Chapter 15
Siderotyping, a Straightforward Tool to Identify Soil and Plant-Related Pseudomonads

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15.1 Introduction

Siderotyping is a method recently developed to characterize bacterial strains by the siderophore(s) they produce when grown under iron deficiency. First applied to fluorescent pseudomonads and their main siderophores, the pyoverdines, the method was primarily used for the recognition of new molecules among pyoverdines. Because of the huge diversity of molecules encountered among this siderophore family, the method became rapidly a useful prerequisite for starting novel structure investigations. Close to 50 structures have been already established and a total of more than 110 structurally different compounds are presently recognized by siderotyping.

Interest for siderotyping considerably increased when it became evident that all strains belonging to a well defined *Pseudomonas* species produce an identical pyoverdine and, furthermore, that most species are characterized by specific pyoverdines. Therefore, beside their interest as powerful siderophores, pyoverdines are also potent taxonomic markers, opening a new and valuable way for bacterial identification and taxonomy within this major genus.

In the present chapter, the chemical as well as the physiological basis of the siderotyping methodology and details on the different methods used to validly differentiate pyoverdines are presented. Our present knowledge on siderophore diversity among *Pseudomonas*, with a particular focus on pyoverdine diversity among fluorescent *Pseudomonas*, as well as a brief overview on what is known on siderophores of non-fluorescent *Pseudomonas*, is summarized. Moreover, a brief analysis of the taxonomic methods presently in use for an efficient *Pseudomonas* identification and classification are developed for comparison purposes with siderotyping methods. As an example, strain clustering obtained by numerical taxonomy

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C.S. Nautiyal, P. Dion (eds.) *Molecular Mechanisms of Plant and Microbe Coexistence.* Soil Biology 15, DOI: 10.1007/978-3-540-75575-3

and illustrated by a dendrogram of phenotypic distances of 85 type-strains and phytopathogen *Pseudomonas* is compared with clusters reached by siderotyping of the same collection. The numerous advantages of the siderotyping method, but also its limits, will be discussed.

15.2 Soil- and Plant-related Pseudomonads: A World within the Microbial World

The genus *Pseudomonas* is widely distributed in nature. These bacteria rank among the major bacterial population in soil and natural water samples, representing very often 2–10% or more of soil isolates as obtained by colony counting (Janssen 2006), whereas close to half of the natural isolates present in mineral waters are pseudomonads (Guillot and Leclerc 1993). Thanks to not yet fully understood attraction mechanisms (Espinosa-Urgel et al. 2002), *Pseudomonas* are also well distributed in plant root environments, thus contributing to a large proportion of the plant-related microbial population. Moreover, many of them demonstrate properties of biotechnological values: *Pseudomonas* isolates are used as biocontrol agents, able to lower or suppress plant diseases of fungal origin (Lemanceau and Alabouvette 1993) thanks to various mechanisms, among them the production of siderophores (Kloepper et al. 1980a,b) or antibiotics (Haas and Defago 2005). Others are successfully competing with saprophytic fungi at the plant rhizosphere level and, therefore, can be used as stimulating agents resulting in crop yield increase. Indeed, thanks to their high metabolic versatility, many pseudomonads have been successfully used in bioremediation of chemicals like nitrates or pesticides, including the degradation of toxic organic compounds such as carbon tetrachloride (Lee et al. 1999).

Pathogenicity to plants and mushrooms is also a trait of interest of many *Pseudomonas* species: 23 species are presently listed by the Taxonomy Committee of the International Society of Plant Pathology. Moreover, some of these species encompass many pathovars: *P. syringae* for instance includes more than 55 pathovars. A huge host range of plant species is attacked by *Pseudomonas* strains with a great variety of symptoms (necrosis, cankers, tumors, maceration). Thus this genus is considered under temperate climates as the major group of phytopathogenic bacteria. Species of the *Pseudomonas syringae* group are known to be epiphytic bacteria whereas most other phytopathogenic *Pseudomonas*, including *P. corrugata*, *P. marginalis,* and *P. tolaasii*, are soil inhabitants.

The multitude of valuable characteristics of *Pseudomonas* has inspired many studies resulting in the isolation of collections of natural isolates from various environments and including in most cases the characterization and identification of the bacterial isolates of interest. However, although the concomitant use of many different phenotypic and genotypic methods (see below for details), the identification at the species level of the numerous isolates worked out in such studies usually fails dramatically. The general conclusion reached after much effort and investment is at the best that a high genetic polymorphism exists within such collections, but

without being able to specify in detail the different bacterial species causing that diversity. This is particularly frustrating when studies reveal specific sub-populations presenting valuable particular features and which would indeed be of interest to characterize precisely.

