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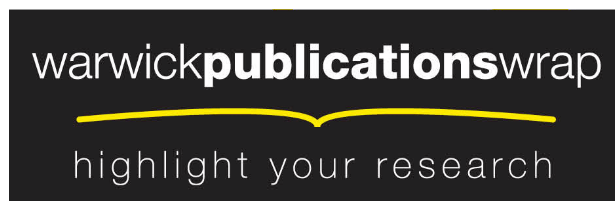

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MOLECULAR PLANT PATHOLOGY

Pathogen profile

Xanthomonas campestris pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops

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Summary: 399	Introduction: 1118
Black Rot control: 770	Pathogen identification and detection: 1091
Pathogen races: 1217	The pathogen genome: 553
Secretion systems: 767	Type III effector genes: 1673
Other virulence and pathogenicity factors: 1046	
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SUMMARY

Background: *Xanthomonas campestris* pv. *campestris* (*Xcc*) (Pammel) Dowson is a Gram negative bacterium that causes black rot, the most important disease of vegetable brassica crops worldwide. Intensive molecular investigation of *Xcc* is gaining momentum and several whole genome sequences are available.

Taxonomy: Bacteria; Proteobacteria; gamma subdivision; Xanthomonadales; Xanthomonas group; *X. campestris* species.

Host range and symptoms: *Xcc* can cause disease in a large number of species of *Brassicaceae* (ex-*Cruciferae*) including economically important vegetable *Brassica* crops and a number of other cruciferous crops, ornamentals and weeds including the model plant *Arabidopsis thaliana*. Black rot is a systemic vascular disease. Typical disease symptoms are V-shaped yellow lesions starting from the leaf margins and blackening of veins.

Race structure, pathogenesis and epidemiology: Collections of *Xcc* isolates have been differentiated into physiological races based on the response of several brassica species lines. Black rot is a seedborne disease. The disease is favoured by warm, humid conditions and can spread rapidly from rain dispersal and irrigation water.

Disease control: The control of black rot is difficult and relies on the use of pathogen-free planting material and the elimination of other potential inoculum sources (infected crop debris and cruciferous weeds). Major gene resistance is very rare in *B. oleracea* (brassica C genome). Resistance is more readily available in other species including potentially useful sources of broad spectrum resistance in *B. rapa* and *B. carinata* (A and AB genomes, respectively) and in the wild relative *A. thaliana*.

Genome: The reference genomes of three isolates have been released. The genome consists of a single chromosome of approximately 5,100,000 bp, with a GC content of approximately 65 and an average predicted number of CDS of 4308.

Important genes identified: Three different secretion systems have been identified in *Xcc*. The gene clusters *xps* and *xcs* encode a type II secretion system and *xps* genes have been linked to pathogenicity. The role of the type IV secretion system (T4SS) in pathogenicity is still uncertain, but it is possible that some of the genes can contribute to the virulence of the pathogen. The *hrp* gene cluster encode a type III secretion system that is associated with pathogenicity. An inventory of candidate effector genes has been assembled based on homology with known effectors; some genes have been associated with avirulence by elicitation of defense in resistant brassicas. A range of other genes have been associated with virulence and pathogenicity.

Useful web-site: http://www.xanthomonas.org/

INTRODUCTION

The genus *Xanthomonas* includes economically important pathogenic bacteria that are generally associated with plants (Vauterin *et al.*, 1990; Hayward, 1993). The taxonomy of this genus was initially determined according to host preference (typically the host of origin), and consequently a large number of species and pathovars have been defined (Burkholder, 1957). Morphological and other physiological and biochemical characters were subsequently used to group the *Xanthomonas* isolates into eight phenotypic groups (Van Den Mooter and Swings, 1990). The *Xanthomonas* species were later re-classified based on DNA-DNA hybridization, leading to *X. campestris* being restricted to comprise only the vascular pathogen *X. campestris* pv. *campestris* (Pammel) Dowson (*Xcc*) which causes black rot of brassica species, and additional pathovars that cause vascular or leaf spot diseases in cruciferous hosts including: *X. campestris* pvs. *aberrans* (Knösel) Dye, *armoraciae* (McCullock) Dye, *barbareae* (Burkholder) Dye, *incanae* (Kendrick & Baker) Dye and *raphani* (White) Dye (Vauterin *et al.*, 1995).

Debate continues in deciding what constitutes different pathovars. For example, some authors like Alvarez *et al.* (1994) have considered that *X. campestris* pv. *raphani*, a pathovar originally described by White (1930) that has a wide broad range of hosts within the *Brassicaceae* and *Solanacea*, and *X. campestris* pv. *armoraciae*, described one year earlier by McCulloch (1929) as a leaf spot disease of horse radish, are synonymous. Other authors like Tamura *et al.* (1994) and Vicente *et al.* (2006) have considered them to be distinct pathovars with different host range. Other *X. campestris* pathovars have received less attention. Some of these pathovars such as *X. campestris* pv. *aberrans* may not be distinct from *Xcc* (Vicente *et al.*, 2001; Fargier and Manceau, 2007; Fargier *et al.*, 2011). Fargier and Manceau (2007)

considered that the species can be restricted to three pathovars (*campestris*, *raphani* and *incanae*), but some isolates from ornamental crucifers, which are currently identified as pv. *campestris* or *incanae,* may still belong to distinct pathovars (Vicente *et al.*, 2006).

THE DISEASE

Black rot was first described by Garman (1894) at the end of the nineteenth century as a disease of cabbage in Kentucky, USA. He isolated two types of bacteria from diseased plants, but could not determine which type of bacteria was causing the disease. In Iowa, USA, Pammel (1895a; 1895b) observed a similar disease in rutabaga and turnip and showed that the disease was caused by a bacterium (named *Bacillus campestris*) with yellow pigmented colonies in culture. Reports from Wisconsin also attributed the disease of turnips and cabbage to the yellow bacterium (Russell, 1898; Smith, 1898). Since then, the disease has been identified in all continents wherever *Brassicaceae* crops are grown (Bradbury, 1986), and is considered the most important disease of vegetable brassica crops worldwide (Williams, 1980).

Brassica oleracea (including cabbage, cauliflower, broccoli, Brussels sprouts and kale), is economically the most important host of *Xcc*. However, the disease also occurs in other brassica crops, radish, ornamental crucifers and related weed species (Bradbury, 1986). Some accessions of *A. thaliana*, the model plant for molecular plant research, are also susceptible when inoculated with *Xcc.*

Life Cycle

Black rot is primarily a seedborne disease (Cook *et al.*, 1952). However, the disease can also be transmitted in infected transplants, infested soil, crop residues, and carry-over in related weed species (Walker, 1953; Schaad and Alvarez, 1993). Schaad and

White (1974) and Dane and Shaw (1996) have showed that *Xcc* can survive in the soil, independently from the host, for approximately 40 days in winter and 20 days in summer. The results of Arias *et al.* (2000) showed that high soil matric potential (saturated soils) can reduce the survival of the pathogen. The pathogen can survive longer in soil within plant tissues than as free living cells. Kocks and Zadoks (1996) showed that crop residues in fresh (2 weeks) refuse piles are more effective in spreading the disease than older (4 months) piles. In some conditions, cruciferous weeds can survive all year round and can provide potentially carry-over inoculum for the crops (Schaad and Dianese, 1981). Arias *et al.* (2000) showed that epiphytic survival of the bacteria on the phylloplane is dependent on the plant species as bacteria survived for 48 days on cabbage, mustard and lettuce, but only 9 days on rice. In some cases, infected crops have also been shown to provide inoculum for the weeds (Dane and Shaw, 1996), and one study has indicated that weeds do not play an important part in the dissemination of black rot (Schaad and Thaveechai, 1983).

The bacteria can disperse over short distances via wind, insects, aerosols, irrigation water, rain, farm equipment and workers. Commercial vegetable brassica crops are raised from transplants. In plant nurseries that produce module-raised transplants, the overhead irrigation system can increase significantly the dissemination of the bacteria and subsequently lead to a high level of disease in the field; changing the irrigation method can therefore limit the spread of the disease (Roberts *et al.*, 2007).

