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Multidrug Efflux System in the Virulence of Plant Pathogenic Bacteria

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Abstract

Plants cannot escape from their enemies, as they are sessile. Plants are also not dependent upon external source of metabolite to get rid of their enemies. Plant defense and immunity is totally inbuilt. Either plant try to self-destroy the part under the pathogen attack or plants use various secondary metabolites to restrict their pathogen. In spite of these protections, diseases are always exception as plant pathogens have developed different strategies to overcome the plant defense and to survive in the hostile host environment. Plant pathogenic bacteria use different protein secretion systems in this regard. While the protein secretion systems such as type II, III, IV, V and VI are known to secrete proteins, the type I secretion system is known to secrete proteins as well as extrude metabolites to adapt and attack the host. This protein secretion system is simple as it is made up of three different proteins, for which it is also known as tripartite efflux system. The role of type I secretion system in virulence plant pathogenic bacteria has not been explored so well unlike the other secretion systems. It may be that the type I secretion system helps the pathogen to efflux out the antimicrobial secondary metabolites produced inside the plant, thereby, helping the pathogen to adapt inside the host. Several studies on the plant pathogenic bacteria such as Erwinia sp. and Ralstonia sp. have given insights regarding the role of type I secretion system in virulence. These findings have given a newer dimension to study plant microbe interactions. In this chapter, we are trying to summarize the finding relating to multidrug efflux system in the phytopathogenic bacteria.

Introduction

Plants suffer from diseases caused by many different organisms, like, bacterial wilt caused by Ralstonia solanacearum [1]; bacterial leaf blight of rice caused by Xanthomonas oryzae [2]; early blight of potato caused by Alternaria solani [3]; fire blight caused by Erwinia amylovora [4]; etc. To counteract pathogens' attacks, plants have developed physical (waxy cuticle, thorns, spines, etc.) and chemical barriers (secrete chemicals to fight the pathogens) [5]. Plants produce secondary metabolites that are anti-microbial in nature. Plant Secondary Metabolites (PSMs) act as defense molecules against pathogenic intrusions. PSMs are produced occasionally in living plant cells and do not play much of significant role in the primary life of plants that produce them. The secondary metabolites are an outcome of the various physiological activities of the plant, used in coping with stressful constraints during challenging and changing environment of growth [5,6]. The PSMs includes terpenoids, phenolics, steroids, flavonoids and alkaloids, which plays significant role in ensuring the survivality of the plant during hostile biotic and

abiotic stress [7]. Apart from the toxicity, the PSMs with color or terpenoids with fragrance have their role in attracting insects for pollination [8]. Although, the PSMs are produced in very low concentration, they are accumulated at the site of pathogenic intrusion and further acts as a barrier for the pathogens.

On the other hand, the plant pathogenic bacteria that cause either localized or systemic infection are well as studied for the mechanism of the disease. They invade their respective hosts by secreting molecules by different secretory systems [9]. According to the structure of the cell membrane, bacteria have evolved secreting molecules and secretory systems as well. Unlike Gram-positive bacteria, Gram-negative bacterial cell envelope is composed of two membranes: Inner membrane and outer membrane separated by the periplasm that contains the peptidoglycan layer. Proteins or molecules secreted by the Gram-negative bacteria to the exterior, therefore, need to cross both the membranes. Gram-negative bacteria secretion systems are more elaborate than that of Gram-positive bacteria. The gram negative phytopathogenic bacteria possess six different types of secretion systems, namely, type I, type II, type III, type IV, type V and type VI secretion systems [10-15]. Type II secretion system secretes out exoproteins into the extracellular environment of the bacteria [11]; while type III secretion system secretes the effector proteins directly into the host cytosol in order to suppress the PAMP- Triggered Immunity (PTI) and to regulate plant metabolism in a way that favors the growth of the pathogen inside the host [12]. The type IV secretion system in Agrobacterium tumificiens is exemplified to transfer T-DNA into plant cells in causing the crown gall disease [16]. The type V secretion system is otherwise known as autotransportes wherein, the proteins are self-transported. Adhesins are mainly transported using the type V secretion system [17]. The type VI secretion system is mainly used for cell-to-cell communication rather than directly attacking the host [18]. While all other secretion system secretes molecules to invade the host, the Type I secretion system is the only secretion system that pumps out toxins from cytosol of the bacterium to its exterior milieu [19]. The major role of these protein secretion systems is either to attack the plant or to adapt the plant hostile environment. However, how the pathogen overcomes the different anti-microbial metabolites has been less elaborately studied. Some of the reports suggest that type I secretion system plays a significant role as it secretes out the metabolites from the cytosol of the bacterial cell [19,20].

