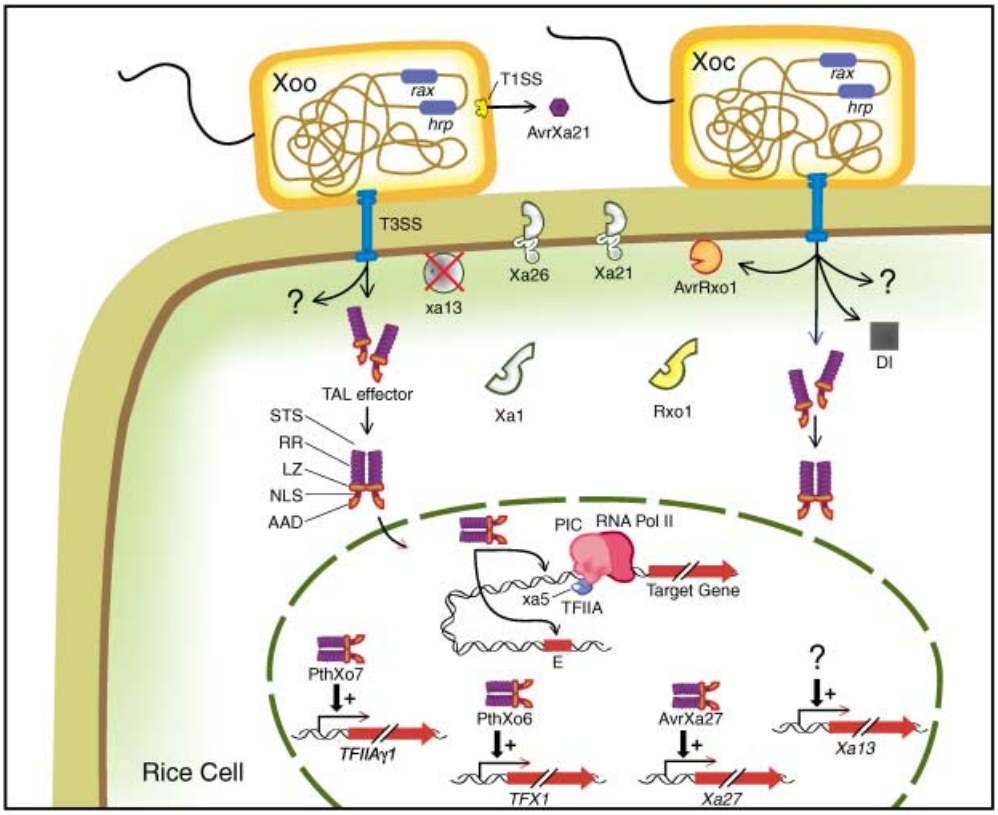


Fig. 4 Molecular interactions involving resistance genes and *Xanthomonas oryzae* effectors in bacterial blight and bacterial leaf streak of rice. Products of the cloned rice genes for resistance to bacterial blight, *Xa21*, *Xa26* (receptor-like kinases), *Xa1* (NBS-LRR protein), *xa5* (an allele of the TFIIA gamma subunit gene on chromosome 5), and *xa13* (a protein 307 amino acids in length of unknown structure), as well as *Rxo1* (NBS-LRR protein), a gene transferred from maize that provides resistance to bacterial leaf streak, localize to diverse parts of the plant cell. Xa21 is activated, presumably in the apoplast, by AvrXa21 of *X. oryzae* pv. oryzae (Xoo). AvrXa21 is a partially characterized molecule dependent on several pathogen *rax* (required for AvrXa21) genes. These include genes with putative roles in gene regulation, peptide sulfation, and the type I secretion system (T1SS). *rax* genes are highly conserved in *X. oryzae* pv. oryzicola (Xoc), but it is not known whether Xoc produces AvrXa21. *Xa21* does not provide resistance against bacterial leaf streak. Cognate avirulence signals for Xa1, Xa26 and xa5 have not yet been identified. Structure and subcellular localization of the product of the cloned *Xa27* resistance gene have not been deduced from its sequence, and the protein is not shown. *Xa27* differs from its susceptible allele only within the putative promoter region. Its expression is specifically induced by AvrXa27, one of several transcription activator-like (TAL) effectors delivered to the host cell by Xoo. Other TAL effectors with avirulence function include AvrXa7 and AvrXa10 (not shown) for which the cognate resistance genes have not been cloned. These and other TAL effectors also function in pathogenesis. All share several distinct structural features: N terminal secretion and translocation signals (STS), a central repeat region (RR) consisting of 5–25 repeats of typically 34 amino acids, a leucine zipper region (LZ), three tandem nuclear localization signals (NLS), and an acidic activation domain (AAD). Xoo TAL effectors PthXo7 and PthXo6 induce expression of *TFIIAγ1*, a paralogue on chromosome 1 of the TFIIA gamma subunit gene, and the *TFX1* transcription factor gene, respectively. These observations provide evidence that TAL effectors alter host gene expression individually as well as more globally by targeting genes encoding components of the host transcription machinery. Delivery of TAL effectors into the host cell depends on the pathogen type III secretion system (T3SS), encoded by *hrp* genes (for the resistance-associated hypersensitivity reaction and for pathogenesis). Inside the plant cell, TAL effectors dimerize and are transported to the nucleus. AvrXa7 binds double-stranded DNA, strengthening the supposition that TAL effectors contribute to pathogenesis by modulating expression of target genes directly, possibly by binding to the promoter region or to an upstream enhancer (E) region. The identity of xa5 suggests that this modulation involves interaction of TAL effectors with the transcriptional pre-initiation complex (PIC). This further suggests that xa5 may disable the virulence functions of TAL effectors by disrupting or modifying their interaction with the PIC. *xa13* provides resistance by being immune to modulation by Xoo. The dominant allele of this gene is induced upon pathogen attack, and this induction is required for disease to ensue. *xa13* remains expressed at only very low levels in leaves, suggesting that absence or low levels of the gene product (indicated by the red 'X') prevents pathogenesis. Induction of *Xa13* presumably is via the action of a specific type III-secreted Xoo effector. Xoc also depends on *hrp* gene-encoded type III secretion for pathogenesis, and it delivers TAL effectors, but this pathovar has not been observed to induce expression of the *TFIIAγ* paralogue or *TFX1*. With the exception of *Rxo1*, no genes for resistance to Xoc have been identified. This fact may be due to the T3SS-dependent ability of Xoc to inhibit resistance gene-mediated responses in rice via the action of one or more presumed type III-translocated defence inhibitors (DI), demonstrated so far for *Xa2*, *Xa7* and *Xa10*. The exception of *Rxo1* suggests the intriguing possibility that AvrRxo1, a membrane-localized, putative cysteine protease, is required for defence inhibition, and its action is precluded when it is recognized via Rxo1. See text for references and further discussion.

Leach, 1991). Phospholipase D, an enzyme involved in the generation of second messengers in signal transduction in animals, accumulated in the plasma membrane of rice cells undergoing either compatible or incompatible interactions, but was concentrated in membrane adjacent to bacterial cells in the incompatible interaction (Young *et al.*, 1996). Whether these responses are necessary or sufficient for resistance remains to be determined, but the results are consistent with their involvement.

Avirulence genes of *X. oryzae* pv. oryzae and bacterial blight resistance genes of rice

The first *avr* genes cloned from Xoo were *avrXa7* and *avrXa10* in the race 2 strain PXO86, corresponding to the *R* genes *Xa7* and *Xa10*, respectively (Hopkins *et al.*, 1992). These encode members of the large AvrBs3/PthA family of *Xanthomonas* effector proteins, which includes proteins with avirulence properties, virulence functions or both (for a review, see Schornack *et al.*, 2006). *X. oryzae* is unique among *Xanthomonas* species characterized so far in the abundance of members of this family present in individual strains. This is true of both pathovars (as detailed under Structural, comparative, and functional genomics of *X. oryzae*, below) (Hopkins *et al.*, 1992; Yang and White, 2004).

In Xoo, different members of the family contribute in a quantitatively different way to virulence (Bai *et al.*, 2000). In Xoo strains PXO86 and KXO85, *avrXa7* acts as a major virulence factor (Bai *et al.*, 2000; Yang and White, 2004). In strains lacking *avrXa7*, other members of the gene family play a dominant role (Yang and White, 2004). Mutations of *avrXa7* detected in field isolates virulent to *Xa7*-containing plants were associated with reductions in virulence on cultivars lacking *Xa7*, relative to isolates with the wild-type *avrXa7* gene. Lineages most aggressive on *Xa7*-containing plants did not persist in the population over 2 years. Based on these observations, it was suggested that relative durability of *R* genes could be predicted according to the fitness or virulence contribution of corresponding avirulence genes (Vera-Cruz *et al.*, 2000).

Members of the AvrBs3/PthA family share a highly conserved N-terminus involved in secretion from the bacterial cell and translocation into the plant cell, a central region of direct, near-perfect repeats, typically of 34 amino acids, and a C-terminal region containing a leucine zipper domain, functional nuclear localization signals (NLSs) and an acidic transcription activation domain (AAD) that functions in yeast and plant assays (Buttner and Bonas, 2002; Szurek *et al.*, 2002; Zhu *et al.*, 1998). The NLSs are required for avirulence activity of some but not all members of the family, indicating that different members are recognized differently (Ballvora *et al.*, 2001; Schornack *et al.*, 2006). The AAD as well as other sequences in the C-terminus determine R protein specificity for some members (Ishihara *et al.*, 2003; Yang and White, 2004; Yang *et al.*, 2005; Zhu *et al.*, 1998, 1999). For AvrBs3 and Avrxa7, the repeats, which vary in number among family members, have also been shown to contribute to their R protein specificity (Herbers *et al.*, 1992; Yang *et al.*, 2005). AvrXa7 was shown to bind double-stranded DNA (Yang *et al.*, 2000), and AvrBs3 to dimerize within the plant cell (Gurlebeck *et al.*, 2005), characteristics of many transcription factors. In light of these features, the AAD and recent evidence demonstrating specific changes in rice transcription due to members of this family (see below), it has been suggested calling these proteins transcription activator-like (TAL) effectors (F. White, personal communication), a name we adopt herein.

