**Xanthomonas gardneri** exoenzymatic activity towards plant tissue

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**Abstract**  Bacterial spot caused by *Xanthomonas* spp. is an important tomato and pepper disease worldwide. Recent outbreaks of bacterial spot disease in Central Brazil and Canada have been attributed to *Xanthomonas gardneri*, which is also recognized as group D of *Xanthomonas campestris pv. vesicatoria*. Carotenoid-like pigments called xanthomonadins, which are diagnostic for yellow *Xanthomonas* spp., were extracted from *X. gardneri*. It was shown that the model plant *Arabidopsis thaliana*, member of the Brassicaceae family, can develop disease symptoms in response to different isolates of *X. gardneri*. Secretion of enzymes has been shown to play an important role in pathogenicity for different pathogens, and to begin to understand the interaction of *X. gardneri* and *A. thaliana*, a biochemical analysis of secreted proteins in the presence of *A. thaliana* leaves was performed. Different enzymatic activities such as for cellulase, α-arabinofuranosidase, pectinase, invertase and xylanase were assayed. In the presence of leaves, cellulase activity was highest after 60 and 72 h of growth and α-arabinofuranosidase activity was detected between 12 and 72 h of growth. Pectinase, invertase and xylanase activities were not detected. Cellulase and α-arabinofuranosidase activities may be important for *X. gardneri* acquisition of plant nutrients through degradation of cellulose fibers and hemicellulose of the cell wall, respectively, to the invasion of the host tissue and/or may generate signal molecules that are recognized by the plant. This is the first study to address how *X. gardneri* responds to host plant tissue.

**Keywords**  Cell-wall degrading enzymes · Processing tomatoes · Plant–pathogen interaction · Xanthomonadins · Enzyme activity

**Introduction**

Bacterial spot disease of tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum annum* L.) is important worldwide. The most visible symptoms are fruit and leaf lesions. Different *Xanthomonas* species have been implicated with the disease; however, the taxonomy of the genus and of the bacterial spot group has been controversial (Jones et al. 2004a; Schaad et al. 2000; Vauterin et al. 2000; Young et al. 2001). Changes in *Xanthomonas* taxonomy have been frequent but are contributing to a better understanding of the genus (Vauterin et al. 1995, 2000; Jones et al. 2004a). There are four phenotypic groups: A, B, C and D able to cause disease in tomatoes and/or pepper, which received a species status in Jones et al. (2004a). Group D is the most distinct from the others and strains from this group are also referred to as *X. gardneri* (Jones et al. 2004a).

The first bacterium from the *X. gardneri* group was identified from a tomato host in the former Yugoslavia and at the time was named *Pseudomonas gardneri* (Šutic 1957). Later it was considered synonymous with *Xanthomonas vesicatoria* based on the fact that *Pseudomonas gardneri* could not be distinguished from the latter both in plants or the laboratory (Dye 1966). It was Hildebrand et al. (1990) who verified by DNA hybridization, that this
entity represented a separated group from \textit{Xanthomonas campestris pv. vesicatoria}. Strain ICPB XG 101, the type strain of \textit{Pseudomonas gardneri} was further mentioned as a pathogen of both tomato and pepper, and was classified as race T2P1 based on the reaction of a differential set of tomato and pepper genotypes (Bouzar et al. 1994). Referred by the epithet \textit{Xanthomonas gardneri}, the organism was reported again occurring in Costa Rica (Bouzar et al. 1999). The picture emerging is that \textit{X. gardneri} is more widespread than previously thought. It has been the major responsible for a bacterial epidemic in Canadian tomato fields in 2003 and 2004 (Cuppels et al. 2006). Furthermore, in a study of 215 strains collected up to the year 2000 in processing tomato fields in central-west Brazil \textit{X. gardneri} was the predominant xanthomonad group recovered (Quezado-Duval et al. 2004). No resistant tomato or pepper genotypes have been reported so far.

The plant cell wall is a natural physical barrier against pathogens and is at the forefront of the interaction between plants and pathogens. To gain access to the contents of plant cells, many phytopathogenic bacteria produce and secrete various cell-wall-degrading enzymes such as cellulases, pectinases and xylanases (Rajeshwari et al. 2005). These enzymes are important because in different pathosystems they were shown to be involved in promoting pathogenesis and virulence (Yakoby et al. 2000; Toth et al. 2003; Valette-Collet et al. 2003; Liu et al. 2005). Degradation of cell-wall polysaccharides can also release products that trigger defense responses by the plant (Hahn et al. 1981; Bruce and West 1982; Gonzalez and Allen 2003).

