Virulence strategies of phytopathogenic bacteria and their role in plant disease pathogenesis

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Phytopathogenic bacteria have evolved several virulence strategies to face hostile environment of the host plant. In this article, we reviewed the recent progress in research on characterization of the virulence factors including secretion system with their protein effectors, toxins production, extracellular polysaccharides, growth regulators, cell wall degrading enzymes, biofilm formation, siderophores and their role in the plant infection and symptom development focusing particularly on a group of bacteria such as Erwinia amylovora, Agrobacterium tumefaciens, Pseudomonas syringae, Ralstonia solanacearum and Xanthomonas campestris that cause different plant diseases including wilts, spots, blights and cankers. The elucidation of each step in pathogenesis may constitute a key step in any design of new molecules for targeting plant pathogenic bacteria for plant disease control.

Key words: Virulence factors, plant disease, phytopathogenic bacteria, phytotoxins, secretion systems, siderophores.

INTRODUCTION

Most phytopathogens must evolve numerous strategies to survive in different environmental conditions to invade and colonize their hosts known as virulence factors. These factors have the ability to modulate the physiological and biochemical mechanisms to enhance the spread of the pathogen, as well as to facilitate the release of nutrients and water from host cells (Toth et al., 2003).

The plant bacterial pathogens, involve many virulence factors that are secreted in the extracellular environment of the host cells. The most studied factors are: (1) adherence to the host cells, with surface adhesins, (2) production of the degradative enzymes that destroy the plant cells walls, (3) toxins that are in the apoplastic cell, (4) other complex molecules are also deployed including the exopolysaccharide (EPS) and those modulating the
plant hormone production. The major intensive studies in the field of plant bacteria interactions are the characterization of the pathogen virulence factors and their main roles in the pathogenicity and the specificity of the host. The elucidation of such aspect may lead to the planning and establishment of new strategy in the plant disease control. In the following section, we overviewed the recent development of the function and mechanism in the plant bacterial pathogens.

SECRETION SYSTEM

Plant pathogenic bacteria have evolved numerous sophisticated strategies for selective transport of proteins and nucleoproteins involved in the virulence across cell membranes in both the apoplastic environment and the cytoplasmic of the plant cells. Currently, six major classes of systems implicated in the virulence have been identified and described in plant pathogenic bacteria named from type I to type VI or T1SS to T6SS. The translocation mechanism of effector proteins from the bacterial cytosol to the external bacterial cell is known as secretion (Alfano et al., 2000).

In plant pathogenic Gram-negative bacteria, two major system are described. The single step process, in which secretion proteins are export ed across the inner and the outer membrane without any periplasmic step, however, the two steps process namely the Sec and the Tat secretion system are first exported in the periplasmic and then transported across the external membrane to the exterior of bacteria cell. In the following section, we summarized the general features of the six identified secretion system known in the phytoopathogenic Gram-negative bacteria (Alfano et al., 2000; Preston et al., 2001).

T1SS secretion sytem

In phytopathogenic Gram-negative bacteria, the type I secretion system also known as the ATP binding cassette (ABC) transporters is involved in the export of various molecules from the cytosol to the external environment without any periplasmic step (Delepelaire et al., 2004). The type I secretion system consists of three distinct proteins that compose a continuous chanel (Ian et al., 2006). The inner membrane ATP binding cassette (ABC) proteins transporters is a specific outer membrane known as outer membrane protein (OMP) and the so called membrane fusion protein (MFP) which is connected to the inner membrane and spans the periplasmic space and extends to the outer membrane(Ian et al., 2006). Many proteins of great importance in pathogenesis are transferred by the ABC secretion system in plant pathogenic bacteria including proteases, lipases or performing toxins. The T1SS is required for numerous plant pathogenic Gram-negative bacteria including both the Erwinia amylovora and Erwinia chrysanthemi (Ian et al., 2006; Liu et al., 2008).