Xa7 and *Xa10* have not yet been cloned, but six other *R* genes for bacterial blight have, and they are diverse in predicted structure and subcellular localization. *Xa21*, the first cloned *R* gene for BB, is a member of a multigene family in rice (Song *et al.*, 1997) and of a larger family of genes in plants and animals involved in recognition of pathogens and innate immunity (Dardick and Ronald, 2006). The gene encodes a receptor-like kinase protein with a predicted extracellular, leucine-rich-repeat (LRR) domain, a transmembrane domain and a cytoplasmic kinase domain (Song *et al.*, 1995). Function but not expression of the gene is developmentally controlled (Century *et al.*, 1999): the gene is effective only in adult plants. This fact is apparently unrelated to the stability of the gene product, as the Xa21 protein is somewhat more stable in younger plants (Xu *et al.*, 2006). Autophosphorylation of residues in the kinase domain stabilizes the protein, but there is conflicting evidence regarding whether kinase activity is essential for function (Andaya and Ronald, 2003; Xu *et al.*, 2006). Xa21D, which encodes the full LRR domain but not the transmembrane and kinase domains owing to a retrotransposon insertion, confers partial resistance with the same race-specificity as Xa21. Partial activity of Xa21D supports the notion that kinase activity is not essential for Xa21 activity, but it is also possible that partial activity of Xa21D could result from interaction with a related, intact receptor kinase. The conservation of race-specificity bolsters the prediction that pathogen recognition is mediated by the LRR (Wang *et al.*, 1998).

The avirulence signal that activates *Xa21*-mediated resistance, 'AvrXa21', has not yet been isolated. Nonetheless, it is dependent on several Xoo genes that provide clues to its molecular nature. The genes required for Avr*X*a21, or '*rax*' genes, include eight genes predicted to contribute to three roles: (1) *raxA*, *raxB* and *raxC* encode proteins with similarity to a membrane fusion protein, an ATP-binding cassette transporter and an outer membrane protein, respectively, for bacterial type I secretion (Goes da Silva *et al.*, 2004); (2) the *raxQ* and *raxP* encode proteins that function in concert to produce phosphoadenosine phosphosulfate (PAPS), an active form of sulfate, and the *raxST* encodes a protein similar to mammalian and bacterial sulfotransferases that use PAPS as the sulfuryl donor (Goes da Silva *et al.*, 2004; Shen *et al.*, 2002); and (3) *raxH* and *raxR* encode proteins with similarity to two-component regulatory systems and regulate *raxST* expression

(Burdman *et al.*, 2004). It is therefore predicted that in response to some environmental cue present *in planta*, AvrXa21, probably a polypeptide, is sulfated and then secreted into the extracellular space where it may function as a ligand of Xa21.

Like *Xa21*, *Xa26* is a broad-spectrum *R* gene to BB, but the spectra of races against which *Xa26* and *Xa21* are effective differ (Table 1). The cloned *Xa26* gene (Sun *et al.*, 2004) encodes a protein structurally similar to Xa21, but with a slightly larger LRR domain (26 vs. 23 repeats), consistent with the prediction that the two proteins detect different signals. In addition, 87% of the solvent-exposed residues in the predicted extracellular parts (including sequences between the signal peptide and the LRR) of Xa21 and Xa26 differ. Like *Xa21*, *Xa26* is expressed constitutively through development, but unlike *Xa21*, its function is not dependent on the age of the plant (Sun *et al.*, 2004). Curiously, based on analysis of *japonica* and *indica* cultivars and transgenic plants carrying *Xa26*, *Xa26*-mediated resistance is affected quantitatively, and qualitatively with regard to race specificity, by the genetic background in which it occurs. (Sun *et al.*, 2004). *Xa26* is also a member of a clustered family of paralogues. Comparisons of the haplotype of this locus in several rice lines revealed an unusually high degree of diversity among paralogues relative to the *Xa21* family. Analysis indicated that point mutations and positive selection were largely responsible for this diversity and may be the most important force in the evolution of new specificities for *R* genes of this type (Sun *et al.*, 2006).

Xa1 is a member of the large NBS-LRR (nucleotide binding site–leucine-rich repeat) class of *R* genes (Meyers *et al.*, 1999; Yoshimura *et al.*, 1998). The corresponding avirulence signal has not been identified. The predicted product has a somewhat unique LRR relative to other members of this class, but the most interesting feature of this gene is that its expression was not constitutive. The gene was up-regulated by clip inoculation with either a virulent or an avirulent strain of Xoo, and by wounding alone (Yoshimura *et al.*, 1998).

xa5 is an allele of a gene on chromosome 5 that encodes the gamma subunit of the general transcription factor TFIIA (TFIIAγ, or Xa5) (Iyer and McCouch, 2004). It differs from the susceptible allele by one codon, resulting in a substitution from valine to glutamic acid at residue 39. TFIIA stabilizes binding of the TATA box binding protein to the TATA box and plays an important role in transcriptional activation, functioning either as an anti-repressor or a co-activator (Hampsey, 1998), but it has not previously been associated with defence in animals or plants.

Several different models for xa5 function have been suggested. Iyer and McCouch (2004) proposed that a TAL effector that activates host genes for susceptibility may function as Avrxa5 and interact directly with xa5. They speculated that this interaction could at the same time reduce the amount of Avrxa5 available to target susceptibility genes and either disrupt host transcription and result in rapid host cell death and resistance, or trigger active defence responses via the action of a guard protein that monitors the status of TFIIA or the pre-initiation complex as a whole. Jiang *et al.* (2006) suggested that xa5 and Xa5 could differ in their affinity for transcriptional activators and repressors of defence-related genes. In their scenario, Avrxa5 would be a transcriptional activator of defence-related genes and bind with greater affinity to xa5 than to Xa5, which would preferentially bind repressors. A. Sugio, B. Yang, T. Zhu and F. White (unpublished data) propose yet another scenario, based on their recent observation that the virulent race 6 strain PXO99A uniquely up-regulates a paralogue of Xa5 on chromosome 1, TFIIAγ1. This up-regulation depends on a TAL effector designated as PthXo7. Their model is as follows. Xoo manipulates host gene transcription to promote infection by targeting transcriptional machinery directly, at least in part, in a TFIIAγ (Xa5)-dependent fashion. The *xa5* allele is immune to this manipulation, resulting in the (recessive) resistant phenotype. PXO99A, which they point out groups with other virulent strains isolated from the same geographical region as the Aus-Boro rice lines from which *xa5* originates, has evolved the capacity to circumvent this immunity of *xa5* to manipulation by activating transcription of the paralogous TFIIAγ gene on chromosome 1, which they observe to be otherwise expressed at low levels relative to the constitutively moderately expressed gene on chromosome 5. This model does not assume the existence of *avrxa5 per se* in avirulent strains, or a direct role of xa5 in pathogen recognition. Resistance could simply be the result of the rice plant being immune to manipulation (i.e. suppression) of its innate defence responses to unspecified bacterial signals by TAL effectors that depend on TFIIAγ.

Depending on the relative strength of the output from interactions involving xa5 vs. Xa5, each of these scenarios is consistent with the recessive behaviour of *xa5* (Blair *et al.*, 2003), or with a semi-dominant character, which has been inferred in some studies (Li *et al.*, 2001). Resolution of the molecular mechanism of action of this unusual resistance gene is an exciting area of ongoing research.

Further evidence for a role of TAL effectors in manipulating host transcription comes from the characterization of *Xa27* (Gu *et al.*, 2005). The functional allele of this *R* gene encodes a protein identical to that encoded by the non-functional allele. Only the functional allele, however, was expressed in rice leaves, and only in response to a strain expressing the corresponding *avrXa27* gene, which encodes a TAL effector. The putative promoter regions of the two alleles differ, consistent with the hypothesis that AvrXa27 activates transcription of *Xa27* directly, or indirectly through activation of a specific host transcription factor. Ectopic expression of *Xa27* induced thickening of secondary cell walls in the xylem and conferred resistance to otherwise compatible strains, confirming that the relevant difference between the alleles was their expression or lack thereof in response to AvrXa27. The cellular function of the *Xa27* gene

product could not be predicted based on its sequence. *Xa27* exemplifies a previously unknown mechanism of activating resistance possibly through 'molecular mimicry' by the plant of the *cis* regulatory element(s) targeted by the pathogen during infection (Gu *et al.*, 2005).

Like *Xa27*, differential expression in response to the pathogen due to polymorphism in the promoter region also distinguishes the functional and non-functional alleles of *xa13*, the sixth and most recently cloned gene for resistance to BB (Chu *et al.*, 2006). Both alleles are normally expressed at low levels in leaves. In contrast to *Xa27*, however, the functional allele of *xa13* remains expressed at low levels following pathogen attack, whereas the non-functional allele is induced. Based on a gene silencing experiment in *Xa13* plants, induction of the gene appears to be required for disease. These observations explain the recessive nature of *xa13*, and suggest that the dominant, non-functional allele can be considered as a 'susceptibility' gene targeted by Xoo. Consistent with this notion, induced expression of an Xa13–β-glucuronidase fusion protein in leaves was detected specifically in parenchyma cells surrounding the vascular bundle. Notably, *xa13* is homologous with *MtN3* of *Medicago truncatula*. *MtN3* is induced during, and required for, the compatible interaction of this legume with the symbiotic bacterium *Sinorhizobum meliloti* that leads to formation of nitrogen-fixing nodules. *xa13* (and *Xa13*) encodes a protein 307 amino acids in length that localizes to the plasma membrane (as a GFP fusion in callous tissue). Curiously, both *xa13* and *Xa13* are highly expressed in male reproductive tissues, and silencing of the locus results in abortive pollen and male sterility. This unexpected finding suggests the intriguing possibility of mechanistic links between reproductive development and disease in plants. The regulatory mechanisms that control *Xa13* expression in different tissues also are of particular interest. It remains to be confirmed that induction of *Xa13* in leaves by the pathogen is the result of TAL effector activity, but it seems likely.

Comprehensive identification of host genes regulated by TAL effectors of both Xoo and Xoc is an important goal for understanding and potentially manipulating both resistance and susceptibility. A. Sugio and colleagues (unpublished data), in addition to identifying TFIIAγ1 as a target of PthXo7, have observed that each of several Xoo strains of different races induces the expression of the rice *TFX1* gene, which encodes a member of the b-zip family of transcription factors. This induction is due to the TAL effector PthXo6. The mechanism of gene induction by TAL effectors, and whether host transcription factors are the primary targets, remains to be determined.

