Genus

Proteobacteria/Gammaproteobacteria/Pseudomonadales/Pseudomonadaceae/

Pseudomonas

Migula 1894, 237[AL] (Nom. Cons., Opin. 5 of the Jud. Comm. 1952, 121)[1]

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Pseu.do'mo.nas or Pseu.do.mo' nas. Gr. adj. *pseudes* false; Gr. n. *monas* a unit, monad; M.L. fem. n. *Pseudomonas* false monad.

Straight or slightly curved rods but not helical, 0.5–1.0 × 1.5–5.0 μm. **Most of the species do not accumulate granules of polyhydroxybutyrate**, but accumulation of polyhydroxyalkanoates of monomer lengths higher than C_4 may occur when growing on alkanes or gluconate. Do not produce prosthecae and are not surrounded by sheaths. No resting stages are known. Gram negative. **Motile by one or several polar flagella**; rarely nonmotile. In some species lateral flagella of short wavelength may also be formed. **Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor; in some cases nitrate can be used as an alternate electron acceptor**, allowing growth to occur anaerobically. **Xanthomonadins are not produced**. Most, if not all, species fail to grow under acid conditions (pH 4.5 or lower). Most species do not require organic growth factors. Oxidase positive or negative. Catalase positive. Chemoorganotrophic. **Strains of the species include in their composition the hydroxylated fatty acids $C_{10:0\ 3OH}$ and $C_{12:0}$, and $C_{12:0\ 2OH}$, and ubiquinone Q-9**. Widely distributed in nature. Some species are pathogenic for humans, animals, or plants.

The mol% G + C of the DNA is: 58–69.

Type species: **Pseudomonas aeruginosa** (Schroeter 1872) Migula 1900, 884 (*Bacterium aeruginosum* Schroeter 1872, 126.)

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Note: The above-mentioned characteristics do not allow an absolute differentiation of this genus from other genera of aerobic pseudomonads[2] belonging to other ribosomal RNA groups. The following approaches add considerable solidity to the decision to assign newly isolated strains to *Pseudomonas*. One is the determination of sequence similarity in ribosomal RNA, which can be demonstrated either by hybridization techniques or, more practically, by nucleotide sequence determination. The second is determination of the fatty acid and ubiquinone composition. These two approaches will be discussed in separate sections of this chapter.

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Number of validated species: 53

Further descriptive information

General Comments

The following sections provide further descriptive information on the properties of species assigned to the genus *Pseudomonas*, beyond the basic characteristics that have been highlighted in the above genus definition. Unfortunately, the comments that follow refer to only a few species, and the available information for these species is uneven. Thus, a literature survey shows that most references on *P. aeruginosa* are focused on its medical importance as an opportunistic pathogen; *P. putida* references are concentrated on its remarkable biochemical versatility; and those on *P. fluorescens* also highlight biochemical properties, some of which are directed to understanding its role in the promotion of plant growth. *P. stutzeri* and *P. mendocina* have attracted attention as denitrifiers, and, in recent years, an infamous side of their reputation is their potential activities as opportunistic pathogens.

Many of the species listed at the end of this chapter or in the tables are little known beyond the details published in the original species descriptions. In addition to these unavoidable limitations, the details that are given for the better known species of the genus represent, by necessity, a radical simplification of the mass of information found in the literature since the date of publication of the first volume of the *Manual* (Palleroni, 1984).

Cell morphology and fine structure

The cells of *Pseudomonas* strains occasionally differ substantially in size and shape from the general definition. For instance, the original description of "*P. ovalis*" (now considered to be a synonym of *P. putida*) highlighted the oval shape of the cells, while the cells of other *P. putida* strains and of some plant pathogenic fluorescent species may far exceed the 5 μm indicated in the definition.

A morphological character that was used to differentiate members of the genus *Pseudomonas* from other aerobic pseudomonads was the inability of the former to accumulate endocellular granules of poly-β-hydroxybutyrate (PHB) when growing in media of low nitrogen content on various carbon sources. More recently, the fluorescent members of *Pseudomonas* were found to be able to accumulate polyhydroxyalkanoates (PHAs) composed of monomers of medium chain lengths (C_6 to C_{12}) when grown on carbon sources such as gluconate, alkanes, and alkenes (Huisman et al., 1989; Anderson and Dawes, 1990; Steinbüchel and Valentin, 1995). Further comments on this subject may be found in the section Procedures for Testing Special Characters, below.

Thin sections of cells of *Pseudomonas* show cell walls and membranes characteristic of Gram-negative bacteria. In freeze-etched preparations of *P. aeruginosa* cells, as many as nine layers can be defined, accounting for all the electron-dense and electron-transparent layers that can be observed in thin sections (Lickfield et al., 1972; Gilleland et al., 1973). Gilleland and collaborators have shown that the outer membrane of the cell wall could be split down the middle when the cells were freeze-etched in the presence of a cryoprotective agent (Gilleland et al., 1973), thus confirming an earlier interpretation of similar experiments. The outer layer thus separated from the outer membrane had in its inner face numerous spherical units, which are made up of protein and in some cases were aggregated in the shape of small rods. *P. aeruginosa* is extremely sensitive to the action of EDTA, which provokes cell lysis. The spherical elements were removed by EDTA treatment but could be restored by addition of magnesium ions, which also restored cell stability.

Flagella and pili (fimbriae)

Typically, *Pseudomonas* cells have polar flagella. The flagellar insertion in some instances is not exactly polar but subpolar, and occasionally it may be difficult to differentiate the latter from the so-called degenerately peritrichous type observed in members of other genera. In addition to the polar flagella, lateral flagella of short wavelength may be produced by strains of some species (*P. stutzeri, P. mendocina*) and are shed much more easily than the polar flagella. Growth on solid media favors the formation of lateral flagella, which suggests that they may be involved in swarming of the population on solid surfaces (Shinoda and Okamoto, 1977).

The number of flagella has taxonomic importance. Based on the work performed at the University of California in Berkeley, the number of flagella (usually expressed as "one" or "more than one") was highly characteristic for all the strains of a species that have been examined. To get good results, it is advisable to follow well-controlled growth conditions and to express the results on a statistical basis, as indicated by Lautrop and Jessen (1964). Some practical recommendations and basic details of the technique

described by these authors will be summarized in the section Procedures for Testing Special Characters. Unfortunately, they have not been followed in the description of most species. In Jessen's classical study of *P. aeruginosa*, 97% of the cells with stained flagella had only one flagellum, 3% two flagella at one pole, and only three cells (about 0.05% of the collection) had three flagella. According to the definition proposed by Lautrop and Jessen (1964), the group can be characterized as polar monotrichous. Only one strain deviated from the limit defined for monotrichous strains in having two flagella on 15% of the flagellated cells, instead of the accepted maximum of 10%.

The amino acid sequence of the flagella of a given species is not always homogeneous. An analysis of the virulence-associated locus *fliC* of *P. aeruginosa* revealed two types of flagellin genes, one variable and the other conserved. Differences in the flagellar sequences amount to about 35%, but the two proteins fold into similar structures during polymerization (Spangenberg et al., 1996). In two strains of *P. putida*, flagellins of apparent molecular masses of 81 kDa and 50 kDa, respectively, were identified. Within the chromosomal fragment encoding the large component, two genes homologous to two flagellin genes of *Salmonella typhimurium* and one gene homologous to a *P. aeruginosa* flagellin gene were identified. The deduced molecular mass of the product of the first flagellin gene was 68 kDa instead of 81 kDa, suggesting posttranslational modification. Both the N- and C-terminal sequences are conservative, while the middle section is variable (Winstanley et al., 1994). PCR amplification of flagellar genes of *P. aeruginosa* gave products that were analyzed for restriction fragment length polymorphism, and 13 groups could be defined, suggesting that this approach may provide a useful genetic marker for the study of genetic variation among closely related species (Winstanley et al., 1996).

Fimbriae (pili) of polar insertion have been reported for *P. aeruginosa* and *P. alcaligenes* in the early studies of Fuerst and Hayward (1969). No fimbriae have been observed in the strains of *P. fluorescens, P. chlororaphis*, and *P. putida* that have been examined.

The *P. aeruginosa* pili are about 6 nm wide, thinner than those of enteric bacteria. They act as receptors for various phages and are retractile (Bradley, 1972a, b), but not all the pili of this species have the property of withdrawing into the cell (Bradley, 1974). Figure 1 shows the polar fimbriae of *P. aeruginosa*, whose genetic determinants are mobilized by the FP plasmids (Bradley, 1980b

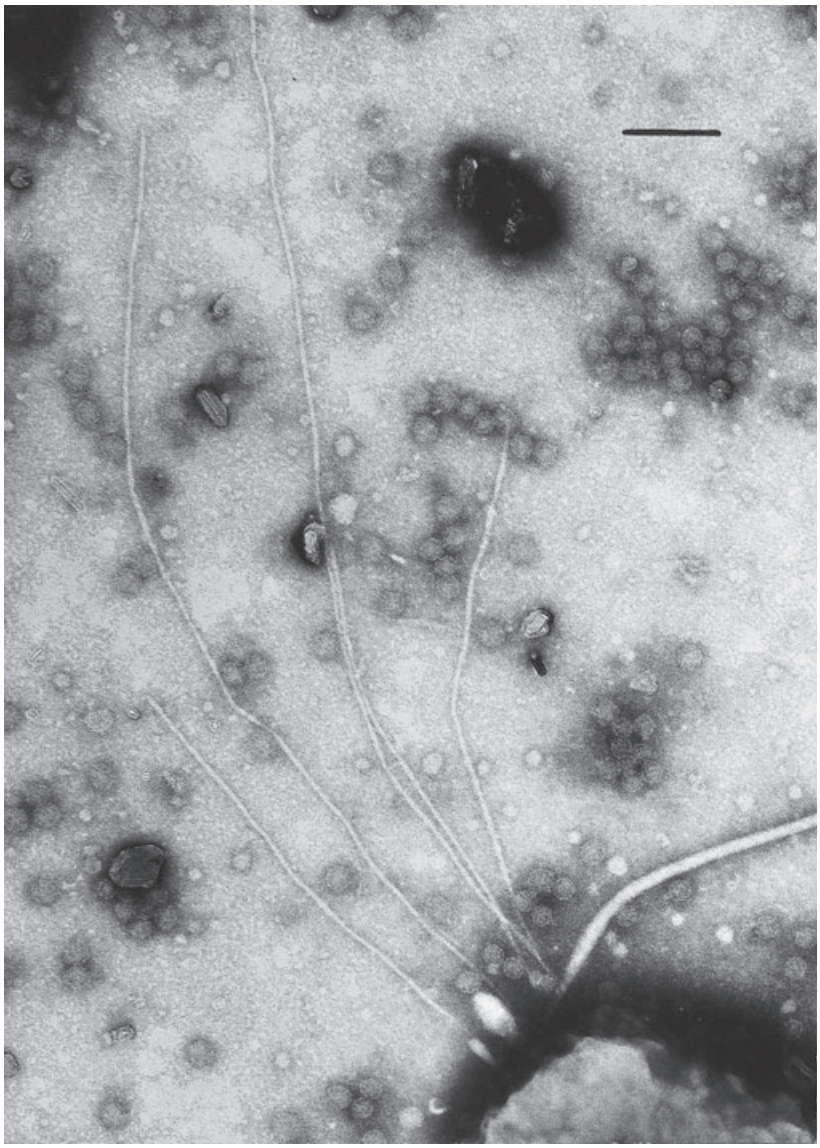
P. aeruginosa produces type-4 pili, which are found in a number of pathogenic bacteria and are involved in cell adhesion to epithelial cells (Woods et al., 1980; Doig et al., 1988). The adhesive region is located at the tip of the pilus, but this mode of attachment is not universal among fimbriae of different species (Smyth et al., 1996). In the C-terminal region of pilin there is a disulfide loop of 12–17 amino acids, and even though pili of different prototypes have little sequence similarity, the loops in all cases are structurally similar. However, the loops do not seem to be essential in maintaining the functionality of the binding domain. In addition to the fimbriae, exoenzyme S and flagella are additional adhesins of importance in the attachment process (Hahn, 1997).

Type-4 pili not only determine attachment to epithelial cells, but also cause a form of surface translocation called twitching motility (Henrichsen, 1975; Bradley, 1980a). Adhesion of *P. aeruginosa* cells to mucin is pilus-independent. A gene *fliO* complemented mutations in motility and adhesion to mucin (Simpson et al., 1995). A gene homologous to the *fliF* gene of other species has been identified in *P. aeruginosa*. It is involved in both motility and adhesion to mucin. Further characterization of the markers involved showed that the basal body structures of flagella also are important for adhesion (Arora et al., 1996).

Pilus formation is genetically very complex and depends on the expression of more than 30 genes distributed in at least six regions of the *P. aeruginosa* chromosome (Mattick et al., 1996; Tan et al., 1996; Watson et al., 1996a). The locations of a number of the pili genes have now been determined. Some of the genes encode proteins highly homologous to factors involved in protein secretion (Alm and Mattick, 1995, 1997; Alm et al., 1996b; Hahn, 1997). At least four different mechanisms are known to participate in the assembly of fimbriae from subunits (Vu-Thien et al., 1996

Some years ago, it was found that the amino terminal sequence of *P. aeruginosa* pili had marked similarity with that of *Neisseria meningitidis* and *Moraxella nonliquefaciens* (Buchanan and Pearce, 1979). One of the gene clusters encodes proteins strikingly similar to chemotaxis components of enteric bacteria and the gliding *Myxococcus xanthus*. In *M. xanthus*, type-4 pili have a role in what has been called "social motility" (Darzins, 1994; Darzins and Russell, 1997; Wu et al., 1997b). Work done with polyclonal antibodies raised against pilins expressed by *P. aeruginosa, Moraxella bovis, Neisseria gonorrhoeae, Dichelobacter nodosus*, and *Vibrio cholerae* showed that these polypeptides have conserved antigenic and, in most instances, immunogenic determinants, which are located in the highly similar amino-terminal domains (Patel et al., 1991).

FIGURE 1. *Pseudomonas aeruginosa* strain PAC5 polar pili, which have been prevented from retracting by the adsorption of a pilus-specific bacteriophage; this can be seen scattered over the field. Bar = 0.1 µm. (Reproduced with permission from D.E. Bradley, Canadian Journal of Microbiology *26*:155–160, 1980, ©National Research Council of Canada.)



AlgR is considered to be a regulator of alginate production in *P. aeruginosa*; in addition, it is required for twitching motility. Adjacent to the *algR* gene, a sensor gene (*fimS*) has been identified, and AlgR and FimS appear to represent another example of the two-component signal transduction systems that have been identified in bacteria. AlgU, an alternate sigma factor, also affects both alginate production and twitching motility, indicating that type-4 fimbriae and alginate, two important virulence determinants, are closely related (Whitchurch et al., 1996).

A gene region identified in nonfimbriated, phage-resistant transposon mutants of *P. syringae* pathovar *phaseolicola* (in this treatment described as a pathovar of *P. savastanoi*) has a high degree of similarity with a *P. aeruginosa* region required for pilus type-4 production (Alm and Mattick, 1995). Pili mutants that have lost the twitching motility characteristic of type-4 fimbriae, could be complemented in *trans* by homologous or heterologous subunits from the same strains (PAO or PAK) and from strains of other species such as *Dichelobacter nodosus* (Watson et al., 1996b).

Composition of cell envelope

The cytoplasmic membrane separates the bacterial cytoplasm from other components of the cell envelopes, and its efficiency as a barrier is related to the lipid bilayer composition, which may undergo relatively rapid changes to adjust its fluidity to changes in the composition of the medium and the physical conditions of the environment.

In contrast to the cytoplasmic membrane, the structure of the outer membrane is asymmetrical. It is composed of a special lipid (lipopolysaccharide, LPS) with hydrocarbon chains giving low fluidity to the inner region of the LPS. Toward the outside region, chains of carbohydrates give to the whole cells a specific antigenic identity. The outer membrane is quite effective as a barrier against passage of molecules such as the antibiotics, which may have a deleterious effect on the cells, but at the same it must be permeable to nutrients. This discrimination is effected by the presence of porins.

The outer membrane proteins of pseudomonads carry the designation Opr followed by a letter or a letter and number (Hancock and Carey, 1980). One monomeric *Pseudomonas* protein, OprF, is similar to OmpA of *E. coli*; it is highly antigenic and not specific toward the solutes that enter the cells (Nikaido, 1992). In *Pseudomonas*, porins are composed of monomeric proteins, and not trimeric proteins as in *E. coli*. The outer membrane of *P. fluorescens* can change its permeability in direct relationship with growth temperature, even though no difference in composition could be found to correlate with this transition. The effect was attributed to a change in the structure of the porins that affects their function, since a decrease in the growth temperature induced a reduction in the conductance in the major component, OprF, suggesting a structural change (De et al., 1997).

Protein fractions representing the OprF of two *P. fluorescens* strains were purified and characterized. A sequence similarity of 94% was found among OprFs of strains from origins as different as milk and soil (De et al., 1995). OprF of *P. aeruginosa* is a major outer membrane component, and as such it is potentially interesting for the preparation of vaccines (Rawling et al., 1995). There are differences in the location of OprFs between the two species. Thus, while in *P. fluorescens* the protein is embedded in the outer membrane in such a way that it offers a surface-exposed region rich in proline, this region is absent in *P. aeruginosa* and in *P. syringae*. In addition, in *P. aeruginosa*, the entire protein is embedded, without an exposed region to the outside (De Mot et al., 1994).

