MINIREVIEW

TAXONOMY OF *PSEUDOMONAS SYRINGAE*

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SUMMARY

The evolution of the processes of classification, identification and nomenclature of *Pseudomonas syringae* are described. Originally referring to a pathogenic species of lilac, in the revision of bacterial names in 1980, *P. syringae* came to represent more than 40 host specific pathogenic populations, as pathovars. DNA-DNA hybridization studies and recent multilocus sequence analyses (MLSA) indicate a '*P. syringae* complex' that now encompasses up to ten *Pseudomonas* species and 60 pathovars of *P. syringae*. A re-evaluation of the complex using poly-phasic approaches could see the revision of these species and the distribution of pathovars in a number of species or genomospecies. Alternative classifications and nomenclature of these taxa is discussed.

INTRODUCTION

Biological discussion is dependent on reliable systems of names (nomenclature) provided by taxonomic studies. Bacterial taxonomy comprises two interdependent activities; classification and identification. These taxonomic activities cannot be conducted in isolation from one another. Classifications are based on comparative studies of authenticated strains and aim to give expression to relationships as natural hierarchies (Young *et al*., 1992). The outcome of classification is the provision of names (nomenclature) to be applied to identified taxa. One expectation of modern systematics has been that genera and species will be precisely circumscribed and that methods would be available for the allocation of isolates to those groups (Murray *et al*., 1990; Vandamme *et al.,* 1996; Stackebrandt *et al*., 2002; Tindall *et al*., 2010). If taxonomists do not provide means of identification then there is no way to differentiate known from unknown taxa and to expand repositories of authentic strains for further study. Informative classifications that indicate bacterial relationships have been reported but, without giving practical means of identification of taxa, they are impractical because they cannot be applied.

An underlying theme of the history of bacteriology is of the uncertainty that has arisen when the taxonomic elements, classification, identification and nomenclature, have not been in synchrony. The taxonomy of plant pathogenic bacteria was subject to the same confusion as in other fields, and within the genus *Pseudomonas*, a pathogenic population identified for part of its history as the '*P. syringae* complex', has given expression to many of the issues. This discussion briefly describes the history of the *P. syringae* complex to show how the problems were resolved, and to outline the principles that may guide taxonomy in future.

EARLY HISTORY OF THE GENUS *PSEUDOMONAS* AND *P. SYRINGAE*

When it was first proposed by Migula (1894), the genus *Pseudomonas* was understood to comprise all bacteria that were Gram-negative rods, aerobic with chemorganotrophic metabolisms and motile by one or more polar flagella. Only recently was this definition refined largely using comparative analyses of 16S rDNA. The 'fluorescent, poly-β-hydroxybutyrate negative pseudomonads' associated with the type species, *P. aeruginosa*, and including *P. syringae* and related species, are now included in the γ-Proteobacteria. Most 'non-fluorescent, poly-β-hydroxybutyrate positive pseudomonads', *Acidovorax*, *Burkholderia* and *Ralstonia*, are now included in the β-Proteobacteria.

With the proposal of the genus, populations of plant pathogenic bacteria were reported as *Pseudomonas* species, beginning with *P. mori* (Boyer and Lambert, 1893) Stevens 1913, followed by *P. syringae* van Hall 1902. Many plant pathogenic *Pseudomonas* spp. followed thereafter. Early on it was established that, with important exceptions, most pathogenic pseudomonads were specific to limited numbers of host taxa. *P. mori* was then, and still is, considered to be specific to *Morus* spp. By contrast, a small number of pathogens affected more than one unrelated host, most notably *P. syringae*,

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first isolated from lilac (see 'The pathogenic structure of *P. syringae*' below).

Until the 1960s, it was believed that a significant component of the physiology of pathogenic bacteria must be devoted to pathogenic activity (Burkholder and Starr, 1948) and it was further assumed that simple nutritional and cultural differences reflected deep-seated metabolic and genetic differences associated with pathogenicity. Early proposals of pathogenic species were based on small numbers of morphological, biochemical and nutritional tests, and colony appearance on different media, many of which are now known to be highly variable or unstable. This assumption, that specific ecological responses involved large components of cell metabolism was generally assumed by bacteriologists and resulted in a proliferation of species names as synonyms for the same pathogen. It was common for synonyms to be used in different parts of the world without understanding that these referred to the same pathogen and disease.