The type III secretion system of *X. oryzae*

TAL and other effectors of Gram-negative plant pathogens are delivered into plant cells via a type III secretion system (T3SS). The T3SS is broadly conserved among Gram-negative pathogens of plants and animals. Some T3SS effectors of plant pathogens share functions with effectors of animal pathogens (Mudgett, 2005). Wild-type secretion of some effectors depends on chaperones, typically small acidic proteins encoded by genes co-transcribed with the gene encoding the effector. The T3SS consists of a supramolecular structure resembling the flagellar biosynthetic complex, but producing a needle-like appendage and conduit, which in phytopathogens is called the Hrp pilus. The T3SS is encoded by *hrp* genes clustered in a pathogenicity island. *hrp* genes are named for their requirement in induction of the hypersensitive reaction in resistant plants or non-hosts, and for pathogenesis (for a review, see Gurlebeck *et al.*, 2006). Specific chaperones do not appear to be required for individual TAL effectors; rather, efficient secretion of these proteins in *Xanthomonas* spp. depends on the global chaperone HpaB, which appears to be involved in targeting of effectors to the secretion apparatus (Buttner *et al.*, 2006).

T3SS-mediated secretion and delivery of AvrXa10 into rice cells by both Xoo and Xoc has been demonstrated using a reporter protein fusion (Makino *et al.*, 2006). The *hrp* gene cluster of Xoo is nearly identical to the well-studied *hrp* cluster of *X. campestris* pv. vesicatoria, but differs within a region between the *hpaB* gene ('*hpa*' is for hrp-associated) and *hrpF*, designated as the *hrpF* peninsula, that varies slightly among *Xanthomonas* species (Oku *et al.*, 2004; Zhu *et al.*, 2000). In Xoo six open reading frames are present between *hpaB* and *hrpF*: four encode transposases or transposase derivatives, and two, designated as *hpa3* and *hpa4* (*xopF1* in *X. campestris* pv. vesicatoria), appear to encode a chaperone and effector pair (Roden *et al.*, 2004; Zhu *et al.*, 2000). Zhu *et al.* (2000) noted that in none of several other xanthomonads for which sequence was compared was both *hpa3* and *hpa4* intact. In light of this, the authors suggested that the *X. oryzae hrpF* peninsula may reflect the structure of the common ancestor of the species examined. Mutagenesis of *hpa4* and *hpa3* caused no detectable change in virulence of Xoo, however, so the role of these genes is unclear (Sugio *et al.*, 2005). The *hrp* cluster in Xoc is practically identical to that of Xoo, except for the *hrpF* peninsula, in which it differs only in the absence of the IS elements (H. Lu and A.J.B., unpublished data).

In *X. campestris* pv. vesicatoria, HrpF, a secreted protein, is required for AvrBs3-dependent hypersensitive reaction (HR) in pepper containing the *Bs3 R* gene, suggesting that HrpF is involved in delivery of the avirulence protein into the plant cell; based on this feature and the function of analogous proteins of animal pathogenic bacteria, HrpF was proposed to function as part of a putative 'translocon' that forms a pore in the plant plasma membrane to allow entry of effectors (Buttner *et al.*, 2002). Mutagenesis of *hrpF* in Xoo, however, reduced but did not abolish the ability to elicit an AvrXa10-dependent HR in rice plants with *Xa10*, as well as the ability to cause watersoaking in susceptible

lines, indicating that HrpF is not strictly required for delivery of effectors into rice cells (Sugio *et al.*, 2005). The *hpa1* gene, present downstream of the *hrpA* in all xanthomonads examined, but conserved to a lesser degree (30–40% identity) than most *hrp* genes (70–100% identity), was first described in Xoo (Oku *et al.*, 2004; Zhu *et al.*, 2000). It encodes a secreted protein (Furutani *et al.*, 2003; Noel *et al.*, 2002) that is required for wild-type levels of virulence and HR elicitation, and is structurally similar to previously characterized harpins of other Gram-negative plant pathogenic bacteria, which can form pores in lipid bilayers and have been proposed to function in translocation of effectors across the plant plasma membrane (Zhu *et al.*, 2000). Even mutation of both *hrpF* and *hpa1* together though did not completely prevent delivery of effectors: the double mutant was weakly virulent and still capable of eliciting an AvrXa10-mediated HR, albeit less well than the wild-type (Sugio *et al.*, 2005). Together the results indicate that proteins other than HrpF and Hpa1 or other mechanisms such as endocytosis or penetration of the plasma membrane by the Hrp pilus may be sufficient for effector entry into plant cells (Sugio *et al.*, 2005). Curiously, the *hrpF* mutant of Xoo transformed with *avrBs3* (in contrast to wild-type Xoo transformed with *avrBs3*) was unable to elicit *Bs3*-dependent HR in pepper. The discrepancy between the results with AvrBs3 and AvrXa10 may be due, among other possibilities, to differing thresholds in the amounts of delivered protein necessary to trigger responses mediated by the respective *R* genes (Sugio *et al.*, 2005).

hrp genes in *Xanthomonas* spp. are expressed *in planta* or in some cases in defined minimal media, but not in rich media. Expression is controlled at least in part by a motif upstream of *hrp* genes known as the plant-inducible promoter (PIP) box (Wengelnik and Bonas, 1996), which is also present upstream of many effectors and some other genes (Lee *et al.*, 2005; Noel *et al.*, 2001). The PIP box is targeted by the transcriptional activator HrpX, a member of the AraC family (Oku *et al.*, 1995; Wengelnik and Bonas, 1996). Expression of *hrpX* is up-regulated by HrpG, which is activated in response to as yet uncharacterized signals. HrpG is homologous with the OmpR response regulator family of bacterial two-component regulatory systems (Wengelnik *et al.*, 1996, 1999). A sensor kinase counterpart or other activator of HrpG has not been identified, but a transcriptional regulator of *hrpG* in Xoo was recently described (Lee *et al.*, 2005; Noel *et al.*, 2001; Tsuge *et al.*, 2006; Wengelnik and Bonas, 1996).

Tsuge *et al.* (2002) have developed an *hrp* gene-inducing minimal medium for Xoo, XOM2. XOM2 was an important technical advance that has facilitated identification of type III-secreted proteins of Xoo (Furutani *et al.*, 2003), discovery of proteins secreted through the general secretory pathway that are Hrp-dependent for expression (Furutani *et al.*, 2004) and an elegant dissection of the sequences essential for activity of the PIP box (Tsuge *et al.*, 2005). The last study identified several classes of degenerate PIP box that are active, and represents a key step toward comprehensive identification of the *hrpX* regulon starting with a bioinformatics approach to locate these motifs in sequenced genomes (see below).

Expression of *hrp* genes by Xoc in XOM2 medium using a reporter construct was not detected (S. Tsuge, personal communication), suggesting that *hrp* genes are regulated differently by the two pathovars. Whether *hrp* genes of Xoo and Xoc are regulated differentially *in planta* and whether differential activation is involved in the tissue specificity of Xoo and Xoc remains to be determined. *hrpX* is interchangeable among Xoo and both vascular and non-vascular pathovars of *X. campestris* (Kamdar *et al.*, 1993), and *hrpG* is interchangeable among Xoo, Xoc and *X. campestris* pv. vesicatoria (Makino, 2005). If there is differential expression in different plant tissues then the key determinants must act upstream of *hrpG*.

Other factors involved in virulence or fitness of *X. oryzae* pathovars

In addition to the *hrp* gene-encoded T3SS, (1) EPS, (2) proteins secreted through the general secretory (type II) system, including several degradative enzymes such as pectate lyases, cellulases, xylanases and proteases, and (3) toxin production contribute to the virulence of phytopathogenic bacteria (reviewed in Alfano and Collmer, 1996). Few studies have investigated Xoc, but both *X. oryzae* pathovars produce copious EPS, and Xoo mutants lacking this ability are severely attenuated in virulence (Dharmapuri and Sonti, 1999; Tang *et al.*, 1996). Type II secretion genes and specific type II-secreted proteins also play important roles in the interaction of Xoo with its host (Chatterjee *et al.*, 2003; Ray *et al.*, 2000; Sun *et al.*, 2005). To date, toxins important in BB or BLS have not been identified.

Studies of Xoo have led to the discovery of new types of genes required for full virulence as well. One of these is *phyA*, encoding phytase, an enzyme involved in degradation of the phosphate storage molecule phytic acid in plants (Chatterjee *et al.*, 2003). Another is *aroE*, encoding shikimate dehydrogenase, an enzyme in the aromatic amino acid biosynthetic pathway. Reduced virulence of an *aroE* mutant suggested that one or more aromatic amino acids may be limiting for Xoo multiplication in rice. The mutant was also deficient in xanthomonadin production, establishing the requirement of an enzyme in a general metabolic pathway for biosynthesis of this unique and characteristic pigment (Goel *et al.*, 2001).

Xanthomonadin, though not required for virulence, protects xanthomonads from photodamage and probably contributes to their survival in the field (Rajagopal *et al.*, 1997). Sequencing and analysis of a 21-kb cluster of genes required for xanthomonadin production in Xoo provided the first detailed characterization of components specific to the xanthomonadin biosynthetic pathway. These included genes for a novel type II polyketide synthase

pathway, and a gene encoding a cytoplasmic protein required for localization of xanthomonadin to the outer membrane (Goel *et al.*, 2002).

TAL effectors and rice defence inhibition by *X. oryzae* pv. oryzicola

As noted above, virulence mechanisms of Xoc have been less well explored than those of Xoo. As in Xoo, the T3SS is essential for pathogenesis (Makino *et al.*, 2006), and large numbers of TAL effector genes are present in individual strains (in two Xoc strains examined, there is an even greater abundance of TAL effector genes than in Xoo strains; see below) (Yang and White, 2004) (A.J.B. *et al.*, unpublished data). Nevertheless, functions for individual TAL effectors of Xoc have not yet been identified. Xoc can deliver AvrXa10 expressed from a plasmid into rice cells, suggesting that its native TAL effectors are expressed and secreted (Makino *et al.*, 2006). However, no Xoc TAL effector gene that complements mutations in Xoo TAL effectors with major virulence contributions could be recovered (Yang and White, 2004). Also, host genes induced by Xoo in a TAL effector-dependent manner are not induced by Xoc (A. Sugio *et al.*, unpublished data). And, of course, no endogenous *avr*/*R* gene combinations have been discerned for BLS. Thus, TAL effectors in Xoo and Xoc may function differently, or, though it seems unlikely, Xoc TAL effectors may not function at all in interactions with rice.