An outer membrane protein of *P. fluorescens* that was inducible under conditions of phosphate limitation was purified and characterized (Leopold et al., 1997). It did not show similarity to any of the known outer membrane proteins. Its distribution among different strains of the species was uneven, i.e., it was not present in all strains, which simply may reflect the internal heterogeneity of *P. fluorescens*. A method of immunofluorescence microscopy involving cell permeability was developed to visualize the specific expression of the protein in cells exposed to limiting phosphate concentrations, in order to use the system to measure phosphate availability (Leopold et al., 1997). In *P. aeruginosa*, exposure to limiting phosphate provoked the expression of porin OprP. Only one of three lysine residues of this protein was required to form the specific phosphate binding site in OprP (Sukhan and Hancock, 1996).

Resistance to polymyxin B, gentamicin, and EDTA is associated with the outer membrane protein OprH in *P. aeruginosa*, which is expressed under magnesium-limiting conditions (Rehm and Hancock, 1996).

P. aeruginosa is able to simultaneously express two different LPS types, which migrate to different positions in electrophoresis and have been named bands A and B. The B band corresponds to the component that confers to the cell its O-antigen serological specificity and the A band to a common antigen. Two of the eight genes that are part of the operon that controls band A biosynthesis and transport encode proteins that are highly similar to a number of proteins that are part of the ABC (acronym for "ATP-binding cassette") transport systems (Rocchetta and Lam, 1997). Of the two LPS O-polysaccharide species, one confers to the cells high hydrophobicity, and the other, hydrophilicity. The factors that provoke a change in the ratio between these two components affect both adhesion and survival (Makin and Beveridge, 1996).

Pigments and siderophores

Early taxonomic treatments of the genus *Pseudomonas* included pigmentation as a generic character, but this is no longer valid. In fact, the colonies and other cell masses always display some colors due to normal cellular components, which, in some instances, become quite apparent. Thus, *P. stutzeri* is grouped with the nonpigmented species, even though the colonies of many strains become dark brown due to high concentration of cytochrome *c* in the cells.

In his comprehensive monograph on the taxonomy of *P. aeruginosa* and other fluorescent pseudomonads, Jessen (1965) listed six pigments produced by the type species:

four phenazines (pyocyanin, pyorubin, chlororaphin, oxiphenazin), the *Pseudomonas* blue protein, and pyoverdine. Several phenazine pigments can be produced by a single strain (Chang and Blackwood, 1969). The best known and most characteristic of these pigments is the phenazine blue pigment pyocyanin, identified many years ago as the cause of the blue color of the pus of wounds infected with *P. aeruginosa*. Synthesis of pyocyanin by *P. aeruginosa* can be stimulated by growth in King A medium (King et al., 1954). It is inducible in a dose-dependent manner and its production is strongly enhanced by addition of L-*N*-(3-oxohexanoyl) homoserine lactone to the medium (Stead et al., 1996).

Phenazine pigments synthesized by other fluorescent pseudomonads are the green, almost insoluble, chlororaphin of *P. chlororaphis*, and the orange phenazine-monocarboxylic acid characteristic of *P. aureofaciens* (in the present treatment these taxa are considered to be subspecies of *P. chlororaphis*). The species "*P. lemonnieri*", which was considered by Stanier et al. (1966) as a biovar of *P. fluorescens*, produces an intracellular blue pigment that has been chemically characterized (Starr et al., 1960, 1967). Some strains of *P. aeruginosa* are able to produce melanin pigments (Mann, 1969).

Other important pigments from the physiological and taxonomic standpoints are the pyoverdines. They are the typical yellow-green pigments of the so-called fluorescent pseudomonads. Jessen (1965) found that his collection of strains of *P. aeruginosa* contained 328 fluorescent strains producing pyocyanin, 18 fluorescent strains not producing pyocyanin, five nonfluorescent strains with pyocyanin, and three nonpigmented strains. Strains of this species lacking one or more of the characteristic pigments, however, could be identified based on other phenotypic properties, and, in general, pigmentation is a most striking but not always dependable characteristic for species identification. In any case, identification of fluorescent *Pseudomonas* species by examination of the fluorescence profiles of pigments diffusing into the medium has been proposed by Shelly et al. (1980).

Pyoverdines are also physiologically important because they function as efficient siderophores (Meyer and Abdallah, 1978; Meyer and Hornsperger, 1978). Their production is enhanced under conditions of iron-starvation. All pyoverdines share a quinoleinic chromophore, which is linked to peptides of different compositions and sizes. This may vary from 6–12 D-and L-amino acid residues. With minor exceptions, each fluorescent *P. seudomonas* type strain produces a pyoverdine with a specific amino acid composition (Budzikiewicz, 1993). However, within a single species there is a diversity of peptide structures.

The presence of D- and L-amino acid in pyoverdines as well as possible cyclic structures suggests a similarity the cyclic peptides of antibiotic properties produced by some Gram-positive bacteria and toxins synthesized by *Pseudomonas syringae* pathovars. These observations are indicative of a nonribosomal type of synthesis (Georges and Meyer, 1995; Merriman et al., 1995). In a search for components of such a system, high molecular weight cytoplasmic proteins have been identified. As expected, they are produced by cells of fluorescent pseudomonads in iron-deficient media, for which they have been named iron-repressed cytoplasmic proteins (IRCPs). They vary from M_r 180–600 kDa, and they give characteristic electrophoresis profiles for strains producing different pyoverdines. On the other hand, they are absent from mutants in pyoverdine synthesis and from cells of nonfluorescent pseudomonads (Georges and Meyer, 1995). As mentioned by Meyer et al. (1997), pyoverdines provide information of use in strain typing and epidemiological applications.

Using a system with a promotorless *lacZ* gene, 24 insertion mutants of *P. aeruginosa* unable to synthesize pyoverdine were isolated. All the mutations could be allocated within a 103-kb region ("pyoverdine region") at 47 min of the *P. aeruginosa* strain PAO genetic map, very near the catechol region (Tsuda et al., 1995). The genetic determinants of pyoverdine production occupy a region of at least 78 kb, but few genes have been characterized at present. *pvdE*, recently identified, codes for a protein that is a member of the ATP-binding cassette group of membrane transporters (McMorran et al., 1996). *pvdA* encodes an enzyme that catalyzes a key step in the synthesis. Three tightly iron-regulated regions are located in a fragment upstream of *pvdA*. Fur (ferric uptake repressor, a protein that controls expression of iron-repressible genes) indirectly controls *pvdA* transcription. When iron is not limiting, Fur blocks the *pvdA* promoter, thus inhibiting transcription of several pyoverdin genes (Leoni et al., 1996). Gene *pvdS* is also required for pyoverdine biosynthesis by *P. aeruginosa*. Under conditions of unlimited iron, Fur also acts here as a repressor, binding to the *pvdS* promoter and preventing expression (Cunliffe et al., 1995).

Because siderophores are iron-scavenging compounds, a large number of strains of *P. aeruginosa* have been analyzed by different methods for pyoverdine-mediated iron incorporation, and in all cases the collection could be subdivided into the same three groups, in spite of the inclusion of strains devoid of pyoverdine production in some of the groups (Meyer et al., 1997). The *P. aeruginosa* cells also are able to incorporate iron combined with siderophores of foreign origin. For instance, they can produce a receptor (PfeA) for the

iron trapped by enterobactin, a siderophore synthesized by enteric bacteria. PfeA production depends on a regulator and a sensor, which are members of the family of two-component regulatory systems. The operon of regulator/sensor is probably regulated by iron, since the regulator gene in this system has a sequence similarity to the iron uptake regulator *fur*, the gene that codes for the above-mentioned Fur protein (Dean et al., 1996). Iron can be transported into *P. aeruginosa* not only by enterobactin but also by at least one of its breakdown products, 2,3-dihydroxybenzoyl-L-serine, in a process that is neither iron-repressible nor strongly energy-dependent (Spangenberg et al., 1995).

Attempts to complement a pyoverdine mutant from a *P. aeruginosa* PAO cosmid bank resulted in the recovery of an apparent wild-type phenotype. Physical mapping indicated that the cloned fragment corresponded to a different region of the PAO chromosome and that the properties of the transconjugants were not the result of a true complementation. The yellow-green fluorescent compound was different in its properties from pyoverdine and it was named pseudoverdine. It lacks a peptide chain, but it resembles pyoverdine in its spectral properties (Stintzi et al., 1996).

Aside from pyoverdines, the fluorescent pseudomonads also produce other strain-specific, but chemically related, siderophores called pseudobactins. A method for isolation of pseudobactin from pseudomonads, and an assay for fluorescent siderophores based on reverse-phase HPLC, have been described (Nowak-Thompson and Gould, 1994). When grown in an iron-deficient medium, each of two strains of *P. putida* and *P. fluorescens* produced two different novel yellow-green fluorescent pseudobactins. All four compounds contained a dihydroxyquinoline-based chromophore. The receptor proteins of the two species are similar but not identical (Khalil-Rizvi et al., 1997). The composition of the corresponding peptides has been described, but the reason for the differences are not apparent, since the uptake system seems to consist of a single receptor in both organisms. Fragments of the peptide component of one pseudobactin have been synthesized. Antifungal peptides called pseudomycins have related structures, but the one mentioned here does not have activity against fungi (Koushik et al., 1997).

A *P. putida* strain WCS358 produces a fluorescent pseudobactin that has been described in detail (von der Hofstad et al., 1986). In spite of the results mentioned in the previous reference, the results here indicate that the ability of *P. putida* WCS358 to use different pseudobactins of various origins is related to the presence of multiple outer membrane receptor proteins (Koster et al., 1995).

In addition to the siderophores mentioned above, the fluorescent pseudomonads produce another one, pyochelin, which is not pigmented (that is, it does not have an absorption spectrum in the visible region), and appears to have low efficiency as an iron-scavenging compound (Liu and Shokrani, 1978). In the composition of pyochelin there is one molecule of salicylic acid (which is by itself a siderophore) condensed to two cysteinyl residues. In *P. aeruginosa*, the pathway of salicylate synthesis has been clarified: conversion of chorismate (which also acts as an intermediate of aromatic amino acid biosynthesis) to isochorismate, which is converted to salicylate plus pyruvate. These steps are catalyzed by two iron-repressible proteins, PchA and PchB (Serino et al., 1995).

The iron trapped by the siderophores is utilized by the producing organism presumably after reduction to ferrous ion, a step catalyzed by ferrisiderophore reductases. Ferripyoverdine reductase is ubiquitous among *Pseudomonas* species (Halle and Meyer, 1992), and since its activity is not under iron regulation, it is not part of the siderophore genetic system (J.-M. Meyer, personal communication).

There are earlier reports of other fluorescent compounds that have been isolated from fluorescent pseudomonads. These include four pteridine derivatives isolated from "*P. ovalis*" (presumably *P. putida*) (Suzuki and Goto, 1971); two fluorescent antibiotics isolated from *P. fluorescens*, which were named fluopsin C and fluopsin F (Shirahata et al., 1970); 6-hydroxymethylpteridine, an intermediate in folic acid synthesis (Viscontini and Frater-Schröder, 1968); and erithroneopterine of *P. putida* (Suzuki and Goto, 1972).

P. fragi, a nonpigmented member of the genus, does not produce siderophores in detectable amounts and it is very sensitive to iron-limiting conditions. Growth can be strongly stimulated by iron combined with siderophores of foreign origin. The siderophores include enterobactin, some pyoverdines, and siderophores of eucaryotic origin, such as transferrin, lactoferrin, and hemoglobin. Probably this property is related to the capacity of *P. fragi* to grow in milk, from which the first strain of this species was isolated many years ago (Gruber, 1905). Iron starvation in this species may induce the synthesis of the siderophore-mediated iron uptake system (Champomier-Vergès et al., 1996).

The following is a useful summary of siderophores produced by *Pseudomonas* species, kindly supplied by J.-M. Meyer from a manuscript submitted for publication. All strains of the fluorescent species *P. aeruginosa, P. fluorescens, P. chlororaphis*, and *P. putida* produce pyoverdines as main siderophores. Some strains also produce pyochelin and/or salicylic acid as secondary siderophores (Cox et al., 1981;

Meyer et al., 1992; Visca et al., 1993). *P. stutzeri* ATCC 17588 produces desferrioxamines E and D2 (Meyer and Abdallah, 1980; Azelvandre, 1993); a different strain of *P. stutzeri* (RC7) gives a catechol-type siderophore (Chakraborty et al., 1990) and an unclassified *Pseudomonas* sp., aerobactin (Buyer et al., 1991). No siderophores have been detected in *P. stutzeri* YPL-1 (Lim et al., 1991), in *P. fragi* (Stintzi et al., 1996), and in *P. mendocina*. It is interesting to note here that the internal heterogeneity of the nonfluorescent species *P. stutzeri* is also reflected in the capacity for siderophore production.

P. alcaligenes, P. mendocina, and *P. flavescens* produce yellow to orange pigments that have not been chemically characterized. Strains of one of the biovars of *P. fluorescens*, which were originally assigned to the species "*P. lemonnieri*", produce an intracellular insoluble pigment of structure related to that of indigoidine, the purple pigment of "*Pseudomonas indigofera*". Both pigments are derivatives of 3,3'-bipyridyl (Kuhn et al., 1965). The pigment of "*P. lemonnieri*" has been reexamined, its chemical structure has been clarified, and it has received the name "lemonnierin" (Ferguson et al., 1980; Jain and Whalley, 1980). Pigments that are not soluble in water and remain associated with the cell mass are found in many former *Pseudomonas* species now classified in other genera.

Nutrition and growth conditions

Strains of *Pseudomonas* species can grow in minimal, chemically defined media with ammonium ions or nitrate as nitrogen source and a single organic compound as the sole carbon and energy source. Some of the species previously assigned to *Pseudomonas* have true growth factor requirements, but none of the species belonging to the rRNA group I (Palleroni, 1984) has an absolute dependence on these nutritional supplements. In media of minimal composition, strains of phytopathogenic *P. syringae* grow very slowly in comparison with strains of the main saprophytic species, and that growth is enhanced by addition of small amounts of complex organic materials (yeast extract, peptones). However, in most cases no true dependence on organic growth factors can be demonstrated. Pantothenate is required by strains of *P. syringae* pathovar *avellanae*, now considered as an independent species, *P. avellanae* (Janse et al., 1996) (see List of Species). In addition, organic growth factors are required by some species of uncertain phylogenetic position (*P. iners, P. lanceolata, P. spinosa*) (see List of Species). Occasionally, attempts have been made to improve the poor growth of phytopathogenic *Pseudomonas* in chemically defined media. In one such proposal, a defined medium for "*P. tomato*" (*P. syringae* pathovar *tomato*) includes L-asparagine, L-glutamine, or L-threonine as a nitrogen source, and D-galactose as carbon source. The organism grows in this medium as well as in complex media (Bashan et al., 1982).

From time to time the capacity for nitrogen fixation has been claimed for some species that later on proved to be unable to perform this function under strictly controlled conditions. In the first edition of this *Manual*, it was assumed that none of the *Pseudomonas* species could be considered legitimate nitrogen fixers; however, this ability is reported to occur in strains of the nonfluorescent species *P. stutzeri* (Krotzky and Werner, 1987; Puente and Bashan, 1994), which is also a vigorous denitrifier.

Requirement for sodium ions has been observed for *Pseudomonas elongata* which, according to Anzai et al. (2000) should be placed in the genus *Microbulbifer* following further taxonomic studies, and for *P. halophila* (described in the section Other Species). This requirement, which is one of the characteristics typical of so-called marine eucaryotes, has not been determined in the majority of *Pseudomonas* species.

P. aeruginosa can utilize one of many different sources of sulfur in the medium. The list includes inorganic and organic compounds, from which the aromatic sulfur compounds are excluded. If sulfur sources other than the preferred ones (sulfate, cysteine, or thiocyanate) are the only ones available, a set of 10 sulfate starvation induced (SSI) proteins are upregulated (Hummerjohann et al., 1998). One of these proteins is periplasmic and has high affinity for sulfate. Even though no similarity of the other nine SSI proteins to other proteins of known function has been detected, they also may represent scavenging elements for the S-sources preferred by the cells. Studies on the genetics of the starvation response have indicated the role of cysteine biosynthetic intermediates and the possibility that at least two independent co-repressors are operative in *P. aeruginosa* (Hummerjohann et al., 1998).

The ability to grow in very simple mineral media at the expense of many organic compounds has served as the basis for extensive nutritional characterization of a large number of strains, providing a mass of phenotypic data ideally suited for taxonomic studies by numerical methods. Aside from their taxonomic implications, nutritional investigations on the utilization of certain groups of compounds have served as the bases for many interesting studies on metabolic pathways, their regulatory mechanisms, and their phylogenetic significance.

The best growth temperature for growth of most strains is approximately 28°C. Some species grow at a substantial rate at 4°C and thus can be considered psychrotrophic. For others the maximum temperature is about 45°C, and therefore they are not true thermophiles. None of the members of the genus

tolerates acidic conditions and growth is invariably negative at pH 4.5.

Metabolism and metabolic pathways

The metabolism of *Pseudomonas* is typically respiratory with oxygen as the terminal electron acceptor, but some species also can use nitrate as an alternate electron acceptor and can carry out oxygen-repressible denitrification (dissimilatory reduction of nitrate to N_2O or N_2). In most cases, denitrification is the property of all members of a given species, and only a few strains may be unable to denitrify. Denitrification is not a species characteristic in *P. fluorescens*; only some biovars are able to denitrify (Stanier et al., 1966).