The bacteria generally enters the plant through hydathodes on the leaf margins, when droplets of gutation contaminated with bacteria are re-absorbed into the leaf (Russell, 1898). This mode of entry is dependent on a combination of environmental, biological and mechanical factors (Meier, 1934). In contrast, stomata

generally do not appear to be important for *Xcc* infection because the disease generally does not spread into surrounding tissues, even though the bacteria can enter the plant through the stomata and produce small dark spots (Cook *et al.*, 1952). This suggests that vascular movement of bacteria is essential for disease development. The bacterium can also enter the plant through wounds caused by machinery, insects, animals, rain, irrigation, wind and machinery.

The typical symptom of blackrot is the formation of V-shaped chlorotic, yellow, lesions with vertices towards the middle vein of the leaves (Figures 1a and 1b) and darkened veins that result from the bacterial movement on the vascular system. The affected tissues can become necrotic, and leaves can fall prematurely; systemic infections can cause stunted growth (Figure 1c) and death of young plants. Secondary infection by other bacterial species can also contribute to further development of severe rotting of vegetable tissue. The infection is often latent when temperatures are low, as the bacteria can persist in the vascular system without producing symptoms and, when the temperature rises, the typical symptoms become evident (Cook *et al.*, 1952; Walker, 1953; Schaad, 1982).

In general, *Xcc* thrives as a severe disease agent in warm, humid climates, and consequently is most serious in tropical, subtropical and humid continental regions (Williams, 1980). Given the global distribution of *Xcc*, black rot will be an increasingly important disease constraint favoured by climate change in more northern latitudes of vegetable production, such as in the southern regions of the UK and warmer regions in Europe.

BLACK ROT CONTROL

Sanitation and management practices that include crop rotation, weed control and use of assayed clean seed can provide significant control of the disease (Schaad and Alvarez, 1993). For example, black rot was a minor disease in the most important production areas in the USA during some decades, probably because growers followed recommended practices including the use of tested, disinfected seed and rotation of seedbeds. However, there was a resurgence of the disease during the 1970s, probably associated with the use of F_1 hybrid seed produced in areas where the disease was endemic (Williams, 1980).

Standard seed testing methods have been developed (Roberts and Koenraadt, 2006). The tolerance for reliable disease control through seed testing needs to be adjusted according to the system of production e.g. the number of seeds tested should be higher for transplants raised with overhead irrigation than for direct-drilled crops (Roberts *et al.*, 2007). Seed treatments including hot water, antibiotics, antibiotics and sodium hypochlorite, hydrogen peroxide and hot acidified cupric acetate or zinc sulphate are available, but no treatment is totally effective. Several methods can be used to reduce the spread of the disease during transplant raising including the use of web and flow irrigation systems instead of overhead irrigation and the use of chlorine dioxide in the irrigation water (Krauthausen *et al.*, 2011).

The development and use of black rot resistant cultivars has long been recognised as an important method of control, but in practice has had only limited success (Taylor *et al.*, 2002). Natural variation and the inheritance of black rot resistance have been studied in several brassica species and, so far, no disease resistance gene has been cloned. Most studies have focused on *B. oleracea* (representing the C genome of brassicas) and a limited number of sources of resistance have been identified including the cabbage cultivar Early Fuji and the

cabbage accession PI 436606 (cv. Heh Yeh da Ping Tou) (Williams, 1972; Dickson and Hunter, 1987; Hunter *et al.*, 1987; Camargo *et al.*, 1995; Taylor *et al.*, 2002; Vicente *et al.*, 2002). Badger Inbred-16, a line derived from Early Fuji, contains quantitative trait loci (QTL) for black rot resistance which have been genetically mapped (Camargo *et al.*, 1995).

The most common and potentially useful sources of black rot resistance occur in the A and B genomes of brassica species and a number of sources of resistance have been identified in the different species containing these genomes (Bain, 1952; Westman *et al.*, 1999; Taylor *et al.*, 2002). The inheritance of major gene resistance has been studied in the diploid *B. rapa* (A genome), and in the tetraploids *B. carinata* (BC genome) and *B. napus* (AC genome) (Guo *et al.*, 1991; Ignatov, 2000; Vicente *et al.*, 2002). A single dominant race-specific gene has been mapped to the A genome in *B. napus* (Vicente *et al.*, 2002), and quantitative loci that control resistance to at least two of the most prevalent races of *Xcc* have been mapped in a Chinese cabbage accession of *B. rapa* (Soengas *et al.*, 2007).

Genes present in the brassica A and B genomes could potentially provide durable black rot control, especially if strong race specific genes (matching the most prevalent races) could be combined in a genetic background of race non-specific genes (*e.g.,* providing quantitative resistance). To achieve this aim, genes from the wild relative *A. thaliana* could potentially be easier and quicker to molecularly characterise, and either be used directly in transgenic brassica crops, or facilitate the identification and interspecific transfer of homologous black rot resistance genes from A or B genome sources into vegetable crops. Interestingly, most *A. thaliana* accessions are resistant to one or more races of *Xcc,* and more than half exhibit broad spectrum resistance to all major races of the pathogen (described below), suggesting

that this wild relative of brassica crops could indeed provide useful sources of durable black rot resistance (Holub, 2007). Tsuji et al. (1991) showed that the resistance to an *Xcc* isolate in the accession Columbia is controlled by a single dominant gene/locus. In addition, Buell and Somerville (1997) described a monogenic and a digenic resistance mechanism in this accession and mapped three genes involved. Plant mutants impaired in resistance to *Xcc* have been isolated and a gene involved in the establishment of the HR and defence response has been identified and mapped (Lummerzheim *et al.*, 2004). However, even though *A. thaliana* and *Xcc* provided one of the earliest experimental models for investigating interactions of *A.thaliana* to a major crop pathogen (Simpson and Johnson, 1990), the molecular basis of natural variation in black rot resistance is largely unexplored in this pathosystem.

PATHOGEN IDENTIFICATION AND DETECTION

The bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson is a Gram negative rod, that occur mostly alone or in pairs and is usually motile by means of a single polar flagellum (Figure 1d). Most strains form yellow, mucoid, glistening colonies (Figures 1e and 1f). The yellow pigments, xanthomonadins (mono- or dibromo arylpolyene structures), and the exopolysaccharide xanthan, responsible for the mucoid or viscous cultures, are typical of the genus (Vauterin *et al.*, 1995), although the existence of atypical pigmented isolates has been reported (Poplawsky and Chun, 1995).

The taxonomy of the genus was mainly based on the hosts of origin and the phenotypic characteristics until the early 1990s. A detailed study of the phenotypic characteristics of the genus has been conducted by Van den Mooter and Swings (1990). Vauterin *et al.* (1995) later reclassified the genus based on DNA-DNA

hybridisation studies. In the new classification, the species *X. campestris* has been restricted to strains that cause disease in *Brassicaceae* (Crucifers) plants (including *X. campestris* pv. *aberrans*, *armoraciae*, *barbarea*, *campestris*, *incanae*, *raphani* and possibly *plantaginis*). The re-classification has been mainly supported with data obtained through other molecular techniques including AFLP and PCR fingerprinting (Rademaker *et al.*, 2000), but there has been some discussion regarding the shifts in the classification of some groups of isolates (Schaad *et al.*, 2000; Vauterin *et al.*, 2000).

The DNA-DNA hybridisation technique is not suitable for routine identification of new pathogen isolates so other molecular methods have been developed. The studies of the 16S rRNA gene (Hauben *et al.*, 1997; Moore *et al.*, 1997a; Moore *et al.*, 1997b) and the 16S-23S intergenic region (Gonçalves and Rosato, 2002) can generally only be used to identify the strains at the genus level. Simões *et al*. (2007) have differentiated species of *Xanthomonas* by PCR-restriction fragment length polymorphism of the genes *rpfB* and *atpD* involved in the regulation of pathogenicity factors and the syntheses of ATP.

Methods based on DNA sequencing have become more popular as the cost of sequencing has decreased. Sequencing of genes that encode conserved proteins involved in essential cell processes and collectively constitute the ‘core genome’ has been developed for identification of pathogens. Parkinson *et al.* (2007; 2009) have shown that sequences of DNA gyrase subunit B (*gyrB*) can be used as an identification tool at the genus, species and possibly pathovar level of *Xanthomonas*; this method is not resolving enough to differentiate isolates within each pathovar. Young *et al.* (2008) showed that multilocus sequence analysis (MLSA) based on partial sequences of four genes, chaperone protein dnaK (*dnaK*), tonB-dependent

receptor (*fyuA*), *gyrB* and RNA polymerase sigma factor (*rpoD*), can differentiate *X. campestris* from other species and can possibly also detect differences between some isolates of the same species. The results of multilocus sequence analysis of eight genes, ATP synthase beta chain (*atpD*), *dnaK*, elongation factor P (*efP*), glutamine synthetase I (*glnA*), *gyrB*, *ropD*, triosephosphate isomerise (*tpiA*) and TonB-dependent receptor (*fyuA*), showed high genetic diversity with *Xcc* isolates forming two distinct groups; the results also support the existence of two other related pathovars (*raphani* and *incanae*) (Fargier *et al.*, 2011).