Another enzymatic activity that is not related to cell wall degradation but that can be important to pathogens is that of invertases which are able to hydrolyze sucrose. Sucrose is the major transportable product of photosynthesis connecting source to sink organs via the phloem. Sucrose is present in the intercellular spaces of source organs and pathogens may use sucrose as a source for carbon as well as energy (Kim et al. 2004).

Despite the growing importance of \textit{X. gardneri} as a pathogen and its distance to other \textit{Xanthomonas} groups, there are still very few studies dedicated to understanding the biology of this pathogen and its interaction with host plants. Here we report on the presence of xanthomonadins, carotenoid-like pigments, that are diagnostic for yellow \textit{Xanthomonas} spp. (Starr and Stephens 1964), in \textit{X. gardneri} which further corroborates its current standing as a bona fide \textit{Xanthomonas}. Also, the model plant \textit{Arabidopsis thaliana} (family Brassicaceae) can develop disease symptoms when inoculated with different isolates of \textit{X. gardneri}. By performing a biochemical analysis of secreted protein activities, we have begun to investigate the interaction between \textit{X. gardneri} and this model plant.

### Materials and methods

#### Origin of \textit{X. gardneri} and growth conditions

The strain CNPH467 of \textit{X. gardneri} (IBSBF 1782 from the Phytobacteria Culture Collection of Instituto Biológico, Campinas, Brazil) was obtained from a field of processing tomatoes showing symptoms of bacterial spot disease in Morrinhos, state of Goiás, Brazil, in 1998 as previously described (Quezado-Duval et al. 2004). Three other \textit{X. gardneri} isolates were also obtained from processing tomato fields in Goiás. Isolate 2006-17 and 2006-21 were obtained in the year 2006, both in the city of Goiânia and isolate 334-T was obtained in 1997 in the city of Itapaci. During the study, \textit{X. gardneri} isolates were stored in phosphate buffer pH 7.0 (1.5 g/l K_2HPO_4, 1 g/l KH_2PO_4) at room temperature. \textit{Xanthomonas campestris pv. campestris} CNPH77, a well-known crucifer pathogen was used as a positive control (Williams et al. 1980; Simpson and Johnson 1990), while \textit{X. vesicatoria} was used as a negative control. Before experiments, bacteria were revived by plating onto nutrient-agar (NA), media containing (per l) 10 g bacto-peptone, 3 g beef extract, 20 g agar, and incubated at 28 °C for 3–5 days, and to guarantee purity were then transferred to another NA plate for 2 days at the same temperature. For enzymatic assays, \textit{X. gardneri} CNPH467 was recovered from NA plates and inoculated into 250 ml of nutrient broth for 3 days at 28 °C and agitation of 200 rev/min. Minimal medium (Dye 1966) containing small cut pieces of \textit{Arabidopsis} accession CS903 leaves at 1%(w/v) was inoculated with \textit{X. gardneri} from nutrient broth to a final O.D._600 of 0.1. At regular intervals, 50 ml aliquots were obtained and the supernatants were separated from bacterial cells and residual substrate by centrifugation at 4,200g. After lyophilization, samples were resuspended in 2 ml distilled water and stored at −20 °C until enzymatic assays were performed in triplicate.

#### Xanthomonadins identification

Xanthomonadins were extracted from \textit{X. gardneri} CNPH467 as previously described by Schaad et al. (2001), however, the extracted pigments were concentrated using a SpeedVac (Eppendorf, Germany) rather than a water bath. Thin layer chromatography was performed using silica gel 60 sheets of 0.2 mm thickness (Merck, Darmstadt, Germany). The anhydrous methanol (J.T. Baker, Mexico) used as a solvent was allowed to move approximately 10 cm. The \(R_f\) value was calculated as the distance traveled by the yellow pigment divided by the distance traveled by the solvent.
Arabidopsis growth conditions and inoculation