Some cytochromes are involved in denitrification through the participation of a special cytochrome oxidase that probably is a remnant of a very primitive mechanism dating from the preaerobic era of the planet (Yamanaka, 1964). An excellent review on the cellular and molecular aspects of denitrification has been published by Zumft (1997). *Pseudomonas* is rich in denitrifying species. The core structures of the denitrification apparatus in these and in denitrifiers belonging to other genera demand the participation of around 50 genes.

Assimilation of nitrate occurs through the reduction of nitrate to ammonia. Although there may be common intermediates in the assimilatory and dissimilatory routes of nitrate reduction (Hartingsveldt et al., 1971), the pathways are encoded by different sets of genes. Some mutations with pleiotrophic effects are due to alterations in a gene involved in molybdenum incorporation into the nitrate reductases (Sias et al., 1980). A comprehensive review on the diversity of enzymes involved in denitrification in the pseudomonads and the genetic organization is available (Zumft and Körner, 1997), as well as a description of the localization of the genes in the *P. aeruginosa* PAO map (Vollack et al., 1998). The genes for nitrate reduction (*nir*), nitric oxide reduction (*nor*), and nitrous oxide reduction (*nos*) have been located in a 30-kb gene cluster in the chromosome of *P. stutzeri* (Braun and Zumft, 1992), and a sequence analysis of a 9.72-kb internal segment that includes the genes is now available (Glockner and Zumft, 1996). Very useful accounts of the enzyme diversity and gene organization of the denitrification genes in denitrifying pseudomonads, as well as the regulatory elements of the denitrification system of *P. stutzeri*, have been published by Zumft and collaborators (Cuypers and Zumft, 1992; Zumft and Körner, 1997). As mentioned before, a review by Zumft (1997) includes much useful information on this subject.

The oxidative degradation of some substrates (particularly the aromatic compounds) or their intermediates by *Pseudomonas* occasionally involves the participation of oxygenases. Both mono- and dioxygenases coupled to a variety of electron donors are well represented in species of the genus. Oxygenases acting on aliphatic compounds such as alkanes may be part of complex systems, and this is also true for the oxidation of some compounds like camphor by *P. putida*, with steps involving oxygenases of considerable complexity that include the iron-sulfur protein putidaredoxin and cytochrome $P\text{-}450_{CAM}$ (Gunsalus et al., 1971). Other systems include the iron protein rubredoxin (Lode and Coon, 1971) and cytochrome *o* (Peterson, 1970).

The classical reactions of the tricarboxylic acid cycle are found in all species of *Pseudomonas* that have been examined. A key reaction is the synthesis of citrate from oxaloacetate and acetyl-CoA, which is under a control system typical of absolute aerobic organisms (Weitzman and Jones, 1968). The control of peripheral catabolic enzymes (amidase, histidase, enzymes of aromatic compounds, and camphor metabolism) by intermediates of the tricarboxylic acid cycle are a manifestation of the central position that it occupies in metabolism. Those intermediates that are used for biosynthetic purposes can be replenished by carboxylation of pyruvate and by the action of the enzymes isocitrate lyase and malate synthase, which are part of the anaplerotic system known as the glyoxylate cycle (Kornberg and Madsen, 1958). A multienzyme complex of tricarboxylic acid cycle enzymes (fumarase, malate dehydrogenase, citrate synthase, aconitase, and isocitrate dehydrogenase) that catalyzes the reactions from fumarate to α-ketoglutarate has been identified in *P. aeruginosa* cells, from which it can be released by gentle osmotic lysis. The complex can be reconstituted from the individual enzymes provided that one of the two citrate synthase isoenzymes is present (Mitchell, 1996).

A variety of macromolecules can be degraded by some strains by means of extracellular enzymes. Hydrolytic enzymes that have been studied in detail include the proteases of *P. aeruginosa* (Morihara, 1964; Morihara et al., 1965), which are important in infections caused by this organism. In addition to these early studies, others will be discussed in relation to pathogenesis to humans and animals.

One of the differences between *P. fluorescens* and *P. putida* is the ability of the former species to produce extracellular proteases, to which the property of gelatin liquefaction can be attributed. A metalloprotease of *P. fluorescens* that is inhibited by EDTA and has a trypsin-like activity has been found to be highly homologous to zinc metalloproteases of diverse origins (Kim et al., 1997a).

Carbohydrates

The amylolytic activity of *P. stutzeri* is responsible for rapid starch hydrolysis, one of the characteristic phenotypic properties of the species. The enzymology of the exoamylase, which is responsible for the formation of maltotetraose as end product, has been examined at the molecular level, and the enzyme has been cloned (Morishita et al., 1997). Phytopathogenic pseudomonads have been found to hydrolyze pectin (Hildebrand, 1971; Ohuchi and Tominaga, 1973, 1975; Wilkie et al., 1973), xylan (Maino et al., 1974), and glycosides (Hayward, 1977).

Common monosaccharides (glucose, fructose, galactose, L-arabinose) are used by strains of most species of the genus, but growth of some of the species (*P. stutzeri, P. mendocina, P. syringae*) may be slow. Most hexoses are degraded by the Entner-Doudoroff pathway, which was discovered in studies on *P. saccharophila* (Entner and Doudoroff, 1952).

The fluorescent pseudomonads have multiple peripheral pathways for glucose oxidation that converge for the synthesis of 6-phosphogluconate, which is further degraded by the Entner-Doudoroff pathway (Eisenberg et al., 1974). Of these routes, one involves direct oxidation of the sugar (oxidative pathway), and either gluconate or 2-ketogluconate can serve as a precursor of 6-phosphogluconate. However, in *P. putida*, 6-phosphogluconate is synthesized preferentially from 2-ketogluconate (Vicente and Cánovas, 1973). Induction of the oxidative pathway in *P. aeruginosa* can only occur in the presence of oxygen. Under denitrifying conditions, only the so-called phosphorylative pathway (starting with the phosphorylation of glucose) is operative in this organism (Hunt and Phibbs, 1981).

A rather peculiar situation is represented by the oxidative degradation of glucose by *P. putida*, which has been shown to oxidize the sugar with a dehydrogenase peripherally located in the cells. This results in gluconate accumulation in the surrounding medium, from which it is taken back into the cells after induction of a specific energy-dependent transport system. The properties of this system are different from a second one specific for glucose uptake by the same cells (Schleissner et al., 1997).

The metabolism of fructose by several species of fluorescent and nonfluorescent pseudomonads occurs by means of a phosphoenol-pyruvate (PEP) phosphotransferase system. The product is fructose-1-phosphate, which may be further phosphorylated and cleaved by an aldolase. An isomerization of free mannose to fructose can be demonstrated in cell-free extracts of strains of many species (Alicia Palleroni and N.J. Palleroni, unpublished), although the significance of this conversion is obscure at present.

An excellent review on alternative pathways of carbohydrate metabolism by pseudomonads is available (Lessie and Phibbs, 1984).

Polyhydroxyalkanoates

At the time the main body of *Pseudomonas* taxonomy was being developed, the general ideas on reserve materials of the species of rRNA group I were vague. As mentioned before, although other groups of aerobic pseudomonads accumulate PHB in granules easily observable under phase microscopy, particularly when grown in media of low nitrogen and high carbon content, with few exceptions the species of rRNA similarity group I (Palleroni, 1984) now classified in the genus *Pseudomonas* did not seem to have the same capacity, and it was even assumed that the reserve material might be dispensable proteins, because of production of ammonia during respirometric studies on the endogenous metabolism of cells. Later it was discovered that the fluorescent pseudomonads (typical representative species of the genus *Pseudomonas*) were able to accumulate PHAs of medium chain length (C_6 to C_{12}) (de Smet et al., 1983; Lageveen et al., 1988). The studies confirmed the inability of these organisms to accumulate PHB, and this inability remains a reliable negative characteristic for differentiation from most other aerobic pseudomonads (Anderson and Dawes, 1990). It has been suggested that the ability to accumulate the medium chain length PHAs, which is not dependent on the presence of a plasmid, may be in itself of taxonomic value (Huisman et al., 1989). Additional comments on this subject will be found in the section Differentiation of the Genus *Pseudomonas* from Other Genera.

Hydrolysis of PHAs for the utilization of these carbon reserve materials can be catalyzed by extracellular depolymerases, of which one, produced by a strain of *P. fluorescens*, has been purified and characterized (Schirmer et al., 1993). The depolymerases are considered to belong to the family of serine hydrolases (Schirmer and Jendrossek, 1994; Schirmer et al., 1995). All lipases and an esterase of *P. fluorescens* had hydrolytic action on triolein, but this property was absent from the PHA depolymerases tested, although these enzymes have the lipase consensus sequence in their structure. The finding confirms the differences in function between lipases and PHA depolymerases (Jaeger et al., 1995).

Biotechnological applications of some pseudomonad lipases have been reported (Tan et al., 1996).

Polyalcohols

Genes involved in the conversion of 2,3-butylene glycol to central metabolites by *P. putida* have been characterized, and there is a high similarity between the genes that encode 2,3-butylene glycol dehydrogenase and those that encode alcohol dehydrogenases (Huang et al., 1994). Degradation of polyethylene glycol has been demonstrated with *P. stutzeri* (Obradors and Aguilar, 1991). The mannitol dehydrogenase of strains of *P. fluorescens* has a broad specificity, since it is also capable of oxidation of other polyalcohols such as sorbitol and arabitol (Brunker et al., 1997).

Acetamide and biochemical evolution

Acetamide is used for growth by several species of aerobic pseudomonads, among them *P. aeruginosa* (Stanier et al., 1966). It is hydrolyzed with liberation of ammonia, which is used then as a nitrogen source, but the enzyme can also catalyze acyl transfer reactions. Amides hydrolyzed by the wild-type enzyme include propionamide, acetamide, formamide, and butyramide, in order of decreasing rates of hydrolysis. Among the amides there are excellent inducers (lactamide) as well as anti-inducers (butyramide). Clarke and collaborators found this system to be admirably suited for studies of experimental evolution, during which it became possible to evolve amidases with altered substrate specificities, and the work of this group, performed during many years, stands out as a beautiful example of experimentation on evolution achieved in the laboratory (Brown et al., 1969; Betz and Clarke, 1972; Brown and Clarke, 1972; Betz et al., 1974). Among the many papers that followed these, a general article by Clarke and Drew (1988) gives a good account of the experimental evolution of the system.

The genetic organization and complete sequence of the amidase operon is now known (Drew and Wilson, 1992), as well as details of its transcription antitermination regulation (Wilson et al., 1996).

Aromatic compounds

For obvious reasons, as in the preceding sections, the short review that follows has been restricted to very few of the many valuable reports available in a well-populated literature on the degradation of aromatic compounds and some derivatives.

Members of the genus *Pseudomonas* are notorious for their capacity of aerobic degradation of a number of hydrocarbons, aromatic compounds, and their derivatives, of which there are natural compounds and final products or intermediates from industrial activities. A considerable number of these compounds are toxic and are found widely dispersed as environmental contaminants. Many of them can be used for growth by *Pseudomonas* species. Compounds as diverse as benzoate, *p*-hydroxybenzoate, mandelate, tryptophan, phthalate, salicylate, polycyclic compounds, and many derivatives may be metabolized by strains of the group following pathways that converge to a common intermediate, β-ketoadipate. This intermediate is formed soon after the last aromatic intermediate is cleaved by a 1,2-dioxygenase, in a type of ring cleavage frequently referred to as an *ortho* cleavage. The attack of the aromatic rings and the mode of cleavage are properties of taxonomic importance that have been of help in the circumscription of the rRNA similarity group I, which comprises the *Pseudomonas* species. In recent times, much information has been added to our basic knowledge of this pathway, but few details will be mentioned here. A transporter and chemoreceptor protein from *P. putida* that is part of the β-ketoadipate regulon, PcaK, was localized in the membrane when it was expressed in *E. coli*, adding to our understanding of active transport in aromatic compound metabolism (Nichols and Harwood, 1997).

The *ortho* ring cleavage, however, is not the only type of ring opening that can be catalyzed by these organisms, and the conditions adopted for the experimental protocols should be precisely stated. Thus, in the *P. putida* type strain, cleavage of the diphenolic intermediate catechol can be caused by either a 1,2-dioxygenase or a 2,3-dioxygenase (which causes a *meta* cleavage) depending on whether the substrate on which the cells had grown was benzoate or phenol, respectively (Feist and Hegeman, 1969). Often the genes encoding for *meta*-cleavage systems are located in plasmids (Austen and Dunn, 1980), as in the case of the well-known plasmid TOL, but this is not always so. Hewetson and collaborators have presented evidence of an *ortho* pathway for *p*-cresol degradation catalyzed by enzymes encoded by plasmid genes (Hewetson et al., 1978). The *ortho*-enzymes (1,2-dioxygenases, also known as pyrocatechases) in different *P. putida* strains have all a common ancestry (Nakai et al., 1995).

The pathways of aromatic compounds degradation are still being extensively analyzed. Regulatory mechanisms, genetic organization of the genes involved, immunological properties of the enzymes, and molecular studies as the bases for strain improvement in possible bioremediation applications are all subjects of great basic and practical importance. Detailed reports on the molecular aspects of the analysis of aromatic hydrocarbon degradation have been published (Zylstra and Gibson, 1991; Zylstra, 1994). Figures 2 and 3 have been taken from one of these sources (Zylstra

FIGURE 2. Pathways for the degradation of toluene by aerobic pseudomonads. The black dot indicates the position of the subsequent hydroxylation that precedes ring cleavage. For more details, see text and also Zylstra and Gibson (1991).

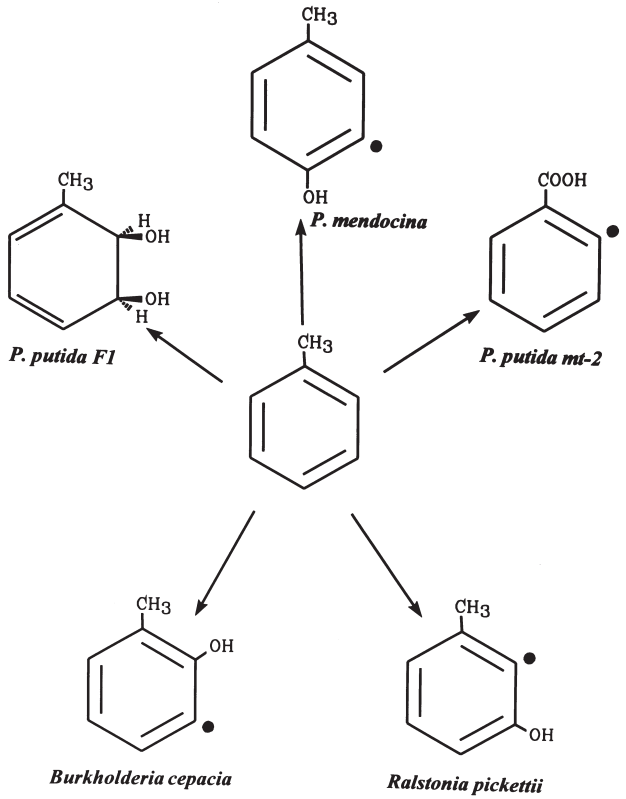
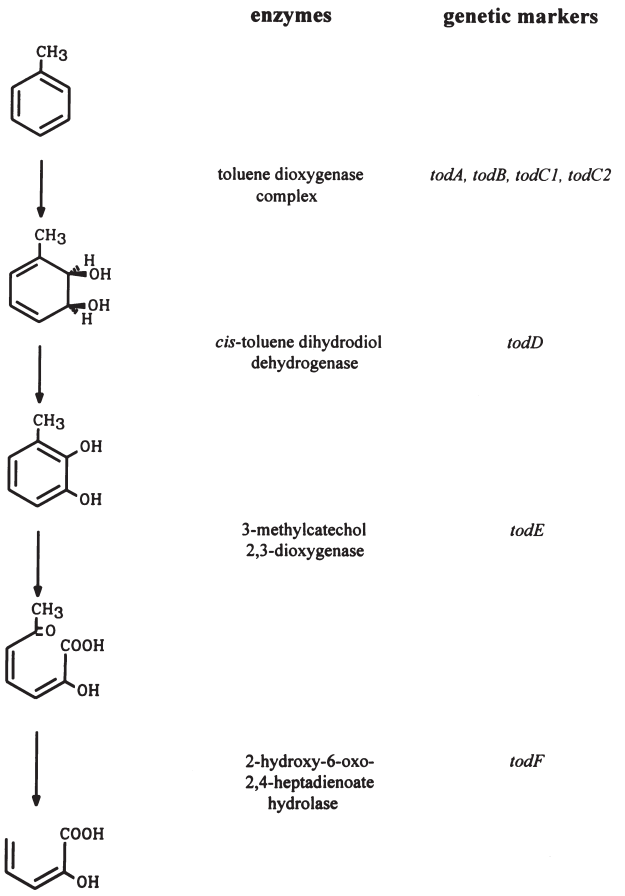


FIGURE 3. Initial steps in the pathway for the aerobic degradation of toluene by *Pseudomonas putida* strain F1. More details are found in Zylstra and Gibson (1991).



and Gibson, 1991). Figure 2 is a graphical representation of the main differences among aerobic pseudomonads in the initial steps of pathways of toluene degradation, and illustrates differences among some members of rRNA groups I and II (represented by *Burkholderia cepacia* and *Ralstonia pickettii*). The ring fissions of the dihydroxy intermediates (catechol, protocatechuic acid, and 3-methyl-catechol) occur in different ways. For organisms carrying the TOL plasmid (A) and *P. putida* F1 (E), the cleavage of the catechols results from the action of a 2,3-dioxygenase (*meta* cleavage), while cleavage of the protocatechuate by *P. mendocina* is of the *ortho* type (1,2-dioxygenase). Further details of the initial steps of toluene degradation by *P. putida* F1 are shown in Figure 3.

