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The post-genomic era: new approaches for studying bacterial diseases of plants

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Abstract. Research in plant pathology is changing dramatically. Genomic and post-genomic investigations are generating large datasets, which allow the formation of holistic and predictive investigations of both pathogens and their plant hosts. The genomes of plant pathogenic bacteria are relatively easy to sequence, thus leading to further investigations on gene expression, proteomics and metabolomics. One of the emerging models for studying bacterial pathogenesis is *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000). This review will focus on the use of 'post-genomic' tools that provide insight into the interaction of *Pst* DC3000 with its various plant hosts.

Introduction

The genomes for over a dozen plant pathogenic bacteria have been completed, and at least 30 additional sequencing projects are underway (Preston *et al*. 2005). Most of the phytopathogenic bacteria that have been sequenced contain a circular chromosome and plasmids of varying sizes (Puhler *et al*. 2004). The data obtained from genomic sequencing projects have expanded the array of genes predicted to control pathogenic interactions with plants, and include genes that are required for adhesion, production of phytotoxins and plant growth hormones, resistance to oxidative stress, plant cell wall degradation, secretory systems, and interference and/or suppression of host defences. Collectively, these represent ~6% of the genes identified in the genomes of bacterial plant pathogens (Puhler *et al*. 2004). It is now obvious that a variety of high throughput techniques will be required to validate the predictions obtained from sequence analysis. Methods that address metabolism, secretion systems and the proteome will be required. The application of some of these techniques has been applied to the study of the model pathogen, *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000.

Pseudomonas syringae pv. *tomato* and close relatives

P. syringae pv. *tomato* DC3000 (*Pst* DC3000) is a pathogen of tomato (Cuppels 1986), *Arabidopsis thaliana* (Whalen *et al*. 1991), and edible *Brassica* spp. (Zhao *et al*. 2000; Keith *et al*. 2003). On tomato, *Pst* DC3000 causes a disease known as bacterial speck, which is characterised by dark brown to black lesions on leaves, fruit and stems; tissue adjacent to the lesions is often chlorotic. On edible, leafy *Brassica* spp. (e.g. broccoli, cabbage, cauliflower, turnip, radish, etc.), *Pst* DC3000 can produce a disease that is indistinguishable from *P. syringae* pv. *maculicola* (*Psm*) (Zhao *et al*. 2000). *Pst* DC3000 also induces water-soaked, spreading lesions, surrounded by a chlorotic margin on the model plant, *Arabidopsis thaliana* (Whalen *et al*. 1991).

Bacterial speck of tomato occurs worldwide wherever tomatoes are grown (Goode and Sasser 1980). Lesions can make the fruit unfit for the fresh market and increase the costs of harvesting and grading. *Pst* can survive on the seed surface for many years in the form of aggregates, especially inside the cavities of the seed surface (Bashan *et al*. 1982). The pathogen can also survive as an epiphyte on greenhouse-grown tomato plants for extended periods of time (Smitley and McCarter 1982). Transplants infested with *Pst* may go undetected because of lack of visible symptoms and thus become an important inoculum source for field epiphytotics (Goode and Sasser 1980).

Life cycle of Pst DC3000

The infection of plants by *Pst* DC3000 initially involves epiphytic (surface) colonisation of leaves and other plant parts. Interestingly, *Pst* DC3000 is not a good epiphyte, and the initial population on the leaf surface seldom exceeds 10^5 cfu/g of leaf tissue (Boureau *et al*. 2002). This is in contrast to the related pathogen, *P. syringae* pv. *syringae* (*Pss*) B728a, which multiplies epiphytically and reaches populations approaching 10^7 cfu/g on the surface of bean leaves (Hirano and Upper 2000). Comparison of the genomes

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of *Pst* DC3000 and *Pss* B728a has shown that the latter strain has many unique traits that contribute to epiphytic fitness, including genes encoding ice nucleation, copper resistance and UV repair (Feil *et al*. 2005). Once in the apoplast of susceptible plants, virulent *Pst* DC3000 must overcome or suppress general antimicrobial defences before they can multiply aggressively (Jin *et al*. 2003).

