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Molecular determinants of the host specificity by *Xanthomonas* spp.

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Abstract

During initial interactions of bacteria with their host plants, most plants recognize the bacterial infections and repel the pathogen by plant defense mechanism. The most active plant defense mechanism is the hypersensitive response (HR) which is the localized induced cell death in the plant at the site of infection by a pathogen. A primary locus induced in gram-negative phytopathogenic bacteria during this initial interaction is the Hrp locus. The Hrp locus is composed of a cluster of genes that encodes the bacteral Type III machinery that is involved in the secretion and translocation of effector proteins to the plant cell. DNA sequence analysis of *hrp* gene in phytopathogenic bacteria has revealed a Hrp pathogenicity island (PAI) with a tripartite mosaic structure. For many gram-negative pathogenic bacteria, colonization of the host's tissue depends on the type III protein secretion system (TTSS) which secrets and translocates effector proteins into the host cell. Effectors can be divided into several groups including broad host range effectors, host specific effectors, disease specific effectors, and effectors inhibit host defenses. The role of effectors carrying LRR domain in plant resistance is very elusive since most known plant resistance gene carry LRR domain. Host specific effectors such as several avr gene products are involved in the determination of the host specificity. Almost all the phytopathogenic *Xanthomonas* spp. carry *avrBs1*, *avrBs2,* and *avrBs3* homologs. Some strains of *X. oryzae* pv. *oryzae* carry more than 10 copies of *avrBs3* homologs. However, the functions of all those *avr* genes in host specificity are not characterized well.

Introduction

In nature plants are resistance to the majority of pathogens, and many bacteria live in close contact with plant without causing any harm. To be a successful pathogen the invading bacterium has to overcome the plants defense. During evolution plant pathogenic bacteria have acquired multiple functions that enable them to colonize and multiply in living tissue. The genus *Xanthomonas* is traditionally known as a taxon of bacterial plant pathogens. This has particularly become the case after the exclusion of *X. maltophilia* from the genus. However, there is growing evidence that many bacterial pathogens, living in close association with plants but causing no apparent disease symptoms on the host from which they were isolated, also belong to the genus *Xanthomonas*. It was reported that *Xanthomonas* infections occur on at least 124 monocotyledonous and 268 dicotyledonous plant species. Among monocotyledons the range of hosts extends across 11 families comprising at least 70 genera, and there are hosts in 57 families of dicotyledons comprising more than 170 genera. The host range of each *Xanthomonas* species or pathovar is in most cases limited to a few species or sometimes a few genera of the same plant family, the name of the pathogen often being derived from the genus or species name of the host plant. In host plants, they produce various symptoms after several days of multiplication, whereas in nonhost plants, they trigger the hypersensitive response (HR), a rapid defense associated programmed death of plant cells at the site of invasion. The initial interactions of bacteria with their plant hosts are critical in determining the final outcome of infection. During this initial interaction, most plants recognize the bacterial infection and repel the pathogen by plant defense mechanism. The hypersensitive response thought to be responsible for limiting the growth of the pathogen is capable of providing resistance to host plants and nonhost plants. Since the bacteria belong to the genus *Xanthomonas* are the major crop pathogen in Korea, we choose the *Xanthomonas* spp. as a model system for plant-microbe interaction.