Tolerance to toluene can develop in cells that normally are killed by contact with solvents. A *P. putida* strain able to grow in the presence of high concentrations of aromatic hydrocarbons was isolated from enrichments. The tolerance was inducible in this strain. Electron micrographs showed that the cells had a periplasmic space wider than that in the nontolerant cells and that the outer membrane integrity was preserved. Tolerance was also acquired to *m*- and *p*-xylene, and the strain gained the capacity of using these two compounds after receiving the plasmid TOL (Ramos et al., 1995). An energy-dependent efflux system may be part of the tolerance of this species to toluene (Isken and De Bont, 1996).

In a personal communication, J.A.M. de Bont has summarized as follows some of the mechanisms of tolerance to solvents: modification of the composition of the head groups of phospholipids (Ramos et al., 1997), of outer membrane proteins (Li et al., 1995; Ramos et al., 1997), of LPS (Pinkart et al., 1996), changes in the rate of turnover of membrane components (Pinkart and White, 1997), and most importantly, alterations in the composition of the fatty acids of phospholipids (Heipieper et al., 1994). This last is a very straightforward response, mostly due to the action of a *cis-trans* isomerase in *P. putida*. This enzyme has been cloned and expressed in *E. coli* (Holtwick et al., 1997). The isomerization is quite fast and does not demand an energy

input. The presence of solvents triggers this activity, but other stress factors (heavy metals, high temperatures, low pH, and water stress due to salt) can act as inducers (Heipieper et al., 1996).

As expected, plasmid TOL, which was discovered in a strain now classified as *P. putida*, has continued receiving much attention. There are interesting relationships between the pathways encoded by TOL genes and those chromosomally located in the host. Thus, when growing on unlimited amounts of toluene, a *P. putida* carrying TOL lost the capacity of using xylenes and toluates, but could grow on toluene by using initially the enzymes of the TOL upper pathway and the chromosomal system for benzoate metabolism (Brinkmann et al., 1994). Catabolite repression by succinate and glucose on the TOL enzymes has been examined (Duetz et al., 1994; Holtel et al., 1994).

The catabolism of toluene and *o*-xylene in *P. stutzeri* has been analyzed by gene cloning (Baggi et al., 1987; Bertoni et al., 1996).

In studies on the degradation of polycyclic aromatic hydrocarbons (PAHs), a mixed natural population in which *P. aeruginosa* and *P. putida* were represented was more efficient in the degradation of the poorly soluble components of the mixture than any of the pure cultures isolated from the population. The latter in turn were more active on the more water-soluble compounds (among them acenaphthene, fluorene, phenanthrene, and fluoranthene) (Trzesicka-Mlynarz and Ward, 1995).

Naphthalene degradation by *P. stutzeri* has been found to be catalyzed by enzymes encoded by highly homologous sets of catabolic genes, although the hosts had been allocated to four different genomovars (Rosselló-Mora et al., 1994c).

Many reports in the literature refer to the degradation by *Pseudomonas* species of a number of derivatives of simple aromatic compounds including *p*-cymene (Eaton, 1996, 1997), nitrophenols (Rani and Lalithakumari, 1994; Meulenberg et al., 1996; Michan et al., 1997), and styrene (O'Connor et al., 1995; Marconi et al., 1996; Beltrametti et al., 1997).

Due to their widespread use in a number of applications based on highly desirable physical and chemical characteristics, the halogenated derivatives of hydrocarbons and aromatic compounds occupy an outstanding position in the studies on environmental microbiology. Many of the multitude of halogenated compounds are toxic and, due to their remarkable stability, they tend to persist in the environment, where they are slowly degraded by a number of organisms, under both aerobic and anaerobic conditions. In the aerobic degradation of these recalcitrant compounds, the pseudomonads are among the most active organisms, and some of them also are involved in the anaerobic degradation when nitrate is present as an alternate electron acceptor. The biodegradation of many halogenated organic compounds has been adequately summarized in reviews (Häggblom, 1992; Fetzner and Lingens, 1994).

In view of the fact that anaerobic bacteria are more active in the initial degradation of haloaromatics that are reductively dehalogenated, attempts have been made to combine this property with the ability of aerobic bacteria for active degradation of the aromatic ring. A proper combination of genes in a *P. putida* strain resulted in the metabolism of polyhalogenated compounds by sequential reductive and oxidative reactions (Wackett et al., 1994).

In *P. putida* chloroaromatic and methylaromatic compounds are degraded via *meta*-cleavage pathways. The chlorocatechol produced in the first case usually inactivates the 2,3-dioxygenase, but one strain was developed with a resistant dioxygenase and had the ability to degrade both toluene and chlorobenzene via the *meta*-cleavage route (Mars et al., 1997).

Phenol, monochlorophenols, monochlorobenzoate, dichlorophenols, 2,3,5-trichlorophenol, and several alkylbenzenes, were oxidized by a strain of *P. putida* by means of an inducible toluene dioxygenase. A study of this property revealed not only the remarkable versatility of the species, but also the influence of other factors (e.g., growth rate) on the competence of the cells for use as bioremediation agents (Heald and Jenkins, 1996).

The degradative enzymes that convert these unusual compounds to intermediates of the central metabolism in distantly related hosts frequently show a high level of similarity. A rather extreme case is illustrated by a comparative study of the deduced amino acid sequences of components of the polychlorinated biphenyl degradation systems of the Gram-positive *Rhodococcus globerulus* and of Gram-negative bacteria, which showed a high similarity between the toluene dioxygenase of *P. putida* and the biphenyl dioxygenase of the Gram-positive organism, suggesting a possible transfer of genes crossing the Gram-positive/Gram-negative barrier (Asturias et al., 1995). This is certainly a reminder of the danger of drawing phylogenetic conclusions based on similarities found in metabolic systems involved in the degradation of these unusual organic substrates.

Amino acid catabolism

One aspect that has attracted the attention of biochemists and bacteriologists for many years is the utilization of arginine by the pseudomonads. Among the degradative pathways, the

arginine deiminase ("dihydrolase") system has been used by taxonomists since the early 1960s for differentiation of species. The reactions catalyzed by the system are the conversion of arginine to citrulline, and of citrulline to ornithine, with liberation of ammonia (Slade et al., 1954). Motility of the cells can be activated by air or arginine (Sherris et al., 1957). In the latter case the required energy is provided by the ATP formed in the citrulline to ornithine step. This system is constitutively produced by fluorescent pseudomonads but it is inhibited under aerobic conditions.

A rise of pH in the medium with arginine, because of ammonia liberation, indicates the presence of the dihydrolase system, and this is the basis of one of the determinative methods (Thornley, 1960). This method is still widely used because it is simpler (although less reliable) than methods based on arginine disappearance. Presence of arginine deiminase is not diagnostic for species of the genus *Pseudomonas* (for instance, *P. stutzeri* is negative, while species now assigned to other genera of aerobic pseudomonads are positive).

Several other pathways of arginine degradation have been described for species of *Pseudomonas*. They are characterized by the following key reactions: arginine oxidase in *P. putida* and arginine decarboxylase in fluorescent *Pseudomonas* species (Stalon and Mercenier, 1984), arginine dehydrogenase in *P. aeruginosa* (Jann et al., 1988), and arginine succinyl transferase in fluorescent *Pseudomonas* species and in *P. mendocina* (Vander Wauven and Stalon, 1985; Stalon et al., 1987). The arginine decarboxylase/agmatine deiminase pathway appears to be characteristic of *Pseudomonas*, and it is the source of polyamines (Stalon and Mercenier, 1984). A brief discussion on the importance of these compounds as taxonomic tools will be given below, in the section on taxonomic considerations.

Pseudomonas species use only one of the various agmatine catabolic pathways, in which there is participation of an inducible agmatine deiminase system. Evidence for the presence of arginase (which converts arginine to ornithine and urea) has not been found in any *Pseudomonas* species. Further studies on arginine metabolism by organisms of this genus have included a number of ureido and guanido compounds, with findings that suggest that the corresponding screening may be of taxonomic value (Tricot et al., 1990).

A study of the distribution and induction of guanidinoacetate amidinohydrolase (GAH), guanidinopropionate amidinohydrolase (GPH), and guanidinobutyrate amidinohydrolase (GBH) among some fluorescent species of *Pseudomonas* has shown the presence of GBH in most strains, with arginine as an inducer (Yorifuji et al., 1983). GPH was detected in *P. aeruginosa* only, and the results further indicate that this species lacks the *P. putida* enzyme(s) required for degradation of L-arginine to 4-guanidinobutyrate.

The information collected on the metabolism of arginine and related compounds suggests an interesting phylogenetic scenario. The arginine deiminase pathway may be a primitive remnant from the time when anaerobic conditions prevailed on our planet, and amino acids may have provided both building blocks and energy (Prieto et al., 1992). If so, the appearance of arginine oxidase may have occurred in more recent times.

In summary, in spite of the variety of arginine catabolic pathways among the aerobic pseudomonads, members of the genus *Pseudomonas* are characterized by the presence of the arginine decarboxylase/agmatine deiminase system. The finding of arginine dehydrogenase in *P. aeruginosa* (Jann et al., 1988) would require a survey of other species not yet tested in order to assess its phylogenetic significance.

Lysine catabolism can occur in *Pseudomonas* by at least three different pathways eventually converging to glutarate, which generates acetyl-CoA. For short, these pathways may be called the "oxygenase", the "pipecolate", and the "cadaverine" routes (Chang and Adams, 1971; Miller and Rodwell, 1971; Fothergill and Guest, 1977), and their distribution in species of *Pseudomonas* is indicated in Table 1.

Amino acid biosynthesis

Catabolic pathways offer a larger variety of metabolic routes than the biosynthetic ones. A good example is the diversity of arginine degradative pathways discussed above. In contrast, similar anabolic routes can be used by widely different organisms. Biosynthetic pathways also are interconnected with other pathways and regulatory systems, and consequently there is a selective pressure favoring their conservation, thus preventing perturbations in pathways belonging to the same network. However, taken in combination with its regulatory mechanisms, a given biosynthetic pathway often can be used as a tool for the exploration of distant relationships among organisms.

The regulatory mechanisms of biosynthetic pathways of some aliphatic amino acids in the pseudomonads have interesting phylogenetic implications. The regulation of activity of aspartokinase and homoserine dehydrogenase is clearly different in organisms of the fluorescent group and species now assigned to other genera. Long ago it was found that *P. stutzeri* and *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) resembled the fluorescent species (Cohen et al., 1969). In fact, this was one of the first findings suggesting a phylogenetic relationship between *P. stutzeri* and other

TABLE 1. Distribution of lysine catabolic pathways in some fluorescent *Pseudomonas* species and *Burkholderia cepacia*[a]

Lysing catabolic pathways	*P. aeruginosa*	*P. fluorescens*	*P. putida* biovar A	*P. putida* biovar B	*Burkholderia cepacia*
Oxygenase	–	+	+	+	–
Pipecolate	+	+	+	–	+
Cadaverine	+	+	–	–	–

[a]For symbols see standard definitions. Taken from Palleroni (1984); data from Miller and Rodwell (1971), Chang and Adams (1971), and Fothergill and Guest (1977).

species of *Pseudomonas*. However, *B. cepacia* also showed similarities with the fluorescent pseudomonads, in spite of its phylogenetic position in a different branch of *Proteobacteria*.

The multibranched pathway of biosynthesis of aromatic amino acids has offered not only more variations in biochemical details, but also a richer source of regulatory information. These are the reasons for which Jensen and his collaborators chose the interconnected pathways as a model for the study of phylogenetic relationships among the aerobic pseudomonads. The results of this work, which has direct taxonomic implications (Byng et al., 1980, 1983; Whitaker et al., 1981b) were in agreement with the subdivision of the pseudomonads into five so-called rRNA homology groups (Palleroni et al., 1973). In fact, the enzymatic assays are simpler than the hybridization methods of ribosomal similarity studies, which makes the former good determinative tools indeed.

Glutamine synthetase, an important enzyme involved in nitrogen metabolism, has been subjected to immunological comparative studies that were expressed in combination with the results of nucleic acid hybridization experiments (Baumann and Baumann, 1978). These studies, aside from representing further confirmation of the internal subdivision of the pseudomonads, have useful determinative applications.

Genetics

Some of the *Pseudomonas* species have attracted a great deal of attention from bacterial geneticists because of their widespread occurrence, their biological and medical importance, their nutritional and biochemical versatility, and the simplicity of conditions required for their cultivation in the laboratory.

Of them, *P. aeruginosa* is by far the best known from the genetic point of view. Strain PAO ("*Pseudomonas aeruginosa* One") is the one that has been most extensively studied. A detailed genomic map including more than 360 genes is now available (Holloway et al., 1994). This map represents the last of a number of reports from the Department of Genetics of Monash University, Clayton, Australia (see below), in combination with work performed elsewhere on the construction of physical maps. Recent additions to the genomic map available for strain PAO include the physical mapping of 32 genes by application of modern molecular techniques. The approach used will increase the number of genes included in databases, where now nearly 40% of the genes of the species are represented (Liao et al., 1996).

A second strain of *P. aeruginosa*, PAT ("*Pseudomonas aeruginosa* Two"), is also familiar to geneticists, and its map closely resembles that of PAO. The strains have different geographical origin, and their similarity is a confirmation of the homogeneity of the species already noticed by many taxonomists.

The three best-known systems of genetic exchange, namely, conjugation, transduction, and transformation, have been observed in strains of *P. aeruginosa*. Conjugation and transduction have been most important in genetic studies of this species. In contrast, transformation occurs in *P. stutzeri, P. mendocina*, and other nonfluorescent members of the genus under natural conditions. Conjugation, which results in transfer of substantial chromosomal segments, has been the most effective source of information for mapping purposes in *P. aeruginosa* genetic studies. The transfer depends on the chromosomal mobilizing ability (cma) of some plasmids, of which FP2 has been used extensively, and to a lesser extent, FP5, FP39, and FP110. Each of these fertility plasmids has one predominant attachment site, and this was the main reason that delayed the demonstration of circularity of the map. This was finally achieved based on 2- and 3-factor crosses with cma plasmids FP2, FP5, FP110, and R68.45 (Royle et al., 1981).

An approach recently introduced in the study of bacterial genomes combines the action of endonucleases that infrequently cut the chromosome, with pulsed-field agarose gel electrophoresis (PFGE), which makes possible the resolution of large DNA fragments. A limited number of cuts per genome is desirable for genomic restriction mapping (McClelland et al., 1987), with clear advantages over frequently cutting endonucleases for the production of fingerprints characteristic of different taxa (Mielenz et al., 1979;

Dobritsa, 1985; Sorensen et al., 1985). Application of this principle has resulted in a practical method for constructing and analyzing macrorestriction patterns of 234 strains of different species of aerobic pseudomonads (Grothues and Tümmler, 1991). The restriction nucleases *AsnI, DraI, SpeI, XbaI*, and *PacI* are among the most appropriate by being specific for AT-rich regions (which are less common than GC pairs in *Pseudomonas*), or for sites including the extremely uncommon tetranucleotide CTAG (McClelland et al., 1987).

The macrorestriction patterns are compared for the number and position of the bands, and use of appropriate equations often indicates a correlation between the estimated similarities of fingerprints and conventional taxonomic groupings. The results obtained by Grothues and Tümmler (1991) in general confirm the classification of pseudomonads based on extensive numbers of phenotypic properties and nucleic acid hybridization studies.

Comparisons involving mol% G + C content of the chromosome, codon usage, and genome size can be used for the calculation of similarity coefficients useful for determinative purposes. The genome restriction patterns also have the practical advantage of helping in epidemiological studies. Differences in these parameters were found between strains susceptible or resistant to multiple antibiotics (Yamashita et al., 1997).

Differences in restriction fragment size distribution may result from various chromosomal rearrangements and/or mutations at the restriction sites, which suggests that the methodology based on restriction fingerprints should be supplemented with other approaches to determine the degree of similarity of the fragments. Large genomic rearrangements have been observed in *P. aeruginosa* strains isolated from clinical samples or from the environment (Schmidt et al., 1996c). The former came mostly from cystic fibrosis cases. A 95-kb plasmid was detected in environmental strains, and it was integrated in the chromosome in cystic fibrosis strains. Exchange of DNA blocks and large DNA inversions led to divergence of clones in this species. The presence of inversions only in cystic fibrosis strains suggests that this niche causes or tolerates substantial changes in the genome (Römling et al., 1997).

A study on pathovars of fluorescent plant pathogenic pseudomonads (Grothues and Rudolph, 1991) indicated that two strains belonging to the same pathovar, but of different origins, can give almost identical restriction fingerprints. However, this is not always the case. Pathogens of wide host range give more diverse restriction patterns than those of restricted host range. The methodology was also used for the examination of representative strains of *P. stutzeri*, a species notorious for its heterogeneity (Stanier et al., 1966; Palleroni et al., 1970). The results show a marked correlation of genome structure with fatty acid composition, and with data of nucleic acid hybridization experiments (Rainey et al., 1994b). A high degree of heterogeneity in macrorestriction patterns was also observed by another group of workers, which did not correlate with the subdivision of the species in genomovars. The marked heterogeneity of this species is attributed, at least in part, to large chromosomal rearrangements (Ginard et al., 1997). The results of these two groups also indicate that the genome size of *P. stutzeri* ranges from 3.4–4.64 Mba for the strains subjected to their studies.