Accessions CS903 (Kas-1), CS1194 (Gö-0), CS1308 (Le-0), CS1566 (Tu-0), CS6100 (Kelsterbach-1), and CS6604 (Na-2) of Arabidopsis thaliana (Le-0), CS1566 (Tu-0), CS6100 (Kelsterbach-1), and CS6604 (Na-2) of A. thaliana were obtained from the Arabidopsis Biological Resource Center (Ohio, USA). Seeds were plated on MS ¼ medium (Sigma, MO, USA), imbibed overnight at room-temperature and cold-treated for 2 days. Plates were exposed to continuous light for germination. One week after germination, seedlings were transplanted to pots, 7 cm in diameter, containing Plantmax Hortaliças HT substrate (Eucatex Agro, SP, Brazil). Plants were grown for 5 weeks under short-day illumination (8 h light/16 h dark) from cool-white fluorescent lamps at approximately 100 μmol m−2 s−1. Temperature was approximately 24 °C and relative humidity 50%. Plants were subirrigated with water as needed. For plant inoculations, bacteria grown on NA plates were resuspended in 10 mM MgCl₂ and the bacterial concentration was adjusted to approximately 5 × 10⁸ c.f.u./ml. For syringe inoculation, the abaxial side of 3 leaves per plant from 6 plants of each Arabidopsis accession was inoculated with the bacterial suspension using a 1 ml syringe without the needle. For spray inoculation, the same number of plants were used but the whole plant was sprayed to saturation with the bacterial suspension. Sprayed plants were kept under high humidity conditions for 2 days. Tomato plants (cv. Bonny Best) were used as positive controls for bacterial virulence.

Protein content

Protein content in lyophilized X. gardneri culture supernatant samples was determined by the Bradford method (1976).

Enzymatic assays

Cellulase activity against crystalline cellulose was measured by the filter paper assay (Lowe et al. 1987). In this assay Whatman no. 1 filter paper strips (6 × 1 cm; ca. 50 mg) were used as a substrate to which 1 ml of 0.2 M sodium–phosphate buffer (pH 7.2) and 1 ml X. gardneri lyophilized culture supernatant were added. This mixture was incubated at 45 °C for 4 h. The reaction was stopped by addition of 1 ml of DNS reagent. The reducing sugar produced in the reaction was quantified with dinitrosalicilic acid (DNS) reagent (Miller 1959) and absorbance at 550 nm was determined. One unit of enzymatic activity was defined as the amount of enzyme that will release 1 μmol of glucose in 1 h of reaction. Trichoderma longibrachiatum crude extract was used as a positive control.

z-Arabinofuranosidase activity was determined according to the method described by Filho et al. (1996). Assays were carried out for 15 min. at 50 °C in a mixture containing 1 mM of the substrate p-nitrophenyl-z-D-arabinofuranose, sodium acetate buffer 100 mM pH 5.0 and 400 μl of X. gardneri lyophilized culture supernatant. The reaction was terminated by the addition of 1 ml 1 M sodium carbonate. The release of p-nitrophenol was determined by measuring absorbance at 410 nm. One unit of enzymatic activity was defined as the amount of enzyme that will increase the absorbance at 410 nm in 0.1 unit of absorbance per minute of reaction. T. longibrachiatum crude extract was used as a positive control for z-arabinofuranosidase activity.

Pectinase activity was measured according to the protocol of Soriano et al. (2005). Briefly, 50 μl of X. gardneri lyophilized culture supernatant was incubated with 75 μl of sodium acetate buffer 50 mM (pH 5.0), 0.12 M NaCl, 6 mM EDTA, and 125 μl of substrate (0.4% of polygalacturonic acid) for 1 h at 45 °C. The reaction was terminated by the addition of 1 ml DNS reagent and absorbance was measured at 550 nm. T. longibrachiatum crude extract was used as a positive control for pectinase activity.

Xylanolytic activity was determined according to the method described by Cardoso and Filho (2003), by incubating 50 μl of X. gardneri lyophilized culture supernatant with 100 μl of 1% (w/v) xylan substrate for 30 min. at 50 °C. The reaction was stopped by the addition of 300 μl of DNS reagent. The reaction was terminated by placing tubes in boiling water for 10 min. and 1.5 ml of distilled water was added to each tube before absorbance at 550 nm was measured. Penicillium corylophilum crude extract was used as a positive control for xylanase activity.