Virulence is multifactorial

One component of virulence in *Pst* DC3000 is the type III secretion system (TTSS), which is required for growth of *P. syringae* in susceptible host plants and the activation of plant defence in nonhost plants (Jin *et al*. 2003). These host responses are elicited when the TTSS delivers effector proteins to the plant cell, presumably via the *hrp* pilus (Collmer *et al*. 2002; He and Jin 2003). The availability of the genomic sequence of *Pst* DC3000 (Buell *et al*. 2003) has resulted in the identification of the entire repertoire of TTSS effectors, and the analysis of pathogen gene expression during infection (Boch *et al*. 2002; Greenberg and Vinatzer 2003; Chang *et al*. 2005). The availability of sequence data from both pathogen and host has, and will continue to, significantly affect research in the coming decade, as this has greatly facilitated the identification of key genes involved in pathogenesis and disease development.

Coronatine, an important aspect of virulence in *Pst* DC3000

Although the TTSS is the most-intensively studied aspect of virulence in *Pst* DC3000, other aspects of virulence include global regulatory genes (Alarcon-Chaidez *et al*. 2003; Chatterjee *et al*. 2003), exopolysaccharides (Keith *et al*. 2003) and phytotoxins (Bender and Scholz-Schroeder 2004). Coronatine (COR) is a chlorosis-inducing phytotoxin produced by several pathovars of *P. syringae* including pv. *alisalensis*, *atropurpurea*, *glycinea*, *maculicola*, *morsprunorum* and *tomato* (Bender and Scholz-Schroeder 2004). COR contributes to the multiplication of *P. syringae in planta* and lesion formation or expansion in several host plants, including ryegrass, soybeans, tomatoes and several crucifers (Bender and Scholz-Schroeder 2004). COR consists of two distinct structural components: (1) the polyketide coronafacic acid (CFA) and (2) coronamic acid (CMA), an ethylcyclopropyl amino acid derived from isoleucine (Ichihara *et al*. 1977; Parry *et al*. 1994). CFA and CMA function as biosynthetic intermediates and are joined together by an amide linkage to form the parent compound, COR (Ichihara *et al*. 1977). CMA is a structural analogue of 1-aminocyclopropane-1-carboxylic acid (ACC), an intermediate in the pathway to ethylene in higher plants. It has also been noted that COR is a structural and functional analogue of jasmonic acid (JA) and related signalling compounds such as MeJA and 12-oxo-phytodienoic acid (12-OPDA), the C_{18} precursor of JA/MeJA (Weiler *et al*. 1994). OPDA, JA, MeJA and other octadecanoids affect the regulation of diverse plant responses including biotic stress (Farmer *et al*. 2003), wounding (Howe and Schilmiller 2002), abscission (Burns 2002) and volatile production (Weber 2002). The identification of the *Arabidopsis coi1* (coronatine insensitive) mutant supports the hypothesis that COR is a functional analogue of MeJA (Feys *et al*. 1994). More recently, a JA insensitive mutant (*jai1*) of tomato, which is also insensitive to COR, was identified as a homologue of the *Arabidopsis COI1* (Zhao *et al*. 2003; Li *et al*. 2004). *jai1* plants, like the *Arabidopsis coi1* mutant, are insensitive to COR and exhibit resistance to strains of *P. syringae* that produce COR (Zhao *et al*. 2003).

Although COR is involved in various physiological responses, we do not understand how COR is perceived in different tissues, precisely how it functions, and to what extent it mimics MeJA. Previous reports have documented the production of ethylene in COR-treated tissue (Kenyon and Turner 1992), a response that may be attributed to the structural similarities between CMA and ACC.

Transcript profiling: a tool to better understand interactions between plants and *P. syringae*

We recently used cDNA microarrays to investigate the molecular processes that are regulated by MeJA, COR, CFA and CMA in tomato leaves (Uppalapati *et al*. 2005). We found that COR and MeJA, but not CMA, induced genes involved in the synthesis of ethylene (Uppalapati *et al*. 2005). Ethylene plays an important role in the symptoms associated with bacterial speck of tomato; for example, plants that are insensitive to ethylene show impaired disease symptoms (Lund *et al*. 1998), and ethylene has been implicated in chlorosis and senescence (Stall and Hall 1984). Ethylene production was observed in COR-treated leaves and was produced when COR-producing bacteria infected susceptible host plants (Kenyon and Turner 1992). In our transcript profiling experiments, COR induced genes associated with ethylene biosynthesis and responsiveness, suggesting that COR may modulate ethylene as a virulence strategy (Uppalapati *et al*. 2005).