Origins of the '*P. syringae* complex'. In a study of 15 determinative tests considered to differentiate fluorescent plant pathogenic *Pseudomonas* spp., Lelliott *et al.* (1966) showed that only five tests: production of levan, oxidase activity, capacity to rot potato, production of arginine dihydrolase, and hypersensitivity reaction in tobacco (LOPAT) differentiated five distinct pathogenic species groups. Species that gave negative reactions in the tests for oxidase activity, capacity to rot potato and production of arginine dihydrolase, and positive hypersensitivity reactions in tobacco were identified as LOPAT Group I pathogens. Subsequently, the more extensive study of Sands *et al.* (1970) showed that many named species in LOPAT Group I could not be distinguished phenotypically and that the distinct pathogenic populations could not be differentiated using the biochemical and nutritional tests then available. Preliminary DNA-DNA hybridization studies (Palleroni *et al.*, 1972; Pecknold and Grogan, 1973) indicated a genomic diversity within LOPAT Group 1, but were inadequate as the basis for significant taxonomic conclusions. The idea evolved that there existed a '*P. syringae* complex' represented as a single species comprising distinct populations capable of infecting limited ranges of hosts (Stolp *et al.*, 1965; Doudoroff and Palleroni, 1974).

THE GENERAL REVISION OF BACTERIAL NOMENCLATURE

The realization that the nomenclature of plant pathogenic bacteria was in a confused state only paralleled the situation for bacteria in general. Many species had been named without adequate descriptions in that representative strains could not identified from them, but without the designation or deposition of type or other reference strains, making reinvestigation and revision impossible. It was only when adequate culture collections of authentic strains were made, allowing the systematic comparison of comprehensive numbers of strains of plant pathogenic bacteria under standardized conditions, that it became clear that many species, including many *Pseudomonas* spp. (Stanier *et al.*, 1966), were not differentiated using any of the biochemical and nutritional tests or other methods then available. A survey (Buchanan *et al.*, 1966) found that most bacterial names, of which there were 20,000-30,000, were illegitimate, were synonyms, or for which there was no record of authentic reference strains, making reinvestigation of taxa impossible. Recognition of the enormity of nomenclatural confusion and the lack of regulation of nomenclature led the International Committee on the Systematics of Bacteria (now the International Committee on the Systematics of Prokaryotes) to propose a complete revision of bacterial nomenclature, embodied in the 1976 Revision of the International Code of Nomenclature of Bacteria (the Code; Lapage *et al.*, 1975, now superseded by Lapage *et al.*, 1992 and amendments). This first edition legislated a new start to bacterial nomenclature based upon an inventory of those bacteria published previously that met the criteria of the revised Code, and specified the requirements for the legitimate publication of correct names, discussed in Young (2008). Central to the revision was the development of the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), which were to include only names that conformed fully to the revised Code. When published, 1791 species in 290 genera were recorded (Euzeby, 1997-2010). As a consequence of this revision, names of most important bacterial plant pathogenic species did not conform to the criteria for listing and would not be included as valid names in the Approved Lists, leaving them without standing in nomenclature. This lacuna led directly to the proposal (Young *et al.*, 1978) that specific pathogens be named at infrasubspecific level as pathovars (a level not covered by the Code), to be regulated under the auspices of the International Society for Plant Pathology. This recommendation was accepted and International Standards for Naming Pathovars (the Standards; Dye *et al.*, 1980) were introduced. The origins of pathovar nomenclature and a critique of it are detailed in Young (2008).

***P. syringae* pathovars.** Because most fluorescent plant pathogenic *Pseudomonas* spp. did not satisfy the criteria for recognition as distinct species, all LOPAT group I species were included in a single species, *P. syringae*, as pathovars. The first record (Young *et al.*, 1978) was of 40 pathovars that included *P. mori* and *P. syringae*. Although the host specific species, *P. mori*, took priority over *P. syringae* because it was first pub-

lished, the name, *P. syringae*, with its many hosts, was chosen as the species name because it would allow flexibility in subsequent refinement of classification of the associated diverse and heterogeneous pathogenic populations of that species with less likelihood of confusing interpretations in nomenclature.

Although *P. syringae* originally represented all bacteria circumscribed as group I in the LOPAT determinative scheme, subsequent studies have shown that the '*P. syringae* complex' comprising the pathovars of *P. syringae* must be expanded to include a number of closely related species (Table 1).