To test for invertase activity, 50 μl of X. gardneri lyophilized culture supernatant were incubated with 950 μl of sucrose 0.4% (w/v) in 50 mM (pH 5.0) sodium acetate buffer previously warmed to 37 °C. The reactions were incubated for 20 min. at 37 °C. Reactions were terminated by the addition of 1 ml DNS reagent and boiled for 10 min. Absorbance at 550 nm was measured after the addition of 2 ml of distilled water. Lysed Saccharomyces cerevisiae cells were used as a positive control.

Results and discussion

Extraction and analysis of pigments

Thin layer chromatography was used to detect the presence of xanthomonadins in X. gardneri. Xanthomonas campes-tris pv. campes tris was used as a positive control. Identification of a yellow spot with an Rf value between 0.42 and 0.49 indicates the presence of xanthomonadins (Schaad
et al. 2001). For the positive control, X. campestris pv. campestris the Rf value was 0.43. For the pigments extracted from X. gardneri, an Rf value of 0.42 was observed, being indicative of the presence of xanthomonadins. The taxonomy of X. gardneri has been object of controversy and has changed over the years. The detection of xanthomonadins in an X. gardneri pigment extract adds to the body of data that places X. gardneri unequivocally in the genus Xanthomonas (Starr and Stephens 1964).

Arabidopsis thaliana can develop disease symptoms in response to X. gardneri

Accessions CS1194, CS1308, CS1566 and CS6604 of A. thaliana were sprayed with X. gardneri CNPH467 suspension to determine if this method of inoculation would produce disease symptoms. Sprayed plants from all the accessions tested developed chlorotic spots 4 days after inoculation (Fig. 1a). Three plants per accession were spray inoculated with 10 mM MgCl2 as a negative control and as expected these remained healthy. To test if syringe inoculation would also produce disease symptoms, plants from the accessions CS903, CS1194, CS1308, CS1566, CS6100 and CS6604 were syringe inoculated with a X. gardneri CNPH467 bacterial suspension. Seven days after the syringe inoculation, chlorosis symptoms were clearly observed on all accessions tested (Fig. 1b), with some leaves also displaying necrosis. Three plants per accession were syringe inoculated with 10 mM MgCl2 as a negative control and as expected these remained healthy. To follow Koch’s postulates, bacteria were isolated from leaf tissue showing disease symptoms 6 days after inoculation. These bacteria showed morphology and color consistent with X. gardneri and were re-inoculated on tomato plants which showed disease symptoms of chlorosis and necrosis.

To further investigate if other isolates of X. gardneri were able to cause disease symptoms in Arabidopsis, accession CS1308 was sprayed with three other isolates (Fig. 2a–c). The same chlorotic spots progressing to necrotic regions obtained with isolate CNPH467 were again observed. Disease symptoms were observed in the X. campestris pv. campestris positive control (Fig. 2d) but not with the X. vesicatoria negative control (not shown) at the same concentration.

These results indicate that A. thaliana can develop disease symptoms when inoculated with X. gardneri and that depending on the mode of inoculation, different disease symptoms develop. Presently it is not clear whether Arabidopsis can be a host to X. gardneri in nature. However, the development of the X. gardneri/Arabidopsis pathosystem may present great advantages as a model system because Arabidopsis is the most well studied plant and that many genomic, genetic and biotechnology tools have been developed for this plant. Many contributions to understanding the molecular details of how a plant interacts and defends itself have come from studies using Arabidopsis (Quirino and Bent 2003). As an example, a bacterial avirulence gene from the tomato pathogen Pseudomonas syringae pv. tomato was identified based on its interaction with Arabidopsis (Whalen et al. 1991) and since then this system has been explored in many ways to dissect the molecular pathways to disease resistance (Quirino et al. 2004; Katiyar-Agarwal et al. 2006; Sato et al. 2007). We have observed that different Arabidopsis accessions present variation in their degree of resistance to X. gardneri (data not shown) and this can be explored in future studies.

Another advantage of the X. gardneri/Arabidopsis pathosystem is that the genome from two other Xanthomonas species, X. axonopodis pv. citri and X. campestris pv. campestris, have been completely sequenced by Brazilian researchers (da Silva et al. 2002). X. axonopodis pv. citri causes citrus canker which affects most commercial citrus cultivars and X. campestris pv. campestris causes black rot in crucifers. Comparison between the genomes of these pathogens with different host specificity showed that more than 80% of the genes are shared. Although X. gardneri has not had its genome sequenced, information from these other Xanthomonas species should be useful to
guide the formulation of gene sequence-based hypotheses for future research.