COR also induced the expression of a set of auxin-related genes, implying that auxin levels also play an important role in pathogenesis (Uppalapati *et al*. 2005). In a study using potato tubers and mung bean hypocotyls (Sakai *et al*. 1979), auxin and COR were speculated to have different primary sites of action but similar physiological activities. Our results support this study and suggest that the COR-induced JA pathway may positively regulate auxin responses in tomato. This is consistent with the hypothesis that JA and auxin may function via a common signalling intermediate that modulates response to multiple plant hormones (Devoto *et al*. 2003).

Our results show that COR modulates genes involved in the pathways to JA, ethylene and auxin. This raises an interesting question: should COR be considered a phytotoxin or a phytohormone mimic? It is not surprising that COR targets these particular phytohormone pathways, as both ethylene and JA are known to positively regulate susceptible interactions between tomato/*Arabidopsis* and *P. syringae* (Kunkel and Brooks 2002). A popular hypothesis is that COR may act as a suppressor of defence response(s), possibly by suppressing salicylic acid-dependent defences in *Arabidopsis* and tomato (Kloek *et al*. 2001; Zhao *et al*. 2003). Although suppression of SA-mediated defences was not observed using exogenously applied COR, genes involved in JA biosynthesis and responsiveness were induced by COR (Uppalapati *et al*. 2005). Mutual antagonism between JA- and SA-mediated defence pathways is well documented (Kunkel and Brooks 2002); consequently, COR may stimulate the JA pathway at the expense of SA-dependent defence responses.

Comparison of transcriptional changes regulated by COR and MeJA

Two recent reports document the existence of JA-modifying enzymes, including a MeJA esterase and a JA amino acid synthetase (Staswick and Tiryaki 2004; Stuhlfelder *et al*. 2004). Presumably, a MeJA esterase could cleave exogenous MeJA to form JA, which could be further metabolised by a JA amino acid synthetase to form JA amino acid conjugates. These metabolised products of MeJA, along with the other MeJA-induced phytohormones (e.g. ethylene, IAA) could contribute to the secondary transcriptional changes in MeJA-treated leaves. This process may enable plant cells to 'fine tune' the chemical signals that regulate plant growth and help maintain jasmonate homeostasis (Staswick and Tiryaki 2004). However, it remains unclear whether COR is metabolised and forms conjugates with amino acids *in planta*. There are very striking differences in the structure of COR and MeJA, and these changes might enable COR to 'evade' MeJA modifying enzymes. If COR is not further metabolised, this could lead to perturbations in JA homeostasis and result in phytotoxicity. Clearly, there are many unresolved questions regarding the activity of COR in modulating phytohormone pathways. Experiments are underway to further analyse COR/MeJA-responsive genes and the potential receptors for these compounds, which will help elucidate the mechanism of action for both COR and MeJA.

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References

Alarcon-Chaidez FJ, Keith L, Zhao Y, Bender CL (2003) RpoN is required for plasmid-encoded coronatine biosynthesis in *Pseudomonas syringae*. *Plasmid* **49**, 106–117. doi: 10.1016/S0147-619X(02)00155-5

Bashan Y, Okon Y, Henis Y (1982) Long-term survival of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper seeds. *Phytopathology* **72**, 1143–1144.

Bender CL, Scholz-Schroeder BK (2004) New insights into the biosynthesis, mode of action, and regulation of syringomycin, syringopeptin and coronatine. In 'The Pseudomonads'. (Ed. JL Ramos) pp. 125–158. (Kluwer Academic Press: Dordrecht)

Boch J, Joardar V, Gao L, Robertson TL, Lim M, Kunkel BN (2002) Identification of *Pseudomonas syringae* pv. *tomato* genes induced during infection of *Arabidopsis thaliana*. *Molecular Microbiology* **44**, 73–88. doi: 10.1046/j.1365-2958.2002.02877.x

Boureau T, Routtu J, Roine E, Taira S, Romantschuk M (2002) Localization of *hrpA*-induced *Pseudomonas syringae* pv. *tomato* DC3000 in infected tomato leaves. *Molecular Plant Pathology* **3**, 451–460. doi: 10.1046/j.1364-3703.2002.00139.x

Buell CR, Joardar V, Lindeberg M, Selengut J, Paulsen IT *et al.* (2003) The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 10181–10186. doi: 10.1073/pnas.1731982100

Burns JK (2002) Using molecular biology tools to identify abscission materials for citrus. *HortScience* **37**, 459–464.