Such difficulty in determining species affiliation is due in part to the lack of precision of taxonomical methods presently in use and also to the great diversity encountered among pseudomonads which, moreover, often has no standing in a nomenclatural frame. Although 16 S rDNA sequencing has clarified the taxonomical position of pseudomonads and limited the number of *Pseudomonas* species to those belonging to the DNA-RNA hybridization group I of Palleroni (1984) (Kersters et al. 1996; Anzai et al. 2000), taking out from the *Pseudomonas* sensu lato listing more than 60 species, the number of sensu stricto species presently recognized is still high with 55 species identified as fluorescent *Pseudomonas* and 53 belonging to the non-fluorescent species (personal compilation of the authors). The total number of 108 species should, moreover, increase considerably in the future. Many species delineated at the early times of phenotypic taxonomy, e.g., *P. fluorescens*, *P. putida*, *P. syringae*, and *P. stutzeri*, have since proved to be very heterogeneous at the genomic level: *P. stutzeri* has recently been split into 18 genomospecies based on DNA-DNA hybridization (Sikorski et al. 2005), while the numerous pathovars of *P. syringae* were separated based on the same criteria into 9 genomovars (Gardan et al. 1999). Moreover, it is well established that *P. fluorescens* and *P. putida* are very heterogeneous species at the phenotypic level as suggested by the recognition of 5 biovars (I–V) within the *P. fluorescens* species and three biovars (A–C) within the *P. putida* species (Palleroni 1984), and also at the genomic level (Hilario et al. 2004). According to the siderotyping method described below, 28 strains belonging to the biovar I of the *P. fluorescens* species are dispatched among 10 siderovars (Meyer et al. 2002), while 144 *P. putida* isolates can be divided into 35 siderovars (Meyer et al., in preparation). The general rule being that one siderovar corresponds usually to one species (Meyer et al. 2002), and even if some already published species are now recognized as junior synonyms (Cladera et al. 2006; Lang et al. 2007), we could easily expect the recognition of close to 200 *Pseudomonas* species in the near future. In such a perspective, siderotyping as a simple and powerful method for strain differentiation, identification and grouping, will be of great interest compared to the taxonomic methods presently in use.

15.3 Conventional Tools for Pseudomonad Characterization and Identification

15.3.1 Phenotypic Tools

Conventional bacteriological tests used to characterize *Pseudomonas* strains include cellular morphology and flagella typing, Gram staining, glucose metabolism, presence of cytochrome C oxidase. Other phenotypic characters of particular interest in this genus are:

- *Accumulation of Endocellular Granules of Poly-Beta-Hydroxybutyrate (PHB)*. Strains of *Pseudomonas* sensu stricto do not accumulate PHB, at the opposite of former pseudomonads which were afterward reclassified in other genera. *P. corrugata* was thought to be an exception, but it was later on demonstrated that this species does not accumulate PHB but medium-chain-length poly-hydroxyalkanoates (Kessler and Palleroni 2000).
- *Production of Specific Pigments*. The best known and first studied is pyocyanin, a phenazine blue pigment that gives its typical blue color to the pus produced in some *P. aeruginosa* infections. Pyocyanin production on King's A medium (King et al. 1954) is a key character in identification of *P. aeruginosa*. Since then, other pigments were characterized and are used in species or biovar identification, among them lemonnierin produced by strains of biovar IV of *P. fluorescens* (Starr et al. 1967) and chlororaphine produced by *P. chlororaphis* (Breed et al. 1957). The most common studied pigment remains pyoverdine, the green fluorescent siderophore produced by fluorescent *Pseudomonas* grown under iron-deficiency, usually detected thanks to the King's B medium (King et al. 1954; Meyer 2000).

To identify plant pathogenic *Pseudomonas*, bacteriologists rely on a combination of five phenotypic tests proposed by Lelliott et al. (1966). This identification key is called LOPAT for *L*evane production from sucrose, presence of cytochrome C *O*xidase, *P*ectinase, *A*rginine dihydrolase and hypersensitive reaction on *T*obacco leaves. It allows one to define five groups: groups I and II correspond to oxidase negative phytopathogenic species (*P. syringae* and related species, and *P. viridiflava*, respectively), groups III and IV to oxidase positive phytopathogenic species (*P. cichorii* and *P. marginalis*, respectively) and group V corresponds to *P. fluorescens* and other saprophytic strains. This determinative key is still very useful but is insufficient and suffers of the failings that characterize identification schemes based on few characters. To identify strains at the pathovar level, phenotypic tests are completed by pathogenicity tests in order to determine host range and symptoms.

Extensive phenotypic studies including 146 nutritional tests (Stanier et al. 1966) demonstrated the extreme nutritional versatility of pseudomonads and allowed their differentiation at the species level with the recognition of biovars for the most heterogeneous *P. fluorescens* and *P. putida* species. Numerical analysis of these data by Sneath et al. (1981) confirmed the discriminative capacity of this approach. Since then, auxanograms were miniaturized and different commercial kits are now available. Assimilation of carbon compounds of three different chemical families could be studied with strips commercialized by BioMérieux: API 50CH (carbohydrates), API 50AA (amino acids) and API 50AO (organic acids). These API systems were replaced by Biotype 100 strips (BioMérieux) which is designed to test carbon assimilation from 99 different sources. The Biolog GN MicroPlate System (Biolog Inc.) allows one to test oxidation of 95 substrates. These kits are very useful for numerical taxonomic analysis and have been used in polyphasic approach to identify discriminative characters for the

description of new species (Grimont et al. 1996; Gardan et al. 2002). Analysis of fatty acid methyl esters of whole cells by high resolution gas chromatography is used in the Microbial Identification System (MIDI, Microbial ID Inc.). The main disadvantage of these kits, beside their cost, is the maintenance of up-to-date databases. Therefore, while descriptions of new species increase, identification scores may decrease.

Methods used for epidemiological purposes involve serotyping, production and sensibility to phages, antibiograms, whole cell protein fingerprints. Most of the time, such methods have been used to characterize species of clinical interest like *P. aeruginosa* (Palleroni 2005).