Exoenzymatic activity of *X. gardneri*

To begin to understand how *X. gardneri* interacts with plants, *X. gardneri* was grown in the presence of *A. thaliana* leaves or in minimal medium only. In minimal medium, *X. gardneri* secreted detectable levels of protein between 36 h and 72 h (Fig. 3a). When growing in the presence of *Arabidopsis* leaves, *X. gardneri* secreted a measurable amount of proteins between 24 h and 72 h after inoculation (Fig. 3b). The peak in secretion occurred at 36 h after inoculation. Overall, the amount of protein secreted by *X. gardneri* in minimal medium or in minimal medium supplemented with leaves was similar.

The array of cell-wall degrading enzymes (CWDE) includes cellulases, xylanases, pectinases and α-arabinofuranosidases which contribute to degrade the typical eudicot cell wall composed of the carbohydrates cellulose, hemicellulose and pectin. Figure 4a shows that *X. gardneri* displayed a low constitutive cellulase activity when grown in minimal medium. In the presence of *Arabidopsis* leaves (Fig. 4b), the highest cellulase activity was detected at the latest time points analysed (60 and 72 h after inoculation). Cellulose fibers are composed of chains of approximately 30–36 chains of β-1,4-glucose that are hydrogen bonded to form a crystalline material (Vorwerk et al. 2004).

Cellulose fibers are crosslinked by hemicellulose and are embedded in a matrix of pectin. Xylans, such as arabinoxyylan and xyloglucan, are the most abundant hemicelluloses. When grown in minimal medium, *X. gardneri* secreted proteins displayed α-arabinofuranosidase activity.
as early as 12 h after inoculation and this activity remained for all the time points tested (Fig. 5a). The α-arabinofuranosidase activity was further induced in the presence of Arabidopsis leaves (Fig. 5b). At the peak of activity, which occurred at 48 h after inoculation, the activity in the presence of Arabidopsis leaves was more than twice that of that observed in minimal medium only. α-L-Arabinofuranoside residues can be a group that is present at C-2 or C-3 or both of the xylose units present in xylans of hemicellulose. α-Arabinofuranosidases remove arabinosyl residues from hemicellulosic polysaccharides. Cellulase as well as α-arabinofuranosidase activities were significantly greater in the presence of Arabidopsis leaves than those observed in minimal medium. Efficient degradation of the intricate cell wall material may require cooperation between these two enzymatic activities.

No enzymatic activity was detected in the assays for pectinase or xylanase when X. gardneri was grown in minimal medium only or in the presence of Arabidopsis leaves although different Xanthomonas are known to have genes for such enzymes (da Silva et al. 2002). Pectins are made of chains of galacturonic acid often branched and decorated by other sugars and are present not only in primary cell walls but also the middle lamella. While our study and that of Bouzar et al. (1999) have identified non-pectinolytic isolates of X. gardneri, work of Cuppels et al. (2006) reports a weak pectinolytic activity. Therefore, there may be some variation with respect to the utilization of this polysaccharide by X. gardneri isolates. Alternatively, the conditions used in assays for pectinase and xylanase activities can affect enzyme expression and/or activity and this may also affect the results obtained.

Invertase activity responsible for sucrose hydrolysis was investigated despite not being related to cell wall degradation, but no activity was detected. Sucrose is present in the intercellular spaces of source organs and pathogens may use sucrose as a source for carbon as well as energy (Kim et al. 2004). Xanthomonas gardneri is known to be able to utilize sucrose as a carbon source; however, we have not detected any secreted invertase activity in this study (Jones et al. 2004b). We conclude that most likely, as reported for other bacteria (Kim et al. 2004), sucrose is transported, with or without concomitant phosphorylation, to the cell interior and then is hydrolyzed by invertase.

