MONICA HÖFTE AND PAUL DE VOS

PLANT PATHOGENIC *PSEUDOMONAS* SPECIES

Abstract. In the current taxonomy, plant pathogenic *Pseudomonas* species are restricted to rRNA group I organisms belonging to the Gamma subclass of *Proteobacteria*. Currently, about 21 validly described plant pathogenic *Pseudomonas* species are known. The most important species is *P. syringae* with more than 50 described pathovars. The pathovar concept is confusing and the taxonomy of *P. syringae* needs revision. *P. syringae* pv. *tomato* has become an important model organism to study host reactions to pathogen infection, mainly because this pathovar can infect the model plant Arabidopsis. Plant pathogenic Pseudomonads cause important diseases on a variety of crops and symptoms include cankers, leaf and stem spots, blight, soft rot and galls. Important pathogenicity and virulence factors are the type III secretion system, ice nucleation activity, the production of secondary metabolites such as phytotoxins, pectolytic enzymes, exopolysaccharides, and hormone production. Complete genome sequences are available for three important *P. syringae* pathovars. Molecular methods are becoming increasingly important in the diagnosis of plant pathogenic *Pseudomonas* species and specific detection techniques aimed at genes involved in pathogenicity are being developed. No single control strategy is effective against the plant pathogenic *Pseudomonas*. Control should be based on a combination of chemical, biological and cultural strategies.

1. INTRODUCTION

In this overview we will deal with plant pathogenic Pseudomonas species *sensu stricto*, this means organisms that belong to the rRNA group I as described by Palleroni et al. (1973). The often confusing taxonomy of this group of organisms will be discussed together with some information about molecular phylogeny. A brief overview will be given of important pathogenicity and virulence factors, together with some more information about the complete genome sequences that are now available for three important *P. syringae* pathovars. The overview will be concluded with recent information about diagnosis and control.

2. TAXONOMY AND MOLECULAR PHYLOGENY

The nomenclature of bacteria in the genus *Pseudomonas* has changed considerably during the last decennia. The genus *Pseudomonas* is currently restricted to those species related to the type species *Pseudomonas aeruginosa*, i.e. the genuine pseudomonads of the rRNA group I (Palleroni et al., 1973), that belong to the Gamma subclass of the *Proteobacteria* (Kersters et al., 1996). Up to now (2005), the genus *Pseudomonas* comprises 18 validly described plant pathogenic species and 3 species that are pathogenic to mushrooms (Tables 1 and 2). An overview of oxidase positive and oxidase negative plant (and mushroom) pathogenic *Pseudomonas* species is given in Table 1 and Table 2.

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Table 1. Validly published names of oxidase positive plant pathogenic pseudomonads[a]

Name	*Host*	*Disease or symptoms* [b]
Pseudomonas agarici	*Agaricus bisporus*	drippy gill
Pseudomonas asplenii	*Asplenum nidus*	leaf spot and blight
Pseudomonas cichorii	wide host range	leaf and stem spots
Pseudomonas constantinii	*Agaricus bisporus*	brown blotch
Pseudomonas corrugata	tomato	pith necrosis
Pseudomonas fuscovaginae	*Oryzae sativa*	leaf sheath brown rot
Pseudomonas marginalis pv. *alfalfae*	*Medicago sativa*	root browning, stunting
Pseudomonas marginalis pv. *marginalis*	wide host range	marginal leaf necrosis, soft rot brown rot of roots, sof rot
Pseudomonas marginalis pv. *pastinacea*	*Pastinaca sativa*	pith necrosis
Pseudomonas mediterranea	*Lycopersicon esculentum*	weakly pathogenic to rice
Pseudomonas palleroniana		café au lait disease
Pseudomonas salomonii	*Oryzae sativa*	brown blotch
Pseudomonas tolaasii	*Allium sativum* *Agaricus* spp.	

[a] Information based on Young et al. (1996) and Young et al. (2004)
[b] Information mainly based on Bradbury (1986) and Smith et al. (1988)

Among the oxidase positive species, *P. chicorii* has a wide host range and causes necrotic leaf and stem lesions. Pectinolytic *P. fluorescens* strains that can cause soft rot on a wide range of hosts, are commonly named *P. marginalis*. *P. marginalis* strains, however, are phenotypically indistinguishable from saprophytic strains of *P. fluorescens* biovar II. The name *P. marginalis* has commonly been used for soft rot bacteria that resemble *P. fluorescens* biovar2 (= *P. marginalis sensu stricto*) or for all fluorescent oxidase positive soft rot bacteria (= *P. marginalis sensu lato*). Janse et al. (1992), however, have shown that within the group of fluorescent oxidase positive pseudomonads various other bacteria show soft rot activity including most biovars of *P. fluorescens*, and isolates identified as *P. putida*, *P. aureofaciens*, and *P. tolaassii*. Since soft rot activity can be demonstrated in so many diverse fluorescent pseudomonads, Janse et al. (1992) concluded that it no longer makes sense to classify all fluorescent oxidase soft rot bacteria in the artificial species *P. marginalis*. All these bacteria appear to belong to the *P. fluorescens* supercluster (Janse et al., 1992).

P. tolaasii, *P. agaraci* and the recently described species *P. constantinii* (Munsch et al., 2002) are pathogenic on the cultivated mushroom *Agaricus*. Also *P. tolaasii* is taxonomically closely related to *P. fluorescens*. *P. corrugata* and the newly established species *P. mediterranea* (Catara et al., 2002) cause pith necrosis on tomato. *P. asplenii* is the causal agent of bacterial leaf blight of bird' nest firn (*Asplenum nidus*) and shows a high similarity to *P. fuscovaginae*, causing leaf

sheath brown rot on rice (*Oryzae sativa*) and other grasses. Based on SDS-PAGE of whole cell proteins, a possible synonymy between *P. asplenii* and *P. fuscovaginae* was suggested (Vancanneyt et al., 1996b).

Table 2. Validly published names of oxidase negative plant pathogenic pseudomonads[a]

Name	*Host*	*Disease or symptoms* [b]
P. amygdali	*Prunus amygdalus*	bacterial canker
P. avellanae	*Corylus avellana*	bacterial canker
P. cannabina	*Cannabis sativa*	
P. caricapapayae	*Carica papaya*	leaf spot
P. ficuserectae	*Ficus erectae*	leaf spot, shoot blight
P. meliae	*Melia azadarach*	galls
P. savastanoi (various pathovars)	see Table 3	
P. syringae (various pathovars)	see Table 4	
P. tremae	*Trema orientalis*	
P. viridiflava	wide host range	leaf necrosis, necrotic spots, stem and root rots

[a] Information based on Young et al. (1996) and Young et al. (2004)
[b] Information mainly based on Bradbury (1986) and Smith et al. (1988)