THE PATHOGENIC STRUCTURE OF *P. SYRINGAE*

As conceived after 1980, *P. syringae* comprised a population composed of pathogenic strains, of which many sub-populations, as pathovars, had highly restricted host ranges, sometimes apparently confined to a single plant genus, e.g. *P. syringae* pv. *pisi*. Others had host ranges that comprised relatively limited but usually related host taxa. *P. syringae* pv. *phaseolicola*, originally assumed to be specific to *Phaseolus*, has also been proved pathogenic to *Dolichos, Macroptilium, Pueraria, and Vigna* spp. Similarly, strains characterized as *P. syringae* pv. *tabaci*, were isolated from more diverse host plants; *Desmodium, Glycine, Phaseolus* and *Tabacum*. In many cases, earlier claims of wide host ranges were probably due to misidentifications (Bradbury, 1986). The major exception is *P. syringae* pv. *syringae*. The structure of the pathogenic populations of this pathovar is complex and not well understood. Bradbury (1986) reported 16 *Pseudomonas* species as synonyms of the pathovar. With the differentiation of the distinguishable pathovars, all other hosts that had previously been allocated to *P. syringae* were ascribed as hosts of *P. syringae* pv. *syringae* (Bradbury, 1986). The pathogen, *P. syringae* pv. *syringae*, takes its name from the host from which it was first isolated, but strains that have been proved to be pathogenic to lilac also infect more than 44 plant species and there are strains with the same determinative characteristics that do not attack lilac (Young, 1991). These latter may have significantly over-lapping host ranges with the pathogen from lilac and therefore be considered to be members of *P. syringae* pv. *syringae*. The capacity or not to infect a single host that is part of a host complex, even if it is the host after which the pathogen was named, should not justify formal differentiation. Furthermore, there are other strains that do not have the specific determinative characteristics of *P. syringae* pv. *syringae*, are not identified as pathovars to their host plant, and the identity of these is unknown. Perhaps they are peripheral members of *P. syringae* pv. *syringae* or perhaps they represent distinct specific pathovars. N. Parkinson (personal communication), in a comparative analysis of *rpoD* sequences from a comprehensive range of strains from the *P. syringae* complex, found that strains from hosts associated with particular pathovars are distributed widely in the *P. syringae* complex. The significance of this in taxonomic or ecological terms is unclear.

Traditionally, the most reliable method of identification of pathogenic species was by proving specific pathogenicity to a suspect host and then applying the appropriate name. However, this method can fail, especially for pathovars of *P. syringae* because *P. syringae* pv. *syringae* can produce symptoms identical to those of several pathovars. For instance, strains isolated from genera in the Cucurbitaceae and proved pathogenic to their original host have been identified as *P. syringae* pv. *lachrymans* (Bradbury, 1986). However, strains from cucurbit species other than those from *Cucumis sativus* were members of *P. syringae* pv. *syringae*, and *P. syringae* pv. *lachrymans* appears to be specific to this species (unpublished information).

AFTER 1980

Following the introduction of the Approved Lists and the Standards, the classification and naming of plant pathogens in fluorescent *Pseudomonas* spp. proceeded in an orderly way, with the proposal of new species and pathovars (Table 1). Demonstrations that pathogens were members of *P. syringae* followed from identification using LOPAT and a small menu of other tests, and for the most part this approach stood the test of time. Occasionally, a more detailed investigation demonstrated heterogeneity within the complex, as when *P. syringae* pv. *avellanae* was shown to be a distinct species (Janse *et al*., 1996).

Application of new methods. Contemporaneously and coincidentally with development of the new nomenclature, new methods were increasingly applied to bacterial classification. Earlier classifications relied almost entirely on data provided by studies of morphology, metabolic reactions, nutrient utilization, pigment production etc. The development of chemotaxonomic tests, those that compared large components of the phenotype, such as cell wall composition, fatty acid and protein profiling, isoprenoid quinone and polyamine comparisons, as well as comparisons of DNA and RNA composition and sequences, offered the advantage in classification that large components of a phenotype could be compared directly. Such methods played an important role in circumscribing specific bacterial groups including plant pathogenic bacteria (Vandamme *et al*., 1996; Gillis *et al*., 2005). A problem with most chemotaxonomic methods is that they use expensive supporting hard-ware, or they require levels of stan-

Table 1. The '*P. syringae* complex'. Column A. List of names of all *Pseudomonas* spp. and pathovars considered in the '*P. syringae*' complex; Column B. Allocation of these to genomospecies by Gardan *et al.* (1999). Column C. Allocation by Yamamato *et al.* (2000) using concatenated *gyrB* and *rpoD* genes. Pathovars of *P. savastanoi* (*P. sav.*) are listed with *P. syringae* (*P. syr.*).