The identification of *Xcc* at the pathovar level is generally based on isolation of the pathogen using semi-selective media. The currently used protocol for detection of the pathogen in seeds uses Fieldhouse-Sasser and mCS20ABN media (Koenraadt *et al.*, 2005; Roberts and Koenraadt, 2006). The morphology of the cultures is generally then checked in subcultures on media like Yeast Dextrose Calcium Carbonate. Classic bacteriological tests, carbon source metabolic fingerprinting (Biolog, Hayward, CA) (Poplawsky and Chun, 1995), fatty acid analysis (MIDI, Newark, DE) (Massomo *et al.*, 2003) and serological tests using polyclonal or monoclonal antibodies (Franken, 1992; Alvarez *et al.*, 1994) have been used to speed the identification of the organisms. All of these methods rely on the availability of databases with the results obtained with representative isolates of different species and pathovars, but frequently problems with the standard isolates used (e.g. misidentification) can complicate the interpretation of new results. The inoculation of susceptible Brassica seedlings is still the most reliable method as it provides the ultimate confirmation of the identification of the pathovar (Roberts and Koenraadt, 2006). However, all of these methods are time consuming and not adequate for high-throughput screening.

Several molecular methods have been used for the identification and characterisation of molecular diversity of *Xcc* and related pathovars. Rademaker *et al.* (2005) have used PCR primers that amplify repetitive sequences that are dispersed across bacterial genomes, to generate a method for distinguishing DNA 'fingerprinting' of isolates. Several studies have demonstrated that rep-PCR (using REP, ERIC and BOX primers) can differentiate isolates at the species, pathovar and intra-pathovar level of *X. campestris* (Rademaker *et al.*, 2005; Vicente *et al.*, 2006). Nevertheless, the comparison of gel profiles and the standardization of the method between laboratories is still difficult to achieve (Parkinson *et al.*, 2007). A DNA probe was developed for the detection of *Xcc*, but although the method worked for infected leaves, it was generally not sensitive enough to detect the pathogen in seeds (Shih *et al.*, 2000).

The *hrp* (hypersensitive response and pathogenicity) genes encode type III secretion systems (see section below). This gene cluster is involved in the plant-pathogen interactions, the growth and development of symptoms in plants and is largely conserved; therefore these genes are good candidates for molecular diagnostics of different species or pathovars. Berg *et al.* (2006) and Zaccardelli *et al.* (2007) developed PCR methods using primers that amplify part of the *hrpF* gene and the *hrcC* secretin-like gene, respectively. These methods allowed the identification of a range of *Xcc* isolates, but were also positive for isolates of the closely related pathovars *aberrans*, *armoraciae*, *raphani*, *barbarea*, *incanae*.

In the near future, the comparison of whole genome sequences might constitute the basis for classification and identification of *X. campestris* and PCR methods with primers related to pathogenicity genes might become part of routine protocols for the identification of *Xcc*. Presently, for the molecular identification to

the genus or species level, MLSA has the advantage of being a cheaper, flexible way of comparing bacteria and it is a practical, easier alternative to hybridisation studies and full sequencing. It is possible that different sets of house-keeping genes need to be selected to target variation between and within different species (Young *et al.*, 2008), but as the number of genes increases, the analysis of results becomes more complicated. At the pathovar level, primers based on *hrp* or other genes linked to pathogenicity might partially substitute the need for testing susceptible plants to confirm the identification.

PATHOGEN RACES

A race structure for *Xcc* was first proposed by Kamoun *et al.* (1992). The authors described five races (numbered 0 to 4) based on the reaction of different brassica species (Table 1). Vicente *et al.* (1998) and Ignatov *et al.* (1998b) have subsequently shown that race 1 can be subdivided into two or three races on the basis of their reaction on several accessions of *B. oleracea* and *B. carinata*. A revised race classification was proposed by Vicente *et al.* (2001) based on a much larger collection of isolates (Tables 2 and 3). Three races (1, 2 and 4) were retained from Kamoun *et al.* (1992), however, no isolate was found that matched race 3 so this race was dropped from the new race classification. Three variant classes were identified amongst the previous race 1 isolates based on the reactions of two *B. oleracea* accessions and an accession of *B. carinata*: a new race 1 that refers to the most commonly found variant, a new race 3 to accommodate a rare variant represented by the type strain of *Xcc* (ATCC33913; NCPPB 528), and an additional race, 5, for three non-UK isolates including an isolate previously included in *X. campestris* pv. *aberrans* (Vicente *et al.*, 2001). It was proposed that race 0 was re-assigned to a new

race 6 to avoid the implication that these isolates lacked avirulence genes; although these isolates are pathogenic in all the differentials currently used, partial resistance to this race has been observed in brassica accessions (Taylor J. D. et al., *unpublished data*). The race 2 is only represented by a single isolate (HRI 3849A), which was used in the earliest molecular investigations of black rot resistance in *A. thaliana* (Kamoun and Kado, 1990; Tsuji *et al.*, 1991; Buell and Somerville, 1997) (Table 3). More recently, race 7 has been added by Brita *et al.* (2007; 2010) and Fargier and Manceau (2007). In addition, Fargier and Manceau (2007) also included races 8 and 9 for classifying isolates that have a narrow host range in the differential cultivars (Tables 2 and 3).

Interestingly, the type strain (ATCC33913; NCPPB528) can cause atypical blight symptoms in brassicas (Alvarez *et al.*, 1994; Chen *et al.*, 1994) characterised by dark necrotic lesions with limited chlorosis and, in this respect, is not typical of the majority of *Xcc* isolates. These symptoms have also been observed in *B. oleracea* accessions inoculated with isolates of races 5 and 6. The ability to elicit blight symptoms may be under genetic control (Chen *et al.*, 1994), but may also be influenced by environmental conditions, especially temperature.

Homozygous brassica lines (*i.e.,* inbred or doubled haploid lines) provide the best means for reproducible, routine identification of *Xcc* races, and seed stocks can be readily re-generated by researchers or public stock centers. However, the current host differential set includes, for example, the cultivar Seven Top Turnip which exhibits variable reactions (possibly due genetic mixture from open pollination in the commercial seed production). F_1 hybrids are also included, which are impossible for researchers to re-generate, and may have limited availability in future years depending on the commercial success of each of these cultivars. To overcome part of these

constraints, doubled haploids from several accessions of *B. oleracea*, *B. napus*, *B. carinata* and *B. juncea* were produced at the University of Warwick, Warwick HRI (now part of the School of Life Sciences) to replace the previous differential lines described by Vicente *et al.* (2001). These include DH lines that replace Cobra, PI199947, Florida Broad Leaf Mustard and Miracle F_1.

Gene-for-gene interactions can be used to explain the relationship between bacterial isolates and differential lines. The proposed gene-for-gene model presented in Table 2 is based on the interaction of at least five matching gene pairs. The genes that confer resistance to the most important races (1 and 4) were designated *R1* and *R4*. The model allows for the possible inclusion of additional gene pairs if new races and differentials are identified. In general, the model was constructed in a way that reflects the origin of the allotetraploid brassica species (N, 1935): *R1* originates from the B genome, *R3* from the C genome and *R4* from the A genome. The proposed model needs to be supported by genetic and molecular data from both the host and the pathogen to be fully validated. In the case of the host, results of crosses made to establish the inheritance of resistance to some of the races indicate that *R1*, *R3* and *R4* are single dominant genes (Vicente *et al.*, 2002).

A simpler gene-for-gene model has been proposed by He et al. (2007) based on the interactions between *Xcc* isolates and cultivars of *Brassica* (*B. juncea*, *B. oleracea*, *B. rapa*), radish (*Raphanus sativus*) and pepper (*Capsicum annuum*) (Table 4). It is possible that some of the resistance/avirulence genes proposed by the authors correspond to genes included in Table 2: the gene Rc1 might correspond to R1, Rc2 to R3 and Rc3 to R4.