In this chapter, we have attempted to put an emphasis on the importance of type I secretion system with regard to virulence of plant pathogenic bacteria. This secretion system can be a potential target for drug discovery against the pathogens.

Different types of protein secretion system and their role in virulence

To interact with other co-existing fellow bacteria and also to invade the host system, bacteria have developed different secretory systems that secretes out molecules, mostly proteins, either to form biofilm for cell-to-cell communication or to have pathogenic association with its host. The Gram-negative pathogenic bacteria possess mainly six different types of protein secretion systems, known as type I, II, III, IV, V and VI secretion systems (Figure 1). All the protein secretion systems contribute significantly to the pathogenicity of the bacteria. Briefly, the role of different protein secretion systems in virulence can be stated as follows:

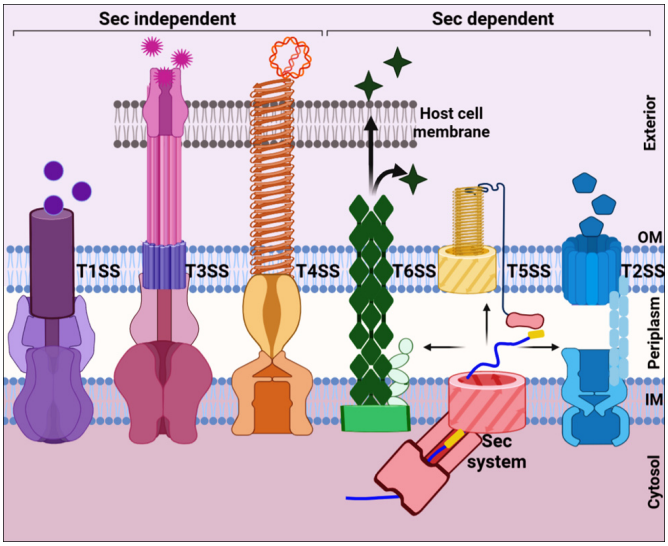

Figure 1: A schematic representation of the different types of protein secretion system in Gram-negative bacteria.

Type I secretion system

The Type I Secretion System (T1SS) secretes out molecules into the extracellular space from the cytosol of the bacteria crossing both the inner and the outer cell membrane. The secreted molecules are not exposed to the periplasm [10]. They are mainly known to extrude out entities (antibiotics, heavy metals, metabolites, etc.) that are toxic to the cell. Thereby, the T1SS is also known as multidrug efflux system as Multidrug Resistance (MDR) in bacteria is an outcome of active extrusion of drugs outside the cell [10]. Their direct role in the virulence of a pathogen is not elaborately studied but some recent reports shows promising role of multidrug efflux system in the virulence of pathogens, which shall be discussed later in this chapter.

Type II secretion system

This protein secretion system is known as general secretory pathway in bacteria. This secretion system originated from bacterial pili. In Gram-negative bacteria, proteins that are secreted outside by the Type II Secretion System (T2SS) follows two steps. First, the proteins are secreted to the periplasm using the signal peptide recognition pathway. From the periplasm, the proteins are secreted through the outer membrane using T2SS [11]. Cell wall degrading enzymes such as cellulase, pectinase, polygalacturonase, endoglucanase in are secreted by T2SS. T2SS mutants are deficient in extracellular enzyme activity and are virulence deficient [21].

Type III secretion system

The Type III Secretion System (T3SS) functions with a syringe-like projection or injectisome through which the molecules from bacterial cytosol are directly transferred to the host cytosol. The T3SS is responsible for the early stage invasion of the pathogen into hosts. It secretes out the Type 3 Effector proteins (T3Es) directly into the host cell through its injectisome that is under the regulation of HrpG master regulator [12]. In brief, the extracellular enzymes secreted by the T2SS induce the plant defense molecules like polyphenols, to accumulate at the site of attachment, which in turn activates the HrpG regulated T3SS to secrete out T3Es. The effector proteins then suppress or regulate the host metabolism such that the bacteria is able to overcome the host defense system. Mutants that are defective in T3SS are nonpathogenic in host plants and unable to elicit

defensive hypersensitive response in resistant hosts. The T3SS in plant pathogenic bacteria is known as Hrp secretory system [22]. The secretion system is originated from bacterial flagellar system. Some bacteria known to secrete more than 50 different effectors through the secretion system.