15.3.2 Genotypic Tools

15.3.2.1 DNA-DNA Hybridization

Since 1987, DNA-DNA hybridization has been the reference method for species delineation (Wayne et al. 1987). First results evidenced very low genomic relatedness within pseudomonads, a result confirmed by rRNA-DNA hybridization (Palleroni et al. 1973) and subsequent affiliation of several species to different classes of *Proteobacteria* (Anzai et al. 2000). DNA-DNA hybridization also showed that some historic species were constituted of several genomospecies (see above). Nowadays, this method – which consists of pair wise comparison of whole genome to determine DNA relatedness – is systematically used in polyphasic taxonomic studies to define new species in the genus. Such defined species represent groups of strains sharing more than 70% of DNA-DNA homology, clearly separated from neighbouring species by lower values. DNA-DNA hybridization, which is cumbersome and time consuming, is not per se an identification tool, but it allows one to delineate species as genetically homogeneous groups for which molecular identification tools can easily be defined.

15.3.2.2 Sequencing of Conserved Genes

Based on *rrs* gene sequences, phylogenetic relationships between *Pseudomonas* species were elucidated (Moore et al. 1996; Anzai et al. 2000). Sequencing of *rrs* gene and comparison with international databases is a convenient way to achieve isolate identification. However, effective identity of *rrs* sequence is not necessarily a sufficient criterion to guarantee species identity because of the highly conservative nature of the *rrs* gene. To overcome this problem and to refine phylogenies, less conserved housekeeping genes were studied. Yamamoto et al. (2000) sequenced *gyrB* and *rpoD* genes. Discrepancies with *rrs* phylogeny were evidenced but the resolution level was correlated with DNA-DNA hybridization data. Recently, Ait Tayeb et al. (2005) showed that the resolution power of *rpoB* tree was three times higher than those of

rrs and that partial sequence of *rpoB* was a good identification marker. There is no doubt that multilocus sequence analysis of housekeeping genes will play a major role in future taxonomic studies and identification schemes, as already shown in a recent study involving 10 housekeeping genes (Frapolli et al. 2007).

15.3.2.3 DNA Fingerprinting

Taxonomic studies within the *Pseudomonas* genus took advantages of the numerous molecular methods developed to investigate genomic polymorphism. Methods based on PCR amplification of whole genome used random priming (RAPD) or primers in repetitive elements (rep-PCR). Others used restriction of the genome (AFLP, PFGE, ribotyping). Among these, ribotyping was developed as an identification tool, with commercialization of an automate (Riboprinter, Qualicon) and development of a database called Taxotron by the Institut Pasteur (Paris, France). Other DNA fingerprinting methods were mainly used to investigate genetic diversity at sub-specific level (Louws et al. 1994; Clerc et al. 1998). In conclusion, characterization and identification of *Pseudomonas* species followed the general evolution of methods used for bacterial identification. Despite the splitting of *Pseudomonas* sensu lato and restriction of *Pseudomonas* genus to species belonging to the DNA-RNA hybridization group I, this genus remains extremely heterogeneous. Identification of species – the majority of which are saprophytic – has for a long time been an awkward task and limits of classical tests for diagnosis were reached. Extensive improvement of characterization and identification of pseudomonads was achieved with the development of molecular methods based on genotypic data like *rrs* gene sequencing. However, at present, there is still a need for routine techniques that allow one to resolve easily the *Pseudomonas* diversity at the species level.

15.4 Siderotyping, or How to Identify Pseudomonads Through a Unique Phenotypic Character

One phenotypic character easy to observe, and quite important since it concerns about half of the *Pseudomonas* sensu stricto species, is the production of pyoverdine, the water-soluble yellow-green and fluorescent pigment previously called fluorescein, produced by the so-called fluorescent *Pseudomonas* (Elliot 1958). This pigment is observable when growing the bacteria on King's B medium where its production results in the appearance of a bright fluorescence in the medium (King et al. 1954). Only a few other genera, e.g., *Azotobacter* spp. or *Azospirillum* spp., are able to produce similar compounds. However, very specific features like nitrogen fixation allow an easy discrimination of these bacteria from the fluorescent pseudomonads.

Pyoverdines are chromopeptides made of a quinolein-based chromophore conferring to the molecule its color and bright fluorescence and, branched to it, a small peptide as well as a dicarboxylic acid side chain (see Fig. 15.1). So far, 50 different