Among the oxidase negative species, *Pseudomonas syringae* is economically the most important with more than 50 pathovars (see Table 3). The pathovar concept was introduced in order to distinguish among bacteria within the species that exhibit different pathogenic abilities (Dye et al., 1980). The term "pathovar" is not part of the taxonomic hierarchy and should therefore be eliminated as the primary name of an organism as soon as sufficient data are obtained to justify species and subspecies ranking (Braun-Kiewnick and Sands, 2001). Within *P. syringae*, the most important and best-studied pathovars are *coronafaciens*, *glycinea*, *lachrymans*, *morsprunorum*, *persicae, phaseolicola, pisi, syringae, tabaci* and *tomato*. In recent years, *P. syringae* pv. *tomato* and the closely related pv. *maculicola* have become important model organisms to study molecular mechanisms of host responses to infection, mainly because many strains of these pathovars are pathogenic on the model plant *Arabidopsis thaliana*. Certain strains exhibit race-cultivar specificity on Arabidopsis, thus providing a model pathosystem for studying both compatible and incompatible host-pathogen ineractions (Preston, 2000). *Pseudomonas savastanoi* is an important tumor or gall inducing species on olive, ash and oleander. Currently, various pathovars are distinguished within this species (see Table 4). Schaad et al. (2000), however, have rejected the inclusion of pv. *phaseolicola* and pv. *glycinea* in the species *P. savastanoi* and propose that pv. *phaseolicola* and pv. *glycinea* should remain as pathovars of the species *P. syringae*. The pectinolytic species *P. viridiflava* has a wide host range and causes necrotic leaf and stem lesions and basal stem and root rots. *P. avellana* caused bacterial canker on hazelnut and has been reported in Greece and Italy (Scortichini et al., 2002). *P. cannabina* and *P. tremae* (Gardan et al., 1999) are

newly established species, but they are of little economic importance. Other species of minor importance are *P. amygdali* on almond trees, *P. caricapapaya* causing leaf spot on *Carica papaya; P. ficuserectae* causing leaf spot on *Ficus erectae* and *P. meliae* causing bacterial galls on chinaberry.

Table 3. Validly described pathovars of *Pseudomonas syringae*[a]

Name	*Host*	*Disease or symptoms* [b]
P.s. pv. *aceris*	*Acer macrophyllum*	leaf spot
P.s. pv. *actinidiae*	kiwi fruits	bacterial canker
P.s. pv. *alisalensis*	*Brassica* spp.	bacterial blight
P.s. pv. *aesculi*	*Aesculus indica*	leaf spot
P.s. pv. *antirrhini*	*Antirrhinum majus*	leaf spot, stem lesions
P.s. pv. *apii*	*Apium graveolens*	leaf spot
P.s. pv. *aptata*	*Beta vulgaris*	leaf spot, foliar blight
P.s. pv. *atrofaciens*	cereals	leaf spot, basal glume rot
P.s. pv. *berberidis*	*Berberis* spp.	leaf spot
P.s. pv. *broussonetiae*	*Broussonetia*	bacterial blight
P.s. pv. *castaneae*	*Castanea crenata*	bacterial canker
P.s. pv. *cerasicola*	*Prunus yedoensis*	galls
P.s. pv. *ciccaronei*	*Ceratonia siliqua*	leaf spot
P.s. pv. *coriandricola*	*Coriandrum sativum*	umbel blight, seed decay
P.s. pv. *coronafaciens*	cereals	halo blight
P.s. pv. *cunninghamiae*	*Cunninghamia lanceolate*	
P.s. pv. *daphniphylli*	*Daphniphyllum*	galls
P.s. pv. *delphinii*	*Delphinium* spp.	leaf spot
P.s. pv. *dendropanacis*	*Dendropanax trifidus*	
P.s. pv. *dysoxyli*	*Dysoxylum spectabile*	leaf spot, shot hole
P.s. pv. *eriobotryae*	*Eriobotrya japonica*	bud blight, twig canker
P.s. pv. *garcae*	*Coffea arabica*	halo blight
P.s. pv. *helianthi*	*Helianthus* spp.	leaf spot
P.s. pv. *hibisci*	*Hibiscus japonica*	leaf spot
P.s. pv. *lachrymans*	cucurbits	angular leaf spot
P.s. pv. *lapsa*	maize, sorghum	stalk rot
P.s. pv. *maculicola*	*Brassica* spp.	bacterial spotting
P.s. pv. *mellea*	*Nicotiana tabacum*	Wisconsin tobacco disease
P.s. pv. *mori*	*Morus* spp.	leaf spots, shoot blight
P.s. pv. *morsprunorum*	*Prunus* spp.	leaf spot and stem canker
P.s. pv. *myricae*	*Myricae rubra*	galls
P.s. pv. *oryzae*	*Oryzae sativa*	halo blight
P.s. pv. *papulans*	*Malus pumila, Pyrus*	blister spot, blister canker
P.s. pv. *passiflorae*	*Passiflora edulis*	necrotic spots

P.s. pv. *persicae*	*Prunus persicae*	leaf spots, cankers, dieback
P.s. pv. *philadelphi*	*Philadelphus* spp.	leaf spot
P.s. pv. *photiniae*	*Photinia glabra*	leaf spot and blight
P.s. pv. *pisi*	*Pisum, Vicia*	bacterial blight
P.s. pv. *porri*	*Allium porrum*	bacterial blight
P.s. pv. *primulae*	*Primula* spp.	leaf spot
P.s. pv. *raphiolepidis*	*Rhaphiolepis umbellata*	galls
P.s. pv. *ribicola*	*Ribes aureum*	leaf spot, defoliation
P.s. pv. *sesami*	*Sesamum indicum*	leaf spot
P.s. pv. *solidagae*	*Solidago altissima*	leaf spot
P.s. pv. *spinaceae*	*Spinacea oleracea*	leaf spot
P.s. pv. *striafaciens*	*Avena sativa*, triticale	stripe blight
P.s. pv. *syringae*	very wide	leaf spots, cankers, dieback
P.s. pv. *tabaci*	*Glycine max, Nicotiana*	wildfire, angular leaf spot
P.s. pv. *tagetis*	*Ambrosia, Helianthus, Tagetes*	leaf spot
P.s. pv. *theae*	*Camellia sinensis*	shoot blight, stem blight
P.s. pv. *tomato*	tomato, *Arabidopsis*	bacterial speck, leaf spot
P.s. pv. *ulmi*	*Ulmus* spp.	leaf and shoot blight
P.s. pv. *viburni*	*Viburnum* spp.	leaf and stem spot
P.s. pv. *zizaniae*	*Zizania aquatica*	

[a] Information based on Young et al. (1996) and Young et al. (2004)
[b] Information mainly based on Bradbury (1986) and on original references in Young et al. (1996, 2004)

Table 4. Validly described pathovars of *Pseudomonas savastanoi*[a]

Name	*Host*	*Disease or symptoms* [b]
P.sav. pv. *fraxini*	*Fraxinus excelsior*	galls
P.sav. pv. *glycinea*[c]	*Glycine max*	bacterial blight
P.sav. pv. *nerii*	*Nerium oleander*	galls
P.sav. pv. *phaseolicola*[c]	*Phaseolus, Pisum, Vigna*	halo blight
P.sav. pv. *retacarpa*	*Retama sphaerocarpa*	galls
P.sav. pv. *savastanoi*	*Olea europaea*	galls

[a] Information based on Young et al. (1996) and Young et al. (2004)
[b] Information mainly based on Bradbury (1986) and on original references in Young et al. (1996, 2004)
[c] Inclusion of these pathovars in the species *P. savastanoi* was rejected by Schaad et al. (2000)