Plant pathogens can be divided in biotrophs, necrotrophs and hemi-biotrophs (Thaler et al. 2004). While biotrophs derive nutrients from living tissue, necrotrophs kill the host and then feed on the dead tissue. Hemi-biotrophs are pathogens that cannot clearly be placed in the two former categories or that change their lifestyles according to conditions or stage of their life cycle (Glazebrook 2005). Although studies with X. gardneri are not yet available, X. campestris pv. vesicatoria which colonizes the susceptible host apoplast, is probably best described as a hemi-biotroph (Thaler et al. 2004). Initially, when it invades the tissue it has a biotrophic lifestyle; however, in the later stages of infection it is necrogenic (Alfano and Collmer 1996). CWDE can have roles in pathogens of different lifestyles as they act to facilitate penetration into host tissue and/or derive substrates from the cell wall for their nutrition. These enzymes may also play a role in triggering plant defenses by generating signal molecules that stimulate host defense (Liu et al. 2005).

Our investigation of exoenzymatic activities of X. gardneri responding to the presence of leaf tissue from a susceptible host plant is important because although the genomes of Xanthomonads have been sequenced (da Silva et al. 2002) only a biochemical approach can answer the question of which enzyme activities are present during the pathogen interaction with a host plant. This is the first report at a biochemical level to address the question of how X. gardneri interacts with host plant tissue. It is possible that these enzymes play a role in the acquisition of nutrients by X. gardneri or have a role in virulence. Future experiments where X. gardneri cell wall degrading enzymes are knocked out should help address this question experimentally.

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References

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

Bouzar H, Jones JB, Stall RE, Hodge NC, Minsavage GV, Benedict AA, Alvarez AM (1994) Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of Xanthomonas campestris pv. vesicatoria strains. Phytopathol 84:663–671

Bouzar H, Jones JB, Stall RE, Louws FI, Schneider JL, de Bruijn FI, Jackson LE (1999) Multiphasic analysis of xanthomonads causing bacterial spot disease on tomato and pepper in the Caribbean and Central America: evidence for common lineages within and between countries. Phytopathol 89:328–335

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bruce RJ, West CA (1982) Elicitation of casbene synthetase activity in castor bean, the role of pectic fragments of the plant cell wall in elicitation by fungal endo-polygalacturonase. Plant Physiology 69:1181–1188

Cardoso OAV, Filho EXF (2003) Purification and characterization of a novel cellulase-free xylanase from Acrophailophora rainiana. FEMS Microbiol Lett 223:309–314

Cuppels DA, Louws FI, Ainsworth T (2006) Development and evaluation of PCR-based diagnostic assays for the bacterial speck and bacterial spot pathogens of tomato. Plant Dis 90:451–458

da Silva AC, Ferro JA, Reinach FC et al (2002) Comparison of the genomes of two Xanthomonas pathogens with differing host specificities. Nature 417:459–463

Dye DW (1966) Cultural and biochemical reaction of additional Xanthomonas species. NZ J Sci 9:913–919

Filho EX, Puls J, Coughlan MP (1996) Purification and characterization of two arabinofuranosidasases from solid-state cultures of the fungus Penicillium capsulatum. Appl Environ Microbiol 62:168–173

Glazbrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

Gonzalez ET, Allen C (2003) Characterization of a Ralstonia solanacearum operon required for polygalacturonate degradation and uptake of galacturonic acid. Mol Plant Microbe Interact 16:536–544

Hahn MG, Davril AG, Albersheim P (1981) Host–pathogen interactions: XIX. The endogenous elicitor, a fragment of a plant cell wall polysaccharide that elicits phytoalexin accumulation in soybeans. Plant Physiol 68:1161–1169

Hildebrand DC, Palleroni NJ, Schrot MN (1990) Deoxyribonucleic acid relatedness of 24 xanthomonads strains representing 23 Xanthomonas campestris pathovars and Xanthomonas fragariae. J App Bacteriol 68:263–269

Jones JB, Lacy GH, Bouzar H, Stall RE, Schaad NW (2004a) Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. Syst Appl Microbiol 27:755–762

Jones AM, Thomas V, Truman B, Lilley K, Mansfield J, Grant M (2004b) Specific changes in the Arabidopsis proteome in response to bacterial challenge: differentiating basal and R-gene mediated resistance. Phytochemistry 65:1805–1816

Kattiar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A, Zhu JK, Staskawicz BJ, Jin H (2006) A pathogen-inducible endogenous siRNA in plant immunity. Proc Natl Acad Sci 103(47):18002–18007