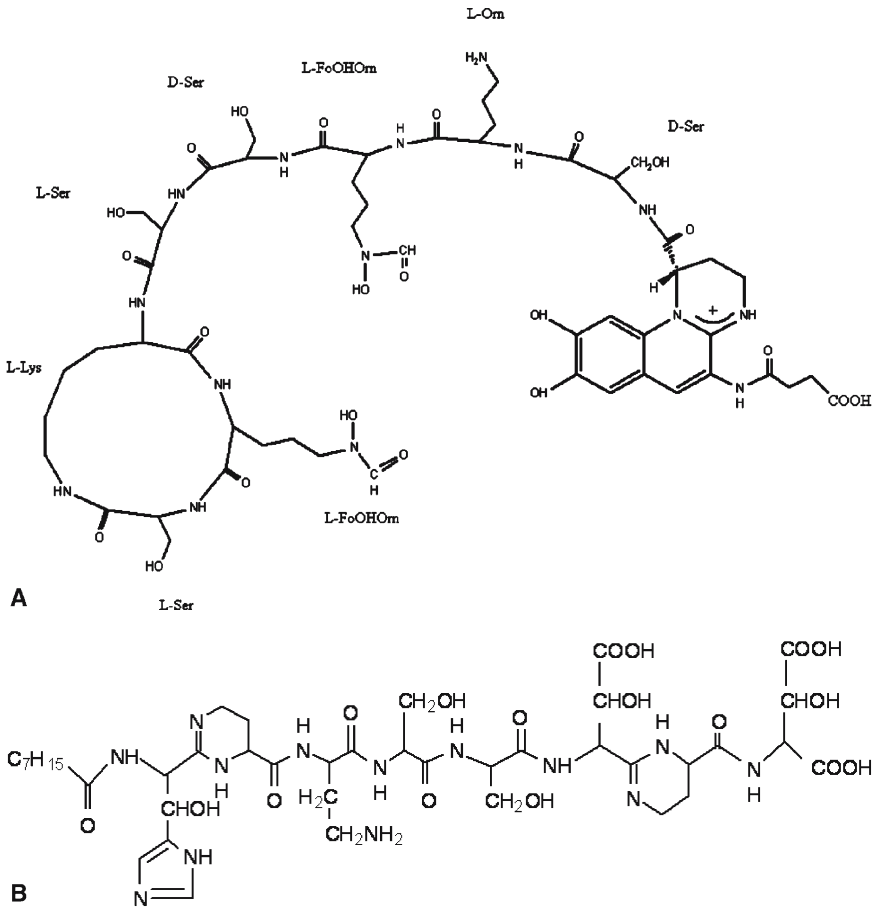

Fig. 15.1 Structures of *Pseudomonas* siderophores. **A** Structure of a pyoverdine. **B** Structure of corrugatin

pyoverdines have been structurally determined. That huge diversity results mainly from variations at the peptide chain level which vary by the number and composition of the aminoacyl residues (for details the reader is referred to the recent exhaustive compilation of Budzikiewicz 2004). It is important to note that pyoverdine structural features are strain specific, which means that one iron-starved strain, whatever the other growth conditions, always produces pyoverdine molecules with the same peptide chain (a very few exceptions have been claimed in the literature; see Budzikiewicz 2004). However, different pyoverdine molecules can be found in a culture supernatant, differing by the nature of the carboxylic acid side chain (succinic or malic acid or their amides, α-ketoglutaric or fumaric acid, for the most frequent ones). Some of these so-called pyoverdine isoforms are produced by iron-starved cells, some others, however, are resulting from chemical modifications

occurring during growth, e.g., the formation of the succinyl isoform resulting from the hydrolysis of the biosynthetised succinamide isoform.

Thus, applying a method which could separate the different isoforms like isoelectrophoresis (electrophoresis in presence of ampholins which determine a pH gradient in the gel), each fluorescent *Pseudomonas* strain producing a particular pyoverdine could be identified through its pyoverdine-isoelectrofocusing (PVD-IEF) pattern, depending on the amino acid content of its pyoverdine and also depending on the number of pyoverdine isoforms present in its culture supernatant. An example is given in Fig. 15.2 which illustrates the PVD-IEF patterns obtained with *P. salomonii, P. palleroniana, P. tolaasii, P. costantinii, P. fuscovaginae* and *P. syringae*.

The experimental procedure to reach such patterns is very simple: 1 mL of a 24-h culture at 25 °C in CAA medium (a Casamino acid-based medium with low iron content, (see Meyer et al. 2002 for detailed formula) is centrifuged and 400 µL of the clear supernatant are lyophilized. The dry residue is dissolved in 20 µL of water and 1 µL solution is deposited on the ampholin-containing polyacrylamide gel for the mini-IEF isoelectrophoresis procedure developed according to the manufacturer recommendations (Biorad). The natural fluorescence of pyoverdines under UV light (350 nm) is used for the revelation of the bands. As experimented in our laboratory, one person using two Mini-IEF gel apparatus can run up to 10 gels, thus determining the PVD-IEF patterns of 140 strains within a day.

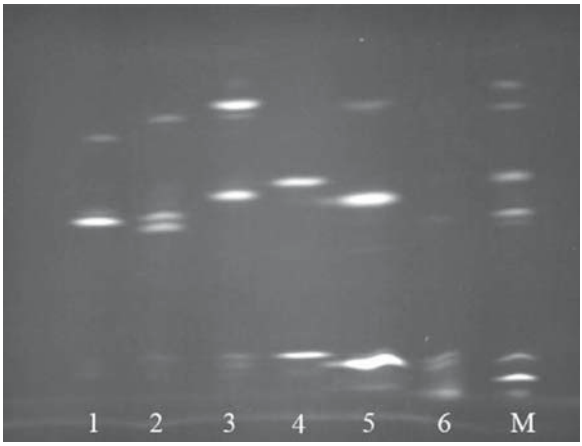

Fig. 15.2 Isoelectrophoretic patterns of some fluorescent *Pseudomonas* pyoverdines. Lane 1, *Pseudomonas salomonii* CFBP 2022^T; lane 2, *Pseudomonas palleroniana* CFBP 4389^T; lane 3, *Pseudomonas tolaasii* CFBP 2068^T; lane 4, *Pseudomonas costantinii* CFBP 5705^T; lane 5, *Pseudomonas fuscovaginae* CFBP 2065^T; lane 6, *Pseudomonas syringae* CFBP 1392^T; lane M corresponds to standard pyoverdine markers for pHi measurements (see Meyer et al. 2002). Abbreviation: CFBP, Collection Française de Bactéries Phytopathogènes (INRA-Angers, France)