Pseudomonas species other than *P. aeruginosa* have been less rewarding subjects for chromosomal mapping. This is unfortunate in the case of a species like *P. putida*, which has been extensively investigated from the biochemical viewpoint. This species has presented serious problems for the development of a satisfactory chromosome transfer system, and, consequently, a less detailed chromosome map is available (Mylroie et al., 1977; Strom and Morgan, 1990). Circularity of the *P. putida* map strain PPN (related to strain ATCC 12633, which was the subject of many metabolic studies for many years) has been demonstrated (Dean and Morgan, 1983). A genetic map of *P. syringae* pathovar *syringae* is also available (Nordeen and Holloway, 1990). The situation is even more regrettable in the case of the very complex species *P. fluorescens*, with many strains extensively known for their phenotypic properties. A good starting point now is the report on a complete physical map of a strain of the species with a genome of 6.63 Mbp. A total of 139 restriction sites and 31 genes have been located in the map (Rainey and Bailey, 1996). Similarly, an efficient mutagenic procedure using electroporation for *P. fluorescens* and a transposon delivery vector has been described (Artiguenave et al., 1997). A *recA* mutant has been obtained, and sequence studies have shown that the chromosomal organization was very similar to that of *P. aeruginosa* and *Azotobacter vinelandii*. The regulatory region and the structural gene differed from those of *Burkholderia cepacia*. By insertion of a kanamycin cassette in the *recA* gene, the mutant obtained had an increased UV sensitivity and was much impaired in its recombinatorial activity (De Mot et al., 1993).

A *recA* mutant strain of *P. stutzeri* is also available (Vosman and Hellingwerf, 1991). It is completely deficient in natural transformation with chromosomal DNA and it is sensitive to UV and methyl-methane sulfonate. The wild-type gene complements an *E. coli recA* mutant (Bennasar et al., 1996).

Plasmids, phages, bacteriocins

Plasmids are important components of the genetic makeup of *Pseudomonas*. Some of them act as fertility factors, some (R plasmids) may impart resistance to various agents, and others confer the capacity of degradation of unusual carbon sources, thus contributing to the nutritional versatility that is a striking feature of many members of the genus. A large number of *Pseudomonas* plasmids have been described in the past decades, and it is impossible to cover all references here. Some of the plasmids are mentioned in other sections of this chapter. Examples of the properties of *Pseudomonas* encoded by plasmid genes are:

1. Resistance characters to antibiotics and other antibacterial compounds, such as carbenicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, tobramycin, sulfonamides;
2. Resistance to chemical and physical agents (borate, chromate, various metal ions, organomercurials, tellurite, ultraviolet radiation);
3. Resistance to bacteriophage propagation; interference with lysogenization by some temperate phages; DNA restriction and modification;
4. Resistance to bacteriocins.

Various other characteristics due to plasmid genetic determinants are the chromosome donor ability, donor-specific phage susceptibility, inhibition of bacteriocin production, fertility inhibition, incompatibility with other plasmids, and, most important for some species, utilization of various organic compounds not normally used for growth by species other than the aerobic pseudomonads. Occasionally plasmids are found that confer on the cells the capacity to use simple organic compounds. Thus, for instance, a *P. fluorescens* plasmid that carries genes of resistance to ampicillin, kanamycin, and streptomycin also allows the host to grow on malonate because of a plasmid gene encoding malonate decarboxylase. The plasmid (pPSF1) can be transferred to *E. coli*, where the ability to grow on malonate can also be expressed (Kim and Kim, 1994).

Plasmids can be classified most effectively by incompatibility, that is, by the incapacity of a given plasmid to coexist in the same cell with other plasmids of the same group. In *Pseudomonas*, at least 10 incompatibility groups have been defined (Jacoby, 1977; Korfhagen et al., 1978). Useful information on *P. aeruginosa* plasmids may be found in a chapter by Jacoby (Jacoby, 1979). In general it can be stated that most of the research with *Pseudomonas* plasmids has been focused on the fluorescent species, and the information on other species is fragmentary.

Plasmids of the various incompatibility groups have different host ranges. The widest range is that of the IncP-1 plasmids, while those of groups 2 and 5–9 are more specific. IncP-2 plasmids include R factors and plasmids carrying genes for the degradation of unusual carbon compounds. Some of them are among the largest of *Pseudomonas* plasmids, exceeding 300 MDa, while the majority range from 10–60 MDa. Outside of the IncP-2, degradative plasmids are also found in the P-9 group.

Degradative plasmids named CAM, OCT, SAL, NAH, TOL, and XYL are involved in the degradation of camphor, *n*-octanol, salicylate, naphthalene, toluates, and xylene, respectively, and have received much attention because they confer to the cells the capacity to degrade environmental pollutants. The naphthalene degradative enzymes of plasmids identified in *P. fluorescens* are also involved in the degradation of high molecular weight polyaromatic hydrocarbons other than naphthalene (Menn et al., 1993).

Of the above-mentioned plasmids, TOL, which was originally isolated from a strain now classified as *P. putida*, is the best known and it still receives a great deal of attention by bacteriologists. An excellent review of the properties of TOL and naturally occurring variants is available (Assinder and Williams, 1990). The upper and lower operons of this plasmid seem to have different origins. In support of this hypothesis, it has been found that their respective codon usages are different, but they are the same for the genes within each operon (Harayama, 1994). TOL can be transferred from cell to cell by conjugation, which occurs at a sufficiently high rate to maintain the plasmid in a dense microbial community without the help of selective pressures (Smets et al., 1993).

Comai and Kosuge (1980) have presented evidence for plasmid involvement in the oxidative degradation of tryptophan to the plant hormone indoleacetic acid (IAA) by *P. savastanoi*. Concentrations of IAA higher than those normally found in plant tissues result in gall formation (Gardan et al., 1992

The range of antibiotic resistance determined by plasmids and the mechanisms by which the resistance is manifested are similar to those found in *E. coli*. Some plasmids are transferred from cell to cell by conjugation; others lack this capacity, but some of them can be mobilized by other conjugative plasmids.

Many different lytic and temperate bacteriophages have been identified in *Pseudomonas*, particularly in *P. aeruginosa*. Their morphological diversity is at least as great as for phages of other bacterial genera. Lysogeny is a very common phenomenon in *P. aeruginosa*, and transducing phages have been very useful in linkage studies. Most *Pseudomonas* phages contain double-stranded DNA, but some are RNA phages,

including one with double-stranded RNA (Semancik et al., 1973; Vidaver et al., 1973). The sequence of *P. aeruginosa* single-stranded RNA phage PP7 has been determined and the results suggest that the phage is related to coliphages but branched off before the coliphages diverged into separate groups (Olsthoorn et al., 1995).

An example of a rather unusual application of a *P. stutzeri* bacteriophage has been its use for the selection of denitrification-negative mutants. The procedure takes advantage of the fact that the phage (phi PS5) adsorbs to the outer membrane protein NosA. Mutants defective in NosA production do not grow with N_2O and are resistant to the phage (Clark et al., 1989).

Even though host specificity is the rule, some bacteriophages attack different but related species, with interesting taxonomic implications (M.P. Starr, personal communication). This approach to bacterial classification was attempted many years ago by Billing, in an effort to improve the methodology for the differentiation of phytopathogenic pseudomonads (Billing, 1963, 1970a). The sensitivity tests were not very useful in themselves, but they were valuable as a complement of the biochemical tests of identification. Pathovars of *P. syringae* isolated from pear could be distinguished by phage sensitivity but not by serological tests from the strains isolated from cherry. On the other hand, pathogenic and saprophytic pseudomonads could not be distinguished by their lysotypes (phage types or phagovars) (Crosse and Garrett, 1963).

Phage sensitivity can also be used successfully as a method of typing *P. aeruginosa* strains, but the procedure is not considered sufficiently reliable to be used by itself as an epidemiological tool without comparison to other typing procedures (Brokopp and Farmer, 1979).

Some factors required for phage nucleic acid replication appear to have been conserved through evolution in some groups of Gram-negative bacteria. One of the elements required for *in vitro* replication of sex-specific single-stranded RNA coliphage Qβ is the so-called host factor (HF). This is a heat-resistant RNA-binding protein of molecular weight 12,000, usually present in *E. coli* as an hexamer (Franze de Fernández et al., 1972). In *P. putida*, a polypeptide of molecular weight 11,000 gives an immunological cross-reaction with HF and allows Qβ replication *in vitro* (DuBow and Blumenthal, 1975). Material cross-reacting with *E. coli* HF antiserum was found in extracts of *P. aeruginosa, P. fluorescens, P. putida*, and in other species of different rRNA similarity groups (Palleroni, 1984)), but the material was heat stable only when it came from organisms of RNA similarity groups I and V (*Pseudomonas, Xanthomonas*, and *Stenotrophomonas*), which are located in neighbor branches in the *Gammaproteobacteria* (DuBow and Ryan, 1977).

Bacteriocins are proteins produced by some bacterial strains, which have a lethal action on other strains of the same species. They have been frequently detected in *Pseudomonas*, and in *P. aeruginosa* they have been named pyocins (from the old name *P. pyocyanea*) and aeruginosins, although both names are improper. Pyocin can be mistaken for pyocyanin, the blue diffusible pigment characteristic of the species, and aeruginosin has been used for two red pigments produced by some strains of *P. aeruginosa* (aeruginosins A and B) (Holliman, 1957; Herbert and Holliman, 1964).

Different types of *P. aeruginosa* bacteriocins have been described. One resembles bacteriophage contractile tails, a second one has the appearance of slender, flexuous rods, and a third (S type) is amorphous and is sensitive to proteolytic attack (Govan, 1974a, b). A novel S-type pyocin has been characterized by molecular techniques (Duport et al., 1995).

Bacteriocin typing can be performed in two ways, which are based on production of these elements, or on sensitivity to them, respectively. The first approach is more commonly used, and it requires a collection of sensitive strains (Govan, 1978; Brokopp and Farmer, 1979). Bacteriocin production by species other than *P. aeruginosa* has been reported in a few instances (Vidaver et al., 1972; Smirnov et al., 1984). In a study on bacteriocin typing of unknown *P. syringae* strains, the specificity was uneven, and the highest level was shown for the case of the phaseolicins of *P. syringae* pathovar *phaseolicola* (in this treatment described as a pathovar of *P. savastanoi*) (Vidaver et al., 1972). In an extension of these studies, the correlation found between bacteriocin type and host plant of origin of phytotoxin production was rather poor (Vidaver and Buckner, 1978). The approach is probably susceptible to further refinements, since 86% of the strains examined were able to produce bacteriocins.

Antigenic structure

Again here, the literature is dominated by the number of papers dedicated to *P. aeruginosa*, reflecting in this case, as in others, the medical importance of this species.

Agglutination of intact *P. aeruginosa* cells can be caused by specific antibodies elicited in animals by specific components that are similar to the O-antigens of other Gram-negative bacteria. The heat-stable O-antigen, considered the most stable marker for *P. aeruginosa*, is represented by one of the components of the lipopolysaccharide (LPS). The specificity is related to the composition of the polysaccharide chains (the O antigens themselves) projecting to the outside of the

cells. The LPS of *P. aeruginosa* has O-specific polysaccharide chains with unbranched oligosaccharides including amino sugars that are not acetylated (Wilkinson, 1983).

P. aeruginosa shows a marked serological diversity. The LPS composition determines at least 17 heat-stable O antigens, and antisera are adequate for serotype identification, in spite of variations in titer and specificity. In experiments using a panel of 48 monoclonal antibodies against eight of the serotypes, various degrees of activity were shown by some of the antibodies, one of which bound to all serotype strains and also to strains of *P. fluorescens* and *P. putida* (Gaston et al., 1986). A further refinement of this approach showed that the antigenic specificity of various parts of the LPS molecule could be clearly demonstrated by preparing monoclonal antibodies recognizing the inner core, outer core, and lipid A regions of LPS. Antibodies to the complex (lipid A + core + one repeat of the O-specific polysaccharide chain) reacted with a lower number of serotypes than the antibodies recognizing the outer core without the oligosaccharide. The specificity was even lower for the antibodies that recognized the inner core, since they reacted not only with the largest number of serotypes tested, but with all the other Gram-negative species included in the experiment (de Kievit and Lam, 1994).

Mild heating of a cell suspension reduces the agglutinability by specific antisera, but the property can be fully recovered by a more intense treatment (100–120°C for 2–2.5 h). The alginate of mucous strains that can be selected under appropriate conditions (Govan and Fyfe, 1978) or isolated from cystic fibrosis cases (Doggett, 1969) does not interfere with the O-antigenicity.

In addition to the immune response against *P. aeruginosa* LPS, which in its early manifestation consists of IgM antibodies (Høiby, 1979), there is a humoral response to cross-reactive antigens that are present in other, mostly Gram-negative, bacteria (Høiby, 1975). A heat labile antigen common to a wide range of bacteria has been isolated from *P. aeruginosa* and shown to be an acidic protein composed of subunits of molecular weight 62,000, present in the cytoplasmic fraction of the cells (Sompolinsky et al., 1980a, b).

Various other antigens have been identified in *P. aeruginosa*. Heat-labile surface antigens are represented by flagella and fimbriae (Bradley and Pitt, 1975; Pitt and Bradley, 1975). An extracellular slime can elicit the production of an agglutinin, and exoenzymes such as phosphatases, proteases, and phospholipases can also act as antigens. The outer membrane protein OprF was subjected to epitope mapping, and, as mentioned before, the fact that it is a major component suggests that it may be a good candidate for use as a vaccine and as a target for monoclonal antibodies for immunotherapeutic applications and for diagnosis (Rawling et al., 1995).

Isolation and characterization of monoclonal antibodies for outer membrane antigens have been described (Hancock et al., 1982). As a practical identification tool for the identification of glucose oxidizing *Pseudomonas* species, Mutharia and Hancock (1985) have proposed the use of a monoclonal antibody (MA1-6), specific for a single antigenic epitope on the outer membrane lipoprotein H2 of *P. aeruginosa*. The epitope was detected in all 17 serotype strains of *P. aeruginosa*, in numerous clinical isolates of the same species, and in other *Pseudomonas* species but not in species of other rRNA similarity groups (Palleroni, 1984). Two strains of *P. aeruginosa* (out of a total of 52) failed to give the reaction. In a *P. aeruginosa* collection that included 30 environmental isolates, no major differences in outer membrane proteins (other than quantitative variations in lipoprotein H2 content) or lipopolysaccharide patterns were observed when compared with those of previously studied clinical isolates (Hancock and Chan, 1988).

Similar conclusions on the advantages of using monoclonal antibodies against outer membrane proteins, in addition to other tools useful for determinative purposes, have been reached from experiments on the detection of the lipoprotein I gene in species of *Pseudomonas* (De Vos et al., 1993), as an extension of the original observations by Saint-Onge et al. (1992).

Many schemes of serotyping have been proposed, but the system of Habs (1957) has gained wide acceptance and, with some modifications, is in general use. Habs defined 12 somatic groups that could be identified by agglutination tests. Different additional proposals and modifications include the addition of 5 O-groups. Standard strains and typing sera are commercially available.

In the opinion of Brokopp and Farmer (1979), serological typing of *P. aeruginosa* based on the O-antigens produces more reliable evidence for relatedness than can be obtained by other, less specific typing methods. Thermolabile surface antigens can also be used for serotyping, and various methods based on flagellar (H) antigens have been proposed (Verder and Evans, 1961; Lányi, 1970). These approaches have not been widely accepted, the main reason being the difficulties encountered in the preparation of specific flagellar antisera (Brokopp and Farmer, 1979).

For epidemiological purposes, serotyping is the most important typing method and, as usually practiced, consists of the reaction of a cell suspension toward a standard set of antibody preparations. Serotyping can be supplemented by

other typing methods, such as production and sensitivity to bacteriocins and lytic phages, biotyping (or characterization of strains by their biochemical and physiological properties) and antibiograms, and sensitivity to antibiotics.

To these procedures we now have to add modern techniques based on molecular concepts. One of them is the arbitrary PCR fingerprinting of strains, which provides a simple and practical typing approach considered to be more discriminatory than the traditional serotyping scheme, although the maximum discriminatory power is achieved by a combination of both methodologies (Hernández et al., 1997). Comparative typing of *P. aeruginosa* also has been carried out by random amplification of polymorphic DNA or pulsed-field gel electrophoresis of DNA macro-restriction fragments (Renders et al., 1996), to which we can add the fingerprinting of whole cell proteins (Khan et al., 1996), and studies on comparative ribotyping and genome fingerprinting (Bennekov et al., 1996).

In the field of fluorescent plant pathogenic pseudomonads, the serological approach has had variable success, and has not helped substantially in the circumscription of nomenspecies. The lack of a solid taxonomic frame of reference has often made it very difficult to interpret the resolving power of the serological techniques. Otta and English (1971) were unable to define a precise serological differentiation of virulent strains from non-virulent ones. On the other hand, species-specific antigens could be identified in *P. syringae* pathovar *phaseolicola* (described here as a pathovar of *P. savastanoi*) (Guthrie, 1968) and in *P. syringae* pathovar *lachrymans* (Lucas and Grogan, 1969a, b). *P. syringae* strains isolated from different groups of host plants could be distinguished immunologically (Otta, 1977). In general, however, the reproducibility of serological data by different authors using different strains of the same nomenspecies appears to be rather poor.