Type IV secretion system

The Type IV Secretion System (T4SS) origin is from bacterial conjugation system [13]. In terms of pathogenicity, it is utilized by A. tumificiens to directly transfer T-DNA into the host cytosol [23]. However, this secretion system also secrete proteins, that is, in B. pertussis, it secretes out pertussis toxin in the extracellular medium [24]. It can be clearly stated that T4SS secretes its molecules in a contact dependent manner.

Type V secretion system

The Type V Secretion System (T5SS) is a simple secretion pathway that can secrete out large protein molecules (>3000aa). This secretion system is also referred to as auto transporters i.e., the molecule formed at its proximal end is transported via a β-barrel structure formed from its distal end. These are the adhesion molecules that are generally transported across the Gram-negative bacterial outer membrane using T5SS [25]. The hemagglutinins help in initial attachment of the pathogen to its host. In plant pathogenic bacteria such as Erwinia and Xyllela, filamentous hemagglutinin role in adhesion and virulence has been demonstrated [26,27].

Type VI secretion system

The Type VI Secretion System (T6SS) is mainly for cell-to-cell communication. It is used as an antibacterial tool during interbacterial competition. The machinery of T6SS is composed of two main parts: A membrane-spanning complex and a double tube structure that resembles a contractile bacteriophage tail. On encountering another bacteria or eukaryote, the inner tube is propelled out, thus, secreting the molecules [15]. For instance, the T6SS of plant pathogen Xanthomonas citri protects it from predatory amoeba dictyostelium iscoideum [28].

The protein secretion systems, altogether, helps the phytopathogenic bacteria to overcome the host defense system and invade the host. The multidrug efflux system apart from effluxing out toxins also serves as a significant virulence determinant.

Multidrug tripartite efflux pump of type I secretion system

Multidrug Resistance (MDR) is widely present in bacterial pathogens, which enables them to withstand lethal doses of drugs [29,30]. Bacteria can become resistant to drug by several tactics such as, altering the cell permeability to avoid the entry of drugs, modifying the molecular targets of the drug, enzymatic modification of the drugs to inactivate them, etc. Multidrug efflux pumps are another such approach to develop resistance. It plays significant role in discharge of toxic substances for developing Multidrug Resistance (MDR) in bacteria. The efflux system extrudes antibiotics, heavy metals, organic pollutants, quorum sensing signals or bacterial metabolites, etc., which are vital for the bacterial survivality in different habitats as well as on their regulation by specific signals. Efflux systems were first reported in Escherichia coli making it resistant towards tetracycline and in the course of evolution, efflux systems are now a ubiquitous type of resistance present in both prokaryotes and eukaryotes. In prokaryotes, five major families of efflux pumps are reported, namely, (i) The ATP-Binding Cassette (ABC) super family (ii) The Resistance-Nodulation-Division (RND) family (iii) The Small Multidrug Resistance (SMR) family (iv) The Major Facilitator Superfamily (MFS) and (v) The Multidrug And Toxic compound Extrusion (MATE) family. In terms of energy source, the ABC transporters are dependent on ATP hydrolysis; MFS, RND and SMR are proton-driven pumps and MATE consists a Na^+/ H^+ drug anti porter system (Figure 2) [30,31]. These efflux pumps have diverse biological roles. For instance, Rhizobium etli uses the MFS efflux pump to withstand the toxic effects of plant flavonoids during the formation of root nodules [32]. Likewise, RND class of efflux pump is used by several bacteria Enterobacteria, Salmonella, Mycobacterium, etc. [33-35], that contribute to their sustainability in the mammalin system. Inactivation or overexpression of efflux pumps leads to attenuation of virulence presumably either due to lack of signaling molecules or the signaling molecule cannot reach the threshold required for Quorum sensing, respectively [36]. Thus, multidrug efflux pumps are vital features of microbes.