Worldwide, races 1 and 4 are predominant, but their relative frequencies in *B. oleracea* crops appear to vary with geographic regions. For example, race 1 appears to

be more common than race 4 in the UK, whereas race 4 was shown to be the predominant race in Portugal (Vicente, 2004), in northwestern Spain (Lema *et al.*, 2011) and in some East African countries like Tanzania and Uganda (Mulema *et al.*, 2012). Other races are generally rare, but may be more common in other host species that are less frequently surveyed. Races 2 and 6 were absent in a collection of isolates from Japan and Russia (Ignatov *et al.*, 1998a). Nepal and the northwest of Spain seem to have diverse populations of *Xcc*, with five different races identified in *B. oleracea* crops plants (Jensen *et al.*, 2010; Lema *et al.*, 2011). The low frequency of race 3 worldwide may be due to the extensive use of cultivars that are resistant to this race. However, this may not have been the case 50 years ago when the type strain of *Xcc* (ATCC33913, NCPPB528) was collected in 1957. Race 5 is also rare, but was recently found in Nepal (Jensen *et al.*, 2010) and in a field in the northwest of Spain (Lema *et al.*, 2011). Race 6 was not found in the UK, but this may have been due to the preponderance of isolates obtained from *B. oleracea*. In Portugal and in the northwest of Spain, race 6 was common in turnip (Vicente, 2004; Lema *et al.*, 2011).

The gene-for-gene model and the availability of defined 'race type strains' should assist in the selection and evaluation of plant material for breeding programs and can be the base for molecular studies. Disease resistance screening should be done with isolates that represent the pathogenic variation of *Xcc*, and therefore should at least include the major races, 1 and 4. Additionally, isolates of race 6 should be useful to detect potential race-non-specific resistance. Monitoring the frequency and distribution of races worldwide is essential to the development of effective strategies for breeding black rot resistant cultivars. Future brassica crops will benefit from combining major genes that confer strong resistance to the most common races of the

pathogen (*R1* and *R4*) and, if possible, race non-specific genes that could confer quantitative resistance to all known races.

THE PATHOGEN GENOME

Three isolates of *Xcc* have been fully sequenced including the type strain ATCC33913 (NCPPB528; LMG568; ICMP13; DSM3586) (da Silva *et al.*, 2002), isolate 8004 (a spontaneous rifampicin-resistant mutant from NCPPB1145) (Qian *et al.*, 2005) and the industrial high xanthan-producing isolate B100 (Vorhöelter *et al.*, 2008). The type strain has been identified as race 3 (Vicente *et al.*, 2001), isolate 8004 has been assigned to race 9 (Fargier and Manceau, 2007) and B100 belongs to race 1 (Vicente, unpublished). The isolate 8004 has featured in several studies of phytopathological properties such as secretion of extracellular enzymes and exopolysaccharides (Tang *et al.*, 1991), cell-cell signalling and biofilm formation (Torres *et al.*, 2007). Further information about the *Xcc* genomes is summarised in Table 5. An isolate of *X. campestris* pv. *raphani* (756C), a closely related pathogen that causes a non-vascular, leaf spot pathogen on *Brassicaceae* and other hosts like tomato, has also been recently sequenced (Bogdanove *et al.*, 2011). These *X. campestris* genomes comprise circular chromosomes of approximately 5,000,000 base pairs (bp), have a high G+C content and do not carry plasmids.

The comparison of the sequence of *Xcc* type strain ATCC33913 and 8004 indicated that significant genomic-scale rearrangement across the replication axis between two IS1478 elements and loss and acquisition of blocks of genes, rather than point mutations, constitute the main genetic variation between the strains (Qian *et al.*, 2005). The sequence of B100 is extensively collinear to the strain 8004, but it differs from the type strain ATCC33913 by the inversion of a large chromosomal fragment

(Vorhöelter *et al.*, 2008). This may indicate that the strains 8004 and B100 have originated via recent recombination events (Qian *et al.*, 2005).

He *et al.* (2007) constructed a whole-genome microarray of the *Xcc* strain 8004 and have used this resource to study the genetic diversity and host specificity this pathogen by array-based comparative hybridization with genomes of 18 pathogenic isolates collected from different host plants and various geographical regions of China. A core set of 3,405 conserved coding sequences was identified and 730 coding sequences that are absent or highly divergent amongst the isolates. Of the 304 proven or postulated pathogenicity genes currently known in Xanthomonas, 258 were conserved and 46 were highly divergent amongst the isolates.

These current reference genomes of *Xcc* are useful for genome-wide comparisons with publically available genomes from other *Xanthomonas* species including strain 306 of *X. axonopodis* pv. *citri* (da Silva *et al.*, 2002) which represents the citrus canker pathogen, strain 85-10 of *X. axonopodis* pv. *vesicatoria* (Thieme *et al.*, 2005) which represents the bacterial spot pathogen which specifically attacks pepper (and not tomato), strains of the rice bacterial blight pathogen *X. oryzae* pv. *oryzae*, KACC10331 (Lee *et al.*, 2005), MAFF 311018 (Ochiai *et al.*, 2005) and PXO99^{A} (Salzberg *et al.*, 2008), strain BLS256 of *X. oryzae* pv. *oryzicola* (Bogdanove *et al.*, 2011), strain GPE PC73 of *X. albilineans* (Pieretti *et al.*, 2009) and strains from *X. vasicola* pv. *vasculorum* and *musacearum* the cause of banana wilt (Studholme *et al.*, 2010). For example, Lu *et al.* (2008) compared six gene clusters associated with pathogenicity across genomes of eight *Xanthomonas* isolates representing vascular and non-vascular diseases of rice, brassicas, pepper and citrus. Interestingly, plasmid DNA was only observed in the reference isolates of *X. axonopodis* pv. *citri* and pv. *vesicatoria*.

SECRETION SYSTEMS

Reference genomes of *Xcc* have begun to reveal an extensive inventory of genes that may be required for plant associations based on homology with known genes from other *Xanthomonas* or *Pseudomonas* species. Most importantly, genome-wide comparisons have revealed three secretion systems (Type II, III and IV) in *Xcc* that have been associated with pathogenic bacteria of plants or animals.

The type II secretion system (T2S) mediates transport of proteins into the extracellular space. This system can secrete plant cell wall-degrading enzymes including cellulose, polygalacturonase, xylanase and protease. Two classes of type II secretion system were identified in *Xanthomonas*, *xps* and *xcs*. The *xps* cluster is present in all *Xanthomonas* genomes currently sequenced and in other genus (e.g. *Xylella*) whilst the *xcs* genes are only present in some of the *Xanthomonas* genomes including *Xcc* and *Xcr* (Lu *et al.*, 2008). Qian *et a*l. (2005) obtained six pathogenicity deficient mutants (*xpsD*, *xpsE*, *xpsF*, *xpsK*, *xpsL* and *xpsM*) related with the *xps* system, whereas no pathogenicity-deficient mutants have been found on the 12 annotated genes related to the *xcs* system; therefore the *xps* genes are related with pathogenicity whilst the *xcs* might have other roles not essential for pathogenicity or might be non-functional.

Mutation of DsbB, a protein involved in disulfide bond formation in the periplasm of *Xcc*, reduced virulence, cell mobility and bacterial growth in planta and resulted in ineffective type II and type III secretion systems (Jiang *et al.*, 2008b).

The Type IV secretion system (T4SS) is important for release of macromolecules and this system is used by gram-negative bacteria to translocate protein and DNA substrates across the cell envelope into target cells (Souza *et al.*, 2011). However, the role of T4SS in pathogenicity of *Xanthomonas* is uncertain. The

T4SS carried in the race 9 isolate 8004 is encoded mainly by genes in the *virD4* and the *virB* cluster that consists of 9 ORFs. Qian *et al*. (2005) identified a mutated gene encoding the channel-forming protein VirB8 and concluded that the T4SS contributes to virulence of the pathogen. However, He *et al*. (2007) have shown that deletion of *virD4* and the *virB* cluster of strain 8004 does not affect virulence of the pathogen.