Type III secretion (TTS) system

To grow in intercellular space, phytopathogenic bacteria sense their environment and induce genes required for host infection. A primary locus induced in Gram-negative phytopathogenic bacteria during recognition phase is the Hrp locus. Several gram-negative phytopathogenic bacteria use type III protein secretion systems to deliver bacterial effector proteins into plant cells. Those *hrp* genes that are broadly conserved in pathogenic *Pseudomonas*, *Erwinia*, *Xanthomonas*, *Ralstonia*, *Yersinia*, *Salmonella*, and *Shigella* spp. are designated *hrc* (HR and conserved). *hrp/hrc* gene clusters of plant pathogens fall into two distinct groups based on their possession of similar genes, operon structures, and regulatory systems. Ths *hrp* clusters of *P. syringae* and *E. amylovora* are in group I, and those of *R. solanacearum* and *Xanthomonas* spp. are group II. Some of the *hrp* genes appear to be completely different between the two groups, the arrangements of genes within some operon are characteristic of each group and the regulatory systems are distinct. A key difference in regulation is that group I operons are activated by HrpL, a member of ECF (extra cytoplasmic functions) subfamily of sigma factors, whereas most group II *hrp* operons are activated by a member of the AraC family. A number of Hrp proteins are most likely associated with or localized in the bacterial membrane. For example, the HrcV protein sequence contains eight transmembrane domains but lacks a signal sequence, suggesting inner membrane localization. Both HrcC1 and HrcJ contain an NH_2-terminal signal sequence and one or two transmembrane domains suggesting that a part of these proteins might be targeted to the outer membrane. The HrcN protein is a putative ATPase with highly conserved nucleotide and magnesium binding domains. Therefore, Hrc proteins presumably constitute the core components of the secretion apparatus in the inner and outer membrane. However, the role of non-conserved Hrp proteins is less clear. Recently, functions of more Hrp proteins were reviled. For example, HrpF was shown to be a putative component of the type III translocon in the plant plasma memebrane and HrpE1 may be a major subunit of the Hrp pilus (Butter et al., 2002).

Phytopathogenic bacteria produce an appendage like structure that is essential for the delivery and regulation of effector proteins to the plant cell through its type III system. The structure of the type III secretion system may explain the question of the existence of the R gene in the cytoplasm instead of the membrane of the plant cell as a receptor. This leads a new hypothesis of the guard model instead of receptor-ligand model for the gene-for-gene concept.

Type III effector proteins

The demonstration that phytopathogenic bacteria use genes that are remarkably similar to genes encoding the type III secretion system in animal pathogenic bacteria provided a hypothetical frame work to explain the molecular mechanisms for pathogen infection and recognition by plants. A general mechanism for bacterial pathogenesis in plants and animals involves the direct deliver of different classes of proteins to the host. These proteins, now referred to as type III effectors, suppress, stimulate, interfere with, or modulate host responses to invading pathogens. The role of type III effectors in plant cells remains elusive. Most phytopathogenic bacterial effectors show no homology to known proteins in existing databases. With the help of the proteomics, about 40 proteins were reported as Type III effectors. The putative effectors secreted by the type III secretion system of the phytopathogenic bacteria can be divided into three groups based on the possible functions of the effectors.

Non-specific effectors

Non-specific effectors such as Harpin or PopA (Pseudomonas out protein) were belonging to the first group (Belbahri et al., 2001). Harpin, a non-specific HR-like elicitor of Erwinia amylovora was the first protein secreted by the Hrp machinery. They are hydrophilic, rich in glycine, heat stable, lack cysteine, and elicit an HR when injected into the apoplast of certain plants. Homologues of Harpin have been found in phytopathogenic bacteria carrying group I hrp system such as Pseudomonas syringae, Erwinia chrysanthemi, but not in bacteria carrying group II hrp system such as Xanthomonas, and Ralstonia. Instead, R. solanacearum secretes PopA, which elicits an HR in tobacco and certain cultivars of petunia. Like the Harpins, the PopA protein is heat stable and glycine rich, but the sequence is entirely different. In contrast to the harpins, the popA gene is not in a hrp gene cluster but is located outside of the large hrp gene cluster. Hpa1A (Hrp associated) is the PopA like protein of X. oryzae pv. oryzae and X. campestris pv. glycines. The hpa1A gene encoded a 13-kDa glycine-rich protein with a composition similar to those of Harpins and PopA. Perfect PIP box (plant inducible promoter) was present in the hpa1A. The mutation on hpa1 showed the reduced disease symptoms and delayed HR on tomato. The phenotype of hpa1 mutants were very similar to those of hopPsyA (hrmA) in Pseudomonas syringae pv. syringae (Heu and Hutcheson, 1993). The HopPsyA protein is secreted in culture by P. syriangae and when expressed transiently in tobacco, it elicits a HR, indicating that its site of action is inside plant cells. The distinct feature of effector proteins belong to this group is that those showed very low host specificity. The injection of any one of these proteins causes a HR in very wide range of plant species. Transgenic rice plants carrying Hpa1A had broad spectrum of resistance against both fungal and bacterial pathogen such as Magnaporthe grisea and X. oryzae pv. oryzae.