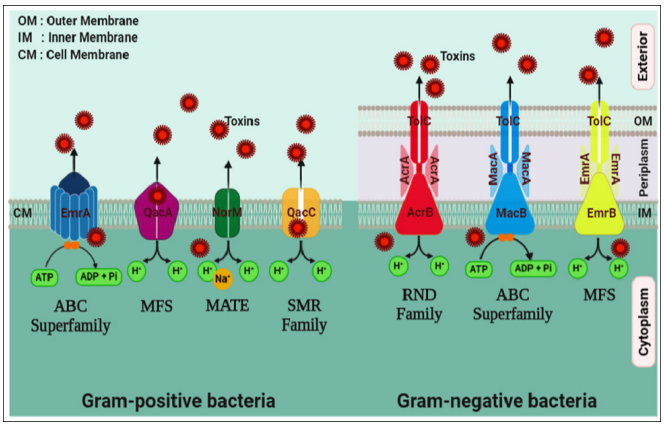

Figure 2: Different classes of multidrug efflux systems in Gram-positive and Gram-negative bacteria. (i) ATP-binding cassette (ABC) Superfamily. (ii) Major Facilitator Superfamily (MFS). (iii) Multidrug And Toxic compound Extrusion (MATE). (iv) Small Multidrug Resistance (SMR) family. (v) Resistance-Nodulation-Division (RND) family [30].

Role of multidrug efflux system in plant-bacteria interaction

Soil is an ecosystem that consists of largest and the most diverse bacterial population. The chemicals present in the soil influence the bacterial repertoire of the soil microbiota. The rhizosphere is the hub where the microorganisms are found in high population and they form contact with plant roots [37]. The microbiota of the rhizosphere is governed by the plant root exudates and their effect upon the microorganisms as well as the relationship amongst the co-existing microbes. Some compounds produced by the plants have antimicrobial effects and the bacteria require efflux pumps to establish the plant-bacteria association [38]. The transcriptomics analysis of Pseudomonas putida grown in the rhizosphere of maize revealed that the expression of different efflux pumps is induced, thereby, suggesting the role of multidrug efflux system in colonization [39]. Another incidence of plant-bacteria interaction has been described in S. maltophilia, wherein, its mutant lacking the SmeDEF efflux pump is not able to colonize the roots of the plant [30]. The efflux system confers the association of A. tumificiens with Medicago sativa. The IfeAB efflux pump of A. tumificiens, induced by flavonoids, is involved in competitive colonization of M. sativa (alfalfa) roots [41]. The EmrAR efflux system in S. meliloti is also induced by flavonoids, which in turn helps in its symbiosis with Medicago tranculata [42]. B. japonicum is another example whose efflux pump BjG30 plays role in early stage of symbiosis

with soyabean [43]. Therefore, multidrug efflux pumps exhibit different functions with regard to bacterial adaptation to different habitats.

Role of multidrug efflux system in virulence of phytopathogenic bacteria

The efflux system has been shown to be involved in virulence of several animal pathogenic bacteria [44]. There is very less study regarding the direct implementation of efflux pumps in the virulence of plant pathogenic bacteria. Among the plant pathogenic bacteria, the role of tripartite efflux proteins in virulence has been reported mainly in Erwinia amylovora and E. chrysanthemi.

Erwinia amylovora is an enterobacterium that causes fire blight in apple and pear. The two essential factors required for causing disease are elucidated as the type III secretion system and production of exopolysaccharide termed as amylovoran [4]. However, the bacterium possesses functions due to which it is able to survive in planta without being out-competed by the secondary metabolites, such as, phytoalexins, produced by the plant. The tripartite efflux system of E. amylovora helps it to bypass the antimicrobial effect of the secondary metabolites [4]. It has been elucidated that the AcrAB efflux pump is indirectly required by the pathogen for causing disease in the rootstock of apple. The mutant of E. amylovora that was lacking in AcrAB efflux system was not able to colonize the host and also impaired in resistance against phytoalexins produced by the plant [45]. This clearly states the importance of efflux system in virulence of the bacterium. E. Chrysanthemi is another pathogenic species of Erwinia that causes bacterial soft-rot disease. The virulence of this bacterium is a result of pectate lyases and other hydrolytic enzymes that leads to degradation of the plant cell wall, causing maceration and necrosis of the plant tissues [46]. A comparative analyses on E. chrysanthemi RND efflux system suggested that the efflux system can contribute to virulence specific to host. The efflux pumps acrAB and emrAB of E. chrysanthemi are involved differentially in host specific virulence with regard to two hosts such as Saintpaulia and Chicory [46]. This indicated that the multidrug efflux systems are specific to plant metabolites and plays significant role in excreting those metabolites from cytosol of the cell.