Susceptibility to antibiotics

Many aerobic pseudomonads are resistant to a number of antibacterial agents. This subject is of particular medical importance because members of this group are serious opportunistic human pathogens and are often isolated from patients and clinical materials. The capacity to resist antibiotics is included in some of the descriptions of new taxa.

As expected, most of the available information on *Pseudomonas* refers to *P. aeruginosa* because of its importance as a serious opportunistic human pathogen. The antibiotics that are most effective in the treatment of *P. aeruginosa* infections include some β-lactams, such as carbenicillin and ticarcillin, third-generation cephalosporins (cefsulodin, cephoperazone, ceftazidime), the synthetic monocyclic β-lactam aztreonam, carbapenems (among them the extremely broad-spectrum semisynthetic imipenem), the aminoglycosides (gentamicin, tobramycin), and the quinolones (ciprofloxacin). In practice, *P. aeruginosa* infections are treated with a mixture of tobramycin and some β-lactams active against *Pseudomonas*, such as piperacillin or azlocillin. Various quinolones are in use for the treatment of chronic or mild corneal or urinary infections.

P. aeruginosa and other fluorescent *Pseudomonas* species are in general resistant to β-lactams other than those indicated above. Carbenicillin is moderately effective and was extensively used in therapeutic applications either by itself or in mixtures with aminoglycosides. Carbenicillin produces cell enlargement and filament formation, but the sensitivity of the cells is not very high, and years ago the emergence of resistant *P. aeruginosa* mutants was reported (Lowbury et al., 1969; Gaman et al., 1976). The mucoid strains that are frequently found in infections in cystic fibrosis patients are somewhat more resistant to the antibiotic.

Resistance to carbenicillin may be due to β-lactamases coded for by genes carried by plasmids of the incompatibility group 1 (IncP-1) that can be transferred to *E. coli* (Lowbury et al., 1969), but similar genes may be found in host restricted IncP-2 plasmids (Bryan, 1979). Seven different kinds of β-lactamases have been identified in 24 *Pseudomonas* plasmids belonging to at least eight of the incompatibility groups (Jacoby and Matthew, 1979). β-lactamase genes are not invariably carried by plasmids and, in fact, there is in *P. aeruginosa* a constitutive penicillinase (Furth, 1975) and an inducible cephalosporinase (Sabath et al., 1965; Sykes and Matthew, 1976) whose genetic determinants are chromosomal.

Even though the third-generation cephalosporin ceftazidime is considered to be highly effective against many *P. putida* strains (Yang et al., 1996b), a metallo-β-lactamase gene *blaIMP* was detected by PCR in a number of Gram-negative organisms, among them resistant strains of *P. fluorescens* and *P. putida* (Senda et al., 1996). It is also interesting that the response to β-lactam antibiotics in *Pseudomonas* can result in their utilization as substrates for growth. A soil strain of *P. fluorescens* was able to grow at the expense of benzylpenicillin as carbon, nitrogen, and energy source (Johnsen, 1977). This property also is present in pseudomonads now assigned to different genera (for instance, *Burk-holderia cepacia*) (Beckman and Lessie, 1979).

Aside from the production of enzymes such as the β-lactamases, the pseudomonads are able to resist many

antibiotics by mechanisms such as a low cell wall permeability, the production of modifying enzymes, and efflux systems. The most abundant porin in the outer membrane, OprF, is probably very important in reducing the permeability to antibiotics, although other porins may be involved.

Enzymes capable of modification of aminoglycosides as a resistance mechanism have been described, and they correspond to the types already known for the enteric bacteria. These enzymes catalyze phosphorylation, adenylation, or acetylation of the antibiotics, although the resistance of *P. aeruginosa* to the aminoglycosides may be largely nonenzymatic.

A useful and comprehensive review on multidrug efflux systems present in both eucaryotes and bacteria has been published by Paulsen et al. (1996). Drug accumulation and efflux system studies suggest that *P. aeruginosa* has at least two different proton motive force-dependent efflux systems (Li et al., 1994, 1995). A chromosomal operon was discovered that confers multidrug resistance to this organism, with three genes, *mexA, mexB*, and *oprM*, encoding for components of a transport system originally shown to be able to correct a defect in iron metabolism (Poole et al., 1993a, b; Ryley et al., 1995). Prior to this work, it was known that OprM was involved in conferring resistance to multiple drugs, and it was later confirmed as a component of the efflux system. The operon *mexAB/oprM* is inducible under iron-limiting conditions, and is co-regulated with components of the pyoverdin-mediated iron transport. Mutants lacking *mexA* or *mexB* are unable to grow under conditions of iron limitation in the medium. All *P. aeruginosa* strains that have been examined have the operon, although its components seem to operate with unequal efficiencies according to the strain.

Mutations in the operon result in intracellular accumulation of chloramphenicol, tetracycline, norfloxacin, benzylpenicillin, carbenicillin, and quinolones. A second efflux system was later identified (*mexGC-mexD-oprJ*) encoding proteins that prevent the cytoplasmic accumulation of quinolones, tetracycline, chloramphenicol, and the cephems (Li et al., 1994,1995; Liu et al., 1995b; Poole et al., 1996).

The most common mechanisms of gentamicin resistance in *P. aeruginosa* are enzymatic modifications of the antibiotic consisting of *N*-acetylation, O-adenylation, and, to a much lesser extent, phosphorylation. These properties are controlled by plasmid genes, mainly IncP-2 plasmids (Bryan et al., 1972, 1973, 1974; Jacoby, 1974a, b). Permeability factors may also be involved in the case of gentamicin and other aminoglycosides (Mathias et al., 1976; Bryan, 1979).

Polymyxins are very active against *P. aeruginosa* and other pseudomonads when tested *in vitro*, but their efficacy *in vivo* is limited. Resistance is unstable, and this condition seems to correlate with an increase in EDTA tolerance. Gilleland and Murray (1976) noted in these variant strains the disappearance of the particles characteristic of the concave cell wall layer separated by freeze-etching (see above in the section on fine structure), but the particles reappeared by growth in the absence of polymyxin, and acquisition of temporary resistance is accompanied by a decrease in the phosphorus content of the outer membrane.

Sensitivity to metals and metalloids

Copper compounds are extensively used in agriculture, and since only minute amounts are needed for nutrition, the excess can affect higher organisms as well as procaryotes, among which the plant pathogens are of particular interest. In *P. syringae* strains that carry the operon *cop*, copper is excluded by combination with components of the periplasm and in outer membrane. A two-component sensory transduction mechanism similar to equivalent systems in other organisms operates in the regulation of expression of copper resistance, and it has strong similarity with a gene that seems to regulate the uptake or efflux of copper in "*Streptomyces lividans*" (Mills et al., 1993).

Inducible copper resistance in *P. aeruginosa* is encoded by chromosomal genes, although the strains that were examined had plasmids (Vargas et al., 1995b).

In *P. fluorescens* isolated from copper-contaminated soil, a chromosomal locus with determinants for copper resistance and competitive fitness was cloned, and genes responsible for conferring copper resistance and production of cytochrome *c* were identified (Yang et al., 1993a, 1996a). A *P. putida* strain isolated from electroplating effluent could accumulate cupric ions in a concentration as high as 6.5% of its dry weight. The capacity was highest when the culture was previously grown under sulfate-limiting conditions (Wong and So, 1993).

Acid-labile sulfide levels were found to be generally higher in silver-resistant *P. stutzeri* strains than in sensitive ones. The resistance to the metal may be due to formation of silver sulfide complexes, since no complex formation with polyphosphate or metal-binding proteins has been found to be the cause (Slawson et al., 1992).

Three plasmids were found in a silver-resistant strain isolated from a silver mine. The largest plasmid (MW 49.4 × 10^6), which specifies silver resistance, is nonconjugative, but it could be transferred to *P. putida* by mobilization with plasmid R68.45 (Haefeli et al., 1984).

P. fluorescens detoxifies aluminum by elaboration of a soluble, aluminum-complexing metabolite. When iron was

present in the medium, the two trivalent metals were immobilized in a lipid-rich complex containing Al, Fe, and P, after an early stage in which aluminum was found to be associated with phosphatidylethanolamine (Appanna et al., 1995; Appanna and Hamel, 1996). Workers from the same group examined the adaptation of *P. fluorescens* to stress caused by excess of cesium (Appanna et al., 1996), and were also involved in determining the basis for resistance of the species to various metals (aluminum, iron, zinc, calcium, and gallium). These metals were supplied as complexes with citrate, which was completely oxidized. As in the case of the resistance to aluminum mentioned above, the metals appeared associated to phosphatidylethanolamine, which later was found in the lipid-rich complex where the metals were located (Appanna and St. Pierre, 1996). In a study on the mechanism of metal-citrate complexes by *P. fluorescens*, the biodegradation depended on the nature of the complex. Thus, the bidentate ferric, nickel, and zinc citrate complexes were readily degraded, while the tridentate cadmium and copper citrates were not. The latter, similarly to uranium citrate, were neither transported inside the cells nor metabolized by cell-free extracts (Joshi-Tope and Francis, 1995).

About one-half of the *P. aeruginosa* known plasmids confer resistance to mercuric ions (Jacoby and Shapiro, 1977). A plasmid (pPB) was found to confer mercury and organomercurial resistance to a *P. stutzeri* strain. The plasmid had two regions of functional and independently regulated *mer* genes, probably transcribed from different promoters (Reniero et al., 1995). Resistance to chromium, boron, and tellurium was also determined by some *Pseudomonas* plasmids (Summers et al., 1978). After growth in the presence of increasing concentrations of tellurite, strains of *P. aeruginosa* and *P. putida* harboring certain plasmids that determine tellurite resistance accumulated crystalline structures containing tellurium in their periplasmic space. From there, these materials were released into the medium in vesicles that are pinched off the outer membrane (Suzina et al., 1995).

Operons of genes coding for arsenic resistance are usually carried in plasmids. Arsenate is reduced to arsenite, which is eliminated by an export system. A chromosomal operon was identified and cloned in *E. coli* and was able to hybridize with chromosomal of other enteric bacteria and *P. aeruginosa*. This chromosomal operon may be the evolutionary precursor of the plasmid operons, with the advantage of a multicopy system as a means of natural amplification (Diorio et al., 1995).

Antibiotic production

Plant root colonization by certain strains of the species *P. fluorescens* and *P. putida* may result in an enhancement of plant growth, an effect that is accompanied by a selective inhibition of other bacterial species and fungi. In part this action is due to the production of siderophores, which remove iron required by organisms that lack systems for utilization of the ferric siderophores. An example is pseudobactin 358 (produced by *P. putida* WBS358) whose structure, resembling that of other siderophores, has been elucidated (Van der Hofstad et al., 1986). The molecular aspects of iron assimilation by the pseudobactin-producing organisms have been analyzed (Leong et al., 1992).

Fragments of the peptide component of pseudobactin do not have fungicidal activity, although they resemble the antifungal compounds pseudomycins (Okonya et al., 1995). However, the fluorescent organisms do produce antifungal antibiotics. Thus, a strain of *P. fluorescens* produces the antibiotics pyrrolnitrin, pyoluteorin, and 2,4-diacetylphloroglucinol, which are able to suppress root diseases due to fungal pathogens. Of the three compounds, the latter represents an important factor in the control of plant diseases. A 6.5-kb chromosomal fragment has been isolated that contains the genes that encode the biosynthetic enzymes (Bangera and Thomashow, 1996). An examination of this and other molecular aspects has led to identifying a sigma factor encoded by gene *rpoS* that has an influence on the production of the antibiotics, on the biological control activity, and on the survival capacity of the strain on plant surfaces (Fedi et al., 1996). Pyrrolnitrin requires the participation of four genes, the only determinants that appear to be absolutely necessary for the complete biosynthesis (Hammer et al., 1997).

In a *P. fluorescens* strain, the production of the above antifungal compounds and of an extracellular protease can be abolished by mutations of an antibiotic production gene (*apdA*). Interestingly, the sequence of this gene is strikingly similar to that of genes that encode sensor kinases required for the pathogenicity of *P. syringae* pathovar *syringae* and *P. viridiflava*, suggesting that *apdA* encodes a putative sensor kinase component of a classical two-component regulatory system that, in this case, is required for the synthesis of a secondary metabolite (Corbell and Loper, 1995).

A different strain of *P. fluorescens* was able to produce three antibiotics: 2,4-diacetyl-phloroglucinol, pyoluterin, and + (S)-dihydroaeruginoic acid. This last compound (detected for the first time in the reported investigations) inhibits phytopathogenic fungi—among them, *Septoria tritici*

(Carmi et al., 1994). Antifungal compounds produced by *P. fluorescens* include chitinase, cyanide, and pyrrolnitrin, all of which have a protective action on a variety of seedlings against damping-off caused by *Rhizoctonia solani* (Gaffney et al., 1994). Brominated derivatives of pyrrolnitrin were synthesized by a strain of *P. aureofaciens* (in this treatment considered to be a subspecies of *P. chlororaphis*) (Van Pée et al., 1983). The characteristics of fluorescent species of the genus *Pseudomonas* that participate in the inhibition of plant root pathogens have been analyzed in a useful review (O'Sullivan and O'Gara, 1992).

Miscellaneous antibiotics produced by fluorescent pseudomonads include (1) karalicin, an antibiotic isolated from a strain in the *P. putida*/*P. fluorescens* cluster, with some inhibitory action on yeasts, and a weak antiviral activity against herpes simplex viruses (Lampis et al., 1996a, b); (2) fluviols, a group of antibiotics with antitumor activity that were isolated from *P. fluorescens* and described (Smirnov et al., 1997); (3) 2-alkyl-quinolones, which inhibit growth of *Helicobacter pylori*, were produced by *P. aeruginosa* strains (Lacey et al., 1995); (4) fluopsins C and F (Shirahata et al., 1970); and (5) obafluorin, a β-lactone produced by *P. fluorescens*, with activity against *Bacillus* species. In spite of its β-lactone structure, this compound is hydrolyzed by β-lactamases (Wells et al., 1984); fosfomycin (phosphonomycin), is produced by a strain of *P. syringae* (Shoji et al., 1986).

An interesting practical application has been found for alginate, which is notorious for causing complications in cystic fibrosis cases. The polysaccharide has been found to be a convenient carrier for seed inoculation with genetically modified *P. fluorescens* strains producing antifungal antibiotics (Russo et al., 1996).

Pathogenicity for plants and mushrooms

Phytopathogenic pseudomonads are allocated to three of the five rRNA similarity groups (Palleroni, 1984). Only the ones in rRNA group I, the present genus *Pseudomonas*, will be discussed here. Various symptoms produced in plants by these organisms such as tumorous outgrowth, rot, blight or chlorosis, and necrosis are the consequence of alterations of the normal metabolism of plant cells by substances excreted by the pathogens. Among these excretions there are toxins, plant hormones, and enzymes capable of attacking various components of plant tissues.

Bibliographic sources of the original papers describing various hydrolytic enzymes produced by phytopathogens can be found in the first edition of the *Manual* (Palleroni, 1984). Various nomenspecies of plant-pathogenic bacteria described in this treatment as pathovars of *P. syringae* have been found to produce phytotoxins capable of producing disease symptoms in susceptible plants. Table 2, taken from published information (Durbin, 1992), presents a summary of well-known examples of toxins as well as their respective targets of mode of action. Several phytotoxins have an amino acid of peptide nature (Leisinger and Margraff, 1979), and one of them, tabtoxin, has a β-lactam structure, a rare example among secondary metabolites of *Pseudomonas* (Stewart, 1971; Durbin et al., 1978).

Toxins do not necessarily parallel the host specificity of the respective phytopathogen (Patil, 1974). Their importance as taxonomic tools is very limited but their practical significance is considerable. In certain instances their production correlates with the presence of plasmids, and this has provoked renewed interest in them (Leisinger and Margraff, 1979). Loss of a plasmid by treatment with acridine orange resulted in loss of syringomycin production (González and Vidaver, 1979), but no correlation was observed between phaseolotoxin production and the presence of plasmids in *P. syringae* pathovar *phaseolicola* (described in this treatment as a pathovar of *P. savastanoi*) (Jamieson et al., 1981).

High concentrations of the plant hormone indole-3-acetic acid produced by *P. savastanoi* are thought to be the cause of tumors in plants of the family Oleaceae (Wilson and Magie, 1964).

The interpretations given to data on host–parasite interactions in plant pathology have been deeply influenced by experiments making use of the genetic variability of the host species and of the pathogenic organisms. The main ideas developed during the first three-quarters of the 20th century, and the consequent conceptual changes in the interpretation of the host-pathogen interactions have been reviewed (Ellingboe, 1981).