Host-specific effectors (avirulence gene products)

Second group effectors are *avr* gene products that restrict host range. *avr* genes have been defined by their ability to induce disease resistance in host plants containing the corresponding R genes. Now, more than 40 *avr* genes have been isolated from different phytopathogenic bacteria. Most Avr proteins are secreted through the type III apparatus. Despite the fact that many bacterial *avr* genes have been isolated, most of them share little or no homology to each other, with the exception of the *avrBs3* families (Van den Ackerveken et al., 1996). A characteristic structural feature of all *Xanthomonas* AvrBs3-like protein, which shares 90-97% sequence identity with each other, is a central region consisting of a variable number of tandem 34-amino-acid repeats. Differences between *avrBs3* family members are largely confined to this central domain. Exchanging the repeat domain alters the avirulence specificity of the protein to the specificity of the gene from which the domain was derived. Likewise, deletions within the repeat domain can give rise to new specificities, indicating that the repeats determine recognition specificity. In addition to the repeat domain, all AvrBs3 family members contain protein signatures that are usually restricted to eukaryotes, namely nuclear localization signals (NLSs) and an acidic transcriptional activation domain (AAD) (Zhu et al., 1998; Zhu et al., 1999). Mutations in the NLSs or AAD of different AvrBs3-like proteins usually abolish recognition by the corresponding R gene. The DNA binding activity of AvrXa7 and the fact that all AvrBs3 homologs contain an acidic C-terminal transcriptional activation domain suggest that these effectors act directly or indirectly as transcription factors, modulating the host transcriptome to the benefit of the bacterial intruder. Some xanthomonads, such as, *X. oryzae* pv. *oryzae*, *X. campestris* pv. *glycines*, and *X. campestris* pv. *malvacarum*, contain multiple copies of *avrBs3* homologs. In the case of *X. oryzae* pv. *oryzae* strain KXO18, up to 14 *avrBs3*-homologs were shown. Sequential addition of *avrBs3* homologs genes in *X. campestirs* pv. *glycines* has increased susceptibility of the pathogen in host soybean plants. This suggests the function of the AvrBs3-homolog as a virulence factor in host plant not only as an elicitor to cause HR in resistant plant. Analysis of AvrBs3 homologs in *X. oryzae* pv. *oryzae* revealed that their virulence function is not merely additive but specific to each family member. This finding rose the question of how these highly conserved effector molecules exert their specific virulence function at the molecular level. Since these effectors were highly host-specific, the analysis of effectors carried by bacterial strains isolated in different area tells us the kinds of cultivars that can be cultivated in that area.

Effectors inhibit host defense system

One major capability of bacterial effector proteins appears to be the suppression of host defense responses. This has been well studied for YpoJ from *Yersinia pestis*, which belong to the YpoJ/AvrRxv family of effectors. YopJ inhibits cytokine production by the host cell and induces apoptosis in macrophages. Homologs of YopJ have been identified in *Xanthomonas campestris* pv. *vesicatoria* (AvrRxv, AvrXv4, AvrBsT and XopJ).

The putative catalytic residues are strictly conserved in all YopJ-like proteins, indicating that they function as proteases. However, besides *X. campestris* pv. *vesicatoria*, most *Xanthomoas* spp. including *X. axonopodis.* pv. *glycines, X. oryzae* pv. *oryzae* and *X. campestirs* pv. *campestris* did not have AvrRxv homologs.

The inhibition of effector proteins with eukaryotic signaling pathways leads to alterations in the host's transcriptome. This appears to be the case for the effector protein YopM from *Y. enterocolitica*, which localized to nuclei of infected host cycles. YopM is a leucine-rich repeat (LR) containing protein that modulates the expression of host genes that are involved in the control of cell growth and the cell cycle. Recently, it was shown that *hpaG* of *X. oryzae* pv. *oryzae*, *X. campestris* pv. *glycines*, *X. campestris* pv. *vesicatoria* and *popC* of *R. solanacearum* are predicted to encode a protein with LRR motifs. LRR motifs are commonly involved in protein-protein interactions and are found in the three major classes of plant-resistance genes (Leister and Katagirl, 2000). This region in *Xanthomonas* spp. carrys two open reading frames. *hpaF* encodes a putative protein of 197 amino acids containing a functional NLS (RPRRR) at amino acid 43. *hpaG* encodes a putative protein of 432 amino acids which contains leucine-rich repeats (LRRs; LXXLXXLXXLXLXXXXXLXXLPXX). The fact that multiple LRR containing proteins are now known to be associated with group II *hrp* systems suggests that *Xanthomonas* spp. and *R. solanacearum* use proteins to directly interact with R proteins inside plant cells to confound the plant defense response. Since HpaF carry the functional NLS domains, HpaF may act in nucleus of the plant cell like YpoM. However, whether the HpaF may inhibit the defense of the plant should be investigated further.