A. Names	B. Genomospecies		C. *gyrB – rpoD*
P. amygdali	1	*P. syringae*	*P. syringae*
P. avellanae	1	*P. syr.* pv. *syringae*	*P. syr.* pv. *syringae*
P. cannabina	1	*P. syr.* pv. *aptata*	
P. caricapapayae	1	*P. syr.* pv. *atrofaciens*	
P. ficuserectae	1	*P. syr.* pv. *dysoxyli*[1]	
P. meliae	1	*P. syr.* pv. *lapsa*	
P. savastanoi	1	*P. syr. pv. panici*[2]	
P. tremae	1	*P. syr.* pv. *papulans*	
P. viridiflava	1	*P. syr.* pv. *pisi*	
P. syringae	2	*P. amygdali*	*P. amygdali*
P. syr. pv. *aceris*	2	*P. ficuserectae*	*P. ficuserectae*
P. syr. pv. *actinidiae*	2	*P. meliae*	*P. sav. pv. savastanoi*
P. syr. pv. *aesculi*	2	*P. savastanoi*	*P. sav.* pv. *glycinea*
P. syr. pv. *alisalensis*	2	*P. sav. pv. savastanoi*[3]	*P. syr.* pv. *lachrymans*
P. syr. pv. *antirrhini*	2	*P. syr.* pv. *aesculi*	*P. syr.* pv. *morsprunorum*
P. syr. pv. *apii*	2	*P. syr.* pv. *ciccaronei*	*P. sav.* pv. *phaseolicola*
P. syr. pv. *aptata*	2	*P. syr.* pv. *dendropanacis*	
P. syr. pv. *atrofaciens*	2	*P. syr.* pv. *eriobotryae*	
P. syr. pv. *atropurpurea*	2	*P. sav.* pv. *glycinea*[3]	
P. syr. pv. *avii*	2	*P. syr.* pv. *hibisci*	
P. syr. pv. *berberidis*	2	*P. syr.* pv. *lachrymans*	
P. syr. pv. *broussonetiae*	2	*P. syr.* pv. *mellea*	
P. syr. pv. *castaneae*	2	*P. syr.* pv. *mori*	
P. syr. pv. *cerasicola*	2	*P. syr.* pv. *morsprunorum*[4]	
P. syr. pv. *ciccaronei*	2	*P. syr.* pv. *myricae*	
P. syr. pv. *coriandricola*	2	*P. sav.* pv. *phaseolicola*[3]	
P. syr. pv. *coronafaciens*	2	*P. syr.* pv. *photiniae*	
P. syr. pv. *coryli*	2	*P. syr.* pv. *sesami*	
P. syr. pv. *cunninghamiae*	2	*P. syr.* pv. *tabaci*	
P. syr. pv. *daphniphylli*	2	*P. syr.* pv. *ulmi*	
P. syr. pv. *delphinii*	3	*P. syr.* pv. *antirrhini*	*P. syr.* pv. *antirrhini*
P. syr. pv. *dendropanacis*	3	*P. syr.* pv. *apii*	*P. syr.* pv. *maculicola*
P. syr. pv. *dysoxyli* [1]	3	*P. syr.*pv. *avii*[5]	
P. syr. pv. *eriobotryae*	3	*P. syr.* pv. *berberidis*	
P. sav. pv. *fraxini*[3]	3	*P. syr.* pv. *delphinii*	
P. syr. pv. *garcae*	3	*P. syr.* pv. *maculicola*	
P. sav. pv. *glycinea*[3]	3	*P. syr.* pv. *passiflorae*	
P. syr. pv. *helianthi*	3	*P. syr.* pv. *persicae*	
P. syr. pv. *hibisci*	3	*P. syr.* pv. *philadelphi*	
P. syr. pv. *japonica*[6]	3	*P. syr.* pv. *primulae*[7]	
P. syr. pv. *lachrymans*	3	*P. syr.* pv. *ribicola*[8]	
P. syr. pv. *lapsa*	3	*P. syr.* pv. *tomato*	
P. syr. pv. *maculicola*	3	*P. syr.* pv. *viburni*	
P. syr. pv. *mellea*	4	*P. syr.* pv. *atropurpurea*	