Gardan et al. (1999) studied a total of 48 pathovars of *P. syringae* and eight related species by DNA-DNA hybridisation and ribotyping and proposed the

delineation of nine discrete genomospecies (see Table 5). Each genomospecies, which may eventually be regarded as a new species, contains strains exhibiting at least 70% DNA/DNA homology with the relevant type strain(s). As a consequence, several pathovars of *P. syringae* and related species have already been, or will be renamed. Genomospecies 1 corresponds to *P. syringae sensu stricto* and also corresponds to the DNA-DNA group "*syringae*" of Pecknold and Grogan (1973). All but one strain of genomospecies 1 belonged to ribogroup A. It has been suggested that the nine pathovars that are grouped in genomospecies 1 may be synonyms of pv. *syringae*. Genomospecies 2 includes 16 different pathovars of *P. syringae* and type strains of the related species *P. savastanoi*, *P. ficuserectae*, *P. meliae* and *P. amygdali*. According to Gardan et al. (1999) *P. amygdali* should be the correct name for this species. This genomospecies corresponds to DNA group "*morsprunorum*" of Pecknold and Grogan (1973). The 20 strains of genomospecies 2 were distributed within four ribogroups, B to E. Genomospecies 3 includes 14 strains of different pathovars of *P. syringae* that demonstrated relatedness to the pathotype strain of *P. syringae* pv. *tomato*. This genomospecies corresponds to the DNA-DNA group "tomato" of Pecknold and

Table 5. Genomospecies in *P. syringae* pathovars and related species (Gardan et al., 1999)

Genomospecies	*Taxon*
Genomospecies 1	*P. syringae, P.s.* pv. *aptata, P.s.* pv. *lapsa, P.s.* pv. *papulans, P.s.* pv. *pisi, P.s.* pv. *atrofaciens, P.s.* pv. *aceris, P.s.* pv. *panici, P.s.* pv. *dysoxyli, P.s.* pv. *japonica*
Genomospecies 2	*P. savastanoi, P. ficuserectae, P. meliae, P. amygdali, P.s.* pv. *phaseolicola, P. s.* pv. *ulmi, P.s.* pv. *mori, P. s.* pv. *lachrymans, P.s.* pv. *sesami, P.s.* pv. *tabaci, P.s.* pv. *morsprunorum, P.s.* pv. *glycinea, P.s.* pv. *ciccaronei, P. s.* pv. *eriobotryae, P.s.* pv. *mellea, P.s.* pv. *aesculi, P.s.* pv. *hibisci, P.s.* pv. *myricae, P.s.* pv. *photiniae, P.s.* pv. *dendropanacis*
Genomospecies 3	*P. s.* pv. *tomato, P.s.* pv. *persicae, P.s.* pv. *antirrhini, P.s.* pv. *maculicola, P.s.* pv. *viburni, P.s.* pv. *berberidi, P.s.* pv. *apii,P.s.* pv. *delphinii, P.s.* pv. *passiflorae, P.s.* pv. *philadelphi, P.s.* pv. *ribicola, P.s.* pv. *primulae*
Genomospecies 4	*P. coronafaciens, P.s.* pv. *porri, P.s.* pv. *garcae, P.s.* pv. *striafaciens, P.s.* pv. *atropurpurea, P. s.* pv. *oryzae, P. s.* pv. *zizaniae*
Genomospecies 5	*P. tremae*
Genomospecies 6	*P. viridiflava*
Genomospecies 7	*P. s.* pv. *tagetis, P.s.* pv. *helianthi*
Genomospecies 8	*P.s.* pv. *theae, P. avellanae, P. s.* pv. *actinidiae*
Genomospecies 9	*P. cannabina*

Grogan (1973). Gardan et al. (1999) recommended *P. syringae* pv. *tomato* CFBP 2212 as the type strain for this genomospecies. Genomospecies 4 includes the type strain of *P. coronafaciens* and seven strains of different pathovars of *P. syringae*. This genomospecies represents "*coronafaciens*". The strains of genomospecies 4 constituted ribogroup F. Genomospecies 5 includes only the pathotype strain of *P. syringae* pv. *tremae* and comprises the species *P. tremae* sp. nov. as described by Gardan et al. (1999). The single strain in this genomotype represents ribogroup K. Genomospecies 6 includes the type strain of *P. viridiflava* and represents *P. viridiflava*. The 3 strains of the genomospecies tested all belonged to ribogroup J. Genomospecies 7 includes *P. syringae* pv. *tagetis* and *P. syringae* pv. *helianthi*; *P. syringae* pv. *tagetis* strain CFBP 1694 is recommended to serve as the reference strain. Both strains belong to ribogroup G. Genomospecies 8 included the type strain of *P. avellanae* (Janse et al., 1996) and thus represents *P. avellanae*. *P. syringae* pv. *actinidiae* strains were not included in the study of Gardan et al. (1999). *P. syringae* pv. *actinidiae* is the causal agent of bacterial canker of kiwifruit and has been reported in Japan, Italy and South Korea. Scortichini et al. (2002) showed that *P.s*. pv. *actinidiae* is genetically related to *P.s.* pv. *theae* and *P.s*. pv. *avellanae* and proposed to include this pathovar in genomospecies 8. Genomospecies 9 includes only the pathotype strain of *P. syringae* pv. *cannabina* and thus comprises *P. cannabina* sp. nov. as described by Gardan et al. (1999). This strain represented ribogroup L. Genomospecies 3 and 8 could not clearly be distinguished by ribotyping.

Sawada et al. (1999) conducted a phylogenetic analysis of *P. syringae* using 56 strains belonging to 19 pathovars. *gyrB* and *rpoD* were adopted as the index genes to determine the course of bacterial genome evolution, while *hrpL* and *hrpS* were selected as the representatives of the pathogenicity-related genes located on the chromosome. The data on all four genes were used to create a genomic tree that showed three distinct monophyletic groups: Group 1, 2 and 3. Pathovar *tomato*, pv. *morsprunorum*, pv. *syringae*, pv. *actinidiae* and pv. *theae* were located in Group 1; pv. *aceris*, pv. *aptata*, pv. *japonica*, pv. *syringae*, pv. *pisi* were in Group 2; and pv. *myricae*, pv. *eriobotryae*, pv. *morsprunorum*, pv. *tabaci*, pv. *lachrymans*, pv. *castaneae*, pv. *phaseolicola*, pv. *glycinea*, pv. *mori* and pv. *broussonetiae* were in Group 3. Three pathovars, pv. *lachrymans*, pv. *morsprunorum* and pv. *syringae*, were distributed over two groups. The pathotype strains of pv. *lachrymans* and pv *morsprunorum* were included in Group 1, but all other strains of these pathovars belonged to Group 3. For *P. syringae*, only a Japanese citrus strain belonged to Group 1, while all other strains were included in Group 2. Group 1 largely corresponds to genomospecies 3 and genomospecies 8 as defined by Gardan et al. (1999). As mentioned before, Gardan et al. (1999) were unable to differentiate these two groups by ribotyping. Group 2 largely corresponds to genomospecies 1 as defined by Gardan et al. (1999), while Group 3 corresponds to genomospecies 2. Sarkar and Guttman (2004) studied the population structure and dynamics of the core genome of *P. syringae* via multilocus sequencing typing (MLST)

of 60 strains, representing 21 pathovars and 2 nonpathogens, isolated from a variety of plant hosts. MLST is a recently developed strain-typing system that focuses strictly on the core genome. In this approach, the DNA sequences from seven housekeeping genes are used to differentiate strains and clonal lineages. The phylogenetic analysis of *P. syringae* revealed four major groups of strains, three of which largely correspond to those identified by Sawada et al. (1999). The fourth group contained only pathogens of monocots (rice, oats and onions) and includes pathovars that correspond with genomospecies 4 as defined by Gardan et al. (1999). An analysis of molecular variance found that host association explained only a small proportion of the total genetic variation in the sample. With respect to the core genome, *P. syringae* is a highly clonal and stable species that is endemic within plant populations. Sarkar and Guttman (2004) concluded that factors outside of the core genome must be maintaining the cohesion of the species and must play very significant roles in determining host suitability.