T2SS secretion system

In Gram-negative bacteria, the T2SS secretion system known as the sec dependant system translocate folded proteins across the inner membrane either by sec pathway or Tat pathway to the periplasm and then the extracellular environment. The plant pathogenic bacteria, uses such a system to export hydrolytic enzymes involved in degrading different plant substrates including cellulases, xylanases, amylases and proteases. Several plant pathogenic bacteria include Pseudomonas fluorescens, Erwinia carotovora pv atroseptica, Xanthomonas compestris pv compestris and X. oryzae pv oryzae (Peabody et al., 2003).

T3SS secretion system

Several plant bacterial pathogens have evolved a strategy of delivering an array of effectors and toxins proteins directly into the cytoplasm of host cells known as the type III secretion systems (Preston et al., 2001; Lindeberg et al. 2012). Theses virulence determinants have the capacity to modulate the physiological functions (Staskawicz et al., 2001; Buttnner and Bonas, 2003). The type III secretion apparatus is composed of more than of 20 proteins consisting of basal body spanning both the inner and the outer membrane of the bacterial cells, and an extracellular needle with a tip complex extending into the host cell (Staskawicz et al., 2001). The TTSS in phytopathogenic bacteria is encoded by hypersensitive response and pathogenesis (hrp) gene involved in the transfer of Avr proteins in the host cell inducing both either pathogenicity on sensitive host or hypersensitive reaction on resistant host. The plant pathogens that use the TTSS system include Xanthomonas spp., Erwinia spp., Pseudomonas syringae and Ralstonia solanacearum (Birch, 2001; Noel et al., 2002; Angot et al., 2006).

T4SS secretion system

The type IV secretion system (T4SS) is present in both the Gram-negative and positive plant pathogenic bacteria (Wallden et al., 2010). This tranlocation system is an important system that deploy the sec gene to transport
the pathogenicity factors from the inner bacterial cell into the extracellular environment or directly into the plant host cell (Judd et al., 2005). The type IV secretion system is involved in the translocation into the plant cell of either the single stranded DNA (ssDNA), the multi subunit toxins or the monomeric proteins including the permeases. This secretion system is related to a conjugation machines. Among the most representative phytopathogenic bacteria that uses the T4SS secretion system is Agrobacterium tumefaciens that target the oncogenic DNA-protein complex in plant cell (Zupan et al., 2000; Juhas et al., 2008; Wallden et al., 2010).

**T5SS secretion system**

The type V secretion system (T5SS) is widely present among the Gram-negative bacteria (Tseng et al., 2009). This translocation system is considered as one of the simplest secretion pathway (Desvaux, 2004; Tseng et al., 2009). The T5SS translocation system is dedicated to transfer a single specific polypeptide known as the passenger domain in two step process (Moreira et al., 2004). The first step is mediated by a sec translocator across the inner membrane. The second step concerns the own transportation of the passenger through the outer membrane by forming a protected module called a β barrel (Van Sluys et al., 2002). During the translocation of passenger domain, the signal sequence can either remain on the bacterial surface or cleave and then released in the extracellular milieu. The type V secretion system can exist in two subtypes which are the autotransporters (AT) system (Type Va) and the TPS nown as AT-2. In Gram-negative bacteria, the virulence factors associated with T5SS passenger are numerous including biofilm formation, adhesins, toxins, enzymes production and cytotoxic activity (Leo et al., 2012; Jacob-Dubuisson, 2013). Among the plant pathogenic Gram-negative bacteria that involve the T5SS secretion system as pathogenicity determinant include Xylella fastidiosa, the causative agent of Pierce’s disease (Igo et al., 2007), the Xanthomonads (Van Sluys et al., 2002; Moreira et al., 2004) and E. chrysanthemi (Tseng et al., 2009).