P. syr. pv. *mori*	4	*P. syr.* pv. *coronafaciens*	
P. syr. pv. *morsprunorum* [5]	4	*P. syr.* pv. *garcae*	
P. syr. pv. *myricae*	4	*P. syr.* pv. *oryzae*	
P. sav. pv. *nerii*[3]	4	*P. syr.* pv. *porri*	
P. syr. pv. *oryzae*	4	*P. syr.* pv. *striafaciens*	
P. syr. pv. *panici*[2]	4	*P. syr.* pv. *zizaniae*	
P. syr. pv. *papulans*	5	*P. tremae*[9]	
P. syr. pv. *passiflorae*	6	*P. viridiflava*	*P. viridiflava*
P. syr. pv. *persicae*	7	*P. syr.* pv. *tagetis*	
P. sav. pv. *phaseolicola*[3]	7	*P. syr.* pv. *helianthi*	
P. syr. pv. *philadelphi*	8	*P. avellanae*	
P. syr. pv. *photiniae*	8	*P. syr.* pv. *theae*	
P. syr. pv. *pisi*	9	*P. cannabina*[9]	
P. syr. pv. *porri*	–[10]	*P. caricapapayae*	*P. syr.* pv. *coriandricola*
P. syr. pv. *raphiolepidis*	–	*P. syr.* pv. *actinidiae*	
P. syr. pv. *ribicola*	–	*P. syr.* pv. *alisalensis*	
P. syr. pv. *retacarpa*	–	*P. syr.* pv. *broussonetiae*	
P. sav. pv. *savastanoi*[3]	–	*P. syr.* pv. *castaneae*	
P. syr. pv. *sesami*	–	*P. syr.* pv. *cerasicola*	
P. syr. pv. *solidagae*	–	*P. syr.* pv. *coriandricola*	
P. syr. pv. *spinaceae*	–	*P. syr.* pv. *coryli*	
P. syr. pv. *striafaciens*	–	*P. syr.* pv. *cunninghamiae*	
P. syr. pv. *syringae*	–	*P. syr.* pv. *daphniphylli*	
P. syr. pv. *tabaci*	–	*P. sav.* pv. *fraxini*[3]	
P. syr. pv. *tagetis*	–	*P. syr.* pv. *japonica*	
P. syr. pv. *theae*	–	*P. sav.* pv. *nerii*[3]	
P. syr. pv. *tomato*	–	*P. syr.* pv. *raphiolepidis*	
P. syr. pv. *ulmi*	–	*P. syr.* pv. *retacarpa*	
P. syr. pv. *viburni*	–	*P. syr.* pv. *solidagae*	
P. syr. pv. *zizaniae*	–	*P. syr.* pv. *spinaceae*	

[1] The pathotype strain is not an authentic strain of the pathogen of *Dysoxylum*, which is proposed as *Xanthomonas dyei* pv. *dysoxyli* (Young *et al*., 2010).
[2] *P. syringae* pv. *panici* is a doubtful name (Young and Fletcher, 1994).
[3] Based on their earlier DNA-DNA hybridization studies, Gardan *et al*. (1992) proposed the new species, *P. savastanoi*, to which these pathovars were allocated. It is now understood that these proposals did not take account of earlier synonyms, of which *P. amygdali* takes priority (Gardan *et al*., 1999).
[4] The pathotype strain of *P. syringae* pv. *morsprunorum* is known not to be representative of the pathovar (Young *et al*., 1996). Gardan *et al*. (1999) showed that this strain was a member of Genomospecies 3. A proved pathogenic strain (CFBP 2116; ICMP 18416) is a member of Genomospecies 2 and is the proposed neopathotype strain.
[5] *P. syringae* pv. *avii* (Ménard *et al*., 2003).
[6] *P. syringae* pv. *japonica* is a later synonym of *P. syringae* pv. *syringae* (Young, 1992).
[7] Gardan *et al*. (1999) reported that the pathotype strain of *P. syringae* pv. *primulae* is a member of *P. viridiflava* and that a proved pathogenic strain (CFBP 4091; ICMP 18417) is a member of Genomospecies 3.
[8] Gardan *et al*. (1999) reported that the pathotype strain of *P. syringae* pv. *ribicola* is a member of *P. viridiflava* and that a proved pathogenic strain (CFBP 4068; ICMP 3883) is a member of Genomospecies 3.
[9] New species proposed in the study of Gardan *et al*. (1999).
[10] Not investigated by Gardan *et al*. (1999).