3. SYMPTOMS

Plant pathogenic Pseudomonas species can cause a variety of symptoms such as cankers, dieback, blossom, twig, leaf or kernel blight and leaf spots caused by *P. syringae* pathovars; soft or brown rot caused by *P. viridiflava* and pectinolytic *P. fluorescens* strains (*P. marginalis*); tumors or galls caused by *P. savastanoi* and mushroom blights caused by *P. tolaasii* and *P. agarici*. See Tables 1 to 4 for a detailed overview.

4. PATHOGENICITY AND VIRULENCE FACTORS

4.1. TTSS and effectors

The type III protein secretion system (TTSS) is key to the plant parasitism of *P. syringae* pathovars and has been found in all of the *P. syringae* strains examined. The TTSS was first discovered in the mammalian pathogenic bacterium *Yersinia* and has since been found in taxonomically diverse Gram-negative bacterial pathogens of plants and animals and in a few non-pathogenic plant-associated bacteria. Most of the *hrp* (hypersensitive response and pathogenicity) and *hrc* (hrp conserved) genes encoding the TTSS system are essential for pathogenicity, which indicates the collective importance of the effector proteins that are injected into plant cells by the system. The reader is referred to several excellent and detailed reviews about TTSS in bacterial plant pathogens (Alfano and Collmer, 2004; Mudgett, 2005). The TTSS in *Pseudomonas syringae* has recently been reviewed by Jin et al. (2003). Functional analysis of the genome of *P. syringae* pv. *tomato* DC3000 has yielded genes for 31 effectors and 7 additional proteins secreted by the Hrp system that likely have a role in translocating effectors across the plant cell wall and plasma membrane (Buell et al., 2003). Effector genes can be dispersed throughout the genome, clustered in a pathogenicity island located

on a plasmid, or associated with the *hrp* gene cluster (Oguiza and Asensio, 2005). Type III effectors are believed to contribute to pathogenesis in two ways: by eliciting the release of water and/or nutrients from the host cell in the apoplastic space; and by suppressing and/or evading plant host defense responses. The type III effectors produced and secreted by the pathogen interact with plant molecules known as virulence targets. In resistant plants, effectors function as avirulence determinants that activate the hypersensitive response (HR), a primary defense response triggered by recognition of the effector-virulence target complex by plant resistance genes. In susceptible plants, effectors avoid specific recognition by the plant host surveillance mechanisms and function as virulence determinants that facilitate pathogenesis and modulate host defense responses and physiology to the benefit of the pathogen. (Oguiza and Asensio, 2005). Based on their localization in plants, type III effector proteins of *P. syringae* can be grouped into two classes: extracellular type III effectors such as HrpZ and HrpW and intracellular type III effectors that are directly transported from the bacterial cell into the plant cytosol. The extracellular type III effectors are glycine-rich, cysteine-lacking, heat-stable proteins, also called harpins, that elicit a hypersensitive-like response when infected into the intercellular space of plant leaves. It is suggested that HrpZ and HrpW may function either in the release of nutrients from the host cell or possibly as mediators of the translocation process, contributing to the effective delivery of effectors in the host cell cytoplasm. Recent studies have firmly established the concept that the suppression of various plant defenses, including basal defense, gene-for-gene resistance, and nonhost resistance, is a major virulence function of intracellular TTSS effectors. This topic has recently been reviewed by Nomura et al. (2005).

4.2. Phytotoxins

Pseudomonas spp. produce a wide spectrum of phytotoxic compounds. Among the most well-characterized bacterial phytotoxins are those produced by *Pseudomonas syringae* (see Bender et al., 1999 for an extensive review). The toxins produced by *P. syringae* include monocyclic lactam (tabtoxin), sulfodiaminophosphinyl peptide (phaseolotoxin), lipodepsipeptide (syringomcins, syringopeptins) and polyketide (coronatine) structures. Although phytotoxins are not required for pathogenicity in *P. syringae*, they generally function as virulence factors and their production results in increased disease severity. *P. syringae* phytotoxins can contribute to systemic movement of bacteria in planta, lesion size, and multiplication of the pathogen in the host. Tagetitoxin is a cyclic hemithioketal molecule that is only produced by strains of *P. syringae* pv. *tagetis*. The toxin interferes with RNA polymerase in protein biosynthesis of chloroplasts. The toxin can rapidly be detected by its ability to elicit apical chlorosis in plant tissues.

Toxins produced by other *Pseudomonas* species include the lipodepsipeptides corpeptin, fuscopeptin, tolaasin and viscosin produced by *P. corrugata*, *P. fuscovaginae*, *P. tolaasii* and *P. fluorescens* (*marginalis*), respectively.

The best studied phytotoxins are coronatine, syringomycin, tabtoxin and phaseolotoxin.

4.2.1. Coronatine

Coronatine is produced by *P. syringae* pv. *atropurpurea, glycinea, maculicola*, *morsprunorum*, and *tomato* (Bender et al., 1999). The structure of coronatine has two distinct components: the polyketide coronafacic acid and coronamic acid, an ethylcyclopropyl amino acid derived from isoleucine. Coronatine shows a remarkable structural and functional homology to methyl jasmonate, a plant hormone which activates the jasmonate (JA) signalling pathway that is involved in plant defense against herbivores and certain pathogens. It has been shown that coronatine activates JA-signalling responses and suppresses salicylic acid (SA) dependent plant defenses. Inoculation of a local leaf of Arabidopsis with the coronatine producing *P. syringae* pv. *maculicola* induced increased susceptibility to secondary infections in systemic leaves (Cui et al., 2005). The action of coronatine in triggering systemic induced susceptibility is probably mediated by cancelling out SA-dependent defenses in systemic leaves through antagonistic cross-talk between the SA and JA signalling pathways (Cui et al., 2005). Recent evidence suggests, however, that coronatine has the ability to stimulate both SA and jasmonates (Block et al., 2005).

4.2.2. Syringomycin and other lipodepsipeptides

Production of syringomycins has been shown in *P. syringae* pv. *syringae*, pv. *aptata*, pv. *atrofaciens* and *P. fuscovaginae* (Bender et al., 1999). Syringomycin is a member of the cyclic lipodepsinonapeptide class of phytotoxins, which are composed of a polar peptide head and a hydrophobic 3-hydroxy fatty acid tail. The amphipathic syringomycin molecule exhibits potent biosurfactant activity. The surface active properties of syringomycin are similar to those of other biosurfactants produced by fluorescent pseudomonads such as viscosin and tolaasin. All strains of *P. syringae* pv. *syringae* analysed produce both syringomycin and syringopeptin. Both compounds are pore-forming cytotoxins that cause necrosis in plants by similar mechanisms.