Chang JH, Urbach JM, Law TF, Arnold LW, Hu A, Gombar S, Grant SR, Ausubel FM, Dangl JL (2005) A high-throughput, near-saturating screen for type III effector genes from *Pseudomonas syringae*. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 2549–2554. doi: 10.1073/pnas.0409660102

Chatterjee A, Cui Y, Yang H, Collmer A, Alfano JR, Chatterjee AK (2003) GacA, the response regulator of a two-component system, acts as a master regulator in *Pseudomonas syringae* pv. *tomato* DC3000 by controlling regulatory RNA, transcriptional activators, and alternate sigma factors. *Molecular Plant–Microbe Interactions* **16**, 1106–1117.

Collmer A, Lindeberg M, Petnicki-Ocwieja T, Schneider DJ, Alfano JR (2002) Genomic mining type III secretion system effectors in *Pseudomonas syringae* yields new picks for all TTSS prospectors. *Trends in Microbiology* **10**, 462–469. doi: 10.1016/S0966-842X(02)02451-4

Cuppels DA (1986) Generation and characterization of Tn*5* insertion mutations in *Pseudomonas syringae* pv. *tomato*. *Applied and Environmental Microbiology* **51**, 323–327.

Devoto A, Muskett PR, Shirasu K (2003) Role of ubiquitination in the regulation of plant defence against pathogens. *Current Opinion in Plant Biology* **6**, 307–311. doi: 10.1016/S1369-5266(03)00060-8

Farmer EE, Almeras E, Krishnamurthy V (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Current Opinion in Plant Biology* **6**, 372–378. doi: 10.1016/S1369-5266(03)00045-1

Feil H, Feil WS, Cain P, Larimer F, DiBartolo G *et al.* (2005) Comparison of the complete genome sequences of *Pseudomonas syringae* pv. *syringae* B728a and pv. *tomato* DC3000. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11064–11069. doi: 10.1073/pnas.0504930102

Feys BJ, Benedetti CE, Penfold CN, Turner JG (1994) *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *The Plant Cell* **6**, 751–759. doi: 10.1105/tpc.6.5.751

Goode MJ, Sasser M (1980) Prevention—the key to controlling bacterial spot and bacterial speck of tomato. *Plant Disease* **64**, 831–834.

Greenberg JT, Vinatzer BA (2003) Identifying type III effectors of plant pathogens and analyzing their interaction with plant cells. *Current Opinion in Microbiology* **6**, 20–28. doi: 10.1016/S1369-5274(02)00004-8

He SY, Jin Q (2003) The Hrp pilus: learning from flagella. *Current Opinion in Microbiology* **6**, 15–19. doi: 10.1016/S1369-5274(02)00007-3

Hirano SS, Upper CD (2000) Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*—a pathogen, ice nucleus, and epiphyte. *Microbiology and Molecular Biology Reviews* **64**, 624–653. doi: 10.1128/MMBR.64.3.624-653.2000

Howe GA, Schilmiller AL (2002) Oxylipin metabolism in response to stress. *Current Opinion in Plant Biology* **5**, 230–236. doi: 10.1016/S1369-5266(02)00250-9

Ichihara A, Shiraishi K, Sato H, Sakamura S, Nishiyama K, Sakai R, Furusaki A, Matsumoto T (1977) The structure of coronatine. *Journal of the American Chemical Society* **99**, 636–637. doi: 10.1021/ja00444a067

Jin Q, Thilmony R, Zwiesler-Vollick J, He SY (2003) Type III protein secretion in *Pseudomonas syringae*. *Microbes and Infection* **5**, 301–310. doi: 10.1016/S1286-4579(03)00032-7

Keith RC, Keith LM, Hernandez-Guzman G, Uppalapati SR, Bender CL (2003) Alginate gene expression by *Pseudomonas syringae* pv. *tomato* DC3000 in host and non-host plants. *Microbiology* **149**, 1127–1138. doi: 10.1099/mic.0.26109-0

Kenyon JS, Turner JG (1992) The stimulation of ethylene synthesis in *Nicotiana tabacum* leaves by the phytotoxin coronatine. *Plant Physiology* **100**, 219–224.