The next step of the IEF-siderotyping procedure is then to group strains presenting the same PVD-IEF pattern as seen by visual comparison. A control is done by co-migrating on a same gel pyoverdines of a same IEF group. A second control is then reached by using another siderotyping procedure, based on the usually high specificity of recognition between ferri-pyoverdines and their respective outer membrane receptors (Hohnadel and Meyer 1988). It is thus controlled that each strain of the IEF group is able to use as iron transporter any of the pyoverdines produced by strains belonging to the same group. Although some pyoverdines have been seen to share very closely related, if not identical, IEF patterns, this second control is usually very discriminative and has allowed so far the discrimination of more than 110 different pyoverdines characterizing as many siderovars, i.e., groups of strains sharing an identical pyoverdine.

One major conclusion reached by siderotyping is that pyoverdine molecules could be used as powerful taxonomic markers. It effectively became evident, once well polyphasic defined species were available to siderotyping analysis, that, as a general rule, strains belonging to one species produce an identical pyoverdine while strains belonging to different species produce structurally different pyoverdines. This has been well established for several well-circumscribed species of fluorescent *Pseudomonas*, i.e., *Pseudomonas monteilii*, *Pseudomonas rhodesiae*, *Pseudomonas mandelii*, *Pseudomonas veronii*, *Pseudomonas tolaasii*, and *Pseudomonas syringae* (Meyer et al. 2002). Moreover, the method successfully contributed to the definition of recently described new species, namely *Pseudomonas brassicacearum* and *Pseudomonas thivervalensis* (Achouak et al. 2000), *Pseudomonas lini* (Delorme et al. 2002), *Pseudomonas mosselii* (Dabboussi et al. 2002), *Pseudomonas salomonii* and *Pseudomonas palleroniana* (Gardan et al. 2002), *Pseudomonas costantinii* (Munsch et al. 2002), and *Pseudomonas lurida* (Behrendt et al. 2007). Furthermore, the assignation, as postulated by siderotyping, of a phenotypic cluster to a given species, was positively verified by DNA-DNA-hybridization (Meyer et al. 2002), proving that the method was particularly efficient for the detection of potential new species. Interestingly, the method was successfully extended to the non-fluorescent species *Pseudomonas corrugata*, *Pseudomonas fredericksbergensis*, *Pseudomonas graminis* and *Pseudomonas plecoglossicida*. Such bacteria do not synthesize pyoverdines as siderophores but other structurally different compounds sharing in common the ability to tightly bind iron(III) and to transport it into the cells thanks to specific outer membrane receptors. These siderophores and their respective iron transport systems are still unknown for a majority of them. Of the four species cited above, only corrugatin, the siderophore of *P. corrugata*, has been identified at the structure level (Risse et al. 1998; Fig. 15.1). The three others, each defined by a specific pHi value (Meyer et al. 2002), remain to be characterized, as well as the ferri-siderophore receptors of the four species. The IEF-analysis procedure for siderophores produced by such non-fluorescent pseudomonads is identical to the one described above for pyoverdines, except that the revelation of siderophores is done by the CAS-overlay method as described by Koedam et al. (1994).

15.5 An Application Within Plant-Pathogen Pseudomonads: Correlation Between Siderotyping and Numerical Taxonomy

A collection of 85 phytopathogenic *Pseudomonas* strains were analyzed through siderotyping and numerical taxonomy. The resulting groups reached by the two methods are compared in Fig. 15.3 as illustrated by PVD-IEF patterns and by a