4.2.3. Tabtoxin

Tabtoxin is a monocyclic β-lactam produced by *P. syringae* pv. *tabaci*, *coronafaciens*, and *garcae*, which cause wildfire on tobacco, and halo blight of oats and coffee, respectively. *P. syringae* pv. *striafaciens*, the causal agent of

bacterial stripe of oats, is tabtoxin-deficient, but further indistinguishable from *P. syringae* pv. *coronafaciens* and pv. *garcae*. Recent evidence suggests that *P. syringae* pv. *coronafaciens, garcae* and *striafaciens* are likely the same pathovar. Introduction of the tabtoxin biosynthetic region in *P. syringae* pv. *striafaciens* resulted in the production of lesions on oat leaves there were indistinguishable from those caused by *P. syringae* pv. *coronafaciens* (Barta and Willis, 2005). Tabtoxin contains tabtoxin-β-lactam linked by a peptide bond to threonine. The chlorosis-inducing activity occurs only after hydrolysis of the peptide bond by aminopeptidases of plant or bacterial origin. Cleavage of the peptide bond releases tabtoxin-β-lactam, the toxic moiety. Tabtoxin-β-lactam irreversibly inhibits glutamine synthetase. This enzyme is the only way to efficiently detoxify ammonia.

4.2.4. Phaseolotoxin

Phaseolotoxin is produced by *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *actinidiae*, which cause halo blight on beans and bacterial canker on kiwifruit, respectively. Phaseolotoxin consists of a sulfodiaminophosphinyl moiety linked to a tripeptide. Phaseolotoxin competitively inhibits ornithine carbamoyl transferase (OCTase), a critical enzyme in the urea cycle, which converts ornithine and carbamoyl phosphate to citrulline. Phaseolotoxin is hydrolysed in planta by peptidases to produce octicidine. Octicidine is an irreversible inhibitor of OCTase and the predominant form of the toxin in infected tissues. Inhibition of OCTase causes an accumulation of ornithine and a deficiency in intracellular pools of arginine, leading to chlorosis.

4.3. Auxin production

Glickmann et al. (1998) studied auxin production by pathovars of *P. syringae* and related species. Most of the analysed strains produced IAA, especially in the presence of tryptophan. The strains *P. syringae* pv. *syringae* 1392 and *P. syringae* pv. *aceris* 2339 (genomospecies 1); P. *savastanoi* pv. *savastanoi* 1670, *P. syringae* pv. *myricae* 2897 and *P. syringae* pv. *photiniae* 2899 (genomospecies 2); *P. syringae* pv. *maculicola* 1657 and *P. syringae* pv. *ribicola* 10971t (genomospecies 3); and *P. syringae* pv. *cannabina* 2341 (genomospecies 9) synthesized IAA at concentrations over 2 μg/ml when grown in modified King B medium without tryptophan and produced high amounts of IAA in the presence of tryptophan. These strains harbor genes homologous to the *iaaM/iaaH* genes of *P. savastanoi*.

The involvement of IAA in pathogenicity has been unambiguously demonstrated for *P. savastanoi* pv. *savastanoi*. For *P. savastanoi* strains, pathogenicity implies biosynthesis of plant growth regulators. The synthesis of hormones such as cytokinins and indole-3-acetic acid (IAA) leads to the formation of the characteristic knots on olive and oleander. *P. syringae* pv. *amygdali* and

P. syringae pv. *myricae* also induce proliferation of plant tissues and also harbor the *iaaM/iaaH* genes. IAA production has also been associated with epiphytic survival or with toxin production as demonstrated for *P. syringae* pv. *syringae* strains on *Phaseolus vulgaris*. There are also indications that IAA may inhibit plant defense mechanisms (Robinette and Matthysse, 1990).

4.4. Ethylene production

Ethylene production has been demonstrated in various pathovars of *P. syringae*, including pvs. *glycinea, pisi* (Weingart and Volksch, 1997a), *cannabina* and *sesami* (Sato et al., 1997). In addition strains of *P. syringae* pv. *phaseolicola* isolated from kudzu (*Pueraria lobata*) also produce ethylene unlike *P.s*. pv. *phaseolicola* strains isolated from bean (Volksch and Weingart, 1997). Volksch and Weingart (1997) have shown that *P.s.* pv. *phaseolicola* strains from kudzu can be clearly differentiated from strains isolated from bean. They utilize mannitol, produce ethylene, and are strongly pathogenic to kudzu, bean, and soybean. It was suggested by Volksch and Weingart (1997) that the strains from kudzu should be separated from the pathovar *phaseolicola* and should represent their own pathovar. The *efe* gene encoding the ethylene-forming enzyme appears to be plasmid-encoded (Watanabe et al., 1998). The role of ethylene production in virulence of *P. syringae* pvs. *glycinea* and *phaseolicola* was studied. Virulence of *P. syringae* pv. *phaseolicola* was not affected by disruption of the *efe* gene, while *efe* mutants of *P. syringae* pv. *glycinea* were significantly reduced in their ability to grow in planta (Weingart et al., 2001).

4.5. Exopolysaccharides

The production of exopolysaccharide polymers by phytopathogenic bacteria has been implicated in several symptoms, including wilting induced by vascular pathogens and the water soaking associated with foliar pathogens (see Denny, 1995 for a review). *P. syringae* pathovars generally produce two EPS molecules: levan, a fructofuranan polymer, and alginate, a co-polymer of O-acetylated β-1,4-linked D-mannuric acid and L-guluronic acid (Gross and Rudolph, 1987). When grown on media with excess sucrose, many *P. syringae* pathovars produce levan (Hettwer et al., 1998). However, alginate appears to be the major EPS produced in water-soaked lesions (Fett and Dunn, 1989; Rudolph et al., 1989). The alginate biosynthetic gene cluster of *P. syringae* pv. *syringae* FF5 was cloned and characterized (Peñaloza-Vàzquez et al., 1997). The arrangement of the alginate gene cluster in *P. syringae* was virtually identical to that described for the human pathogen *P. aeruginosa*. However, the regulation and signals for transcriptional activation of alginate biosynthesis differed in the two species, presumably because of their adaptation to plant and animal hosts, respectively (Peñaloza-Vàzquez et al., 1997). An alginate deficient mutant of *P. syringae* pv. *syringae* was significantly impaired in its ability to colonize tomato leaves (a non-host) compared

with the wild type strain, indicating that alginate plays a role in epiphytic fitness. The mutant retained the ability to form lesions on bean leaves, but symptoms were less severe and the mutant population was significantly reduced in comparison with the wild type. Apparently, alginate contributes to the virulence of *P. syringae* pv. *syringae*, perhaps by facilitating colonization or dissemination of the bacterium in planta (Yu et al., 1999).

P. syringae pv. *ciccaronei*, which causes leaf spots on carob plants produces a mannan exopolysaccharide. The pure polysaccharide showed phytotoxic effects, i.e., chlorosis and necrosis on tobacco leaves (Corsaro et al., 2001).

4.6. Pectinolytic enzymes

Soft-rotting *Pseudomonas fluorescens* (*marginalis*) strains are capable of degrading pectic components of plant cell walls by producing a wide variety of pectolytic enzymes, including pectin methyl esterase, pectin lyase, polygalacturonase and two pectate lyase isozymes. *P. viridiflava* produces a single pectate lyase (PelV), which has a very alkaline PI, like the major Pel enzyme of *P. fluorescens* (*marginalis*) (Liao et al., 1994). *pel* genes have been cloned from *P. fluorescens* and *P. viridiflava* and DNA sequence analysis has revealed that *P. fluorescens* and *P. viridiflava* Pels are members of the *Erwinia chrysanthemi* PelADE family (Liao et al., 1996). The *P. viridiflava pelV* gene has been mutated revealing it to be essential for soft-rot pathogenesis (Liao et al., 1988). At least some of the *P. syringae* pathovars also produce pectic enzymes and *pel* gene sequences are available in the database for *P. syringae* pv. *lachrymans*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *tabaci*, and *P. syringae* pv. *glycinea*. *P. syringae* pv. *glycinea* produces two alkaline pectate lyase isozymes with pIs of 9.0 and 9.5 and an alkaline polygalacturonase (Magro et al., 1994). *P. syringae* pv. *lachrymans* produces a single pectate lyase enzyme with a pH optimum between 8.0 and 8.5 which is encoded by the *pelS* gene (Bauer and Collmer, 1997).