One or more Xoc TAL effectors may be involved in the recently discovered ability of this pathovar to inhibit rice *R* gene-mediated defence, as this ability was T3SS-dependent (Makino *et al.*, 2006), and TAL effectors (of Xoo and *X. axonopodis citri*) have been shown to suppress defence responses in tobacco (Fujikawa *et al.*, 2006). The activity of a defence inhibitor (TAL or other) could explain the lack of observed major gene resistance to BLS in rice. Identification and targeted mutagenesis of the defence inhibitor(s) of Xoc might reveal underlying gene-for-gene interactions involving TAL effectors (Makino *et al.*, 2006).

Defence inhibition by Xoc operated whether avirulence signal was delivered by Xoc or by co-inoculated Xoo. The ability of Xoc to block defence responses in co-inoculations raises the possibility that *R* genes to BB and perhaps other diseases may not be effective when BLS is present (Makino *et al.*, 2006).

Defence inhibition by Xoc was tested only for *Xa7* and *Xa10*, for which the corresponding avirulence signals are TAL effectors, and for *Xa2*, for which the corresponding avirulence signal has not been identified. Whether defence inhibition is effective against *R* genes that respond to avirulence signals other than TAL effectors is unknown. Intriguingly, however, each of the Xoo *rax* genes is highly conserved in Xoc (our unpublished observation), suggesting the possibility that Xoc secretes AvrXa21 but quells the *Xa21*-mediated response (*Xa21* does not provide resistance to Xoc).

The maize *Rxo1* gene for resistance to BLS of rice

Xoc does not successfully inhibit defence mediated by the cloned maize gene *Rxo1*. In maize, *Rxo1* mediates a non-host defence response to Xoc, and as a transgene in rice it prevents the development of BLS. The gene encodes an NBS-LRR protein, and curiously, it is also the *Rba1* gene which confers resistance in maize to some strains of the bacterial stripe pathogen *Burkholderia andropogonis* (Zhao *et al.*, 2004b, 2005).

avrRxo1 has also been cloned. It is present in all strains of Xoc tested, indicating that it may play a critical role in fitness (including virulence). Activity of *avrRxo1* is T3SS-dependent, but its product is not a TAL effector and indeed lacks similarity to any known protein. It does contain a eukaryotic cysteine protease active site motif, as well as a putative ATP/GTP binding motif. *avrRxo1* does not hybridize to *B. andropogonis* DNA. It is conceivable that Rxo1 directly recognizes AvrRxo1 as well as a distinct effector in *B. andropogonis*, or, as was suggested by Zhao *et al.* (2004a), that Rxo1 responds to modification of a host protein, perhaps proteolytic cleavage, by either AvrRxo1 or an effector from the leaf stripe pathogen. AvrRxo1 contains several internal matches to the consensus eukaryotic N-terminal myristylation motif. Whether the protein is processed to expose a myristylation site at the N-terminus is not yet known, but an AvrRxo1–green fluorescent protein fusion localized to the plant plasma membrane (Zhao *et al.*, 2004a).

As a transgene, *Rxo1* holds great promise for rice breeding as a simply inherited source of resistance to BLS. The immunity of *Rxo1* function to inhibition by Xoc could derive from the presumably distinct nature of AvrRxo1 recognition, but it also suggests that this effector may be required for defence inhibition and its action precluded by direct or indirect interaction with Rxo1. Notably, *avrRxo1* is absent from Xoo; it probably was acquired independently by Xoc through horizontal transfer as the locus in which it resides is flanked by IS elements and its G+C content is lower than average for the Xoc genome (Zhao *et al.*, 2004a).

STRUCTURAL, COMPARATIVE AND FUNCTIONAL GENOMICS OF *X. ORYZAE*

The genomes of two Xoo strains, Japanese strain MAFF 311018 (also called T7174) and Korean strain KACC10331 (also called KXO85), have been sequenced (Lee *et al.*, 2005; Ochiai *et al.*, 2005). Sequencing of the genomes of Philippine race 6 strain PXO99 (C. He, personal communication) and a more genetically tractable derivative, PXO99A (Choi and Leach, 1994; A.J.B. *et al.*, available in the Comprehensive Microbial Resource, http://www.tigr.org) is nearing completion. The genome sequences are approximately 4.9 Mbp in length and have a G+C content of *c.* 64 mol%, agreeing with previous estimates (Ochiai *et al.*,

2001; Vauterin *et al.*, 1992; Vera-Cruz *et al.*, 1984). Multiple families of both previously identified and novel IS elements and the large family of TAL effector genes (Hopkins *et al.*, 1992) with their tandem repeat regions are scattered throughout the genomes. As mentioned, the abundance of TAL effector genes is characteristic of *X. oryzae* relative to other *Xanthomonas* spp. Likewise, the *X. oryzae* genomes have greater numbers of IS elements than other published *Xanthomonas* genomes (Ochiai *et al.*, 2005). It should be noted that these features present challenges in computational assembly of the genome sequences, and indeed, in the published KACC10331 genome (Lee *et al.*, 2005), fewer TAL effector genes are reported than expected based on published Southern blots (12 reported vs. at least 15 expected). These blots were carried out such that no more than one band appeared per gene (Yang and White, 2004). For T7174, 17 TAL effector genes are reported (Ochiai *et al.*, 2005), in agreement with estimates based on Southern hybridization (Yang and White, 2004).

Preliminary comparisons suggest significant rearrangements in the PXO99A genome relative to the T7174 and KXO85 genomes, and relatively more minor rearrangements between the latter two. Also, the genomes show numerous rearrangements and inversions relative to those of *X. axonopodis* pv. citri, *X. campestris* pv. campestris and *X. campestris* pv. vesicatoria (Ochiai *et al.*, 2005; Thieme *et al.*, 2005; H. Lu and A.J.B., unpublished data). A role of IS elements and TAL effector genes in generating rearrangements through recombination seems plausible, but due to the possibility of artefacts in the assemblies, confirmation of these rearrangements awaits conclusive corroborative evidence. Ochiai *et al.* (2005) have suggested that activity of IS elements and recombination among TAL effector genes have contributed to the diverse race structure within Xoo. Consistent with this suggestion, Rajeshwari and Sonti (2000) identified active IS elements responsible for generating genetic variation in culture, and Ponciano *et al.* (2004) garnered evidence suggesting recombinations within the TAL effector gene family among field isolates.

Sequencing of the genome of Xoc strain BLS256 also has recently been completed (A.J.B. *et al.*, available in the Comprehensive Microbial Resource, http://www.tigr.org). This genome overall is similar to the Xoo genomes with respect to size, G+C content, abundance of IS elements and TAL effector genes (28, in agreement with estimates for strain BLS303 based on Southern hybridization) (Yang and White, 2004), and the presence of inversions and rearrangements, but has notable unique features. For example, a gene cluster in this genome with predicted functions in synthesis and transport of lipopolysaccharide (LPS), and bordered by two highly conserved genes, is distinct both in gene order and gene content from the corresponding locus in the Xoo genomes. Half of the cluster is orthologous with the *X. axonopodis* pv. citri cluster present at this locus, and half is apparently unique (P. Patil and R. Sonti, unpublished data). Interestingly, Patil and Sonti (2004) have shown that among several Indian strains and a Philippine strain, the Xoo cluster at the locus is highly conserved, and functions in virulence, but in two strains, the Indian BXO8 strain and a Nepalese strain, the cluster was different. Recent analysis indicates that the locus in BXO8 and the Nepalese strain is completely orthologous with the *X. axonopodis* pv. citri locus, and further, that disruption of *wzt*, a putative ATP binding protein-encoding gene within the cluster, causes severe attenuation in virulence and defects in the lipopolysaccharide profile (P. Patil and R. Sonti, unpublished data). The hypervariation at this *lps* locus in the xanthomonads at the species, pathovar and strain levels suggests an important adaptive role for LPS variation in the evolution of these bacteria (Patil and Sonti, 2004).

Further comparative and functional analyses among the *X. oryzae* genome sequences, and among these and other available and pending *Xanthomonas* genomes sequences promises to shed further light on the nature and evolution of bacterial pathogenesis of plants, especially host-species specificity and host-tissue specificity, as well as host cultivar specificity. Highly efficient transposon mutagenesis strategies have been used to generate high-coverage mutant libraries for Xoo and *X. campestris* pv. campestris strains; these represent valuable resources for functional genomic studies (Sun *et al.*, 2003). The genome sequences are a requisite resource for comprehensive identification of the type III effector inventory of Xoo and Xoc, and for a comprehensive evaluation of pathogenesis-related gene regulation in these bacteria. We have recently developed a combined whole-genome spotted oligonucleotide microarray (available at http://www.ricearray.org) for Xoo and Xoc that should be particularly useful for this purpose.

CURRENT PERSPECTIVE AND FUTURE PROSPECTS

The interactions of *X. oryzae* pathovars with rice constrain production of the staple food of more than half the world's population. Since the discovery of BB and BLS early in the last century, great progress has been made in characterizing the modes of pathogen infection, identifying the factors that influence disease development, delineating the remarkable diversity within the populations of Xoo and Xoc, breeding for resistance, and understanding the interactions among major resistance genes and quantitative loci. Sustainable control measures for BB and BLS, however, require a better understanding of the molecular basis of Xoo and Xoc pathogenicity, the processes that generate diversity within the pathogen population, and the mechanisms involved in pathogen perception, signalling and defence in rice.

Recent progress has revealed a central role for TAL effectors and suggests that pathogen manipulation of host transcription and interference with host defences are important mechanisms in

pathogenesis. TAL effector gene sequences and IS elements seem likely to be involved in the evolution of new pathotypes. Cloning and characterization of six rice *R* genes for BB and one from maize effective against BLS have uncovered a remarkable diversity of structure and function, and hinted at the processes that have occurred in the evolution of resistance and resistance-breaking mechanisms. Nonetheless, these exciting findings are only a beginning.