The type III secretion system (T3SS) is generally thought to be essential for pathogenicity and initiation of disease in susceptible plant hosts, and can be involved in elicitation of cell death and other defence responses in resistant plants. The T3SS is encoded by the *hrp* (*h*ypersensitive *r*esponse and *p*athogenicity) cluster of genes and translocates effector proteins directly into the plant cells; it is also possible that effectors can exit the bacterial phytopathogens via the Hrp pathways (Lindgren, 1997). Individual genes have been named *hrp*, *hrp*-conserved (*hrc*) or *hrp*-associated (*hpa*). Most *hrp* gene sequences are only found in *Xanthomonas* and related genera while *hrc* genes are conserved in many plant and animal pathogens. In *Xcc*, the *hrp* cluster consists of 26 genes extending from *hpa2* to *hrpF* (da Silva *et al.*, 2002). The *Xcc* genomes do not contain *hpa3* and the N-terminal region of *xopF1* although these genes are present in other *Xanthomonas* including *Xcr* (Lu *et al.*, 2008). Four mutants of genes encoding T3SS machinery (*hrcJ*, *hrcU*, *hrcV*, *hrpE*) and one encoding a regulatory protein (*hrpG*) were non-pathogenic (Qian *et al.*, 2005). Mutations in the regulator *hrpG* of strain 8004 can make all the *hrp* genes express constitutively and enhance the intensity of the HR reaction in pepper (Jiang *et al.*, 2006).

Wei *et al.* (2007) showed that *hpaR*, a putative *marR* family transcriptional regulator, is essential for the pathogenicity of strain 8004 on cabbage, is required for the HR response on non-host pepper and is under the positive control of the two key *hrp* gene regulators HrpG and HrpX. The GntR family is a frequent group of helix-

turn-helix transcription regulators in bacteria. One of the six GntR regulators, HpaR1 positively and negatively affects the expression of HR and pathogenicity *hrp* genes that encode the type III secretion via hrpG (An *et al.*, 2011).

Several genes that are regulated in a HrpX-dependent manner possess a consensus nucleotide sequenced TTCGB-N_{15}-TTCGB (B can be base C, G or T), which has been termed plant-inducible-promoter box, or PIP box. The detection of PIP boxes provides a refined tool for identification of candidate genes in the hrpX regulon as well as effector protein genes of the type III pathway. For example, 17 putative PIP box sequences have been described in the promoter regions of *Xcc* ATCC33913 (da Silva *et al.*, 2002) and 56 genes were predicted to have PIP boxes in strain 8004 (Jiang *et al.*, 2009).

TYPE III EFFECTOR GENES

There has been considerable research effort to identify and understand T3 effector function in *Xanthomonas* species. White (2009) reviewed in detail the type III effectors from *Xanthomonas* and grouped them in 39 different groups (called Xop) reflecting sequence similarity. From these, 21 appear to be present in the *Xcc* strain ATCC33913. Nine effectors considered to be core effectors, are present in almost all *Xanthomonas* isolates with the exception of *X. albilineans* and in some cases *Xcr*; other effectors are found in more limited number of species/isolates (Bogdanove *et al.*, 2011; Ryan *et al.*, 2011). From the extensive list of genes encoding putative T3SS proteins in *Xcc* and *X. campestris* pv. *raphani* summarised in Table 6, effort has begun to identify potential effector proteins (which are important for pathogenicity in a susceptible host) and avirulence determinants (which elicit defence in a resistant host). Effector proteins are generally thought to be involved in suppressing the host

defence system or in altering host metabolism to favour nutrient uptake of the pathogen. However, experimental verification of effector function can be difficult. For example, deletion of individual effector genes can often result in little or no reduction in pathogenicity because of the presence of alternative functional (redundant) effector proteins (Cunnac *et al.*, 2009). Some T3SS effectors may activate defences (*e.g.,* rapid host cell death) when infiltrated into plant tissue and thus indicate a potential role as avirulence (Avr) proteins in a resistant host (Flor, 1971; Keen, 1990).

Xu *et al.* (2006) showed that mutagenesis of eight candidate genes from strain 8004 had no affect on pathogenicity in Chinese radish and cabbage. However Xu *et al.* (2006) and He *et al*. (2007) showed that mutants of *avrBs1* lost the ability to elicit an HR in the pepper line ECW10R that contains the corresponding R gene *Bs1* (Table 4). The AvrBs1 effector is responsible for eliciting HR on pepper dependent on T3SS (Xu *et al.*, 2006). However Castañeda *et al*. (2005) did not detect an HR variation between the *avrBs1* mutants and the wild type 528T. He et al. (2007) suggested that it is possible that the function of *avrBs1* is redundantly encoded in the *Xcc* type strain and that the expression is different in the two strains.

Ignatov *et al.* (2002) reported that an *avrBs2* homolog, designated *avrRxc1/3*, determined avirulence of *Xcc* 512/2 (a natural mutant strain of a race 3 isolate) on *Brassica* plants with the B genome, including *B. juncea* cv. Florida Mustard and *B. carinata* line PI199947 and on some *B. oleracea* cultivars (including the lines SR1/3, Badger Inbred-16/2 and cv. Miracle F1). This gene was also responsible for a mesophyllic HR on leaves of Florida Mustard and non-host pepper plants carrying *Bs2*. Xu *et al*. (2006) showed that *avrBs2* is required for full virulence of strain 8004 on Chinese radish and cabbage. In contrast, Castañeda *et al*. (2005) showed that the

deletion of the entire putative *avrBs2* homolog in the type strain had no effect on the interaction with Florida Mustard and pepper. The strains used by Ignatov *et al*. (2002) are probably derived from the type strain that was used by da Silva *et al*. (2002) and Castañeda *et al*. (2005) and therefore it is difficult to conciliate these results and the role of the *avrBs2* homolog on pathogenicity/race specificity still need to be clarified.

Genes from the AvrBs3 family have been found in a number of *Xanthomonas* species and pathovars and in *Ralstonia solanacearum* (White *et al.*, 2009). This large family of closely related T3 effectors constitute the TAL effector family. All members of the family have a common structure with tandem repeats of a sequence of amino acids typically of 34 amino acids in the central part of the proteins, imperfect heptad leucine zipper (LZ) repeats, nuclear localization signals (NLSs) and an acidic activation domain (AAD); the avirulence and virulence specificity of an AvrBs3 member depend on the number and order of the repeats and the NLSs and the AAD (Gurlebeck *et al.*, 2006).

Interestingly, *Xcc* does not contain genes from the *avrBs3/pth* family, whereas three members of this family have been identified in an isolate identified as *X. campestris* pv. *armoraciae*. These three genes were designated *hax2*, *hax3* and *hax4* (homolog of *avrBs3* in *Xanthomonas*) (Kay *et al.*, 2005). The three Hax proteins are translocated via the T3SS. The Hax3 and Hax4 proteins have the typical structure of the AvrBs3-like effectors with 34 amino-acid repeats in the central part, but Hax2 has 35 amino acid repeats that contain an additional proline residue. The three *hax* genes have an additive effect on disease symptom in radish with hax2 having the strongest influence and two *hax* genes (*hax3* and *hax4*) have a *Bs4*-dependent avirulence activity in tomato (Kay *et al.*, 2005).

Transconjugants of Chinese isolates containing *avrXccE1* (*xopE*) became avirulent on the Chinese cabbage (*B. rapa*) cv. Zhongbai-83 (He *et al.*, 2007) (Table 4). In contrast Castañeda *et al.* (2005) did not notice any effect on pathogenicity on the plants tested, but it is possible that the lines tested by those authors did not possess the *R* gene responsive to *avrXccE1*.

Jiang *et al.* (2008a) showed that XopN from strain 8004 (designated XopXccN) is required for full virulence. A mutant of the strain 8004 with an insertion in *xopXccN* was significantly weaker than strain 8004 when inoculated on Chinese radish (*Raphanus sativus* var. *radiculus*) cv. Manshenhong. The expression of *xopXccN* is regulated by the key *hrp* regulators HrpG and HrpX.