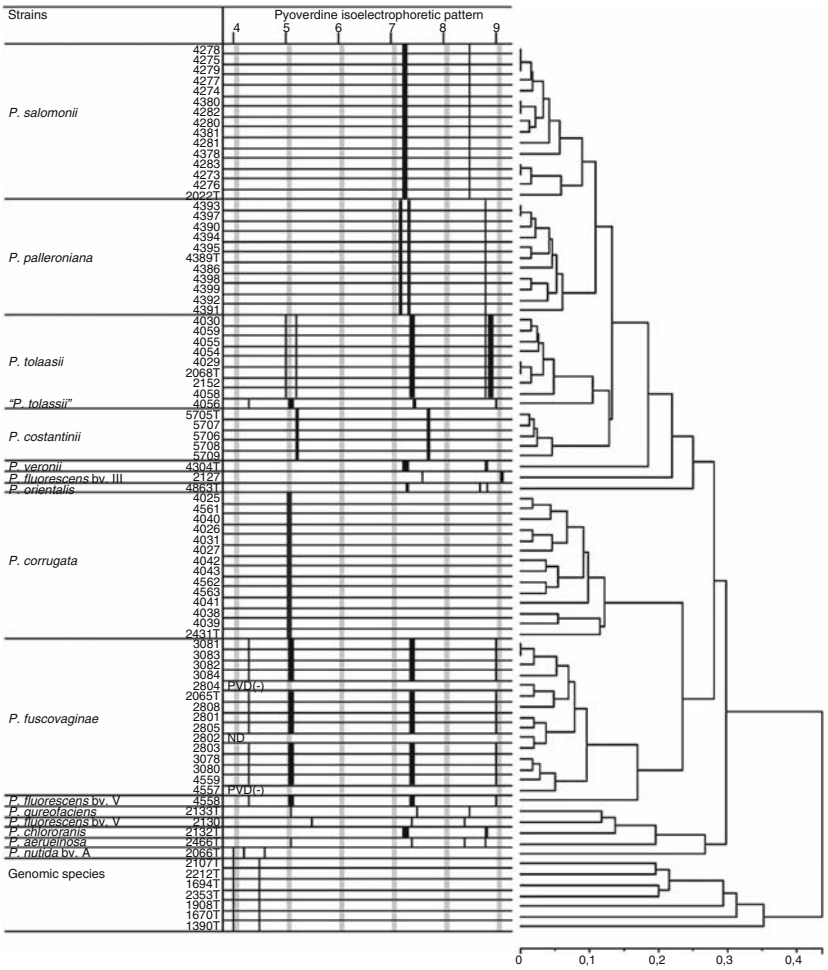

Fig. 15.3 Correlation between siderotyping and numerical taxonomy. The pyoverdine-isoelectrophoretic patterns of 85 *Pseudomonas* strains are shown in the *middle panel* by *bars* representing the different pyoverdine isoforms found in the respective CAA culture supernatants. The *thickness of the bars* reflects the intensity of fluorescence of the pyoverdine-isoform bands, as visualized under UV light at 350 nm after electrophoresis. Phenotypic dendrogram is depicted in the *right panel*. For details on pyoverdine isoelectrophoresis and on the construction of the dendrogram, see Meyer et al. (2002) and Gardan et al. (2002), respectively

dendrogram representing phenotypic distances between strains. Six phenotypic clusters can easily be distinguished at a phenotypic distance of 0.11 in the dendrogram, corresponding to the species *P. salomonii*, *P. palleroniana*, *P. tolaasii*, *P. costantinii* and *P. fuscovaginae*. It is evident that these groups match perfectly with the grouping reached by siderotyping. This conclusion is also valid for the non-fluorescent *P. corrugata* species. Some discrepancies, however, concerns *P. syringae* strains which were characterized by a unique PVD-IEF pattern while demonstrating some phenotypic heterogeneity (phenotypic distance of 0.35) which is in agreement with the genomovar multiplicity found in this group (Gardan et al. 1999). It is also evident that unclustered strains with high phenotypic distances, e.g., strains identified in Fig. 15.3 as *P. veronii, P. fluorescens* bv. III*, P. orientalis*, *P. fluorescens* bv. V*, P. aureofaciens, P. chlororaphis, P. putida* bv. A and *P. aeruginosa*, are also characterized by as many specific PVD-IEF patterns, thus confirming the strong correlation between siderovars and phenotypic clusters. Interestingly, among the *P. tolaasii* isolates, strain CFBP 4056 demonstrated a surprisingly high phenotypic distance when compared to the other strains of the group. According to its PVD-IEF pattern, it is evident that the strain is related to the *P. fuscovaginae* siderotype, suggesting that its present taxonomic position as a *P. tolaasii* isolate needs to be revised.

A difficulty encountered in siderotyping concerns isolates that are deficient in siderophore production and which cannot be characterized, indeed, by a siderophore-IEF profile. This problem can be overcome by developing siderophore-mediated (^{59}Fe) iron uptake experiments, as was done for the pyoverdine-deficient *P. fuscovaginae* strains. These strains were grouped within the *P. fuscovaginae* siderovar based on their capacity to specifically use the *P. fuscovaginae* pyoverdine as iron transporter. This grouping was consistent with the dendrogram which shows that these strains are closely related within the species.

15.6 Conclusions

By its simplicity and rapidity of execution, siderotyping is a particularly promising method for the characterization and identification at the species level of fluorescent and non-fluorescent *Pseudomonas*. As illustrated here, the method could advantageously replace a numerical analysis of phenotypic data, thus saving time and money while results obtained by the two methods are in most cases in full agreement.

We are presently developing the use of the method in studies relevant to different ecological topics which are usually investigated through conventional methods such as the study of survival capacity of biocontrol strains (Molina et al. 1998; Nautiyal et al. 2002), the search and characterization of strains with specific biological features (Landa et al. 2002), the influence of soil factors and agricultural practices on the diversity and distribution of pseudomonads in soils (Lemanceau et al. 1995; Frey et al. 1997; Aagot et al. 2001; Kwon et al. 2005), or the selection of specific pseudomonad populations (Ross et al. 2000; Founoune et al. 2002).

References

Aagot N, Nybroe O, Nielsen P, Johnsen K (2001) An altered *Pseudomonas* diversity is recovered from soil by using nutrient-poor *Pseudomonas*-selective soil extract media. Appl Environ Microbiol 67:5233–5239

Achouak W, Sutra L , Heulin T, Meyer JM, Fromin N, Degreave S, Christen R, Gardan L (2000) Description of *Pseudomonas brassicacearum* sp. nov. and *Pseudomonas thivervalensis* sp. nov., root-associated bacteria isolated from *Arabidopsis thaliana* and *Brassica napus*. Int J Syst Evol Microbiol 50:9–18

Ait Tayeb L, Ageron E, Grimont F, Grimont PAD (2005) Molecular phylogeny of the genus *Pseudomonas* based on *rpoB* sequences and application for the identification of isolates. Res Microbiol 156:763–773

Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H (2000) Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. Int J Syst Evol Microbiol 50:1563–1589