dardization that can only be met within a single laboratory and therefore are not portable. This increase in the numbers of such methods in the 1980s posed the need for some coordination if a multiplicity of alternative classifications generated by the diverse methods was to be avoided, especially in relation to novel molecular methods. Wayne *et al*. (1987) gave guidance to the practices associated with the proposal of new species. They proposed a quantitative definition of the bacterial species as the population whose strains share more than 70% DNA-DNA hybridization and have a T_m of less than 5°C. This definition has come to form the standard for species circumscriptions. Wayne *et al*. (1987) also urged that hybridization data be supported by phenotypic data. In proposing this, they anticipated that phenotypic data would support the genomic framework;

that there would be a congruence of systematics using different methods and that this would give expression to phylogenetic relationships. Murray *et al*. (1990) gave strong endorsement to the need for supporting evidence for species proposals based on DNA-DNA reassociation data and this principle has formed the basis of species classification to the present day (Stackebrandt *et al*., 2002; Tindall *et al*., 2010).

DNA-DNA hybridization studies and genomospecies. In a comprehensive investigation of DNA-DNA reassociation in the *P. syringae* complex, Gardan *et al*. (1999) identified nine 'genomospecies' (Table 1). Four of these represented the majority of species and pathovars of the complex. These results have largely been confirmed by sequence studies (see below). Because genomospecies were not supported by their study of ribotyping or comparisons of carbon source utilization using Biotype 100 (BioMerieux), Gardan *et al*. (1999) refrained from making formal species proposals. Had they successfully published formal species proposals on the basis of their genomic data, then further identifications of pseudomonad pathovars might have been paralysed in the way that occurred in *Xanthomonas* following the publication of the revision of Vauterin *et al*. (1995) because of the lack of easy methods of identification. By contrast, pathogens could be allocated to *P. syringae sensu lato* by phenotypic methods and about 13 pathovars have been reported since Gardan *et al*. (1999) (Table 1; Column B).

The genomospecies structure provides important insights into the relationships of the complex. Genomospecies 1 includes *P. syringae* pv. *syringae* and five recognized pathovars; *P. syringae* pv. *aptata, P. syringae* pv. *atrofaciens, P. syringae* pv. *lapsa, P. syringae* pv. *papulans* and *P. syringae* pv. *pisi*. *P. syringae* pv. *aptata, P. syringae* pv. *atrofaciens* and *P. syringae* pv. *lapsa* all of which produce syringomycin, a characteristic found only in *P. syringae* pv. *syringae* in the *P. syringae* complex (Hu *et al*., 1998) and *P. syringae* pv. *aptata* and *P. syringae* pv. *atrofaciens* conform to the determinative tests of *P. syringae* pv. *syringae* (Young and Triggs, 1994). These pathovars may all be synonyms of *P. syringae* pv. *syringae.* If confirmed, then the genomospecies, *P. syringae*, is restricted to *P. syringae* pv. *syringae* comprising strains with wide host ranges and only the pathovars *P. syringae* pv. *papulans* and *P. syringae* pv. *pisi*. Genomospecies 2 includes *P. amygdali, P. ficuserectae* and *P. meliae* and 17 pathovars. If confirmed as an authentic species by further studies, then according to the Code the genomospecies would be named *P. amygdali*, which is the earliest synonym, *P. ficuserectae* and *P. meliae* becoming pathovars of the species. *P. amygdali* pv. *amygdali*, would be named to represent the population pathogenic to almond. Genomospecies 3 contains no named species. Genomospecies 4 is represented mainly by pathovars of graminaceous species, but includes *P. syringae* pv. *porri* from leek, a member of the Liliaceae, and *P. syringae* pv. *garcae*, from coffee, a member of the Rubiaceae. Genomospecies 5, 6, 7, 8 and 9 are represented by small numbers of pathogens, and *rpoD* sequence data (N. Parkinson, personal communication) suggest that some of these may be subject to revision.