Mutagenesis of the eight candidate effector genes from type strain ATCC33913 (syn. 528^T) had no effect on pathogenicity when mutants were used to inoculate six crucifer species, and none of the mutated genes singly or in any combination affected the non-host HR elicited by the type strain in pepper. However, insertion or deletion mutants in a locus of a gene designated *avrXccFM* (XopAH) became virulent on Florida Mustard, and therefore changed the race specificity of the isolate (Castañeda *et al.*, 2005). The T3SS effector AvrXccC of strain 8004 that belongs to the AvrB effector family of *Xanthomonas* has been shown to have a dual effect: this effector is required for full bacterial virulence in a susceptible *B. oleracea* cv. Jingfeng 1 and for avirulence in a *B. napiformis* L.H. Bailey (syn. *B. juncea* (L.) Czern. var. *napiformis* (Pailleux & Bois) Kitam.) accession (not specified) (Wang *et al.*, 2007). The *avrXccC* gene was expressed in the race 2 strain HRI3849A (that causes symptoms on the mustard accession used by these authors) and the resulting strain was avirulent. He *et al*. (2007) also showed that an avrXccC mutant of 8004 became pathogenic on mustard (*B. juncea* var. *megarrhiza* Tsen et Lee) cv. Guangtou

and Chinese cabbage (*B. rapa* subsp. *pekinensis*) cv. Zhongbai-83. The *avrXccC* gene of strain 8004 that confers avirulence on some mustard cultivars is identical to *avrXccFM* of strain ATCC33913 (syn. 528T) that confers avirulence on Florida Mustard (He *et al.*, 2007). Wang *et al*. (2007) showed that AvrXccC is anchored to the plant plasma membrane and its avirulence function for host recognition depends on its location. The expression of *avrXccC* is *hrpG/hrpX*-dependant.

Xcc also contains genes encoding leucine-rich-repeat (LRR) proteins. These motifs are commonly involved in protein-protein interactions and are found in several classes of plant disease resistance genes. Xu *et al*. (2008) showed that one of these genes, *xopAC*, named $avrAC_{Xcc8004}$, encodes a protein containing LRRs; this gene is a type III effector that appears to be restricted to strains of *X. campestris*. The *A. thaliana* accession Col-0 is resistant to the wild-type 8004 strain and to an $avrAC_{Xcc8004}$ mutant when the leaf mesophyll was infiltrated with bacterial suspensions, but the Col-0 ecotype became susceptible to the mutant when the bacteria was introduced into the vascular system by piercing the central vein of leaves indicating that the product of $avrAC_{Xcc8004}$ is recognised in vascular tissues and this gene might be related to the ability to colonize the xylem of a host.

Some *avr*/effector genes have also been identified in the closely related pathovars including isolates identified as *X. campestris* pv. *armoraciae* and *raphani* (possibly synonymous). Parker *et al.* (1993) cloned an avirulence gene of an isolate identified as *X. campestris* pv. *raphani* (isolate 1067). This isolate was avirulent in all the *A. thaliana* accessions tested by the authors. When the *avrXca* gene was transferred to *Xcc* strain 8004, it strongly reduced symptom development and bacterial growth in Columbia plants, but did not affect virulence to *Brassica* plants. The *avrXca* gene encodes a protein of 67 kDa that had no homology with known

sequences and conferred avirulence in a number of *A. thaliana* accessions except one ('Kas-1'). The avirulence phenotype is not *Hrp* dependent and the interaction with *A. thaliana* did not lead to a characteristic HR, indicating that this may not be a type III effector. More recently, Corbett *et al*. (2005) have characterised a virulence factor designated Svx of *E. carotovora* subsp. *atroseptica* that shows homology to AvrXca. This protein is secreted by type II secretion apparatus and the transcription of the *svx* gene is regulated by quorum-sensing; the function of this protein is unknown, but the authors consider that it may play a role in pathogenicity of *E. carotovora* pv. *atroseptica*.

So far, there is some evidence that ten of the putative effector genes from *Xcc* are phenotypically functional in a very restricted number of isolates. Some of the other putative effectors could be pseudogenes or poorly expressed genes in the isolates studied or the genes might have small effects on pathogenicity or fitness that have not been detected in the assays (Castañeda *et al.*, 2005). Further research into the putative effectors using other isolates and hosts might still show that they have a function in pathogenicity and/or race specificity. It is also possible that some effectors were once pathogenicity genes that determine the host range of the pathogen; the function of some of these effectors could be gratuitous or even detrimental, but their structure might allow rapid adaptive selection for pathogenic function (Gabriel, 1999).

OTHER VIRULENCE AND PATHOGENICITY FACTORS

The surface structure and appendages of phytopathogenic bacteria are important for the attachment, colonization and infection of the host. Type IV pili may contribute to bacterial pathogenesis by affecting surface motility, micro-colony and biofilm formation, adhesion, immune evasion and cell signalling (Craig *et al.*, 2004). The comparison of genomes from isolates ATCC33913 and 8004 showed that at least 26

genes related to the pili assembly are highly conserved and mutations in two of the assembly genes (*pilB* and *pilC*) have reduced virulence (Qian *et al.*, 2005).

Many phytopathogenic bacteria produce a large number of factors that might be essential or contribute to cause disease. The bacteria from the genus Xanthomonas typically produce yellow, membrane-bound pigments called xanthomonadin. These pigments have a role in maintain the ecological fitness of the bacteria, protecting the cells against photooxidative stress. The xanthomonadin biosynthesis is encoded by the *pig* cluster of genes. Poplawsky and Chun (1998) showed that mutation of *pigB* result in a reduction of epiphytic survival and decrease in infection via the hydathodes. The biosynthesis of xanthomonadin is regulated by 3-hydroxybenzoic acid, a diffusible factor (DF) that is associated with a range of biological functions; DF-deficient mutants are non pigmented, impaired in survival ability and less virulent (He *et al.*, 2011).

Xcc produces a range of extracellular enzymes (including proteases, pectinases and endoglucanase). The extracellular enzymes are capable of degrading the plant cell components and may be required to overcome plant defence responses, to allow bacteria to move into uncolonized plant tissues and to mobilize plant polymers for nutritional purposes (Torres *et al.*, 2007).

In *Xcc*, the synthesis of extracellular degrading enzymes and exopolysaccharides is regulated by products of the *rpf* genes (rpfABFCHGDIE) (da Silva *et al.*, 2002). The *rpf* gene cluster of *Xcc* regulates genes involved in the synthesis of extracellular hydrolytic enzymes, extracellular polysaccharides (including xanthan) and is involved with motility, toxin, oxidative-stress resistance, aerobic respiration and biofilm formation (Wilson *et al.*, 1998; Dow *et al.*, 2003). A diffusible signal factor (DSF) responsible for cell-to-cell signalling is involved in the

transcriptional control of these genes (Barber *et al.*, 1997). Some genes under control of the rpf/DSF system are required for the first stages of endophytic colonization (Gudesblat *et al.*, 2009). The expression levels of proteases and endoglucanases are reduced when the *rpfI* gene is inactivated in *Xcc*, suggesting that *rpfI* may have a function in the extensive tissue degeneration that is characteristic of black rot (Dow *et al.*, 2000). Mutants deficient in RpfG, a phosphodiesterase that degrades c-di-GMP or the elevation of c-di-GMP via overexpression of a GGDEF domain protein WspR caused aggregation of cells, reduction in mobility and decrease of production of virulence factors, extracellular enzymes and exopolysaccharides (Hsiao *et al.*, 2011).

The Mips (macrophage infectivity potentiators) genes encode proteins reported as virulence factors in human pathogens. Zang *et al.* (2007) showed that a *mip*-like gene of *Xcc* is involved in pathogenicity through an effect on the production of exopolysaccharides and on the activity of extracellular proteases.

Extracellular polysaccharides (EPS) play an important role in the pathogenicity and virulence of many bacteria both in terms of direct interactions with host cells and in conferring protection against hostile environments (Coplin and Cook, 1990). The most important EPS secreted by *Xcc* is xanthan gum, an extracellular polysaccharide (EPS) that has many industrial applications. Xanthan is a complex extracellular polysaccharide with a cellulosic back-bone and trisacchride side chains of two mannose and one glucoronate residues that are attached to every second glucose in the backbone (Jansson *et al*, 1975) (Becker *et al.*, 1998; Yun *et al.*, 2006; Vorhöelter *et al.*, 2008). Yun *et al.* (2006) have shown that a xanthan minus mutant strain and a mutant strain that produced truncated xanthan failed to cause disease in *A. thaliana* and *Nicotiana benthamiana* and induced the deposition of callose in these plants; pre-treatment of the plant with xanthan restored the pathogenicity of both

strains. The results indicate that xanthan induces susceptibility to *Xcc* by suppressing callose deposition.