Behrendt U, Ulrich A, Schumann P, Meyer JM, Spröer C (2007) *Pseudomonas lurida* sp. nov., a fluorescent species associated with the phyllosphere of grasses. Int J Syst Evol Microbiol 57:979–985

Breed RS, Murray EGD, Smith NR (1957) Bergey's manual of determinative bacteriology, 7th edn. The Williams and Wilkins Co. Baltimore, p 103

Budzikiewicz H (2004) Siderophores of the Pseudomonadaceae sensu stricto (fluorescent and non-fluorescent *Pseudomonas* spp.) Prog Chem Org Nat Prod 87:81–235

Cladera AM, Garcia-Valdes E, Lalucat J (2006) Genotype versus phenotype in the circumscription of bacterial species: the case of *Pseudomonas stutzeri* and *Pseudomonas chloritidismutans*. Arch Microbiol 184:353–361

Clerc A, Manceau C, Nesme X (1998) Comparison of randomly amplified polymorphic DNA with amplified fragment length polymorphism to assess genetic diversity and genetic relatedness within genospecies III of *Pseudomonas syringae*. Appl Environ Microbiol 64:1180–1187

Dabboussi F, Hamzé M, Singer E, Geoffroy V, Meyer JM, Izard D (2002) *Pseudomonas mosselii* sp. nov., a new species isolated from clinical specimens. Int J Syst Evol Microbiol 52:363–376

Delorme S, Lemanceau P, Christen R, Corberand T, Meyer JM, Gardan L (2002) *Pseudomonas lini* sp. nov., a novel species from bulk and rhizospheric soils. Int J Syst Evol Microbiol 52:513–523

Elliot RP (1958) Some properties of pyoverdine, the water-soluble pigment of the *Pseudomonas*. Appl Microbiol 6**:**241–246

Espinosa-Urgel M, Salido A, Ramos JL (2002) Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. J Bacteriol 182:2363–2369

Founoune H, Duponnois R, Meyer JM, Thioulouse J, Masse D, Chotte JL, Neyra M (2002) Interactions between ectomycorrhizal symbiosis and fluorescent pseudomonads on *Acacia holosericea*: isolation of Mycorrhiza Helper Bacteria (MHB) from a soudano-sahelian soil. FEMS Microbiol Ecol 41:37–46

Frapolli M, Défago G, Moënne-Loccoz Y (2007) Multilocus sequence analysis of biocontrol fluorescent *Pseudomonas* spp. producing the antifungal compound 2,4-diacetylphloroglucinol. Env Microbiol (in press)

Frey P, Frey-Klett P, Garbaye J, Berge O, Heulin T (1997) Metabolic and genotypic fingerprinting of fluorescent pseudomonads associated with the Douglas Fir-*Laccaria bicolor* myccorrhizosphere. Appl Environ Microbiol 63:1852–1860

Gardan L, Shafik H, Belouin S, Grimont F, Grimont PAD (1999) DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and *Pseudomonas cannabina* sp. nov. (ex Sutic and Dowson 1959). Int J Syst Bacteriol 49:469–478

Gardan L, Bella P, Meyer JM, Christen R, Rott P, Achouak W, Samson R (2002) *Pseudomonas salomonii* sp. nov. pathogenic on garlic, and *Pseudomonas palleroniana* sp. nov., isolated from rice. Int J Syst Evol Microbiol 52:2065–2074

Grimont PAD, Vancanneyt M, Lefevre M, Vandemeulebroecke K, Vauterin L, Brosch R, Kersters K, Grimont F (1996) Ability of Biolog and Biotype-100 systems to reveal the taxonomic diversity of the pseudomonads. Syst Appl Microbiol 19:510–527

Guillot E, Leclerc H (1993) Bacterial flora in natural mineral waters: characterization by ribosomal ribonucleic acid gene restriction patterns. Syst Appl Microbiol 16:483–493

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hilario E, Buckley TR, Young JM (2004) Improved resolution on the phylogenic relationship by the combined analysis of *atp*D, *car*A, *rec*A and 16SrDNA. Antonie van Leeuwenhoek 86:51–64

Hohnadel D, Meyer JM (1988) Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains. J Bacteriol 170:4865–4873

Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16s rRNA and 16S rRNA genes. Appl Environ Microbiol 72:1719–1728

Kersters K, Ludwig W, Vancanneyt M, De Vos P, Gillis M, Schleifer KH (1996) Recent changes in the classification of the pseudomonads: an overview. Syst Appl Microbiol 19:465–477

Kessler B, Palleroni NJ (2000) Taxonomic implications of synthesis of poly-beta-hydroxybutyrate and other poly-beta-hydroxyalkanoates by aerobic pseudomonads Int J Syst Evol Microbiol 50:711–713

King EO, Ward MK, Raney DF (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 44:301–307

Kloepper JW, Leong J, Teintze M, Schroth MN (1980a) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:885–886

Kloepper JW, Leong J, Teintze M, Schroth MN (1980b) *Pseudomonas* siderophores: a mechanism explaining disease suppressive soils. Curr Microbiol 4:317–320

Koedam N, Wittouck E, Gaballa A, Gillis A, Höfte M, Cornelis P (1994) Detection and differenciation of microbial siderophores by isoelectric focusing and chrome azurol S overlay. BioMetals 7:287–291