In recent times, work performed by H.H. Flor with a fungal pathogen of flax gave origin to the gene-for-gene hypothesis. According to this hypothesis, the development of disease or the production of a well-circumscribed hypersensitive reaction in plant tissues depends on matching genes of resistance in the host plants with avirulence genes in the pathogens. In genetic experiments, host resistance was found to be dominant over susceptibility, and the pathogen avirulence dominant over virulence. Matching host resistance with avirulence in the pathogen results in "incompatibility", manifested in a hypersensitive reaction in the host tissues. In the other three combinations (resistant host/virulent pathogen, susceptible host/avirulent pathogen, and susceptible host/virulent pathogen), a "compatible" association is established, which results in disease. These concepts are

TABLE 2. Some toxins produced by phytopathogenic *Pseudomonas* species and pathovars[a]

Species	Toxins	Target or mechanism
P. syringae		
P. syringae pathovar *atropurpurea*	Coronatine	
P. syringae pathovar *coronafaciens*	Tabtoxinine-β-lactam	Glutamine synthetase
P. syringae pathovar *garcae*	Tabtoxinine-β-lactam	Glutamine synthetase
P. syringae pathovar *glycinea*	Coronatine, polysaccharide	
P. syringae pathovar *lachrymans*	Polysaccharides	
P. syringae pathovar *maculicola*	Coronatine	
P. syringae pathovar *morsprunorum*	Coronatine	
P. syringae pathovar *phaseolicola*[b]	Phaseolotoxin	Ornithine transcarbamylase
P. syringae pathovar *savastanoi*[b]	Indole acetate, cytokinins	
P. syringae pathovar *syringae*	Syringomycins	Plasma membrane
	Syringopeptins	
	Syringotoxins	Plasma membrane
P. syringae pathovar *tabaci*	Tabtoxinine-β-lactam	Glutamine synthetase
P. syringae pathovar *tagetis*	Tagetitoxin	Chloroplastic RNA polymerase
P. syringae pathovar *tomato*	Coronatine	
P. tolaasii	Tolaasin	Plasma membrane

[a]For symbols see standard definitions. Modified from Durbin (1992). The nomenclature used is the one preceding the proposal by Gardan et al. (1992).
[b]*P. syringae* pathovar *savastanoi* is described in this treatment as an independent species, *P. savastanoi*, and pathovar *phaseolicola* is considered as a pathovar of *P. savastanoi*; see description of this species in the list of species).

being subjected to scrutiny in the genetic study of a number of pathovars of the fluorescent species *P. syringae* (Vivian, 1992), which has opened new horizons in attempting to understand the role played by the avirulence genes in the gene-for-gene hypothesis.

Pathogenicity for humans and animals

Some references on this subject have been cited on sections of this chapter dealing with outer membrane composition, genetics, pili, and flagella.

As stated by Mekalanos (1992) in an excellent review on environmental signals that control virulence in bacteria, an expanded view of virulence comprises not only the properties directly linked to the pathogenic ability of microorganisms (adherence to host tissues, production of toxins, invasion mechanisms, host defenses) but also other factors involved in "house-keeping" functions. In regard to opportunistic pathogens such as the fluorescent pseudomonads and, in particular, *P. aeruginosa*, the functions in this "gray area" can lead to different interpretations. Thus, competition of *P. aeruginosa* for iron in the host tissues by production of a strong chelator such as pyochelin is on the whole comparable to the reaction of this organism in an iron-deficient artificial medium. However, the fact that pyoverdine, the main iron-scavenging compound produced by *P. aeruginosa*, allows normal growth of pyoverdine-negative mutants in a medium containing human iron-transferrin complex justifies considering pyoverdine production as a component of *P. aeruginosa* virulence complex (Meyer et al., 1996). In addition, iron-bound pyochelin acts as an efficient catalyst for hydroxyl radical (HO·) formation, and it contributes to endothelial cell damage from exposure to the superoxide radical (O_2^-) and H_2O_2 (Britigan et al., 1997).

P. aeruginosa seldom infects healthy individuals outside the hospital environment, and the condition of the host is essential in determining the clinical relevance of this opportunistic pathogen. Strains of *P. aeruginosa* can be isolated from a bewildering variety of sources, since perhaps it is one of the most, if not the most, common species in nature. Numbers are very low in human feces, which may be due to competition

with other species, since the numbers increase significantly because of antibiotic treatments (Levison, 1977).

In chronically debilitated and immunocompromised patients, *P. aeruginosa* is capable of causing serious and even fatal infections. Individuals with extensive burns, or those who have been subjected to surgical procedures, catheterization, and treatment with broad-spectrum antibiotics, are particularly vulnerable targets. Factors involved in the transmission of *P. aeruginosa* in hospitals have been reviewed and analyzed, and preventive measures are being recommended (Doring et al., 1996).

Iron and osmolarity are mentioned by Mekalanos as environmental signals inducing in *P. aeruginosa* the expression of dissimilar virulence determinants. Control of these and other factors provoked by environmental signals usually act at the transcription level (Mekalanos, 1992). *P. aeruginosa* controls the expression of multiple genes coding for virulence factors by means of LasR, a transcriptional activator, a key component that acts in a cell-density manner ("quorum sensing"), with participation of a *Pseudomonas* autoinducer and an *N*-acylhomoserine lactone. Production of virulence factors (exotoxin A, elastase, alkaline protease, alginate, phospholipases, and rhamnolipids acting as extracellular surfactants) can be stimulated in mutants by gene transfer, or by addition of the synthetic *N*-acylhomoserine lactones (Ochsner and Reiser, 1995). Characteristically, quorum sensing becomes maximal at the time the culture is entering the stationary phase of growth (Rust et al., 1996; Albus et al., 1997).

A global activator, GacA, is responsible for the production of exoenzymes and secondary metabolites in *P. aeruginosa*. Its inactivation resulted in reduced formation of the cell density signal and of LasR. Amplification of the *gacA* gene carried on a multicopy plasmid causes early and enhanced production of LasR and of the lactone. GacA is important in the regulation of the synthesis of virulence factors (Reimmann et al., 1997).

P. aeruginosa exotoxin A is a virulence factor resembling diphtheria toxin in its mode of action. It is able to transfer an ADP-ribosyl group to elongation factor 2, causing translation termination, inhibition of protein synthesis, and cell death (Vasil et al., 1977). The toxin is a protein of 613 amino acid residues, of which residues 60–120 are of importance for excretion and contain information for interaction with eucaryotic cells (Lu and Lory, 1996). The toxin is excreted when the cells grow under iron-limiting conditions, and its production is repressed when iron is not limiting, which suggests that the ferric uptake regulator Fur is involved, as in pyoverdine synthesis. However, the situation is more complicated, and accessory iron regulatory systems may be involved (Barton et al., 1996; Ochsner et al., 1996). The genetic picture is quite complicated. A regulatory gene (*ptxR*) is involved, and when introduced in a multicopy plasmid, it increases toxin production four- to fivefold, while other virulence factors remain unchanged.

P. aeruginosa is able to produce several proteases. In fact, the organism can cause serious corneal infections and conditions that complicate diseases of the respiratory tract and burn cases, and in all these instances proteases are responsible for the tissues alterations. The most notorious of the proteases as a virulence factor is elastase, with activity on elastin, which lines the blood vessels. The protease is produced in the cell as a precursor of 54 kDa, but it is excreted as a smaller molecule (39.5 kDa) (Morihara and Homma, 1985). Various other proteases produced by the same organism are the cause of lesions in different human and animal tissues. Their importance in pathogenesis is not easy to determine, but mutants defective in protease formation are known to be less virulent.

Alginate is an exopolysaccharide produced by several species of *Pseudomonas*. Various aspects of this extracellular product already have been mentioned, one of which refers to the relationship to fimbriae, which, like alginate, are involved in adherence to tissues (Baker, 1990). A recent review covers important aspects of alginate biosynthesis by bacteria (Gacesa, 1998). The effect that environmental sensory signals have on alginate production as a virulence factor has been examined by DeVault et al. (1989). Specific factors that have been examined include osmolarity, which affects alginate formation by various fluorescent pseudomonads (Singh et al., 1992), and metals (Kidambi et al., 1995).

The *P. aeruginosa* alginate has been the one most thoroughly studied, but production of the polysaccharide also has been detected and characterized in *P. mendocina* (Govan et al., 1981; Hacking et al., 1983; Anderson et al., 1987; Sengha et al., 1989), *P. putida* (Govan et al., 1981), *P. fluorescens* (Govan et al., 1981; Conti et al., 1994; Smit et al., 1996), and even *P. syringae* (Kidambi et al., 1995). Sequences of alginate genes have been detected in other alginate producers and nonproducers belonging to rRNA similarity group I and in species of other rRNA similarity groups (Palleroni, 1984; Fialho et al., 1990; Fett et al., 1992). Similarity of the genetic determinants of alginate in *P. aeruginosa* and *Azotobacter vinelandii* (Ertesvag et al., 1995; Rehm et al., 1996) suggests either a transfer of these determinants among related members of *Proteobacteria*, or else a relic of common ancestry.

Interest in the *P. aeruginosa* alginate, which was first identified in *Pseudomonas* more than three decades ago (Linker and Jones, 1966), derives mostly from its importance as a virulence

factor causing respiratory tract infections that aggravate the condition of many cystic fibrosis patients.

Alginate is composed of alternating units of mannuronic and L-guluronic acids joined by a β (1,4) linkage, where part of the carboxyl groups of mannuronic acid are acetylated. The polymer does not show the repetition of a certain type of unit, but instead there are regions where one or the other uronic acid predominates.

The steps of alginate biosynthesis are formulated starting with fructose-6-phosphate, going through mannose-6-phosphate, mannose-1-phosphate, GDP-mannose, and GDP-mannuronic acid (steps catalyzed by enzymes encoded by the genes *algA, C, A,* and *D,* respectively), followed by polymerization, acetylation, epimerization, and final export of the alginate polymer (Chakrabarty, 1998). The genetic aspect of the process is quite complicated because it includes participation of many biosynthetic and regulatory proteins encoded by many more genes than the ones above indicated. The genes have been mapped in three regions of the *P. aeruginosa* chromosome (Goldberg, 1992).

The polymer is not used as a reserve material, and its synthesis demands energy that is not recovered by the cells. It is hard to think of a role other than protection of the pathogen against the host defenses. Secretion of alginate represents a detoxification mechanism to protect the bacterial cells against accumulation of toxic energy-rich nucleoside triphosphates under conditions that prevent active multiplication (Chakrabarty, 1998).

P. stutzeri is frequently isolated from clinical materials but rarely causes disease. In the few cases in which it was the cause of infections, the patients often had another serious underlying disease, and they responded to antibiotics (aminoglycosides, some of the β-lactams, or cephalosporins) (Noble and Overman, 1994).

Ecology

Numerous natural materials are good sources for the isolation of strains of *Pseudomonas*, as well as of strains of species of other rRNA groups now assigned to different genera. It is unfortunate that few procedures can be recommended for the selective isolation of *Pseudomonas* species with exclusion of these other organisms. In some of the habitats, strains of *Pseudomonas* species may represent a minority of the microbial flora; however, certain conditions—a pH close to neutrality, organic matter in solution, a temperature in the mesophilic range, a good supply of dissolved oxygen—together with a capacity for rapid growth in the absence of complex organic factors can favor their predominance. Even in media of extremely low nutrient content, pseudomonads occasionally multiply to a considerable extent. Thus, *P. aeruginosa* has been found capable of growth at the expense of minor impurities present in hospital distilled water (Favero et al., 1971).

Strains of many species are ubiquitous, and isolation data often throw little light on their ecology. When dealing with organisms of such versatility, ecological conclusions are particularly difficult to draw. Of the 57 strains of fluorescent *Pseudomonas* species isolated by den Dooren de Jong, 23 had their origin in soil, and with one exception all were classified as *P. putida*, while all other strains were gelatin-liquefiers isolated from water, and were assigned to *P. fluorescens* (den Dooren de Jong, 1926). However, the conclusion that *P. putida* is a soil organism whereas *P. fluorescens* predominates in water could not be supported by an analysis of isolation data of many other strains from the collections of the Department of Bacteriology at Berkeley, California, and of the Seruminstitut in Copenhagen (unpublished observations). Obviously, a decision on this point is not easy in view of the possibility of cross-contamination of materials from the two habitats.

In recent years, more precise data regarding habitats have become available. An early observation suggests that the predominant fluorescent pseudomonads in wheat rhizosphere comprise one of the biotypes of *P. fluorescens* (Sands and Rovira, 1971). Many other reports have confirmed the fact that these organisms proliferate in general in plant rhizospheres, where they seem to have a stimulating effect on plant growth. In part, this may be due to inhibition of plant pathogenic organisms by iron starvation (Kloepper et al., 1980; Sarniguet et al., 1995) and to the utilization of the iron-siderophore complex by the plants (Powell et al., 1980). An additional beneficial effect is suggested by studies on the rhizosphere of plants growing in the Canadian Arctic, in which *P. putida* can grow and stimulate root elongation of plants during spring and winter at 5°C, as shown by using a strain that can survive exposure to −20°C to −50°C. Following growth at 5°C, the strain secreted a protein with antifreeze activity (Sun et al., 1995). A numerical taxonomic analysis of strains of fluorescent pseudomonads associated with the roots of tomato plants has been published (Stenström et al., 1990).

A recent survey indicates that of the species of bacteria isolated from the rhizosphere of wheat, 40% stimulated plant growth, 40% were inhibitory, and 20% had no effect (Lugtenberg and De Weger, 1992). The properties of fluorescent *Pseudomonas* species that are involved in the suppression of

pathogenic species in the root system of plants have been reviewed (O' Sullivan and O'Gara, 1992).

A gnotobiotic system for the study of colonization of plant rhizospheres by *Pseudomonas*, based on seed inoculation and subsequent estimation of different parameters, has shown that many strains were outcompeted by a chosen *P. fluorescens* strain. Slow-growing and autoxotrophic mutants of this strain were at a disadvantage, indicating that growth rate and the capacity for multiplication in the absence of exogenous growth factors were important for the persistence of a strain in the rhizosphere community (Simons et al., 1996).

Many species of *Pseudomonas* have been isolated from *Lolium* leaves (Stout, 1960). *P. fluorescens* has been found frequently, and *Burkholderia cepacia* and *Stenotrophomonas maltophilia* less so (Austin et al., 1978). In their review of the literature, these authors mention isolation of *P. fluorescens* from leaves of *Phaseolus vulgaris, Fagus*, and *Pinus* by various workers, and consider that this species is indigenous to leaf surfaces. Whether the strains can become opportunistic plant pathogens is an open question, but some of the epiphytic pseudomonads have definite pathogenic potentialities, as was suggested by Billing (1970b) for *P. viridiflava*.

Plant pathogenic pseudomonads are normally isolated from lesions in plant hosts, which are natural enrichment cultures. Because of their ecological niches, these bacteria may offer some of the most interesting materials for the study of bacterial speciation (Palleroni and Doudoroff, 1972). In general, animals are not as good sources of *Pseudomonas* species, unless these are involved in infections as opportunistic pathogens.

In different areas, speciation of plant pathogens may proceed at different rates according to the selective forces imposed by various environmental factors. Some pathovars of pathogenic species may develop as distinctive ecotypes according the selective pressures predominating in a given area (Garrett et al., 1966).

Continued association with living hosts is important to the survival of members of the *P. syringae* group (Schroth et al., 1981). The association does not necessarily have to involve lesions, since the pathogen may be able to survive as an epiphyte on hosts as well as on nonhosts (Ercolani et al., 1974). Survival of the *P. syringae* pathovars in soil may not be long, in contrast with other fluorescent species (Schroth et al., 1981).

A brief discussion of a few points related to modern biotechnological applications of *Pseudomonas* strains may be pertinent to the present discussion. It has been reassuring to observe that the introduction of a plant growth-promoting strain of *P. fluorescens* and a genetically modified derivative did not appreciably disturb the indigenous microflora when introduced to the soil (Mahaffee and Kloepper, 1997), and that carbon-starved *P. fluorescens* had a good survival capacity and resistance to stress in this environment (Van Overbeek et al., 1995).

The attractive possibility of using genetic means to improve the metabolic versatility of *Pseudomonas* species for the degradation of environmental pollutants has raised questions about the convenience of keeping the lateral spread of genes in the environment under control. These questions have stimulated the development of ingenious methodologies to quantify the degree of horizontal transfer of genes (Jaenecke et al., 1996), to prevent such transfer (Diaz et al., 1994), and to contain the spread of inoculated strains (Jensen et al., 1993b). Modern biotechnological developments also have resulted in improvements for observation of cells in natural environments. These improvements include the combined use of an appropriate probe and scanning confocal laser microscopy (Moller et al., 1996b), as well as in the construction of a biosensor for monitoring metabolic activities *in situ* with a bioluminescent reporter *P. fluorescens* strain (Heitzer et al., 1994).

Pseudomonas species are important members of natural microbial communities. The capacity of these organisms to react to environmental changes is in part related to their capacity to exchange genetic material. An evaluation of this process may be attempted using a strategy that tries to avoid selecting for the characters acquired by the recipients during exchange. The core of the procedure is the use in donor cells of an engineered *lazZ* reporter gene whose expression is shut down by chromosomal repressors at the levels of transcription and translation. The gene will be freely expressed when it can escape repression in the new host, which, consequently, can be identified (Jaenecke et al., 1996).

The consequences of the high surface/volume ratio in procaryotes are manifested, among other things, in concentrating on their surface components of the liquid medium. Fine-grained minerals precipitate around the cell clusters as consequence of their activities when the cells are either freely suspended ("planktonic"), or attached to surfaces with formation of a biofilm. LPS is important in the attachment, which results in a biofilm. Membrane vesicles containing degradative enzymes may bleb off the cell surface and have a predatory action against cells of surrounding populations, with liberation of nutrients that help the growth of the biofilm, as suggested by results obtained with a *P. aeruginosa* model system (Beveridge et al., 1997).

Enrichment and isolation procedures

Direct isolation of *Pseudomonas* species often can be achieved from many natural materials, especially soil and water. From these materials, direct isolation in solid complex or minimal media is feasible. When heavy contamination with fungi is expected, these media can be supplemented with antifungal compounds such as cycloheximide (Actidione), which is added to the medium after autoclaving (final concentration, 20–50 μg/ml), from a concentrated solution that does not need to be sterilized.