Fortunately, the interactions of *X. oryzae* pathovars with rice also continue to serve as a powerful and uniquely valuable model for research toward solutions in disease control. Moreover, research in BB and BLS promises to continue generating findings of fundamental relevance to plant pathology and plant and microbial biology generally, as well as pathogenesis and innate immunity in animals. A superb infrastructure and body of resources is available for rice, including among others an expansive, well-characterized germplasm collection, completed genome sequence, whole genome microarrays and a growing collection of mutant libraries (see http://www.iris.irri.org/IRFGC). Xoo and Xoc are genetically tractable, and as mentioned above, complete genome sequences are now or will soon be available for four strains of Xoo and one strain of Xoc, and concomitant resources for functional and comparative genomics are accruing. Large collections of geographically distinct isolates and pathotypes are also available for Xoo and to a lesser extent for Xoc.

Several areas of inquiry will be propelled by the ongoing use of these resources in pathogen, host, and integrated comparative and functional genomic studies. Some that promise to be most fruitful in the near future include: (1) the nature of tissue specificity in plant–bacterial interactions, which is pertinent to many bacterial diseases of plants; (2) the evolution of race–cultivar specificity, which is likely to carry practical implications and shed light on forces that shape evolution of both bacterial and plant genomes generally; (3) the diversity of mechanisms in plant resistance gene function, which as described above promises to reveal fundamental aspects of plant biology (especially gene transcription) as well as signalling mechanisms relevant to innate immunity in humans; (4) the function of TAL and other bacterial effectors, essential for disease, but also useful as tools for exploring molecular processes in plants; and (5) epidemiology and diagnostics for disease prevention, diagnosis and risk assessment, which will aid in effective quarantine and rational legislation. Each of these areas holds promise for discoveries that will lead to sustainable measures for disease management, e.g. chemical or plant-derived inhibitors of essential pathogenicity mechanisms, manipulation of function and specificity of *R* genes, rapid and cost-effective means of detection of Xoo and Xoc, and other potential measures. With continued progress and coordinated effort, the threat of BB and BLS to food security may eventually be diminished, and the importance of *X. oryzae* pathovars limited to their usefulness as models.

ACKNOWLEDGEMENTS

We thank H. Kaku, J. Leach, T. Mew, K. Tsuchiya, C. Vera-Cruz and V. Verdier for providing photographs, R. Sonti and F. White for sharing data prior to publication, C. Vera-Cruz and G. Carrillo for assistance compiling information for Table 1, L. Darnielle for photography and K. Costello for graphics.

NOTE ADDED IN PROOF

Yang *et al.* [**Yang, B., Sugio, A. and White, F.F.** (2006) Os8N3 is a host disease-susceptibility gene for bacterial blight of rice. *Proc Natl. Acad. Sci. USA* **103**, 10503–10508] recently demonstrated that Xoo TAL effector PthXo1 induces expression of *Os8N3*, a member of the *MtN3* family of plant genes. Their work also suggests that Os8N3 is the dominant allele of *xa13*.

REFERENCES

Adhikari, T.B., Basnyat, R.C. and Mew, T.W. (1999a) Virulence of *Xanthomonas oryzae* pv. oryzae on rice lines containing single resistance genes and gene combinations. *Plant Dis.* **83**, 46–50.

Adhikari, T.B., Mew, T.W. and Leach, J.E. (1999b) Genotypic and pathotypic diversity in *Xanthomonas oryzae* pv. oryzae in Nepal. *Phytopathology*, **89**, 687–694.

Adhikari, T.B., Vera-Cruz, C.M., Zhang, Q., Nelson, R.J., Skinner, D.Z., Mew, T.W. and Leach, J.E. (1995) Genetic diversity of *Xanthomonas oryzae* pv. oryzae in Asia. *Appl. Environ. Microbiol.* **61**, 966–971.

Alfano, J.R. and Collmer, A. (1996) Bacterial pathogens in plants: life up against the wall. *Plant Cell*, **8**, 1683–1698.

Andaya, C.B. and Ronald, P.C. (2003) A catalytically impaired mutant of the rice Xa21 receptor kinase confers partial resistance to *Xanthomonas oryzae* pv. oryzae. *Physiol. Mol. Plant Pathol.* **62**, 203–208.

Awoderu, V.A., Bangura, N. and John, V.T. (1991) Incidence, distribution and severity of bacterial diseases on rice in West Africa. *Trop. Pest Manag.* **37**, 113–117.

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.

Ballvora, A., Pierre, M., van den Ackerveken, G., Schornack, S., Rossier, O., Ganal, M., Lahaye, T. and Bonas, U. (2001) Genetic mapping and functional analysis of the tomato *Bs4* locus governing recognition of the *Xanthomonas campestris* pv. vesicatoria AvrBs4 protein. *Mol. Plant–Microbe Interact.* **14**, 629–638.

Bennetzen, J.L. and Ma, J. (2003) The genetic colinearity of rice and other cereals on the basis of genomic sequence analysis. *Curr. Opin. Plant Biol.* **6**, 128–133.

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.

Bradbury, J.F. (1984) Genus II. *Xanthomonas* Dowson. In: *Bergey's Manual of Systematic Bacteriology* (Krieg, N.R. and Holt, J.G., eds), pp. 199–210. Baltimore: Williams & Wilkins.

Brar, D.S. and Khush, G.S. (1997) Alien introgression in rice. *Plant Mol. Biol.* **35**, 35–47.

Burdman, S., Shen, Y., Lee, S.W., Xue, Q. and Ronald, P. (2004) RaxH/RaxR: a two-component regulatory system in *Xanthomonas oryzae* pv. oryzae required for AvrXa21 activity. *Mol. Plant–Microbe Interact.* **17**, 602–612.

Buttner, D. and Bonas, U. (2002) Getting across—bacterial type III effector proteins on their way to the plant cell. *EMBO J.* **21**, 5313–5322.

Buttner, D., Lorenz, C., Weber, E. and Bonas, U. (2006) Targeting of two effector protein classes to the type III secretion system by a HpaC- and HpaB-dependent protein complex from *Xanthomonas campestris* pv. vesicatoria. *Mol. Microbiol.* **59**, 513–527.

Buttner, D., Nennstiel, D., Klusener, B. and Bonas, U. (2002) Functional analysis of HrpF, a putative type III translocon protein from *Xanthomonas campestris* pv. vesicatoria. *J. Bacteriol.* **184**, 2389–2398.

Century, K.S., Lagman, R.A., Adkisson, M., Morlan, J., Tobias, R., Schwartz, K., Smith, A., Love, J., Ronald, P.C. and Whalen, M.C. (1999) Developmental control of *Xa21*-mediated disease resistance in rice. *Plant J.* **20**, 231–236.

Chatterjee, S., Sankaranarayanan, R. and Sonti, R.V. (2003) PhyA, a secreted protein of *Xanthomonas oryzae* pv. oryzae, is required for optimum virulence and growth on phytic acid as a sole phosphate source. *Mol. Plant–Microbe Interact.* **16**, 973–982.

Chen, H., Wang, S. and Zhang, Q. (2002) New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. *Phytopathology*, **92**, 750–754.

Choi, S.H. and Leach, J.E. (1994) Genetic manipulation of *Xanthomonas oryzae* pv. oryzae. *Int. Rice Res. Notes*, **19**, 31–32.

Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., Li, X., Fu, B., Li, Z., Bennetzen, J.L., Zhang, Q. and Wang, S. (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* **20**, 1250–1255.

Curtis, L.C. (1943) Deleterious effects of guttated fluid on foliage. *Am. J. Bot.* **30**, 778–781.

Dardick, C. and Ronald, P. (2006) Plant and animal pathogen recognition receptors signal through non-RD kinases. *PLoS Pathog.* **2**, e2.

Das, S.R. (1977) Assessment of rice varieties for resistance to bacterial leaf streak disease caused by *Xanthomonas translucens* f. sp. *oryzicola*. *Sci. Culture*, **43**, 93.

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

Dye, D.W. (1978) Genus IX. *Xanthomonas* Dowson 1939. *NZ J. Agric. Res.* **21**, 153–177.

Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* **9**, 275–296.

Fujikawa, T., Ishihara, H., Leach, J. and Tsuyumu, S. (2006) Suppression of defense response in plants by the *avrBs3/pthA* gene family of *Xanthomonas* spp. *Mol. Plant–Microbe Interact.* **19**, 342–349.

Furutani, A., Tsuge, S., Ohnishi, K., Hikichi, Y., Oku, T., Tsuno, K., Inoue, Y., Ochiai, H., Kaku, H. and Kubo, Y. (2004) Evidence for *HrpXo*-dependent expression of type II secretory proteins in *Xanthomonas oryzae* pv. oryzae. *J. Bacteriol.* **186**, 1374–1380.

Furutani, A., Tsuge, S., Oku, T., Tsuno, K., Inoue, Y., Ochiai, H., Kaku, H. and Kubo, Y. (2003) Hpa1 secretion via type III secretion system in *Xanthomonas oryzae* pv. oryzae. *J. Gen. Plant Pathol.* **69**, 271–275.

Gao, D., Liu, A., Zhou, Y., Cheng, Y., Xiang, Y., Sun, L. and Zhai, W. (2005) Molecular mapping of a bacterial blight resistance gene *Xa-25* in rice. *Yi Chuan Xue Bao*, **32**, 183–188 (in Chinese).

Gao, D.Y., Xu, Z.G., Chen, Z.Y., Sun, L.H., Sun, Q.M., Lu, F., Hu, B.S., Liu, Y.F. and Tang, L.H. (2001) Identification of a new gene for resistance to bacterial blight in a somaclonal mutant HX-3 (*indica*). *Rice Genet. Newslett.* **18**, 66.

Gnanamanickam, S., Brindha Priyadarisini, V., Narayanan, N., Vasudevan, P. and Kavitha, S. (1999) An overview of bacterial blight disease of rice and strategies for its management. *Curr. Sci.* **77**, 1435–1443.

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.

Goel, A.K., Rajagopal, L. and Sonti, R.V. (2001) Pigment and virulence deficiencies associated with mutations in the *aroE* gene of *Xanthomonas oryzae* pv. oryzae. *Appl. Environ. Microbiol.* **67**, 245–250.

Goes da Silva, F., 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.

Gossele, F., Cruz, C.M.V., Outryve, M.F.V., Swings, J. and Ley, J.D. (1985) Differentiation between the bacteria causing bacterial blight (BB), bacterial leaf streak (BLS), and bacterial brown blotch on rice. *Int. Rice Res. Newslett.* **10**, 23–24.

Goto, M. (1992) *Fundamentals of Bacterial Plant Pathology*. San Diego: Academic Press.