**T6SS secretion system**

The type VI secretion system (T6SS) has been recently discovered as new mechanism for effectors transportation across the cell membrane in the Gram-negative bacteria (Bingle et al., 2008; Filloux et al., 2008; Shrivastava and Mande, 2008; Pukatzki et al., 2010). The structure of the T6SS secretion system presents a significant similarity with the bacteriophage tails which inject their effector protein proteins either directly into the host cell or in the extracellular milieu (Tseng et al., 2009; Pukazki et al., 2010). The T6SS secretion system is involved in the translocation of numerous pathogenicity determinants including the biofilm formation, the quorum sensing and antibacterial toxins. This secretion mechanism has been identified in many Gram-negative bacteria including Agrobacterium tumifaciens, Pectobacterium atrosepticum and Pseudomonas syringae (Wu et al., 2008; Records and Gross, 2010) and Xanthomonas oryzae (Fillouw et al., 2008).

**PECTIN DEGRADING ENZYMES**

Pectin substrate is a complex polysaccharide presents in all plants in the middle lamella of primary cell wall consisting mainly of galacturonic acid residues linked with an α(1-4) glucosidic bond (Pedrolli et al., 2009; Kothari and Baig, 2013). The acid groups are largely esterified with methyl groups. Plant pathogenic bacteria are known to produce an array of inducible extracellular enzymes that degrade plant cell wall constituents (Collmer and Keen, 1986). These enzymes are thought to play a key role as virulence factors. The most enzymes in bacteria plant pathogen and fungi are those degrading the pectin substances which are also the widely studied as determinants (Collmer et al., 2002). Among the widely pectic enzymes in phytopathogenic bacteria are two important classes namely the pectate lyases (PL) and polygalacturonases (PG) (Collmer and Keen, 1986; Saile et al., 1997). The plant pathogens that secrete complexes of pectic enzymes such as the pectate lyases (PL) (Boch et al., 2002; Collmer et al., 2002) and polygalacturonases (PG) includes the soil rot Erwinias namely E. carotovora and E. chrysanthemi (Barras et al., 1994; Carpita and McCann, 2000; Collmer et al., 2002).

**SIDEROPHORES**

Iron is an essential element for nearly all microorganisms including the plant pathogenic bacteria as it participates in numerous process such as redox reactions, oxygen binding and as cofactors for vital enzymes (Buyer and Leong, 1986). To maintain the availability of the free iron at acceptable concentration to limits the growth of invading bacterial pathogen, the host uses two major proteins for the transport and storage of free iron including the transferins and ferritins (Dave and Dube, 2000; Gull and Hafeez, 2012). Many plant pathogenic bacteria secrete molecular weight for ferric ion (Fe³⁺) chelate and transfer agent known as siderophores from the host then pumped in the bacteria cytosol by specific
membrane receptors (Leong and Neilands, 1981, Williams and Griffiths, 1992). Siderophores have been shown to play a major role as virulence factors for numerous plant pathogenic bacteria in plant disease. Among the compounds secreted include chrysobactin which is a catechol by E. chrysanthemi and E. carotovora (Perswerk et al., 1989; Alfano and Collmer, 2004) and the hydroxamate which is a siderophore produced by Agrobacterium tumefaciens (Leong and Neilands, 1981). Another iron transportation system mediated by specific proteins is known as NRAMP activated by infected plant particularly in response to biotic stress or iron limitation in plant host. The NRAMP are now known to be involved in innate immunity and to be the basic resistance for plant towards the pathogens( Expert et al., 2012; Dellagi et al., 2009).

ANTIMICROBIAL COMPOUNDS DETOXIFICATION

For the defense mechanisms, most plants produce antimicrobial compounds as secondary metabolites in response to pathogen infection (Glazebrook et al., 1997). Phytoalexins are among these antimicrobial substances which are considered as molecules at sufficient concentration that limit and reduce the growth and multiplication of pathogenic microorganisms (Hammond-Kosack and Jones, 1996). Among the major studied and illustrated compounds are pisatins in peas (VanEtten et al., 1975; Van Etten et al., 1989); saponins in oats, isoflavonoids in legumes and terpenoids in Solanaceae (Turbe et al., 1992). On the other hand, different mechanisms were described particularly in fungi which counter these antimicrobial substances. The pisatin are detoxified by cytochrome CP 450 monooxygenase (Matthews and Van Etten, 1983). Similarly, Fusarium oxysporum f.sp. Lycopersicum produce an inducible extracellular enzyme known as tomatinase which detoxifies the alpha tomatine. Furthermore, most Xanthomonads detoxify reactive oxygen and superoxide species using catalases (Qian et al., 2005). Recently, two inducibles enzymes were secreted by Pseudomononas syringaeare involved in the isothiocyanates detoxification (Fan et al., 2011).