Xanthan has a broad range of applications in food and non-food products from the oil, pharmaceutical, cosmetic, paper, paint and textile industries and is mainly used as a thickening, stabilising, gelling and emulsifying agent (Becker *et al.*, 1998). The biosynthesis of xanthan production and the genes that encode for the enzymes involved have been studied (Ielpi *et al.*, 1993; Vanderslice *et al.,* 1990). The xanthan synthesis is encoded by twelve *gum* genes, (*gumBCDEFGHIJKLM*) which are located in a single gene cluster of 12 kb that is mainly expressed as an operon from a promoter upstream of the first gene, *gumB* (Vojnov *et al.*, 2001). The genes required for the synthesis of xanthan and its nucleotide sugar precursors are highly conserved among the three sequenced strains (ATCC33913, 8004 and B100), but are significantly different when compared with the other sequenced *Xanthomonas*; a model for the biosynthesis of xanthan based on the genome annotation has been proposed (Vorhöelter *et al.*, 2008). The disruption of the *rmlA, xanA* and *gumK* genes that are responsible for the biosynthesis of intermediates for xanthan production, can lead to loss of pathogenicity (Qian *et al.*, 2005).

The lipopolysaccharide (LPS) is an essential component of the outer membrane of gram negative bacteria and is an important virulence factor in *Xanthomonas* (Dow *et al.*, 1995; He *et al.*, 2007; Patil *et al.*, 2007). Over 20 genes for LPS synthesis have been characterized in *Xcc* including *xanAB*, *rmlABCB*, *rfaXY*, *lpsIJ* and 15 genes that constitute the *wxc* cluster (Vorholter *et al.*, 2001). The *wxc* gene cluster is involved in the synthesis of the LPS O-antigen, the most variable part of LPS. The *wxc* genes are highly divergent between *Xcc* strains indicating that different isolates might produce varied LPSs (He *et al.*, 2007). The LPS gene cluster

of *Xcc* B100 is significantly different from 8004 and ATCC33913, but it is almost identical to the LPS cluster of *Xcr* 756C. This could indicate that the LPS cluster of B100 was acquired recently via horizontal gene transfer from *Xcr* 756C or, more likely, that the *Xcr* strain diverged recently from an *Xcc* with a B100 type LPS locus (Patil *et al.*, 2007). Qian *et al.* (2005) showed that the disruption of a number of *wxc* genes resulted in significant reduction of virulence.

FUTURE PERSPECTIVES

Research into *Xcc* and closely related pathovars has now reached the genomic age, although it is still lags behind progress from investigation of *Pseudomonas* pathogens such as *P. syringae* pv. *tomato* and *maculicola*. Our understanding of *Xcc* is increasing rapidly through functional and comparative genomic studies and we are starting to understand the role of some of the key genes involved in pathogenicity. Nevertheless there are still many areas that need further work including the study of the mode of entry of the pathogen, such as comparisons between the vascular pathogen *Xcc* that generally penetrate the hosts via the hydathodes and the non-vascular pathogen *Xcr* that generally penetrate through stomata. The effect of the environment and genetic factors in determining the preferred mode of entry of these pathogens is still under-studied. The importance of epiphytic survival and factors that contribute for it are also not well understood.

Recent studies have shown the role of some effector proteins, but most of them are still not characterised and their molecular targets and function are still unknown. Most of the effector/avirulence genes in the postulated gene-for-gene model have not been molecularly identified yet.

With the advances in sequencing, additional sequences of different *Xcc* isolates, including multiple isolates from each race and also additional sequences of isolates of closely related pathovars should be available soon. The comparison of multiple sequences should then provide more clues on the important genes that determine pathogenicity including effectors; presence and absence of particular genes and the variability between isolates will give some clues on the role and evolution of these genes. Functional analysis of the genes can then confirm their role in the plant-pathogen interactions.

On the host side, a number of disease resistance genes in brassicas and *A. thaliana* have been postulated, some have been mapped, but none has been cloned. The effort to identify more disease resistance genes should be continued and some of the most important crops could be improved with the incorporation of disease resistance genes from closely related species via interspecific crosses and embryo rescue or via transformation. It is also possible that engineering the promoters of known R genes to recognise multiple effectors could contribute to the development of broad spectrum disease resistant crops (Ryan *et al.*, 2011). The control of the disease could be improved by pyramiding disease resistance genes and/or using multiple lines with different gene combinations that could be mixed and balanced to match the avirulence genes present in the bacterial population (Pink and Puddephat, 1999).

Application of functional genomics and proteomics to bacteria in planta to identify virulence factors and application of functional genomics and proteomics to both resistant and susceptible host plants inoculated with *Xcc* will provide key information on the interaction between the bacteria and the hosts. Research on the diversity of *Xcc*, pathogenicity factors and evolution together with host-pathogen

interaction studies should lead to improvements in the prevention and control of black rot of crucifers.

TABLE 1. Differentiation of *Xanthomonas campestris* pv. *campestris* races according to Kamoun *et al.* (1992).

Differential cultivars	Races				
	0	1	2	3	4
Early Jersey Wakefield (*B. oleracea*)	+	+	+	+	+
Just Right Turnip, Tokyo Turnip Hybrid (*B. rapa*)	+	+	+	-	-
Seven Top Turnip (*B. rapa*)	+	+	-	+	-
Florida Broad Leaf India Mustard (*B. juncea*)	+	-	+	-	-

TABLE 2. Postulated gene-for-gene model to explain the relationship between *Brassica* lines and races of *Xanthomonas campestris* pv. *campestris* (adapted from Vicente *et al.* (2001), Fargier and Manceau (2007) and Jensen *et al*. (2010)).

Differential cultivars or accessions	**Resistance genes (R)**					**Races / Avirulence genes (A)**								
						1	2	3	4	5	6	7	8	9
						A1	.	A1	A1?	.	.	.	A1	A1
						.	A2	.	.	.	.	.	A2	.
						.	A3	A3	.	A3	.	.	A3	A3
						.	.	.	A4	.	.	.	.	A4
						.	.	.	.	.	.	A5	.	.
Wirosa F1 *(B. oleracea)*	.	.	.	.	.	+	(+)/+	+	+	+	+	+	+	+
Just Right Hybrid Turnip *(B. rapa)*	.	.	.	R4	.	+	+	+	-	+	+	+	+	-
COB60 (*B. napus*)	.	R2?	.	R4	.	+	(+)	+	-	+	+	+	nt	nt
Seven Top Turnip *(B. rapa)*	.	R2	.	R4	.	+	-	+,v	-,v	+,v	+	+	-	-
PIC1 (*B. carinata*)	R1	.	.	R4?	.	-	(+)	-	-	+	+	+	-	-
FBLM2 (*B. juncea*)	R1	.	.	R4?	R5	-	+	-	-	(+)	+	-	-	-
Miracle F1 *(B. oleracea)*	.	R2?	R3	.	.	+	-/(+)	-/(+)	+	-	+	+	-	-
SxD1 (*B. oleracea*)	.	.	R3	.	.	+	-/(+)	-	+	-	(+)/+	+	nt	nt

+, compatible interaction (susceptibility); -, incompatible interaction (resistance); (+), weakly pathogenic.
nt, not tested; v, variable

Table 3. Race-type strains of *Xanthomonas campestris* pv. *campestris* (adapted from Vicente *et al*. (2001) and Fargier and Manceau (2007)).

HRI/UW Isolate number	Other collections (number)	Host	Country (Year)	Race
3811	P. Williams (PHW1205)	*B. oleracea*	USA	1
3849A	C.I. Kado (2D520)	*B. oleracea* var. *botrytis*	USA	2
5212	NCPPB (528), CFBP5241, LMG568, ATCC33913	*B. oleracea* var. *gemmifera*	UK (1957)	3
1279A	-	*B. oleracea* var. *capitata*	UK (1984)	4
3880	NCPPB (2986)	*B. oleracea* var. *capitata*	Australia (1975)	5
6181	-	*B. rapa*	Portugal (1996)	6
-	CFBP (4953)	*B. oleracea* var. *botrytis*	Belgium (1999)	7
8444A	B. D. Jensen (N35)	*B. oleracea* var. *capitata*	Nepal	7
-	CFBP (1124), LMG (8032)	*B. oleracea* var. *botrytis*	France (1967)	8
3961	NCPPB (1145), LMG (8004), CFBP (6650)	*B. oleracea* var. *botrytis*	UK (1958)	9

HRI/UW = The University of Warwick (ex-HRI department), Wellesbourne, UK; NCPPB = National Collection of Plant Pathogenic Bacteria, Sand Hutton, UK; CFBP = Collection Française de Bactéries Phytopathogènes, INRA, France; LMG = Laboratorium voor Microbiology, Gent, Belgium

TABLE 4. Postulated gene-for-gene model to explain the relationship between *Brassica* spp., radish and pepper lines and isolates of *Xanthomonas campestris* pv. *campestris* (adapted from He *et al*. (2007)).