Gu, K., Tian, D., Yang, F., Wu, L., Sreekala, C., Wang, D., Wang, G.-L. and Yin, Z. (2004) High-resolution genetic mapping of *Xa27* (t), a new bacterial blight resistance gene in rice, *Oryza sativa* L. *Theor. Appl. Genet.* **108**, 800–807.

Gu, K.-Y., Yang, B., Tian, D.-S., Wu, L.-F., Wang, D.-J., Sreekala, C., Yang, F., Chu, Z.-Q., Wang, G.-L., White, F.F. and Yin, Z.-C. (2005) *R* gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*, **435**, 1122–1125.

Gurlebeck, D., Szurek, B. and Bonas, U. (2005) Dimerization of the bacterial effector protein AvrBs3 in the plant cell cytoplasm prior to nuclear import. *Plant J.* **42**, 175–187.

Gurlebeck, D., Thieme, F. and Bonas, U. (2006) Type III effector proteins from the plant pathogen *Xanthomonas* and their role in the interaction with the host plant. *J. Plant Physiol.* **163**, 233–255.

Hampsey, M. (1998) Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol. Mol. Biol. Rev.* **62**, 465–503.

He, Q., Li, D.-B., Zhu, Y.-S., Tan, M.-P., Zhang, D.-P. and Lin, X.-H. (2006) Fine mapping of *Xa2*, a bacterial blight resistance gene in rice. *Mol. Breed.* **17**, 1–6.

Herbers, K., Conrads-Strauch, J. and Bonas, U. (1992) Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. *Nature*, **356**, 172–174.

Hilaire, E., Young, S.A., Willard, L.H., McGee, J.D., Sweat, T., Chittoor, J.M., Guikema, J.A. and Leach, J.E. (2001) Vascular defense responses in rice: peroxidase accumulation in xylem parenchyma cells and xylem wall thickening. *Mol. Plant–Microbe Interact.* **14**, 1411–1419.

Ho, B.L. and Lim, W.C. (1979) Effects of nitrogen and planting density on bacterial blight *Xanthomonas oryzae* of rice. *Malays. Agric. J.* **52**, 171–176.

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.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J. and Khush, G.S. (1997)

Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* **95**, 313–320.

Ishihara, H., Ponciano, G., Leach, J.E. and Tsuyumu, S. (2003) Functional analysis of the 3′ end of *avrBs3/pthA* genes from two *Xanthomonas* species. *Physiol. Mol. Plant Pathol.* **63**, 329–338.

Ishiyama, S. (1922) Studies of bacterial leaf blight of rice. *Report Imp. Agric. Stn. Konosu*, **45**, 233–261.

Iyer, A.S. and McCouch, S.R. (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant–Microbe Interact.* **17**, 1348–1354.

Jiang, G.-H., Xia, Z.-H., Zhou, Y.-L., Wan, J., Li, D.-Y., Chen, R.-S., Zhai, W.-X. and Zhu, L.-H. (2006) Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog *TFIIAγ1*. *Mol. Gen. Genomics*, **275**, 354–366.

Johri, B.N., Sharma, A. and Virdi, J.S. (2003) Rhizobacterial diversity in India and its influence on soil and plant health. *Adv. Biochem. Eng. Biotechnol.* **84**, 49–89.

Jones, R.K., Barnes, L.W., Gonzalez, C.F., Leach, J.E., Alvarez, A.M. and Benedict, A.A. (1989) Identification of low-virulence strains of *Xanthomonas campestris* pv. oryzae from rice in the United States. *Phytopathology*, **79**, 984–990.

Kaku, H. and Ogawa, T. (2000) The relationship between browning reaction and bacterial blight resistance gene *Xa3* in rice. *Rice Genet. Newslett.* **17**, 76–78.

Kamdar, H.V., Kamoun, S. and Kado, C.I. (1993) Restoration of pathogenicity of avirulent *Xanthomonas oryzae* pv. oryzae and *X. campestris* pathovars by reciprocal complementation with the *hrpXo and hrpXc* genes and identification of HrpX function by sequence analyses. *J. Bacteriol.* **175**, 2017–2025.

Khush, G.S. and Angeles, E.R. (1999) A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa*. *Rice Genet. Newslett.* **16**, 92–93.

Koch, M. and Parlevliet, J.E. (1991a) Residual effects of the *Xa-4* resistance gene in three rice cultivars when exposed to a virulent isolate of *Xanthomonas campestris* pv. oryzae. *Euphytica*, **55**, 187–193.

Koch, M.F. and Parlevliet, J.E. (1991b) Genetic analysis of, and selection for, factors affecting quantitative resistance to *Xanthomonas campestris* pv. oryzae in rice. *Euphytica*, **53**, 235–245.

Lee, K.S., Angeles, E.R. and Khush, G.S. (2000) Inheritance of resistance to race 6 of bacterial blight in rice. *Rice Genet. Newslett.* **17**, 73.

Lee, K.S. and Khush, G.S. (2000) Genetic analysis of resistance to bacterial blight, *Xanthomonas oryzae* pv. oryzae, *Rice*. *Rice Genet. Newslett.* **17**, 72.

Lee, B.M., Park, Y.J., Park, D.S., Kang, H.W., Kim, J.G., Song, E.S., Park, I.C., Yoon, U.H., Hahn, J.H., Koo, B.S., Lee, G.B., Kim, H., Park, H.S., Yoon, K.O., Kim, J.H., Jung, C.H., Koh, N.H., Seo, J.S. and Go, S.J. (2005) The genome sequence of *Xanthomonas oryzae* pathovar oryzae KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res.* **33**, 577–586.

Lee, K.S., Rasabandith, S., Angeles, E.R. and Khush, G.S. (2003) Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology*, **93**, 147–152.

Li, Z.K., Arif, M., Zhong, D.B., Fu, B.Y., Xu, J.L., Domingo-Rey, J., Ali, J. and Vijayakumar, C.H.M., Yu, S.B. and Khush, G.S. (2006) Complex genetic networks underlying the defensive system of rice (*Oryza sativa* L.) to *Xanthomonas oryzae* pv. oryzae. *Proc. Natl Acad. Sci. USA*, doi: 10.1073/pnas.0507492103.

Li, Z.K., Luo, L.J., Mei, H.W., Paterson, A.H., Zhao, X.H., Zhong, D.B. and Wang, Y.P., Yu, X.Q. and Zhu, L. (1999) A 'defeated' rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. oryzae. *Mol. Gen. Genet.* **261**, 58–63.

Li, Z.K., Sanchez, A., Angeles, E., Singh, S., Domingo, J., Huang, N. and Khush, G.S. (2001) Are the dominant and recessive plant disease resistance genes similar? A case study of rice *R* genes and *Xanthomonas oryzae* pv. oryzae races. *Genetics*, **159**, 757–765.

Liu, H.-X., Liu, F.-Q., Hu, B.-S., Yang, W.-F., Chen, Z.-Y. and Xu, Z.-G. (2004) Virulence of *Xanthomonas oryzae* pv. oryzae on rice near-isogenic lines with single resistance gene and pyramiding lines in China. *Agric. Sci. China*, **3**, 764–769 (in Chinese).

Makino, S. (2005) *Molecular mechanisms of pathogenesis in pathovars of the rice pathogenic bacterial species Xanthomonas oryzae*. MSc thesis. Ames, Iowa: Iowa State University.

Makino, S., Sugio, A., White, F.F. and Bogdanove, A.J. (2006) Inhibition of resistance gene–mediated defense in rice by *Xanthomonas oryzae* pv. *oryzicola*. *Mol. Plant–Microbe Interact.* **19**, 240–249.

Mew, T.W. (1987) Current status and future prospects of research on bacterial blight of rice. *Annu. Rev. Phytopathol.* **25**, 359–382.

Mew, T.W., Alvarez, A.M., Leach, J.E. and Swings, J. (1993) Focus on bacterial blight of rice. *Plant Dis.* **77**, 5–12.

Mew, T.W., Vera Cruz, C.M. and Medalla, E.S. (1992) Changes in race frequency of *Xanthomonas oryzae* pv. oryzae in response to rice cultivars planted in the Philippines. *Plant Dis.* **76**, 1029–1032.

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W. and Young, N.D. (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant J.* **20**, 317–332.

Mizukami, T. and Wakimoto, S. (1969) Epidemiology and control of bacterial leaf blight of rice. *Annu. Rev. Phytopathol.* **7**, 51–72.

Moffett, M.L. and Croft, B.J. (1983) *Xanthomonas*. In: *Plant Bacterial Diseases* (Fahy, P.C. and Persley, G.J., eds), p. 393. New York: Academic Press.

Mudgett, M.B. (2005) New insights to the function of phytopathogenic bacterial type III effectors in plants. *Annu. Rev. Plant Biol.* **56**, 509–531.

Murty, V.S.T. and Devadath, S. (1982) Studies on forecasting of bacterial blight of rice. *Indian Phytopathol.* **35**, 241–246.

Nakai, H., Nakamura, K., Kuwahara, S. and Saito, M. (1988) Genetic studies of an induced rice mutant resistant to multiple races of bacterial leaf blight. *Rice Genet. Newslett.* **5**, 101–103.

Narayanan, N.N., Baisakh, N., Vera-Cruz, C.M., Gnanamanickam, S.S., Datta, K. and Datta, S.K. (2002) Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. *Crop Sci.* **42**, 2072–2079.

Noda, T. and Kaku, H. (1999) Growth of *Xanthomonas oryzae* pv. oryzae in planta and in guttation fluid of rice. *Ann. Phytopathol. Soc. Japan*, **65**, 9–14.

Noda, T., Li, C., Li, J., Ochiai, H., Ise, K. and Kaku, H. (2001) Pathogenic diversity of *Xanthomonas oryzae* pv. oryzae strains from Yunnan province, China. *Jpn. Agr. Res. Q.* **35**, 97–103.

Noda, T., Yamamoto, T., Kaku, H. and Horino, O. (1996) Geographical distribution of pathogenic races of *Xanthomonas oryzae* pv. oryzae Japan in 1991 and 1993. *Ann. Phytopathol. Soc. Japan*, **62**, 549–553.

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.

Noel, L., Thieme, F., Nennstiel, D. and Bonas, U. (2002) Two novel type III-secreted proteins of *Xanthomonas campestris* pv. vesicatoria are

encoded within the *hrp* pathogenicity island. *J. Bacteriol.* **184**, 1340–1348.