References

Belbahri, L., Boucher, C., Candresse T., Nicole, M., Ricci, P., and Keller, H. 2001. A local accumulation of the *Ralstonia solanacearum* PopA protein in transgenic tobacco renders a compatible plant-pathogen interaction incompatible The Plant J. 28:419-430

Buttner D. and U. Bonas. 2002. Getting across-bacterial type III effector proteins on their way to the plant cell. EMBO J. 21:5313-5322

Changsik Oh, Sunggi Heu, and Cho, Y. 1999. An *hrc*U-homologous gene mutant of *Xanthomonas campestris* pv. *glycines* 8ra that lost pathogenicity on the host plant but was able to elicit the hypersensitive response on nonhosts. Mol. Plant-Microbe Interact. 12:633-639

Collmer, A., Badel, L., Charkowski, O., Deng, W., Fouts, E., Ramos, R., Rehm, H., Anderson, M., Schneewind, O., van Dijk, K., and Alfano, JR. 2000. *Pseudomonas syringae* Hrp type III secretion system and effector proteins. Proc. Natl. Acad. Sci. USA 97: 8770-8777

Gueneron, M., Timmers A., Boucher, C., and Arlat, M. 2000. Two novel proteins, PopB, which has functional nuclear localization signals, and PopC, which has a large leucine-rich repeat domain, are secreted through the *hrp*-secretion apparatus of *Ralstonia solanacearum*. Mol. Microbiology 36:261-277

Guttman, D., Vinatzer, B., Sarkaar, S., Ranall, M., Kettker, G., and Greenberg, J. 2002. A functional screen for the type III (hrp) secretome of the plant pathogen *Pseudomonas syringae.* Science 295:1722-1726

Heu, S. and Hutcheson, S. 1993. Nucleotide sequence and properties of the *hrm*A locus associated with the *Pseudomonas syringae* pv. *syringae* 61 *hrp* gene cluster. Mol. Plant- Microbe Interact. 6: 553-564

Hutcheson, S. W., and Heu, S. 1996. Regulation and function of *hrp* genes in *Pseudomonas syringae* strains. In (N. Keen and G. Stacey) Plant-Microbe Interactions Vol. 3, Chapman & Hall

Kim, F., and Alfano, R. 2002. Pathogenicity islands and virulence plasmids of bacterial plant pathogens. Current topics in microbiology and immunology 264:127-147

Lahaye T., and Bonas U. 2001. Molecular secretes of bacterial type III effector proteins. Trends in Plant Science 6: 479-485

Leister, RT. and Katagirl, F. 2000. A resistance gene product of the nucleotide binding site-leucine rich repeats class can form a complex with bacterial avirulence proteins *in vivo*. Plant J. 22(4): 345-354

Leyns, F., De Cleene, M., Swings, J., and De Ley, J. 1984. The host range of the genus Xanthomonas. The botanical review, 50:308-355

Noel L., Thieme F., Buttner D., and U. Bonas. 2003. XopC and XopJ, Two novel type III effector proteins from Xanthomonas cmpestris pv. vesicatoria. J Bact. 185:7092-7102

Silva, R., et al. 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. Nature 417:459-463

Stuiver, MH. and Custers, V. 2001. Engineering disease resistance in plants. Nature 441: 865-868

Van den Ackerveken, G., Marois, E., and Bonas, U. 1996. Recognition of the bacterial avirulence protein AvrBs3 occurs inside the host plant cell. Cell 87:1307-1316

Xiao, Y., Y. Lu, S. Heu and Hutcheson, H. 1992. Organization and environmental regulation of *hrp* expression in *Pseudomonas syringae* pv. *syringae* 61. J. Bacteriol. 174:1734-1741

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

Zhu, W., Yang, B., Wills, N. Johnson, L., and White, F. 1999. The C terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus. The plant cell 11:1665-1674