Another phytopathogenic bacterium is Ralstonia solanacearum, which is a Gram-negative, broad host range pathogen that causes bacterial wilt [47]. It is a systemic pathogen and it might be possible that this bacterium uses type I secretion system more elaborately to adapt to different host. There is only one report about the characterization of the type I secretion system in this pathogen. In an in vivo gene expression study, two genes are acrA and dinF are found to be expressed inside the host belongs to the type I secretion system. The gene when knocked out in the pathogen, it was demonstrated that the mutant is reduced for virulence in tomato [48]. However, the virulence of the mutant on other host has not been studied. Our laboratory is working on R. solanacearum F1C1 strain [49]. We have demonstrated the virulence of the bacterium in tomato and eggplant seedlings [50-52]. We have isolated a transposon-induced mutant in a tripartite system (homolog of RSc2727; unpublished result from the lab) which we have observed to exhibit differential virulence between tomato and eggplant seedlings. In tomato, it is highly virulence deficient, while in eggplant its virulence is comparable to wild type. The mutant has pleiotropic phenotype, deficient in several phenotypes, including sensitivity to heavy metal (unpublished result from the lab). Therefore, we are proposing a schematic model based on our hypothesis (Figure 3).

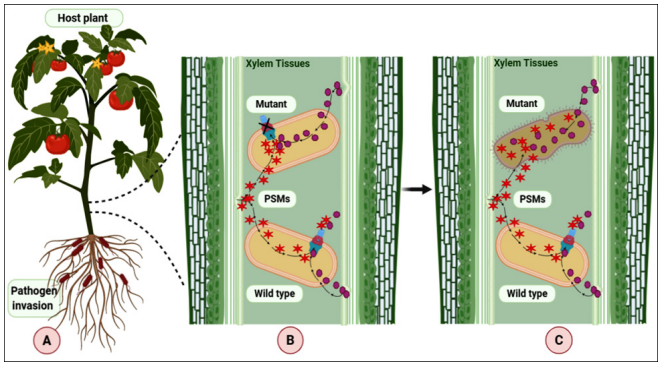

Figure 3: A schematic model explaining the role of multidrug efflux system in the virulence of plant pathogenic bacteria.
A. A host (tomato) plant invaded by phytopathogenic bacteria (shown in maroon color) through the roots.
B. Magnified image of the L.S. of stem showing the wild type and mutant (impaired in efflux system) in the xylem tissues. In the presence of pathogen, the host plant produces Plant Secondary Metabolites (PSMs). The wild type bypasses the host defense by extruding out the metabolites from its cytosol. The mutant is unable to efflux out the metabolites as it is impaired in the multidrug efflux system.
C. The mutant is outcompeted due to the accumulation of metabolites that are toxic to the cell. The wild type survives the hostile environment inside host and thrives further to cause disease.

Conclusion

In this chapter, we have summarized the significance of multidrug efflux system in plant pathogenic bacteria. Unlike animals, plants are rich source of secondary metabolites, which are most likely used to protect them from various pathogens. However, plant pathogenic bacteria with type I secretion system or multidrug efflux system can overcome this plant defense response by extruding out the metabolites from inside of its cell. This is a relatively newer dimension to study plant microbe interactions. In future, more research on Type I secretion in phytopathogenic bacteria will shed more light on their host specific function. How their expression is regulated? How do they specify the metabolite to secrete out from bacteria? What is the role of endophytes, whose host range and adaptation are less studied? Novel drug molecule targeting the type I secretion system can be designed such that to enhance resistance in plants against their bacterial pathogens. A new approach of combating the pathogens can be up taken wherein, the plants are engineered such that, the structures of the metabolites are altered while their functionality remains unchanged. This will ensure the influx of the metabolites into the bacterial cell membrane but due to altered structure, the type I secretion system might not be efficient to efflux out the metabolites. This can be a novel strategy to fight against the plant pathogens.

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