					Avirulence genes (A)			
					xop AH (avrXccC)	.	.	.
					.	*avrRc2*[a]	.	.
					.	.	*xopE (avrXccE1)*	.
					.	.	.	*avrBs1*
Differential cultivars or lines	**Resistance genes (R)**							
Guangtou *(B. juncea* var. *megarrhiza*)	*Rc1*	.	.	.	-	+	+	.
Jingfeng-1 *(B. oleracea)* and Huaye (*R. sativus* var. *longipinnatus*)	.	*Rc2*	.	.	+	-	+	.
Zhongbai-83 *(B. rapa* var. *pekinensis)*	.	.	*Rc3*	.	+	+	-	.
ECW10R (*Capsicum annuum* v. *latum*)	.	.	.	*Bs1*	+	+	+	HR

[a] This gene has not been identified yet.

+, compatible interaction (susceptibility); -, incompatible interaction (resistance); HR, hypersensitive response.

Table 5. *Xanthomonas campestris* pv. *campestris* strains that have been completely sequenced and features of the genome (adapted from Vorhölter *et al*. (2008)).

Isolate	ATCC 33913	8004	B100
Other designations	NCPPB (528), HRI5212, LGM 568, CFBP 5241	Rifampicin resistant lab strain derived from NCPPB 1145, CFBP 6650	
Host of origin	Brussels sprouts (*B. oleracea* var. *gemmifera*)	Cauliflower (*Brassica oleracea* var. *botrytis)*	
Country of origin	UK	UK	
Year of isolation	1957	1958	
Race	3	9	1
Size (bp)	5,076,187	5,148,708	5,079,002
G+C content (%)	65.0	64.9	65.0
CDS – predicted number	4,181	4,273	4,471
CDS – function assigned	2,708	2,671	2,878
Ribosomal RNA operons	2	2	2
Transfer RNAs	54	54	54
Insertion sequence elements	109	115	59
Reference	Da Silva (2002)	Qian et al. (2005)	Vorhöelter at al. (2008)

Table 6. Occurrence, features and function of putative and proven effector/avirulence genes of *Xanthomonas campestris* pv. *campestris*.

Effector class	Synonyms	Gene ID in ATCC33913	Gene ID in 8004	Gene ID in B100	Gene ID in Xcr 756C	PIP box	Features and function on pathogenicity
Core effectors[1]							
AvrBs2		XCC0052	XC 0052	xcc-b100 0057	no	Yes	Glycerophosphoryl diester phosphodiesterase Avirulence in *B. juncea, B. carinata* and *B. oleracea* and HR on pepper (Ignatov *et al.*, 2002) or no effect (Castañeda *et al.*, 2005)
XopK		XCC2899	XC 1210	xcc-b100 1254	no		Unknown
XopL	XopLR	XCC4186	XC 4273	xcc-b100 4400	no	Yes	Leucine-rich protein Reduced virulence on radish (Jiang *et al.*, 2009)
XopN		XCC0231	XC 0241	xcc-b100 0253	no		ARM/HEAT repeat Reduced virulence on radish (Jiang *et al.*, 2008a)
XopP		XCC1247	XC 2994	xcc-b100 3057	XCA_1500	Yes	Unknown Reduced virulence on radish (Jiang *et al.*, 2009)
XopQ		XCC1072	XC 3177	xcc-b100 3274	no	Yes	Putative inosine-uridine nucleotide N-ribohydrolase Reduced virulence on radish (Jiang *et al.*, 2009)
XopR		XCC0258	XC 0268	xcc-b100 0280	XCA_4254		Unknown
XopX		XCC0529 XCC0530	XC 0541 XC 0542	xcc-b100 0558 and 0559	no		Methionine-rich protein
XopZ		XCC1975	XC 2210	xcc-b100 2274	no		Unknown
Other effectors[2]							
AvrBs1		XCC2100	XC 2081	xcc-b100 2396	no	No	HR on pepper (He *et al.*, 2007)
XopD		XCC2896	XC 1213	xcc-b100 1256	no		SUMO cysteine protease (C48 family), EAR motif, DNA binding, nuclear localisation
XopE	AvrXccE1	XCC1629	XC 2602	no	no	Yes	Putative transglutaminase Avirulence on *B. rapa* (He *et al.*, 2007)
XopF		XCC1218	XC 3024	xcc-b100 3087	XCA_1470		Unknown
XopG		XCC3258	XC 0967	xcc-b100 2655	no		M27 family peptidase (Clostridium toxin)
XopH	AvrBs1.1	XCC2099	XC 2082	xcc-b100 2395	no	No	Putative tyrosine phosphatase
XopJ	AvrXccB	XCC3731	XC 3802	no	no	Yes	Putative cysteine protease (C55 family) or Ser/Thr acetyltransferase. Ubiquitin-like protease. No effect on pathogenicity (Jiang *et al.*, 2009)
XopAC	AvrAC	XCC2565	XC 1553	xcc-b100 1596	XCA_2914	Yes	Leucine-rich protein Avirulence recognised in vascular tissues of Arabidopsis (Xu *et al.*, 2008)
XopAD		no	no	no	XCA_1464		SKWP repeat protein
XopAG		XCC3600	XC 0563	xcc-b100 0580	no		Unknown
XopAH	AvrXccC	XCC2109	XC 2004	xcc-b100 2071	no	Yes	Dual function. Avirulence in *B. juncea* and *B. rapa*; virulence in *B. oleracea* (Castañeda *et al.*, 2005; He *et al.*, 2007; Wang *et al.*, 2007)
XopAL1	AvrPhpE	XCC1246	XC 2995	xcc-b100 3058	XCA_1499	Yes	Reduced virulence on radish (Jiang *et al.*, 2009)
XopAL2		Downstream XCC3574	Downstream XC_3916	xcc-b100 0616	no		Unknown
XopAM	XopR1	XCC1089	XC 3160	xcc-b100 3256	no	Yes	Reduced virulence on radish (Jiang *et al.*, 2009)
XopAT		no	no	no	XCA_1464a	Yes	Unknown. No similarity with other known proteins (Bogdanove *et al.*, 2011)
XopA		XCC1240	XC 3002	xcc-b100 3065	XCA_1492		Harpin, maybe not a t3e
HpaA		XCC1224	XC 3018	xcc-b100 3081	XCA_1476		T3 secretion control protein, maybe not a t3e
HrpW		XCC1219	XC 3023	xcc-b100 3086	XCA_1471		Pectate lyase, maybe not a t3e
AvrXccA1	AvrXca	XCC4229	XC 4318	xcc-b100 4450	XCA_4581	No	Maybe not a t3e. Virulence of Xcr in Arabidopsis (Parker *et al.*, 1993).
AvrXccA2		XCC2396	XC 1716	xcc-b100 1770	XCA_2696	No	Unknown, maybe not a t3e

Adapted from da Silva *et al*. (2002), White *et al*. (2009), Jiang *et al*. (2009) and Ryan *et al*. (2011)

[1] Nine core effectors are found in most *Xanthomonas* spp. with the exception of *X. albilineans* and, in some cases, *Xcr*.

[2] Other effectors are found in a more limited number of *Xanthomonas* spp. isolates.

Figure 1. a) Symptoms of black rot on a cabbage field; b) Typical black rot V-shape lesion on a cabbage leaf; c) Two plants of Savoy cabbage with symptoms of systemic infection following incolulation of *Xanthomonas campestris* pv. *campestris* and a healthy control plant; d) Electron microscopy image of a *X. campestris* pv. *campestris* rod-shaped cell showing a single polar flagellum; e) *X. campestris* pv. *campestris* culture growing on King's medium B; f) *X. campestris* pv. *campestris* culture growing on Yeast Dextrose Calcium Carbonate medium.

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