4.7. Ice nucleation

The ability of bacteria to nucleate supercooled water to form ice is uniquely limited to *P. syringae* and a few other bacterial species including strains of *P. fluorescens* and *P. viridiflava*. Ice nucleation-active (INA) bacteria are usually phyllosphere inhabitants. Ice nucleation has been used as a trait to distinguish strains among some of the *P. syringae* pathovars. For example strains within pv. *syringae* frequently exhibit the ice phenotype, while none of the strains tested thus far within pv. *tomato* or *morsprunorum* are ice nucleation active. The presence of INA bacteria on leaf surfaces can destroy leaf habitats at subzero temperatures. Frost-sensitive plants are injured when ice forms within plant tissues. In the absence of heterogeneous ice nuclei, water associated with leaves will supercool. Supercooling in the temperature range of 0 to about –5°C is primarily limited by

the presence of INA bacteria. Below –5°C, other heterogeneous ice nuclei, probably also limit supercooling. Thus, INA bacteria are responsible for ice formation, and hence injury to plants, mainly in the range from 0 to –5°C (Hirano and Upper, 2000). Within the plant pathogenic pseudomonads, *ice* (or *ina*) genes have been cloned and sequenced from strains of *P. syringae* (Green and Warren, 1985) and *P. fluorescens* (Warren et al., 1986). Recently it was shown that *P. syringae* pv. *syringae* B728a also has an unlinked gene encoding an antifreeze protein (Feit et al., 2005). Antifreeze proteins are secreted into the medium, where they inhibit the growth of external ice by adsorbing onto the ice surface and lowering the temperature at which it can grow. It is possible that the ice nucleation activity of *P. syringae* strains, which are quantitatively quite variable, is modulated by the acivity of such antifreeze proteins.

5. COMPLETE GENOME SEQUENCES

Within the group of plant-associated fluorescent pseudomonads, complete genome sequences are available of the plant commensal *P. fluorescens* Pf-5 (Paulsen et al., 2005), the Arabidopsis and tomato pathogen *P. syringae* pv. *tomato* DC3000 (Buell et al., 2003), the epiphytic pathogen *P. syringae* pv. *syringae* B728a (Feil et al., 2005) and the bean pathogen *P. syringae* pv. *phaseolicola* 1448A (Joardar et al., 2005). As expected, *P. fluorescens* Pf-5 lacks a number of virulence factors found in plant pathogens. There is no evidence in the *P. fluorescens* Pf-5 genome for the biosynthesis of known *P. syringae* phytotoxins or enzymes associated with degradation of plant cell walls and cell wall components. In addition, no evidence was found for a type III protein secretion system, frequently found in bacterial pathogens of animals and plants (Paulsen et al., 2005).

The *P. syringae* pv. *tomato* DC3000 genome is composed of one circular chromosome of 6,397,126 bp and two plasmids of 73,661 bp and 67,473 bp. DC3000 encodes a wide range of proteins that are implicated in virulence. Buell et al. (2003) identified 298 genes (5% of the total) in the virulence category. Functional analysis of the DC3000 genome has yielded genes for 31 effectors and 7 additional proteins secreted by the Hrp system that likely have a role in translocating effectors across the plant cell wall and plasma membrane. Genes for the phytotoxin coronatine are chromosomally encoded in DC3000. There is no evidence in de DC3000 genome for the biosynthesis of any of the known *P. syringae* lipodepsinonapeptide phytotoxins. DC3000 contains two genes *iaaH* and *iaaM* required for IAA production. All of the genes required for alginate biosynthesis in P. aeruginosa are present in DC3000. Three genes encoding levansucrases, required for the biosynthesis of the polysaccharide levan, were also identified in DC3000. Genes encoding cell-wall-degrading enzymes are present in DC3000 and include a pectin lyase, a polygalacturonase, and three enzymes predicted to have cellulolytic activity. DC3000 lacks a gene encoding the outer-membrane ice nucleation protein.

P. *syringae* pv. *syringae* B728a is distinct from DC3000 because it exhibits a very pronounced epiphytic phase on plants, while DC3000 is a poor colonizer of the exterior of plants and may be considered as an "endophyte" (Feil et al., 2005). P. *syringae* pv. *syringae* is composed of one circular chromosome of 6,093,698 bp harboring 5,127 genes. *P. syringae* pv. *syringae* B728a has 27 type III secretion effectors, five of which are not found in DC3000. B728a is known to synthesize two syringopeptins and syringostatin and gene clusters for both phytotoxins as well as a gene encoding an ABC transporter for export of boh metabolites are present in the genome of B728a. P. *syringae* pv. *syringae* strains are also capable of producing a family of peptide derivatives called syringolins. Syringolins have no known impact on the interaction of bacteria with their host plants, but they are recognized by nonhost plants, where these peptides activate defense-related genes and induce resistance to fungal pathogens. Orthologs of the genes participating in biosynthesis and export of syringolin A are present in the B728a genome. B728a has an operon for the biosynthesis of IAA, which includes *iaaM* (Psyr1536) and *iaaH* (Psyr1537).

P. *syringae* pv. *phaseolicola* 1448A encodes 5,353 open reading frames on one circular chromosome (5,928,787 bp) and two plasmids (131,950 and 51,711 bp). Searches of the 1448A genome using the DC3000 virulence ORFs revealed that 81% of the DC3000 virulence ORFs are present in 1448A, including genes for many Hop effectors, secretion pathways I, II and III, and cell wall-degrading enzymes (Joardar et al., 2005).

6. DIAGNOSIS

A combination of using (semi)selective media, biochemical/nutritional, pathogenicity, and genetic tests is recommended for the precise identification of phytopathogenic pseudomonads. The reader is referred to Lopez et al. (2003) and Alvarez et al. (2004) for a general overview about detection and diagnosis of plant pathogenic bacteria and to Braun-Kiewnick and Sands (2001) for a detailed overview of diagnostic techniques useful for plant pathogenic pseudomonads.

Usually, a preliminary identification can be made by the use of semiselective media in combination with disease symptoms and host of origin. A semiselective media useful for the isolation of Pseudomonas is the iron-limiting modified King B medium on which fluorescent pseudomonads produce their characteristic yellow-green pigment pyoverdine. Pathovars of *P. syringae* usually produce less pigment then saprophytic *Pseudomonas* strains. Soft-rotting pseudomonads such as *P. marginalis* and *P. viridiflava* can be identified by pit production on Crystal Violet Pectate medium. In addition, MP medium is a general purpose agar for detecting pectate lyases. Some selective media for specific *P. syringae* pathovars can be found in Braun-Kiewnick & Sands (2001). LOPAT characters (Leliott et al., 1966) are still very useful for species identification within the fluorescent pseudomonads

and include **Levan** production on sucrose medium, **Oxidase** reaction, **Pectolytic** activity on potato slices or pectate gel, **Arginine** dihydrolase activity, and hypersensitive reaction on **Tobacco** leaves. Additional tests can be found in Braun-Kiewnick and Sands (2001). Some characteristics useful for the differentiation of the most important plant pathogenic *Pseudomonas* species can be found in Table 6.