TOXINS (PATHOTOXINS)

Plant pathogenic bacteria are known to produce a wide range of both specific and nonspecific host phytoxins. Some are polypeptids, glycoproteins others are secondary metabolites that are required as virulence factors in plant disease (Alfano and Collmer, 2004). These toxins acts by using diverse mechanisms from modulating and suppressing plant defense response to alteration and inhibition of normal host cellular metabolic process (Thomas et al., 1983). These toxins act also directly on the expression and development of disease symptoms. Among the most well studied pathotoxins known also as phytoxins include syringomycins, syringopeptins, tabtoxins, phaseolotoxins and coronatine described particularly in P. syringae pathovars(Thomas et al., 1983).

Syringomicins and syringopeptins

Both syringomycins and syringopeptins are a group of polar cyclic peptide known as lepodepsipeptides toxins which are secreted by several pathovars of P. syringae (Lu et al., 2005). These toxins act by disrupting the host cell membrane forming small pores leading to the electrolyte leakage from plant cell cytoplasm inducing necrosis of plant tissue of affected plant (Blender et al., 1999).

Coronatine

Plant pathogenic coronatine is produced by several pathovars of P. syringae and contribute as virulence factor. Coronatine consists of two major polyeptide components, the coronafacic acid and coronamic acid molecules. Coronatine share similarity in structure with jasmonic acid-isoleucine(JA-Ile) and hence mimic them (Brooks et al., 2005; Katsir et al., 2008). Coronatine plays a key role in early stage of infection by inhibition of the stomatal immune defense leading to the entry of the pathogen. This toxin counteract the pathogen associated molecular patterns (PAMPs) induced stomatal closure in both P. syringae and X. compestris (Hutchison and Gross, 1997; Gommez-Gomez and Boller, 2002). In fact, PAMPs consists of conserved components motifs that include flagellin and lipopolysaccharide (LPS). These molecular patterns are recognized by plant pathogen recognition receptors (PRRs). These perception of PAMPs activates the basal defenses mechanisms in early stages of interaction of plant pathogen interaction (Bittel and Robatzek, 2007; Melotto et al., 2008; Nurnberger and Kemmerling, 2009). On the other hand, these pathotoxin also contribute to expression of other diseases including the chlorosis symptoms, hypertrophy and lesion formation (Sekai et al., 1979; Brooks et al., 2005). Another well studied class of siderophores, are those synthetized by fluorescent pseudomonads. Pyoverdine play a key role in controlling iron availability in the rhizosphere (Visca et al., 2002; Expert et al., 2012). On the other hand, the pyoverdine was recently identified as virulence factors in P. syringae pv.tabaci (Tagushi et al., 2010).

Phaseolotoxins

Phaseolotoxins are synthetized by different pathovars of
Pseudomonas syringae including the pathovars phaseolicola and actinidia. The phaseolotoxin is a tripeptide which is hydrolyzed to produce an octicidine metabolite that is an irreversible inhibitor of ornithine carbonyl transferase (OCTase) (Arrebola et al., 2003; Melotto and Kunkel, 2013). The OCTase enzyme is considered to play a major role in the urea cycle of the plant (Arrebola et al., 2003).