Kwon SW, Kim JS, Crowley DE, Lim CK (2005) Phylogenetic diversity of fluorescent pseudomonads in agricultural soils from Korea. Lett Appl Microbiol 41:417–423

Landa BB, Mavrodi OV, Raaijmakers JM, NcSpadden Gardener BB, Thomashow LS, Weller DM (2002) Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. Appl Environ Microbiol 68:3226–3237

Lang E, Griese B, Spröer C, Schumann P, Steffen M, Verbarg S (2007) Characterization of '*Pseudomonas azelaica*' DSM 9128, leading to emended descriptions of *Pseudomonas citronellolis* Seubert 1960 (Approved Lists 1980) and *Pseudomonas nitroreducens* Iizuka and Komagata 1964 (Approved Lists 1980), including *Pseudomonas multiresinivorans* as its later heterotypic synonym. Int J Syst Evol Microbiol 57:878–882

Lee CH, Lewis TA, Paszczynski A, Crawford RL (1999) Identification of an extracellular catalyst of carbon tetrachloride dehalogenation from *Pseudomonas stutzeri* strain KC as pyridine-2, 6-bis(thiocarboxyate). Biochem Biophys Res Commun 261:562–566; erratum 265:770

Lelliott RA, Billing E, Hayward AC (1966) A determinative scheme for the fluorescent plant pathogenic pseudomonads. J Appl Bacteriol 29:470–489

Lemanceau P, Alabouvette C (1993) Biological control of *Fusarium* diseases by fluorescent *Pseudomonas* and non-pathogenic *Fusarium*. Crop Prot 10:279–286

Lemanceau P, Corberand T, Gardan L, Latour X, Laguerre G, Boeufgras JM, Alabouvette C (1995) Effect of two plant species, Flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent pseudomonads. Appl Environ Microbiol 61:1004–1012

Louws FJ, Fulbright DW, Stephens CT, Debruijn FJ (1994) Specific genomic fingerprints of phytopathogenic *Xantomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. Appl Environ Microbiol 60:2286–2295

Meyer J-M (2000) Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* species. Arch Microbiol 174:135–142

Meyer J-M, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P et al. (2002) Siderophore typing, a powerful tool for the identification of fluorescent and non-fluorescent *Pseudomonas*. Appl Environ Microbiol 68:2745–2753

Molina L, Ramos C, Ronchel MC, Molin S, Ramos JL (1998) Construction of an efficient biologically contained *Pseudomonas putida* strain and its survival in outdoor assays. Appl Environ Microbiol 64:2072–2078

Moore ERB, Mau M, Arnscheidt A, Böttger EC, Hutson RA, Collins MD, Van De Peer Y, De Watcher R, Timmis KN (1996) The determination and comparison of the 16S rRNA gene sequences of species of the genus *Pseudomonas* (sensu stricto) and estimation of the natural intrageneric relationships. Syst Appl Microbiol 19:478–492

Munsch P, Alatossava T, Meyer JM, Marttinen N, Christen R, Gardan L (2002) *Pseudomonas costantinii* sp. nov., another causal agent of brown blotch disease, isolated from cultivated mushroom sporophores in Finland. Int J Syst Evol Microbiol 52:1973–1983

Nautiyal CS, Johri JK, Singh HB (2002) Survival of the rhizosphere-competent biocontrol strain *Pseudomonas fluorescens* NBRI2650 in the soil and phytosphere. Can J Microbiol 48:588–601

Palleroni NJ (1984) *Pseudomonas*. In: Krieg NR (ed.) Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore, pp 141–199

Palleroni NJ (2005) Genus I. *Pseudomonas* Migula 1894. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 2, pt B, 2nd edn. Springer, Berlin Heidelberg New York, pp 323–379

Palleroni NJ, Kunisawa R, Contopoulos R, Doudoroff M (1973) Nucleic acid homologies in the genus *Pseudomonas*. Int J Syst Bacteriol 23:333–339

Risse D, Beiderbeck H, Taraz K, Budzikiewicz H, Gustine D (1998) Corrugatin, a lipopeptide siderophore from *Pseudomonas corrugata*. Z Naturforsch 53c:295–304

Ross IL, Alami Y, Harvey PR, Achouak W, Ryder MH (2000) Genetic diversity and biological control activity of novel species of closely related pseudomonads isolated from wheat field soils in South Australia. Appl Environ Microbiol 66:1609–1616

Sikorski J, Lalucat J, Wackernagel W (2005) Genomovars 11 to 18 of *Pseudomonas stutzeri*, identified among isolates from soil and marine sediment. Int J Syst Evol Microbiol 55:1767–1770

Sneath PHA, Stevens M, Sackin MJ (1981) Numerical taxonomy of *Pseudomonas* based on published records of substrate utilization. Antonie van Leeuwenhoek 47:423–448

Stanier RY, Palleroni NJ, Doudoroff M (1966) The aerobic pseudomonads: a taxonomic study. J Gen Microbiol 43:159–271

Starr MP, Knackmuss HJ, Cosens G (1967) The intracellular blue pigment of *Pseudomonas lemonnieri*. Arch Mikrobiol 59:287–294

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG (1987) Report of the Ad Hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Yamamoto S, Kasai H, Arnold DL, Jackson RW, Vivian A, Harayama S (2000) Phylogeny of the genus *Pseudomonas*: intrageneric structure reconstructed from the nucleotide sequences of *gyrB* and *rpoD* genes. Microbiology 146:2385–2394