Kim HS, Park HJ, Heu S, Jung J (2004) Molecular and functional characterization of a unique sucrose hydrolase from Xanthomonas axonopodis pv. glycines. J Bacteriol 186:411–418

Liu H, Zhang S, Schell MA, Denny TP (2005) Pyramiding unmarked deletions in Ralstonia solanacearum shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. Mol Plant Microbe Interact 18:1296–1305

Lowe SE, Theodorou MK, Trinci AP (1987) Cellulases and xylanase of an anaerobic rumen fungus grown on wheat straw, wheat straw holocellulose, cellulose, and xylan. Appl Environ Microbiol 53:1216–1223

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Quedoz-Duval AM, Leite RP, Trufetti D, Camargo LEA (2004) Outbreaks of bacterial spot caused by Xanthomonas gardneri on processing tomato in central-west Brazil. Plant Dis 88:157–161

Quirino BF, Bent AF (2003) Deciphering host resistance and pathogen virulence: the Arabidopsis/Pseudomonas interaction as a model. Mol Plant Pathol 4:517–530

Quirino BF, Genger R, Ham JH, Zabala G, Bent A (2004) Identification and functional analysis of Arabidopsis proteins that interact with resistance gene product RPS2 in yeast. Physiol Mol Plant Pathol 65:257–267

Rajeshwari R, Jha G, Soni TV (2005) Role of an in planta-expressed xylanase of Xanthomonas oryzae pv. oryzae in promoting virulence on rice. Mol Plant Microbe Interact 18:830–837

Sato M, Mitra RM, Coller J, Wang D, Spivey NW, Dewdney J, Denoux C, Glazebrook J, Katagiri F (2007) A high-performance, small-scale microarray for expression profiling of many samples in Arabidopsis-pathogen studies. Plant J 49(3):565–577

Schaad NW, Jones JB, Chun W (eds) (2001) Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, Minnesota

Schaad NW, Vidaver AK, Lacy GH, Rudolph K, Jones JB (2000) Evaluation of proposed amended names of several pseudomonads and xanthomonads and recommendations. Phytopathology 87:807–813

Simpson RB, Johnson LJ (1990) Arabidopsis thaliana as a host for Xanthomonas campestris pv. campestris. Mol Plant Microbe Interact 3:233–237

Soriano M, Diaz P, Pastor FJ (2005) Pectinolytic systems of two aerobic sporogenous bacterial strains with high activity on pectin. Curr Microbiol 50:114–118

Starr MP, Stephens WL (1964) Pigmentation and taxonomy of the genus Xanthomonas. J Bacteriol 87:293–302

Šutic D (1957) Tomato bacteriosis. Rev Appl Mycol 36:734–735

Thaler JS, Owen B, Higgins VJ (2004) The role of the jasmonate pathway in Arabidopsis/Pseudomonas pathogen interactions. Mol Plant Pathol 4:17–30

Valette-Collet O, Cimerman A, Reinig M, Levis C, Boccara M (2003) Disruption of Botrytis cinerea pectin methyl-esterase gene Bpme1 reduces virulence on several host plants. Mol Plant Microbe Interact 16:360–367

Vauterin L, Rademaker J, Swings J (1995) Synopsis on the taxonomy of the genus Xanthomonas. J Bacteriol 87:807–813

Vauterin L, Rademaker J, Swings J (2000) Synopsis on the taxonomy of the genus Xanthomonas. Phytopathology 90:677–682

Vorwerk S, Somerville S, Somerville C (2004) The role of plant cell wall polysaccharide composition in disease resistance. Trends Plant Sci 9:203–209

Whalen MC, Innes RW, Bent AF, Staskawicz BJ (1991) Identification of Pseudomonas syringae pathogens of Arabidopsis and a
bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3(1):49–59

Williams PH (1980) Black rot: a continuing threat to world crucifers. Plant Dis 64(8):736–742

Yakoby N, Freeman S, Dinoor A, Keen NT, Prusky D (2000) Expression of pectate lyase from *Colletotrichum gloeosporioides* in *C. magna* promotes pathogenicity. Mol Plant Microbe Interact 13:887–891

Young JM, Bull CT, de Boer SH, Firrao G, Gardan L, Saddler GE, Stead DE, Takikawa Y (2001) Classification, nomenclature, and plant pathogenic bacteria—a clarification. Phytopathol 91:617–620