Gardan *et al*. (1992) re-established *P. savastanoi* (with the pathovars *savastanoi, glycinea* and *phaseolicola*) on the basis of DNA-DNA reassociation data but subsequently (Gardan *et al*., 1999) reported that *P. amygdali, P. ficuserectae* and *P. meliae* all shared high reassociation values with *P. savastanoi* and were all earlier synonyms, with *P. amygdali* as the earliest synonym. This highlights a common problem; that limited studies that do not take account of all relevant taxa may lead to classifications that are subsequently shown to be inadequate and new names that are confusing.

Polyphasic studies. Perhaps it is remarkable that, for a genus that has been studied in many ecological contexts, there is very little understanding of the basic physiology and metabolism of *Pseudomonas*; most studies have been based on *P. aeruginosa* and *P. fluorescens* (Palleroni, 2005). Several methods have been applied to differentiate *Pseudomonas* spp.; quinone systems, fatty acid, protein, polar lipid or polyamine profiles, but these only give satisfactory results when the species are clearly distinct (Peix *et al*., 2009). The interrelationships of poly-phasic studies were illuminated by a multi-laboratory investigation of the genus *Pseudomonas* reported in *Systematic and Applied Microbiology* (1996). Many species, including plant pathogenic species, were examined by ribotyping (Brosch *et al*., 1996), fatty acid content of whole-cell hydrolysates and phospholipid fractions (Vancanneyt *et al*., 1996a), SDS-PAGE of whole-cell protein (Vancanneyt *et al*., 1996b), westprinting (Tesar *et al.,* 1996), and Biolog and BioMerieux API Biotype-100 systems (Grimont *et al*., 1996). Using DNA-DNA reassociation as the basis for congruence, the notable feature of the accumulated data is the different relationships indicated between species by these different polyphasic methods as discussed in Young (2000). Few of these methods have been used to compare many plant pathogenic *Pseudomonas* spp. A review of studies of fatty acid profiles (Stead, 1990, 1992; Weller *et al*., 2000) showed that they do not correspond to the genomospecies and a recent investigation of the fatty acid profiles of almost all members of the *P. syringae* complex (Table 1) with the genomic groups indicated little or no correspondence between genomospecies and fatty acid groups (J. Elphinstone, personal communication). This failure may be rooted in the character of genomospecies of the *P. syringae* complex (Gardan *et al*., 1999). With few exceptions, they indicate measurable and high reassociation values, usually greater than 30% (Gardan *et*

al., 1999). Such values indicate very high sequence similarities (Young *et al*., 1992) and this may be the reason why no differentiating phenotypic characters can be identified.

IDENTIFICATION OF *P. SYRINGAE* AND PATHOVARS

Immediately after 1980, the identification of plant pathogenic species could still be based on simple phenotypic tests because these formed the basis of species differentiation. With primary reliance for classification being increasingly orientated towards poly-phasic and molecular methods, novel species were proposed for which there were fewer simple or portable methods by which they could be identified, as noted for *Xanthomonas* above. For pathovars of *P. syringae*, the use of determinative tests was shown to be of limited value for the differentiation of pathovars (Young and Triggs, 1994; Palleroni, 2005), most of which could be identified by these means. However, it was usually possible to make successful identifications of isolates because the host is known and therefore identification requires only the differentiation of a few pathogenic species or pathovars (Young, 2000).

PCR primers can offer a reliable method for the confirmation of identity of pathovars. Palacio-Bielsa *et al*. (2009) record a total of 246 papers describing primers for plant pathogenic bacteria. Of these, 30 describe primers for 19 members of the *P. syringae* complex: the species *P. avellanae, P. cannabina* and *P. fuscovaginae,* and the pathovars *actinidiae, alisalense, atropurpurea, coryli, glycinea, maculicola, morsprunorum, papulans, phaseolicola, pisi, savastanoi, sesami, syringae, tagetis, theae,* and *tomato*. Clearly a full inventory of primers for all pathovars is desirable. There is always a need for comprehensive studies to confirm specificity if false positive and false negative results are to be avoided (Bereswill *et al*., 1994).