Biolog and Biotype-100 systems can also be used to differentiate *Pseudomonas* species (Grimont et al., 1996). These methods, however, do not allow correct identification at the pathovar level. Other identification methods use a chemotaxonomic approach such as whole-cell fatty acid composition, which is useful for differentiation of major phylogenetic groups (Vancanneyt et al., 1996b) and SDS-PAGE of whole-cell proteins, which yield species-specific protein profiles (Vancanneyt et al., 1996a). These methods, however, do not give differentiation at the pathovar level.

Pathovar identification is more complicated than species identification, since it relies on more tests and host specificity. Braun-Kiewnick and Sands (2001) have listed a series of tests that can be used to distinguish the most important pathovars of *P. syringae* with good accuracy. Identification, however, should always be confirmed by a pathogenicity test. In addition, the use of toxin bioassays as described by Braun-Kiewnick and Sands (2001) can be helpful to differentiate toxin producing pathovars of *P. syringae*. Presumptive pathovar identification can also be based on serological tests by using specific antibodies raised against the lipopolysaccharides of bacterial cell walls. Antibody-based diagnostic kits and reagents are commercially available for various *P. syringae* pathovars including pv. *glycinea*, pv. *lachrymans*, pv. *phaseolicola*, pv. *tomato*, pv. *pisi* and pv. *syringae*.

Molecular techniques are rapidly overtaking serology, enzymology and metabolic analyses for the identification of plant pathogenic bacteria (Louws et al., 1999; Lopez et al., 2003). Most used in taxonomy and detection are the conserved ribosomal genes (Widmer et al., 1998; Miller et al., 2002). Other target sequences are situated in non-coding regions such as the ribosomal spacers, short repetitive regions, insertion sequences or sequences with unknown function such as determined by SCAR. Using rep-PCR primers, several species and/or pathovars can be identified (Louws et al., 1994; Manceau and Horyais, 1997; Weingart and Volksch, 1997b). In some cases plasmid-based sequences are targeted (Takahashi et al., 1996). When genes involved in pathogenicity are known, species or pathovar species sequences can be determined. Several PCR-based detection techniques are based on *hrp* gene sequences and have been developed for pathogens such as *P. syringae* pv *tomato* (Zaccardelli et al., 2005), *P. syringae* pv. *papulans* (Kerkoud et al., 2002), and *P. avellanae* (Loreti & Gallelli, 2002). DNA-analysis of toxin genes can be helpful to differentiate pathovars of *P. syringae*. Some PCR primers that can be used to detect toxin genes are listed in Braun-Kiewnick and Sands

(2001). PCR detection techniques have been developed for pathogens that produce coronatine (Ullrich et al., 1993; Bereswill et al., 1994; Zhao et al., 2002), lipodepsinonapeptides (Sorensen et al., 1998; Bultreys and Gheysen, 1999), phaseolotoxin (Prosen et al., 1993; Schaad et al., 1995), tabtoxin (Lydon and Patterson, 2001) and tagetitoxin (Kong et al., 2004). In some cases, however, non-toxigenic strains that escape detection based on toxin genes, can cause disease as has been reported for *P. syringae* pv. *phaseolicola* (Rico et al., 2003). Production of indole acetic acid (IAA) is useful for identification of the gall producing pseudomonad *P. savastonoi* and can be identified by serological or molecular techniques (Braun-Kiewnick and Sands, 2001; Penyalver et al., 2000).

Table 6. Differentiation of some important plant pathogenic *Pseudomonas* species[d]

	PHB[a]	Levan	oxidase	pectolytic activity	arginine dihydrolase	fluorescent pigment	Growth at 37°C
P. syringae	-	+[b]	-	-	-	+	-
P. savastanoi	-	-	-	V[c]	-	+	+
P. viridiflava	-	-	-	+	-	+	-
P. cichorii	-	-	+	-	-	+	-
P. marginalis	-	+	+	+	+	+	-
P. corrugata	+	+	+	-	-	-	+
P. agarici	-	-	+	-	-	+	-

+, 80% or more strains positive; -, 80% or more strains negative

[a] Poly β hydroxybutyrate

[b] pathovars *delphinii, papulans* and *passiflorae* are negative

[c] between 21 – 79% of strains positive

d information based on Braun-Kiewnick and Sands (2001) and Smith et al. (1988)

6.1. Pyoverdins

A common characteristic of almost all phytopathogenic pseudomonads is the production of Fe(III)-chelating siderophores, called pyoverdins that are fluorescent under UV light and are typically produced on iron-limiting media such as King's Medium B (King et al., 1954). The only exceptions are the species *P. corrugata,* some strains of *P. amygdali*, *P. cannabina*, *P. meliae*, and *P. fuscovaginae*, and strains of the *P. syringae* pathovars *persicae, morsprunorum, sesami,* and *garcae* (Bultreys et al., 2003). Pyoverdins have a quinoline chromophore, responsible for the colour of the molecule that is bound to a peptide chain and to a dicarboxylic acid or to a dicarboxylic amide. Pyoverdins can be useful in systematics and identification because of the variation found in the peptide part of the molecule. Pyoverdins typically contain three iron-binding ligands; one ligand is located in a catechol moiety in the chromophore, while the other two are located in the peptide chain and are hydroxamic acids derived from ornithine or β-hydroxyaspartic acid. Interestingly, more than 40 pyoverdin peptide chain compositions have been identified in the group containing the arginine dihydrolase-positive, saprophytic or

opportunistic animal-pathogenic fluorescent *Pseudomonas* species, but only one composition has been found in the group containing the arginine dihydrolyase-negative phytopathogenic fluorescent *Pseudomonas* species. Arginine dihydrolyase-negative, phytopathogenic, fluorescent Pseudomonas species produce atypical pyoverdins in which two β-hydroxyaspartic acid residues and no derivates of ornithine are involved in iron chelation. Bultreys et al. (2003) have shown that within the group of arginine dihydrolase negative pseudomonads, the oxidase negative species *P. viriflava*, *P. ficuserectae* and the 38 pathovars of *P. syringae* tested, produce the same atypical pyoverdin. The oxidase positive species *P. cichorii* produces a similar atypical pyoverdin that contains a glycine instead of a serine. The more distantly related species *P. asplenii* and *P. fuscovaginae* both produced a less similar atypical pyoverdin. In contrast arginine dihydrolase positive species such as *P. fluorescens*, *P. putida*, *P. chlororaphis*, *P. marginalis* and *P. agarici* produce different typical pyoverdins.

7. CONTROL

7.1. Antibiotics

Antibiotic use in plant agriculture has recently been reviewed by McManus et al. (2002). The use of antibiotics for the treatment of bacterial diseases on plants is modest relative to applications in human and veterinary medicine. Because they are relatively expensive, antibiotics are used primarily on high-value fruit and vegetable crops and ornamental plants. Streptomycin, an aminoglycoside antibiotic, has been the major antibiotic used on plants in the USA. In Europe, streptomycin is either not permitted, only used on an emergency basis, or used regularly, depending on the country. Streptomycin is used to control various pathovars of *Pseudomonas syringae*, which cause fruit-spotting or blossom-blast symptoms on apple, pear and related landscape trees. On tobacco streptomycin is used to control wildfire, caused by *Pseudomonas syringae* pv. *tabaci*. Another *Pseudomonas* pathogen that is targeted is *P. cichorii* on celery, where it causes bacterial blight (McManus et al., 2002). Oxytetracycline, a tetracycline antibiotic and gentamcyin, an aminoglycoside antibiotic, are used to control *Pseudomonas* spp. on several vegetable crops in Latin American countries.