Nyvall, R.F. (1999) *Field crop diseases.* Ames, Iowa: State University Press.

Ochiai, H., Inoue, Y., Hasebe, A. and Kaku, H. (2001) Construction and characterization of a *Xanthomonas oryzae* pv. oryzae bacterial artificial chromosome library. *FEMS Microbiol. Lett.* **200**, 59–65.

Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. and Kaku, H. (2005) Genome sequence of *Xanthomonas oryzae* pv. oryzae suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *Jpn. Agr. Res. Q.* **39**, 275–287.

Ochiai, H. and Kaku, H. (1999) Genetic relationships among *Xanthomonas* species and pathovars based on RFLP analyses of PCR-amplified 16S, 23S rDNA and rDNA internal transcribed spacer. *Ann. Phytopathol. Soc. Japan*, **65**, 437–446.

Ogawa, T., Busto, G.A.J., Tabien, R.E., Romero, G.O., Endo, N. and Khush, G.S. (1991) Grouping of rice cultivars based on reaction pattern of Philippine races of bacterial blight pathogen *Xanthomonas campestris* pv. oryzae. *Jpn. J. Breed.* **41**, 109–120.

Ogawa, T., Yamamoto, T., Khush, G.S. and Mew, T.W. (1986) The *Xa-3* gene for resistance to Philippine races of bacterial blight of rice. *Rice Genet. Newslett.* **3**, 77.

Oku, T., Alvarez, A.M. and Kado, C.I. (1995) Conservation of the hypersensitivity-pathogenicity regulatory gene *hrpX* of *Xanthomonas campestris* and *X. oryzae*. *DNA Seq.* **5**, 245–249.

Oku, T., Tanaka, K., Iwamoto, M., Inoue, Y., Ochiai, H., Kaku, H., Tsuge, S. and Tsuno, K. (2004) Structural conservation of the *hrp* gene cluster in *Xanthomons oryzae* pv. oryzae. *J. Gen. Plant Pathol.* **70**, 159–167.

Oryzabase. (2006) *Integrated Rice Science Database.* http://www.shigen.nig.ac.jp/rice/oryzabase/.

Ou, S.H. (1972) *Rice Diseases.* Kew, Surrey: Commonwealth Mycological Institute.

Ou, S.H. (1973) *A Handbook of Rice Diseases in the Tropics.* Los Baños: International Rice Research Institute.

Ou, S.H. (1985) *Rice Diseases.* Kew, Surrey: Commonwealth Agricultural Bureau.

Patil, P.B. and Sonti, R.V. (2004) Variation suggestive of horizontal gene transfer at a lipopolysaccharide (*lps*) biosynthetic locus in *Xanthomonas oryzae* pv. oryzae, the bacterial leaf blight pathogen of rice. *BMC Microbiol.* **4**, 40.

Ponciano, G., Webb, K., Bai, J., Cruz, C.-V. and Leach, J.-E. (2004) Molecular characterization of the *avrXa7* locus from *Xanthomonas oryzae* pv. oryzae field isolates. *Physiol. Mol. Plant Pathol.* **64**, 145–153.

Porter, B.W., Chittoor, J.M., Yano, M., Sasaki, T. and White, F.F. (2003) Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. *Crop Sci.* **43**, 1484–1492.

Qi, Z. and Mew, T. (1985) Adult-plant resistance of rice cultivars to bacterial blight. *Plant Dis.* **69**, 896–898.

Rajagopal, L., Sundari, C.S., Balasubramanian, D. and Sonti, R.V. (1997) The bacterial pigment xanthomonadin offers protection against photodamage. *FEBS Lett.* **415**, 125–128.

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.

Rao, Y.P., Shekhawat, G.S., Mohan, S.K. and Reddy, P.R. (1972) Evaluation of rice varieties for resistance to bacterial leaf-streak (*Xanthomonas translucens*). *Ind. J. Agr. Sci.* **42**, 502–505.

Ray, S.K., Rajeshwari, R. and Sonti, R.V. (2000) Mutants of *Xanthomonas oryzae* pv. oryzae deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. *Mol. Plant–Microbe Interact.* **13**, 394–401.

Raymundo, A.K., Briones, A.M. Jr, Ardales, E.Y., Perez, M.T., Fernandez, L.C., Leach, J.F., Mew, T.W., Ynalvez, M.A., McLaren, C.G. and Nelson, R.J. (1999) Analysis of DNA polymorphism and virulence in Philippine strains of *Xanthomonas oryzae* pv. oryzicola. *Plant Dis.* **83**, 434–440.

Reddy, A.P.K., Mackenzie, D.R., Rouse, D.I. and Rao, A.V. (1979) Relationship of bacterial leaf blight *Xanthomonas oryzae* severity to grain yield of rice *Oryza sativa*. *Phytopathology*, **69**, 967–969.

Reimers, P.J., Guo, A. and Leach, J.E. (1992) Increased activity of a cationic peroxidase associated with an incompatible interaction between *Xanthomonas oryzae* pv. oryzae and rice *Oryza sativa*. *Plant Physiol.* **99**, 1044–1050.

Reimers, P.J. and Leach, J.E. (1991) Race-specific resistance to *Xanthomonas oryzae* pv. oryzae conferred by bacterial blight resistance gene *Xa-10* in rice *Oryza sativa* involves accumulation of a lignin-like substance in host tissues. *Physiol. Mol. Plant Pathol.* **38**, 39–56.

Roden, J.A., Belt, B., Ross, J.B., Tachibana, T., Vargas, J. and Mudgett, M.B. (2004) A genetic screen to isolate type III effectors translocated into pepper cells during *Xanthomonas* infection. *Proc. Natl Acad. Sci. USA*, **101**, 16624–16629.

Ronald, P. and Leung, H. (2002) THE RICE GENOME. The most precious things are not jade pearls … *Science*, **296**, 58–59.

Ryba-White, M., Notteghem, J.L. and Leach, J.E. (1995) Comparison of Xanthomonas oryzae pv. oryzae strains from Africa, North America, and Asia by restriction fragment length polymorphism analysis. *Int. Rice Res. Notes*, **20**, 25–26.

Sakthivel, N., Mortensen, C.N. and Mathur, S.B. (2001) Detection of *Xanthomonas oryzae* pv. oryzae in artificially inoculated and naturally infected rice seeds and plants by molecular techniques. *Appl. Microbiol. Biotechnol.* **56**, 435–441.

Sanchez, A.C., Brar, D.S., Huang, N., Li, Z. and Khush, G.S. (2000) Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci.* **40**, 792–797.

Schornack, S., Meyer, A., Romer, P., Jordan, T. and Lahaye, T. (2006) Gene-for-gene-mediated recognition of nuclear-targeted AvrBs3-like bacterial effector proteins. *J. Plant Physiol.* **163**, 256–272.

Shen, Y., Sharma, P., Goes da Silva, F. and Ronald, P. (2002) The *Xanthomonas oryzae* pv. oryzae *raxP and raxQ* genes encode an ATP sulphurylase and adenosine-5′-phosphosulphate kinase that are required for AvrXa21 avirulence activity. *Mol. Microbiol.* **44**, 37–48.

Sheng, Z.-J., Zhen, L.-Y. and Jun, F.-X. (2005) Detection of QTL conferring resistance to bacterial leaf streak in rice chromosome 2 (*O. sativa* L. spp. *indica*). *Scientia Agric. Sinica*, **38**, 1923–1925.

Shimamoto, K. and Kyozuka, J. (2002) Rice as a model for comparative genomics of plants. *Annu. Rev. Plant Biol.* **53**, 399–419.

Sidhu, G.S., Khush, G.S. and Mew, T.W. (1978) Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice, *Oryza sativa* L. *Theor. Appl. Genet.* **53**, 105–111.

Sigee, D.C. (1993) *Bacterial Plant Pathology: Cell and Molecular Aspects.* Cambridge, UK: Cambridge University Press.

Singh, D.V., Banerjee, A.K., Kishun, R. and Abidi, A.B. (1980) Effect of bacterial leaf streak on the quantitative and qualitative characters of rice. *Indian J. Mycol. Plant Pathol.* **10**, 67–68.

Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S. and Khush, G.S. (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13 and Xa21*) using marker-assisted selection into *indica* rice cultivar PR106. *Theor. Appl. Genet.* **102**, 1011–1015.

Singh, K., Vikal, Y., Singh, S., Leung, H., Dhaliwal, H.S. and Khush, G.S. (2002) Mapping of bacterial blight resistance gene *xa8* using microsatellite markers. *Rice Genet. Newslett.* **19**, 94–97.

Sivamani, E., Anuratha, C.S. and Gnanamanickam, S.S. (1987) Toxicity of *Pseudomonas fluorescens* towards bacterial plant pathogens of banana (*Pseudomonas solanacearum*) and rice (*Xanthomonas campestris* pv. oryzae). *Curr. Sci.* **56**, 547–548.

Song, W.Y., Pi, L.Y., Wang, G.L., Gardner, J., Holsten, T. and Ronald, P.C. (1997) Evolution of the rice *Xa21* disease resistance gene family. *Plant Cell*, **9**, 1279–1287.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*, **270**, 1804–1806.

Sugio, A., Yang, B. and White, F.F. (2005) Characterization of the *hrpF* pathogenicity peninsula of *Xanthomonas oryzae* pv. oryzae. *Mol. Plant–Microbe Interact.* **18**, 546–554.

Sun, X., Cao, Y. and Wang, S. (2006) Point mutations with positive selection were a major force during the evolution of a receptor-kinase resistance gene family of rice. *Plant Physiol.* **140**, 998–1008.

Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S. and Zhang, Q. (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. oryzae in rice, encodes an LRR receptor kinase-like protein. *Plant J.* **37**, 517–527.

Sun, Q.H., Hu, J., Huang, G.X., Ge, C., Fang, R.X. and He, C.Z. (2005) Type-II secretion pathway structural gene *xpsE*, xylanase- and cellulase secretion and virulence in *Xanthomonas oryzae* pv. oryzae. *Plant Pathol.* **54**, 15–21.

Sun, Q., Wu, W., Qian, W., Hu, J., Fang, R. and He, C. (2003) High-quality mutant libraries of *Xanthomonas oryzae* pv. oryzae and *X. campestris* pv. campestris generated by an efficient transposon mutagenesis system. *FEMS Microbiol. Lett.* **226**, 145–150.

Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T.W. and Kersters, K. (1990) Reclassification of the causal agents of bacterial blight *Xanthomonas campestris* pathovar oryzae and bacterial leaf streak *Xanthomonas campestris* pathovar oryzicola of rice as pathovars of *Xanthomonas oryzae* new species Ex Ishiyama 1922 Revived Name. *Int. J. Syst. Bacteriol.* **40**, 309–311.

Szurek, B., Marois, E., Van Bonas, U., Rossier, O., Hause, G. and Bonas, U. (2002) Type III-dependent translocation of the *Xanthomonas* AvrBs3 protein into the plant cell. *Mol. Microbiol.* **46**, 13–23.

Tan, G.-X., Ren, X., Weng, Q.-M., Shi, Z.-Y., Zhu, L.-L. and He, G.-C. (2004) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. *Yi Chuan Xue Bao*, **31**, 724–729 (in Chinese).

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.

Tang, D., Wu, W., Li, W., Lu, H. and Worland, A.J. (2000) Mapping of QTLs conferring resistance to bacterial leaf streak in rice. *Theor. Appl. Genet.* **101**, 286–291.

Thieme, F., Koebnik, R., Bekel, T., Berger, C., Boch, J., Buettner, D., Caldana, C., Gaigalat, L., Goesmann, A., Kay, S., Kirchner, O., Lanz, C., Linke, B., McHardy, A.-C., Meyer, F., Mittenhuber, G., Nies, D.-H., Niesbach-Kloesgen, U., Patschkowski, T., Rueckert, C., Rupp, O., Schneiker, S., Schuster, S.-C., Vorhoelter, F.-J., Weber, E., Pueh-ler, A., Bonas, U., Bartels, D. and Kaiser, O. (2005) Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. vesicatoria revealed by the complete genome sequence. *J. Bacteriol.* **187**, 7254–7266.

Tsuge, S., Furutani, A., Fukunaka, R., Oku, T., Tsuno, K., Ochiai, H., Inoue, Y., Kaku, H. and Kubo, Y. (2002) Expression of *Xanthomonas oryzae* pv. oryzae *hrp* genes in XOM2, a novel synthetic medium. *J. Gen. Plant Pathol.* **68**, 363–371.

Tsuge, S., Nakayama, T., Terashima, S., Ochiai, H., Furutani, A., Oku, T., Tsuno, K., Kubo, Y. and Kaku, H. (2006) Gene involved in transcriptional activation of the *hrp* regulatory gene *hrpG*. *Xanthomonas oryzae* pv. oryzae. *J. Bacteriol.* **188**, 4158–4162.

Tsuge, S., Terashima, S., Furutani, A., Ochiai, H., Oku, T., Tsuno, K., Kaku, H. and Kubo, Y. (2005) Effects on promoter activity of base substitutions in the cis-acting regulatory element of *HrpXo* regulons in *Xanthomonas oryzae* pv. oryzae. *J. Bacteriol.* **187**, 2308–2314.

Vasudevan, P., Kavitha, S., Priyadarisini, V.B., Babujee, L. and Gnanamanickam, S. (2002) Biological control of rice diseases. In: *Biological Control of Crop Diseases* (Gnanamanickam, S., ed.), pp. 11–32. New York: Marcel Dekker.

Vauterin, L., Hoste, B., Kersters, K. and Swings, J. (1995) Reclassification of *Xanthomonas*. *Int. J. Syst Bacteriol.* **45**, 472–489.

Vauterin, L., Yang, P., Hoste, B., Pot, B., Swings, J. and Kersters, K. (1992) Taxonomy of xanthomonads from cereals and grasses based on SDS-PAGE of proteins, fatty acid analysis and DNA hybridization. *J. Gen. Microbiol.* **138**, 1467–1477.

Vera-Cruz, C.-M., Bai, J., Ona, I., Leung, H., Nelson, R.-J., Mew, T.-W. and Leach, J.-E. (2000) Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proc. Natl Acad. Sci. USA*, **97**, 13500–13505.

Vera-Cruz, C.M., Gossele, F., Kersters, K., Segers, P., Van den Mooter, M., Swings, J. and De Ley, J. (1984) Differentiation between *Xanthomonas campestris* pv. oryzae, *Xanthomonas campestris* pv. oryzicola and the bacterial 'brown blotch' pathogen on rice by numerical analysis of phenotypic features and protein gel electrophoregrams. *J. Gen. Microbiol.* **130**, 2983–2999.

Wakimoto, S. and Mew, T.W. (1979) Predicting the outbreak of bacterial blight of rice by the bacteriophage method. *Philippine Phytopathol.* **15**, 81–85.

Wang, G.L., Ruan, D.L., Song, W.Y., Sideris, S., Chen, L., Pi, L.Y., Zhang, S., Zhang, Z., Fauquet, C., Gaut, B.S., Whalen, M.C. and Ronald, P.C. (1998) *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. *Plant Cell*, **10**, 765–779.

Wang, C., Tan, M., Xu, X., Wen, G., Zhang, D. and Lin, X. (2003) Localizing the bacterial blight resistance gene, *Xa22(t)*, to a 100-Kilobase bacterial artificial chromosome. *Phytopathology*, **93**, 1258–1262.

Wang, W., Zhai, W., Luo, M., Jiang, G., Chen, X., Li, X., Wing, R.A. and Zhu, L. (2001) Chromosome landing at the bacterial blight resistance gene *Xa4* locus using a deep coverage rice BAC library. *Mol. Gen. Genomics*, **265**, 118–125.

Wengelnik, K. and Bonas, U. (1996) HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. vesicatoria. *J. Bacteriol.* **178**, 3462–3469.

Wengelnik, K., Rossier, O. and Bonas, U. (1999) Mutations in the regulatory gene *hrpG* of *Xanthomonas campestris* pv. vesicatoria result in constitutive expression of all *hrp* genes. *J. Bacteriol.* **181**, 6828–6831.

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.

Xie, G.L. and Mew, T.W. (1998) A leaf inoculation method for detection of *Xanthomonas oryzae* pv. oryzicola from rice seed. *Plant Dis.* **82**, 1007–1011.

Xie, G., Sun, S., Chen, J., Zhu, X., Chen, J.YeY., Feng, Z. and Liang, M. (1990) Studies on rice seed inspection of *Xanthomonas campestris* pv. oryzicola: immunoradiometric assay. *Chinese J. Rice Sci.* **4**, 127–132.

Xu, W.H., Wang, Y.S., Liu, G.Z., Chen, X., Tinjuangjun, P., Pi, L.Y. and Song, W.Y. (2006) The autophosphorylated Ser686, Thr688, and Ser689 residues in the intracellular juxtamembrane domain of XA21 are implicated in stability control of rice receptor-like kinase. *Plant J.* **45**, 740–751.

Yang, B., Sugio, A. and White, F.F. (2005) Avoidance of host recognition by alterations in the repetitive and C-terminal regions of AvrXa7, a type III effector of *Xanthomonas oryzae* pv. oryzae. *Mol. Plant–Microbe Interact.* **18**, 142–149.

Yang, Z., Sun, X., Wang, S. and Zhang, Q. (2003) Genetic and physical mapping of a new gene for bacterial blight resistance in rice. *Theor. Appl. Genet.* **106**, 1467–1472.

Yang, B. and White, F.F. (2004) Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol. Plant–Microbe Interact.* **17**, 1192–1200.

Yang, B., Zhu, W., Johnson, L.B. and White, F.F. (2000) The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. oryzae is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. *Proc. Natl Acad. Sci. USA*, **97**, 9807–9812.

Yoshimura, A., Mew, T.W., Khush, G.S. and Omura, T. (1983) Inheritance of resistance to bacterial blight in rice cultivar Cas 209. *Phytopathology*, **73**, 1409–1412.

Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z.X., Kono, I., Kurata, N., Yano, M., Iwata, N. and Sasaki, T. (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl Acad. Sci. USA*, **95**, 1663–1668.

Young, S.A., Guo, A., Guikema, J.A., White, F.F. and Leach, J.E. (1995) Rice cationic peroxidase accumulates in xylem vessels during incompatible interactions with *Xanthomonas oryzae* pv. oryzae. *Plant Physiol.* **107**, 1333–1341.

Young, S.A., Wang, X. and Leach, J.E. (1996) Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with *Xanthomonas oryzae* pv. oryzae. *Plant Cell*, **8**, 1079–1090.

Zhang, Q., Lin, S.C., Zhao, B.Y., Wang, C.L., Yang, W.C., Zhou, Y.I., Li, D.Y., Chen, C.B. and Zhu, L.H. (1998) Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. oryzae) from *O. rufipogon*. *Rice Genet. Newslett.* **15**, 138.

Zhang, Q., Wang, C., Zhao, K., Zhao, Y., Caslana, V., Zhu, X.D., L. and Jiang, Q. (2001) The effectiveness of advanced rice lines with new resistance gene *Xa23* to rice bacterial blight. *Rice Genet. Newslett.* **18**, 71.

Zhao, B.Y., Ardales, E., Brasset, E., Claflin, L.E., Leach, J.E. and Hulbert, S.H. (2004b) The *Rxo1/ Rba1* locus of maize controls resistance reactions to pathogenic and non-host bacteria. *Theor. Appl. Genet.* **109**, 71–79.

Zhao, B., Ardales, E.Y., Raymundo, A., Bai, J., Trick, H.N., Leach, J.E. and Hulbert, S.H. (2004a) The *avrRxo1* gene from the rice pathogen *Xanthomonas oryzae* pv. oryzicola confers a nonhost defense reaction on maize with resistance gene *Rxo1*. *Mol. Plant–Microbe Interact.* **17**, 771–779.

Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J. and Hulbert, S. (2005) A maize resistance gene functions against bacterial streak disease in rice. *Proc. Natl Acad. Sci. USA*, **102**, 15383–15388.

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.

Zhu, W., Yang, B., Chittoor, J.M., Johnson, L.B. and White, F.F. (1998) AvrXa10 contains an acidic transcriptional activation domain in the functionally conserved C-terminus. *Mol. Plant–Microbe Interact.* **11**, 824–832.

Zhu, W., Yang, B., Wills, N., Johnson, L.B. and White, F.F. (1999) The C terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus. *Plant Cell*, **11**, 1665–1674.