The tabotoxinine beta lactam (TBL), the active form of the tabotoxin is produced in the host plant after hydrolysis with an aminopeptidase (Moore et al., 1984; Arrebola et al., 2007). The TBL pathotoxin plays a key role as an inhibitor of the glutamic synthetase (Thomas et al., 1983). Both the tabotoxines and phaseolotoxins contribute to the virulence of the P. syringae by inhibiting the host response defense at early stage and by inhibiting the photosynthesis process leading to severe chlorosis of the affected plant tissues (Arrebola et al., 2007).

ADHESINS AND EXTRACELLULAR POLYSACCHARIDES

Adhesins are considered as biomolecules such as proteins and glycoproteins that mediate the binding of the bacteria to the host cell (Katzen et al., 1996). The adherence is the first step interaction between the pathogen and the plant host which lead to the attachment and colonization of foliage or root tissues of the host plant (Kao et al., 1992; Alfano and Collmer, 1996). The plant pathogenic bacteria utilize several types of adhesins including a proteinous fimbrial or non fimbrial adhesions. Another group of adhesins which play key role in numerous plant pathogen interaction are the exopolysaccharides (EPS) (Kim et al., 2003; Melotto and Kunkel, 2013). The EPS are carbohydrate compounds secreted and maintained tightly associated with the bacterial capsule or released around the bacterial matrix. The importance of adhesins as virulence factors has been studied in numerous plant pathogens. Hence, X. compestris produce a major exopolysaccharide known as Xanthan gum implicated in infection (Denny, 1995; Melotto and Kunkel, 2013). The EPS amylovorin is another example of adhesins produced by E. amylovora, the causal agent of fire blight. P. syringae, the causal agent of several plant diseases produces different EPS such as alginate, levan (Denny, 1995). Proteinous fimbrial adhesins are also implicated in the infection caused by P. syringae (Yu et al., 1999).

QUORUM SENSING AND BIOFILM PRODUCTION

Quorum sensing is a bacterial communication mechanism that regulates the density of microbial population using the gene expression in response to the environmental and chemical sensing system (Kanda et al., 2011; Melotto and Kunkel, 2013). The signal molecules known as autoinducers that are detected by different bound receptors of bacterial cells are produced in coordinate manner at a specific bacterial stage such as disease physiological function including epiphytic growth, competition or colonization and virulence stage (von Bodman et al., 2003; Kanda et al., 2011). Quorum-sensing signal N-acyl homoserine lactones are known to regulate numerous virulence factors including enzymes production and exopolysaccharides in many plant pathogenic bacteria (Teplicki et al., 2000). Among the quorum-sensing regulator detected in P. syringae PsrA, is Pel regulator in E. chrysanthemi. However, a series of regulators namely MqsR, QseBC and exporter TqsA, could be present in E. amylovora (Hugouvieux-Cotte-Pattat et al., 1992).

Biofilm is a complex multilayer cellular structure attached to an inanimate surface or tissues and embedded within an exopolysaccharide material (Welch et al., 2000; Dow et al., 2003). Biofilm provides a protection for bacterial cell from a wide range of hostile and extreme environmental conditions including deshydration, extreme pH and UV radiation (Welch et al., 2000; Melotto and Kunkel, 2013).

Biofilm also shield bacteria cell from host immune response and antimicrobials compounds (Dow et al., 2003). Several plant pathogenic bacteria have been considered as biofilm producer as virulence factors including X. compestris (Dow et al., 2003) and P. syringae (Keith et al., 2003).

CONCLUSION

Based on several advances in literature on bacterial disease, it is clear that plant bacteria expresses virulence factors in each specific stage of pathogenesis. The virulence of plant pathogens is a multifactorial phenomenon which involves host-pathogen interactions that must be largely explored. In this review, we summarized the major bacterial virulence determinants that are required for establishing infection and disease development. On the other hand, an efficient strategy for bacterial disease control needs further studies of the virulence factors at the molecular levels in order to know their contribution in the plant pathogen interaction.

Conflict of Interest

The authors have not declared any conflict of interest.

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island has a tripartite mosaic structure composed of a cluster of type III secretion genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. Proc. Natl. Acad. USA 97:4856-4861