SEQUENCING

It is unlikely that further comprehensive DNA-DNA reassociation studies of the *P. syringae* complex will be conducted; the expertise required and the time and cost being prohibitive (Vauterin *et al*., 1997; Martens *et al*., 2008). One way forward will be by comparative sequencing analyses using appropriate genes. The criteria for such selection is that they should reflect classifications based on DNA-DNA reassociation in order to maintain continuity of nomenclature (Stackebrandt *et al*., 2002; Tindall *et al*., 2010). Because comparative analyses of genes in general often do not produce concordant relationships, a further criterion should be that genes selected should indicate relationships that correspond to that of the overall genome and it is obvious that consideration of several genes is preferable to reliance on a single gene (Stackebrandt *et al*., 2002). A relatively small number of comparative sequence analyses have been made for the *P. syringae* complex. Those that have are incomplete. A comprehensive comparative analysis of 16S rDNA of *Pseudomonas* (Anzai *et al*., 2000) demonstrated a group comprising *P. amygdali, P. avellanae, P. caricapapayae, P. cichorii, P. ficuserectae, P. meliae, P. savastanoi, P. syringae* and *P. viridiflava.* Because 16S rDNA is so highly conserved, no discrimination within the group was achieved. A study of *Pseudomonas* by Yamamoto *et al*. (2000) using concatenated *gyrB* and *rpoD* sequences demonstrated the grouping of a few species of the *P. syringae* complex in accord with the work of Gardan *et al*. (1999) (Table 1). Recently, N. Parkinson (personal communication) conducted a comprehensive comparative analysis of the *P. syringae* complex based on a partial sequence of *rpoD*. In an ecological study, Sarkar and Guttman (2004) conducted a multilocus sequence analysis (MLSA) involving seven sequences from 21 pathovars. They found that the commonly used genes *gyrB* and *rpoD* were among those that shared least correspondence with the other five genes. Although pathotype strains were not included as reference strains their results supported the four major genomospecies of Gardan *et al*. (1999).

THE FUTURE

The direction of bacterial classification and the application of formal names is unclear. One possibility will see an insistence on the continuation of descriptions based on polyphasic classification (Vandamme *et al*., 1996; Gillis *et al*., 2005) with comprehensive phenotypic descriptions (Tindall *et al*., 2010). As noted, polyphasic classifications do not necessarily give coherent support for species descriptions established using molecular methods. With the increasing focus on molecular studies, it is an open question whether the chemotaxonomic databases necessary for polyphasic support of novel *Pseudomonas* species as closely related as are the members of the *P. syringae* complex are likely to be produced. If this is so, then it is possible that the genomospecies of Gardan *et al*. (1999) or those indicated by sequencing data may never be translated into formal species that meet the criteria outlined above. An alternative approach is that of Lindström and Martínez-Romero (2005) who suggested that the characterization of species could be based on sequence data alone. Such an approach would be contrary to all previous taxonomic intention (Wayne *et al*., 1987; Murray *et al*., 1990; Stackebrandt *et al*., 2002; Gevers *et al*., 2005; Tindall *et al*., 2010) and, whatever its merit, it is unlikely to

be adopted in the foreseeable future.

Regulated genomospecies? Rather than have taxonomic paralysis, an alternative might be to accept the present approach, requiring phenotypic and molecular data as the basis for formal species classifications and nomenclature, but to give greater formal support to the genomovar concept described by Ursing *et al*. (1995). They proposed that, where genomic groups could be delimited by DNA-DNA hybridization, but could not be differentiated by phenotypic means, they might best be referred to as genomovars, if only as a temporary measure. Extending on this proposal, where genomic groups are established by DNA-DNA hybridization or equivalent MLSA, or other by genomic comparisons, then they could be named as genomospecies, with some recognition in the Code. This would provide a nomenclature giving expression to informative genotypic differences until such time as phenotypic characters could be identified that were correlated to the genomic differences. The term, '*Candidatus*', is applied informally at present to unculturable bacteria for which phenotypic descriptions cannot be established and whose circumscriptions are based on molecular methods (Murray *et al*., 1995). It is intended to formulate specific rules in the Code to give clear guidance to their nomenclature (Stackebrandt *et al*., 2002). A similar formulation for 'genomospecies' could usefully provide a bridge in the absence of useful polyphasic descriptions and the development of complete species circumscriptions for formal species proposals. Robust guidance, as for pathovar nomenclature, might be needed for a genomospecies nomenclature for such groups as those indicated in the *P. syringae* complex, in perpetuity.

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