Kloek AP, Verbsky ML, Sharma SB, Schoelz JE, Vogel J, Klessig DF, Kunkel BN (2001) Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (*coi1*) mutation occurs through two distinct mechanisms. *The Plant Journal* **26**, 509–522. doi: 10.1046/j.1365-313x.2001.01050.x

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology* **5**, 325–331. doi: 10.1016/S1369-5266(02)00275-3

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell* **16**, 126–143. doi: 10.1105/tpc.017954

Lund ST, Stall RE, Klee HJ (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. *Plant Cell* **10**, 371–382. doi: 10.1105/tpc.10.3.371

Parry RJ, Mhaskar SV, Lin M-T, Walker AE, Mafoti R (1994) Investigations of the biosynthesis of the phytotoxin coronatine. *Canadian Journal of Chemistry* **72**, 86–99.

Preston GM, Studholme DJ, Caldelari I (2005) Profiling the secretomes of plant pathogenic Proteobacteria. *FEMS Microbiology Reviews* **29**, 331–360. doi: 10.1016/j.femsre.2004.12.004

Puhler A, Arlat M, Becker A, Gottfert M, Morrissey JP, O'Gara F (2004) What can bacterial genome research teach us about bacteria-plant interactions? *Current Opinion in Plant Biology* **7**, 137–147. doi: 10.1016/j.pbi.2004.01.009

Sakai R, Nishiyama K, Ichihara A, Shiraishi K, Sakamura S (1979) The relation between bacterial toxic action and plant growth regulation. In 'Recognition and specificity in plant host-parasite interactions.' (Ed. JM Daly) pp. 165–179. (University Park Press: Baltimore)

Smitley DR, McCarter SM (1982) Spread of *Pseudomonas syringae* pv. *tomato* and role of epiphytic populations and environmental conditions in disease development. *Plant Disease* **66**, 713–717.

Stall RE, Hall CB (1984) Chlorosis and ethylene production in pepper leaves infected by *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* **74**, 373–375.

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *Plant Cell* **16**, 2117–2127. doi: 10.1105/tpc.104.023549

Stuhlfelder C, Mueller MJ, Warzecha H (2004) Cloning and expression of a tomato cDNA encoding a methyl jasmonate cleaving esterase. *European Journal of Biochemistry* **271**, 2976–2983. doi: 10.1111/j.1432-1033.2004.04227.x

Uppalapati SR, Ayoubi P, Weng H, Palmer DA, Mitchell RE, Jones W, Bender CL (2005) The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. *The Plant Journal* **42**, 207–217. doi: 10.1111/j.1365-313X.2005.02366.x

Weber H (2002) Fatty acid-derived signals in plants. *Trends in Plant Science* **7**, 217–224. doi: 10.1016/S1360-1385(02)02250-1

Weiler EW, Kutchan TM, Gorba T, Brodschelm W, Niesel U, Bublitz F (1994) The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. *FEBS Letters* **345**, 9–13. doi: 10.1016/0014-5793(94)00411-0

Whalen M, Innes R, Bent A, Staskawicz B (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis thaliana* and a bacterial gene determining avirulence on both *Arabidopsis* and soybean. *The Plant Cell* **3**, 49–59. doi: 10.1105/tpc.3.1.49

Zhao Y, Damicone JP, Demezas DH, Rangaswamy V, Bender CL (2000) Bacterial leaf spot of leafy crucifers in Oklahoma caused by *Pseudomonas syringae* pv. *maculicola*. *Plant Disease* **84**, 1015–1020.

Zhao Y, Thilmony R, Bender CL, Schaller A, He SY, Howe GA (2003) Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. *The Plant Journal* **36**, 485–499. doi: 10.1046/j.1365-313X.2003.01895.x

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