The emergence of streptomycin-resistent plant pathogens has complicated the control of bacterial diseases of plants. Resistance to streptomycine has been reported in *P. cichorii*, *P. syringae* pv. *lachrymans*, *P. syringae* pv. *papulans* and *P. syringae* pv. *syringae* (McManus et al., 2002). Resistance to streptomycin in *Pseudomonas* bacteria is plasmid/transposon determined. The linked *strA-strB* genes that encode streptomycin-inactivating phosphotransferases are located on variants of transposon Tn5393 which are present in *P. syringae* pv. *syringae*. The streptomycin resistance transposon Tn5393a, which carries a *strA-strB* determinant

(Psyr2669-2670) is found in the *P. syringae* pv. *syringae* B728a genome (Feil et al., 2005).

7.2. Copper-based fungicides

Since the use of antibiotics is restricted in most European countries, copper-based fungicides are the only effective compounds available to the farmer to control bacterial plant diseases. Copper-based fungicides such as Bordeaux mixtures are used extensively to control bacterial pathogens on fruit trees such as *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum* on stone fruit trees. The use of copper, however, has several disadvantages. Phytotoxicity can occur and resistance to copper develops rapidly in bacteria. Hwang et al. (2005) have recently shown that most *P. syringae* strains are copper resistant. Copper resistance genes, including the *copABCD* operon and a *copRS* two-component regulatory system are present in the genome of *P. syringae* pv. *syringae* B728a (Feil et al., 2005). These proteins appear to be 92-96% identical to plasmid-encoded CopABCDRS proteins found in other strains of *P. syringae.*

7.3. Plant activators

Plant activators such as 1,2,3-benzothiadiazole (or acibenzolar-S-methyl; also known as Actigard or Bion) and probenazole (Yoshioka et al., 2001; Nakashita et al., 2002; which induce systemic resistance in plants (Sticher et al., 1997; Vallad and Goodman, 2004) can be used to control bacterial leaf pathogens. Louws et al. (2001) have shown that Acibenzolar-S-methyl can be integrated as a viable alternative to copper-based bactericides for field management of bacterial speck, caused by *P. syringae* pv. *tomato*, particularly where copper-resistant populations predominate. Actigard was also used to control P. *syringae* pv. *tabaci* on tobacco in field trials (Cole, 1999).

7.4. Seed treatment

Various pathovars of *P. syringae* are seedborne including *P.s*. pv. *coronafaciens* on cereals, *P.s.* pv. *glycinea* on soybean, *P.s*. pv. *lachrymans* on cucurbits, *P.s*. pv. *maculicola* on brassicas*, P.s*. pv. *phaseolicola* on bean, *P.s*. pv. *pisi* on pea, *P.s*. pv. *porri* on leek, *P.s*. pv. *tabaci* on tobacco, and *P.s.* pv. *tomato* on tomato (Smith et al., 1988). The first consideration in controlling these pathogens is to obtain pathogen-free seed. This can be achieved by seed production in arid regions, seed certification by serological or molecular techniques, chemical treatment of seeds with antibiotics or copper-based compounds or heat treatment of seeds (Kritzman, 1993; Bashan and de Bashan, 2002).

7.5. Biological control

Perhaps the best known example of biological control against plant pathogenic bacteria, including plant pathogenic Pseudomonads, is the use of ice nucleation-deficient deletion mutants of *P. syringae* and *P. fluorescens* to prevent or reduce the growth of frost-forming bacteria on leaves and blossoms (Lindemann and Suslow, 1987; Wilson and Lindow, 1993, Skirvin et al., 2000, Lindow & Brandl, 2003). This research has led to commercial products such as Frostban that can be used on fruit crops, almond, potato, and tomato crops.

There are various examples of plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas* and *Bacillus* spp. (Kloepper et al., 2004) that can control leaf pathogens including pathovars of *P. syringae* via induced systemic resistance (van Loon et al., 1998, Vallad and Goodman, 2004, Meziane et al., 2005). Mixtures of PGPRs, mainly *Bacillus* strains, have been used in field trials to control angular leaf spot caused by *P. syringae* pv. *lachrymans* on cucumber (Raupach and Kloepper, 2000). Induced systemic resistance used in combination with other strategies was effective in controlling bacterial speck on tomato (Bashan and de Bashan, 2002).

Mainly bacterial antagonists, have been tested to control pathovars of *P. syringae* under field conditions. Volksch and May (2001) describe the use of near isogenic or ecologically similar antagonistical strains to target *P. syringae* pv. *glycinea* under field conditions. Strains of *Pantoea agglomerans* suppressed the development of basal kernel blight of barley, caused by *Pseudomonas syringae* pv. *syringae*, Under field conditions, 45 to 74% kernel blight disease reduction was observed (Braun-Kiewnick et al., 2000). A non-pathogenic *P. syringae* strain gave some control in field trials at various locations in the USA and Canada against bacterial speck (Wilson et al., 2002).

7.6. Genetic resistance

Host-specific *P. syringae* pathovars show a typical gene-for-gene interaction with their host and resistance against them is generally mediated by major resistance genes. Breeding programmes and tolerant or resistant host cultivars have been developed for various economic important pathovars of *P. syringae* including pv. *morsprunorum* (Garrett, 1979; Santi et al., 2004), pv. *phaseolicola* (Taylor et al., 1978; Zaiter and Coyne, 1984), pv. *pisi* (Taylor et al., 1989), pv. *tabaci*, pv. *tomato* and pv. *glycinea* (Smith et al.,1988). Resistance genes against *P. syringae* pathovars have been mapped or cloned in tomato (Pedley and Martin, 2003), bean (Ariyarathne et al., 1999), pea (Hunter et al., 2001) and soybean (Ashfield et al., 2003). In addition, various resistance genes against *P. syringae* pathovars have been cloned in Arabidopsis (see Hammond-Kosack and Parker, 2003 for an overview).

7.7. Cultural practices

Free water on susceptible leaves and optimal temperature for bacterial growth are the best combination for promoting disease by plant bacteria. It is possible to reduce bacterial diseases in greenhouses by controlling the environment, by maintaining low relative humidity values using periodic aeration of the greenhouse and drip irrigation, and by holding suboptimal temperatures for pathogen proliferation. In Israel, farmers have been able to reduce damage caused by *P. syringae* pv. *lachrymans* on cucumber and other vegetable crops by using rounded greenhouse structures made of plastic treated with anticondense chemicals. These plastics avoid that water drops fall on leaves (Okon, 1990).

In general disease incidence can be lowered by avoiding excess use of nitrogen fertilizers. Other sensible practices are crop rotation, disinfection of pruning tools and destruction of possibly affected host debris (Okon, 1990; Smith et al., 1988).

7.8. Integrated control

Control of plant pathogenic bacteria is difficult and there is not one strategy that is 100% effective. However, sanitary measures, combined with cultural, chemical and/or biological strategies may lead to satisfactory disease control (Bashan and de Bashan, 2002).

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9. AFFILIATIONS

Monica Höfte, *Lab. Phytopathology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Gent, Belgium. Tel. 0032 9 2646017, e-mail: monica.hofte@ugent.be*

Paul De Vos, *Lab. Microbiology, Department of Biochemistry, Physiology and Microbiology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium. Tel. 0032 9 2645110, e-mail: paul.devos@ugent.be*