A medium that is frequently used for direct isolation especially by plant pathologists is medium B^3ofKing et al. (1954), which enhances pyoverdin production by fluorescent organisms but is also satisfactory for nonpigmented strains as a general purpose medium. Some selective solid media have been developed based on medium B. An example is a medium that contains penicillin G, novobiocin, and cycloheximide (Sands and Rovira, 1970). These compounds do not inhibit the fluorescent pseudomonads, and their colonies can be identified on the plates by the characteristic diffusible, fluorescent pigment. A modification of this medium later was published for the isolation of *Pseudomonas* species with pectolytic properties (Sands et al., 1972). The medium contains sodium polypectate, and the pectolytic activity is detected by flooding the plates after growth with a solution of hexadecyltrimethylammonium bromide, which precipitates intact pectin. Once identified, the colonies are isolated as soon as possible since the compound is toxic to the cells.

Two selective media containing the detergent lauroyl sarcosine and the antibiotic trimethoprim, and differing in having either Casamino acids (medium S1) or L-asparagine (medium S2), gave good selectivity and detection of fluorescence on initial plating. Of the two media, S1 gave the highest recovery, although S2 was more selective (Gould et al., 1985).

Selective media for the oxidase-negative fluorescent phytopathogens have been described (Sands et al., 1980).

Some methods for the isolation of certain species may go through an initial enrichment step. The lesions produced by plant pathogenic pseudomonads on various plant organs represent a natural enrichment, from which direct isolation can be attempted. The lesions selected should not be too old, when other organisms may have gained access to the diseased tissues. Isolation of denitrifying pseudomonads (*P. aeruginosa, P. stutzeri, P. mendocina*, some biovars of *P. fluorescens*, etc.) after enrichment is often successful. The denitrification experiment can be performed using particular carbon sources as electron donors, and the incubation can be done at a temperature that favors multiplication of the desired organism. However, due to our ignorance of the proper conditions, or unpredicted transformations by other organisms in the microflora, these experiments may fail to give the expected results.

It is convenient to keep in mind that that species of aerobic pseudomonads other than the ones mentioned above may predominate in the enrichment cultures. In the chapter on *Burkholderia* in this *Manual*, a table has been included that summarizes the properties of *Pseudomonas* species that are able to denitrify, together with selected species of denitrifiers of other rRNA groups.

Methods of enrichment and isolation are dispersed in a number of publications, and some of them are very specific for species originally assigned to *Pseudomonas* but now allocated to newly created genera and phylogenetically belonging to other rRNA groups (Palleroni, 1984) than group I. Additional details may be found in the second edition of the treatise *The Prokaryotes* (Palleroni, 1992a), and in the first edition of this *Manual* (Palleroni, 1984).

Maintenance procedures

Most *Pseudomonas* strains can be maintained on slants of common bacteriological media (nutrient agar or other standard complex media, or various chemically defined media with the addition of 0.5% yeast extract and 0.1% lactate or glycerol), with transfers every 1–2 months. Slants can be stored at 4–8°C. The collection examined by Stanier et al. (1966) included a number of strains of fluorescent and nonfluorescent *Pseudomonas* species that had been kept on slants of ordinary media with periodic transfers since the 1920s, and their phenotypic properties appeared to have remained essentially unchanged. In contrast, some strains of *Pseudomonas* species as well as members of other rRNA groups (Palleroni, 1984) are not so easy to maintain for long periods in slants in the refrigerator.

Lyophilization is generally reliable for long-term preservation, particularly when proteinaceous material (e.g., skim milk) is used as an ingredient of the suspending fluid. There are many variations of lyophilization procedures traditionally followed by various laboratories, with good results. A substantial proportion of the cell mass of cultures of *Pseudomonas* species does not survive even the most careful lyophilization procedure, and lyophilization has been replaced in many laboratories by freezing cultures at a temperature of −20°C or

lower (usually from about −70° to −80°C in mechanical freezers or at lower temperatures in liquid nitrogen) after addition of a cryoprotective agent to the liquid before freezing (for instance, 5–10% glycerol, final concentration).

Procedures for testing special characters

Flagellar number and insertion

The observation of these characteristics is best done on smears prepared by the method described by Jessen (1965), combined with results expressed on a statistical basis, as mentioned earlier (Lautrop and Jessen, 1964). On clean microscope slides, thin smears are prepared from dilute suspensions in distilled water of bacteria that have grown in broth agar cultures at 23°C or 30°C for about 18 h. Best results are obtained using freshly poured solid media whose surface has been dried in an incubator for no more than 1 h. The number of cells with flagella in the smears often varies with the degree of dryness of the agar medium in which the strains have been growing. The smears are stained for flagella by the method of Leifson (1960)[4]Only slides with well-dispersed cells and well stained for flagella should be used. The number of flagella on each of the first 50–100 flagellated cells is noted. Cells with flagella at both ends are disregarded, since they probably represent cells in an early stage of their division. Some results of this technique have been mentioned in the early section on flagella and pili (fimbriae).

Screening of nutritional and other physiological properties

The medium described by Stanier et al. (1966) has been used extensively for nutritional screenings and for testing various physiological properties of cultures of pseudomonads. One disadvantage of this medium is that not all cultures tolerate well the heavily chelated composition of the medium. More satisfactory results are obtained with a formulation that is both simpler and safer, such as the medium recommended by Palleroni and Doudoroff (1972),[5]which is most satisfactory for autotrophic and heterotrophic enrichments and as a general medium for cultivation and short-term preservation. Minimal media of similar composition have been described (Schlegel and Lafferty, 1971; Zavarzin and Nozhevnikova, 1977). These last authors recommend supplementation of the medium with minor elements for best results with fastidious organisms, but such additions are unnecessary when dealing with *Pseudomonas* strains.

Good heterotrophic growth in all these minimal media can be obtained by addition of a single organic compound as a carbon and energy source (usually 0.1%, final concentration). Experimental details of the nutritional analysis have been extensively discussed elsewhere (Stanier et al., 1966; Palleroni and Doudoroff, 1972). Ideally, the analysis should be performed under conditions that prevent cross-feeding or competition among the various strains when the medium is solidified with agar and several strains are patched on a single plate. Organic impurities in the agar may also be a serious problem. For critical tests, liquid media may be preferable, although the amount of work involved and the facilities required may be considerably greater. Carbon compounds that are to be tested in different concentrations or that require special treatments for their use in nutritional screenings have been discussed by Palleroni and Doudoroff (1972). Description of many species of aerobic pseudomonads performed at Berkeley included nutritional studies using many organic compounds as substrates. The studies were extended to the plant pathogenic pseudomonads in two laboratories (Misaghi and Grogan, 1969; Sands et al., 1970).

At present, the studies on nutritional properties of various prokaryotic groups are more often performed using commercial kits designed to reduce the labor involved in traditional procedures. Some of them (API, Biotype-100 strips, Biolog GN Micro-plate System, Hayward, CA) have been used in studies on *Pseudomonas*, and the results will be briefly discussed below in the section on taxonomic comments.

Biosynthesis of medium-chain-length polyhydroxyalkanoic acids (mcl-PHAs)

The following observations on the biosynthesis and detection of mcl-PHAs have been provided by Birgit Kessler, a member of B. Witholt's group at the Swiss Federal Institute of Technology in Zürich.

1. As with poly-β-hydroxybutyrate (PHB), accumulation of mcl-PHAs can be elicited in a medium of high C/N ratio, and the polymer can be detected after staining with Sudan Black or by direct observation under phase contrast microscopy. However, granules of the two polymers cannot be differentiated by either procedure, and the identification procedure may involve either gas chromatographic analysis (Timm and Steinbüchel, 1990) or a solubility test. The mcl-PHAs are soluble in acetone, while PHB is not.
2. Assimilation of medium- and long-chain-length fatty acids (saturated, unsaturated, or certain branched and substituted acids) enables the cells to accumulate mcl-PHAs. Some *Pseudomonas* strains (*P. aeruginosa* PAO and *P. putida*

KT2442) accumulate mcl-PHAs when grown on carbohydrates. In contrast, *P. oleovorans* lacks this capacity, but it accumulates mcl-PHAs when grown on alkanes. Growth on butyrate and β-hydroxybutyrate induces production of small amounts of PHAs, and the granules can be seen under phase contrast directly or after staining with Sudan Black.

3. Several species of aerobic pseudomonads have been tested for their capacity of PHB and/or PHA accumulation (Timm and Steinbüchel, 1990). Production of both types of polymers by a single strain did not receive special attention, but recently a strain of an unidentified *Pseudomonas* sp. was found with such capacity when grown on fatty acids or carbohydrates.

Pigment production

Perhaps the most widely used medium for pyoverdine production is the medium B of King et al. (1954). Paton (1959) has described a medium whose preparation involves extensive treatment of the solution and the agar with iron-chelating agents, but the removal of iron is so complete that it often results in inhibition of bacterial growth (Garibaldi, 1967). Garibaldi recommends instead a medium that is based on the well-known iron-chelating capacity of conalbumin, a component of egg-white.[6]Luisetti and collaborators (1972) recommend a medium[7]forthe enhancement of fluorescent pigment production by plant pathogenic and other pseudomonads that fail to fluoresce in the medium B of King and collaborators.

A point frequently incorrectly stated or disregarded altogether in descriptions of procedures for the detection of pyoverdins by fluorescent pseudomonads is the type of ultraviolet lamp to be used for observing fluorescence. Only the "true" fluorescent pigment will fluoresce under a source of short wavelength ultraviolet light (around 254 nm). Some species belonging to rRNA group II (Palleroni, 1984) (*Burkholderia cepacia, B. gladioli, B. caryophylli*) produce diffusible yellow-green pigments that are sometimes mistaken for the fluorescent siderophores.

Medium A of King et al. (1954) is generally recommended for the production of phenazine pigments to which pyocyanin belongs. In our experience, this medium is not always very effective, but unfortunately no alternative can be recommended. Phenazine pigment production, therefore, appears erratic, particularly with cultures that have been kept for a long time under ordinary laboratory conditions.

The blue pigment of *P. fluorescens* biovar IV ("*P. lemonnieri*") can be produced abundantly by fresh cultures in the potato medium[8]familiar to mycologists. As mentioned earlier, the pigment is related to indigo iodine (Starr et al., 1967). Interestingly, this same medium induces the production of indigo iodine by an unrelated organism, *Corynebacterium insidiosum* (Starr, 1958).

Ring fission mechanisms

The *ortho* or *meta* cleavage of two central intermediates in the metabolism of aromatic compounds (catechol and protocatechuate) can be tested by the method originally suggested by K. Hosokawa (Stanier et al., 1966). After growth in a chemically defined medium with an aromatic substrate, the cells are suspended in 0.02 M Tris buffer (pH 8). To each 2-ml portion of suspension are added a drop of toluene and 0.2 ml of either 0.1 M catechol or 0.1 M protocatechuate. A bright yellow color appearing in a short time (usually within a few seconds) indicates a *meta* cleavage. The tubes are shaken for 1 h at 30°C, and then tested for the appearance of β-ketoadipate (indicative of an *ortho* cleavage) by the Rothera reaction, as follows. Solid ammonium sulfate is added to saturation and the pH is brought up to about 10 by the addition of two drops of 5 N ammonium hydroxide. One drop of freshly prepared 25% sodium nitroprusside in water is added. A deep purple color indicates a positive reaction.

Arginine dihydrolase reaction

The method giving the most unequivocal results is the direct one, based on an estimation of arginine disappearance under anaerobic conditions. A bacterial suspension (200 Klett units, as measured with green filter 54) is incubated for 2 h at 30°C in the presence of arginine (2.5×10^{-4} M) under anaerobic conditions. The tubes are immersed in a boiling water bath for 15 min and arginine is estimated by the method of Rosenberg in a sample of the supernatant after removing the cells. A control, without addition of arginine, is included in the experiment (Rosenberg et al., 1956).

The Rosenberg procedure is cumbersome and time-consuming, and other methods appear to be much simpler and sufficiently reliable. Among them, the method of Thornley (1960) is perhaps the most convenient. As recommended by Lelliott et al. (1966), Thornley's medium[9]canbe conveniently dispensed in 3-ml portions into 5-ml screw cap vials. After autoclaving, the vials are inoculated by stabbing and sealed with melted paraffin. Anaerobic formation of ammonia from arginine can be detected by a change of color of the indicator within 3 d.

Acid production from carbohydrates

As pointed out by Palleroni and Doudoroff (1972), the reaction of acid production from carbohydrates is not necessarily correlated with nutritional data, since the acid produced by oxidation of a sugar may not always be a suitable carbon source for growth. However, this should not diminish the taxonomic value of the test. More serious, though, is the objection of redundancy, in that a single enzyme may affect the oxidation of several different sugars (Weimberg, 1962).

Acid production from carbohydrates has been used very extensively for descriptive purposes since the last century, and it is still being used in various laboratories. It is described here among the recommended procedures, provided that the results are interpreted with proper reservations. The method of choice is that described by Hugh and Leifson (1953), which gives clear and reproducible results. The main advantage of this method is the use of a medium containing a low concentration of peptone.[10]Ifthe concentration is high, the deamination can neutralize a positive acid reaction from the sugar.

Hydrolysis of Tween 80

This reaction is indicative of lipolytic activity, and is carried out in the medium of Sierra (1957)..[11]Themedium is usually dispensed in Petri dishes and is spot-inoculated with the cultures. During the incubation, the plates are observed daily for opacity around the patches, due to formation of insoluble calcium soaps (Sierra, 1957). It the reaction has been negative for 10 d, the bacterial mass may be scraped off to observe the medium under the patch. The presence of a precipitate, however, may be due to release of endocellular lipases by lysis of part of the population, and therefore, for practical purposes, the reaction is considered negative.

Nitrate reduction and denitrification

Reduction of nitrate to nitrite can be tested according to Lelliott et al. (1966), and denitrification according to Stanier et al. (1966). Some of the problems encountered in the denitrification test have been discussed by Palleroni and Doudoroff (1972).

Miscellaneous tests

Additional tests that are performed in the identification of *Pseudomonas* species include the following, with references to bibliographic sources for the methods: oxidase test (Kovács, 1956; Stanier et al., 1966); levan formation from sucrose (Lelliott et al., 1966; Stanier et al., 1966); gelatinase (Skerman, 1967); and the egg yolk reaction (Lelliott et al., 1966; Stanier et al., 1966). In testing for catalase, the usual procedure of placing a drop of hydrogen peroxide solution on top of a colony and observing the formation of bubbles is usually satisfactory, but occasionally the reaction may be so weak as to require observation under a dissection microscope, or sensitive detection methods involving the use of an oxygen electrode (Auling et al., 1978).

A number of additional techniques have been added as taxonomic tools to supplement the phenotypic analysis of the aerobic pseudomonads. The results of many of these methods will be summarized below under general taxonomic comments, and the papers cited are sources of information on the methodologies followed in each case. The methods described in these bibliographic references cover various aspects of nucleic and protein studies, the analysis of the fatty acid composition, as well as the description of numerical procedures for data processing.

Differentiation of the genus *Pseudomonas* from other genera

The first edition of the *Manual* included a discussion of the properties that distinguish the aerobic pseudomonads of the genus *Pseudomonas sensu lato* from other phenotypically similar Gram-negative, nonsporulating aerobic bacteria. The reader is referred to this source for comments on this subject in its broader sense (Palleroni, 1984).

Over a quarter of a century ago, strains of *Pseudomonas* species were subjected to experiments of ribosomal RNA–DNA hybridization. Based on the conservative nature of the ribosomal RNA cistrons that had been demonstrated in species of the genus *Bacillus* (Doi and Igarashi, 1965; Dubnau et al., 1965), these experiments attempted to disentangle the phylogenetic complexity that was suspected in the unrelated genus *Pseudomonas*. The results were indeed very striking. The rRNA–DNA hybridization experiments clearly suggested an internal subdivision of *Pseudomonas* into five rRNA groups that seemed "to be very distantly related to each other phylogenetically … [deserving] at least independent genus (and possibly family or order) assignment" (Palleroni et al., 1973). One of these groups, which included the type species, *P. aeruginosa* (rRNA group I), was to retain the genus name. It showed a closer relationship to *Xanthomonas* and to *E. coli* than to other groups of pseudomonads.

It was soon realized by other workers that extension of this approach to comparative studies of other bacterial taxa could yield results of use "as an index of general relatedness [to] provide a sound base on which to construct taxonomic or

maybe identification schemes" (Johnson and Francis, 1975). This prophetic statement found ample corroboration in the years that followed.

Pseudomonas species were examined again at Gent (Belgium) on a much broader basis, but using essentially the same experimental approach, nucleic acid hybridization, and the results, published 10 years later (De Vos and De Ley, 1983), confirmed the conclusions of the original observations. In view of the phylogenetic diversity demonstrated in the genus *Pseudomonas* as classically defined, one of the striking consequences from the taxonomic point of view was that the set of basic properties that had been considered characteristic for this group of organisms—i.e., rod shaped, Gram negative, nonsporeforming, motile by means of polar flagella, and aerobic—was inadequate for practical diagnosis of the genus *Pseudomonas*. The circumscription of *Pseudomonas* is now restricted to the members of rRNA similarity group I of Palleroni et al. (1973), and it is unfortunate that the similarities to members of other groups (which, in the first place, was the basic reason for their original assignment to the genus *Pseudomonas*) make it difficult to define discrete sets of differential phenotypic characters.

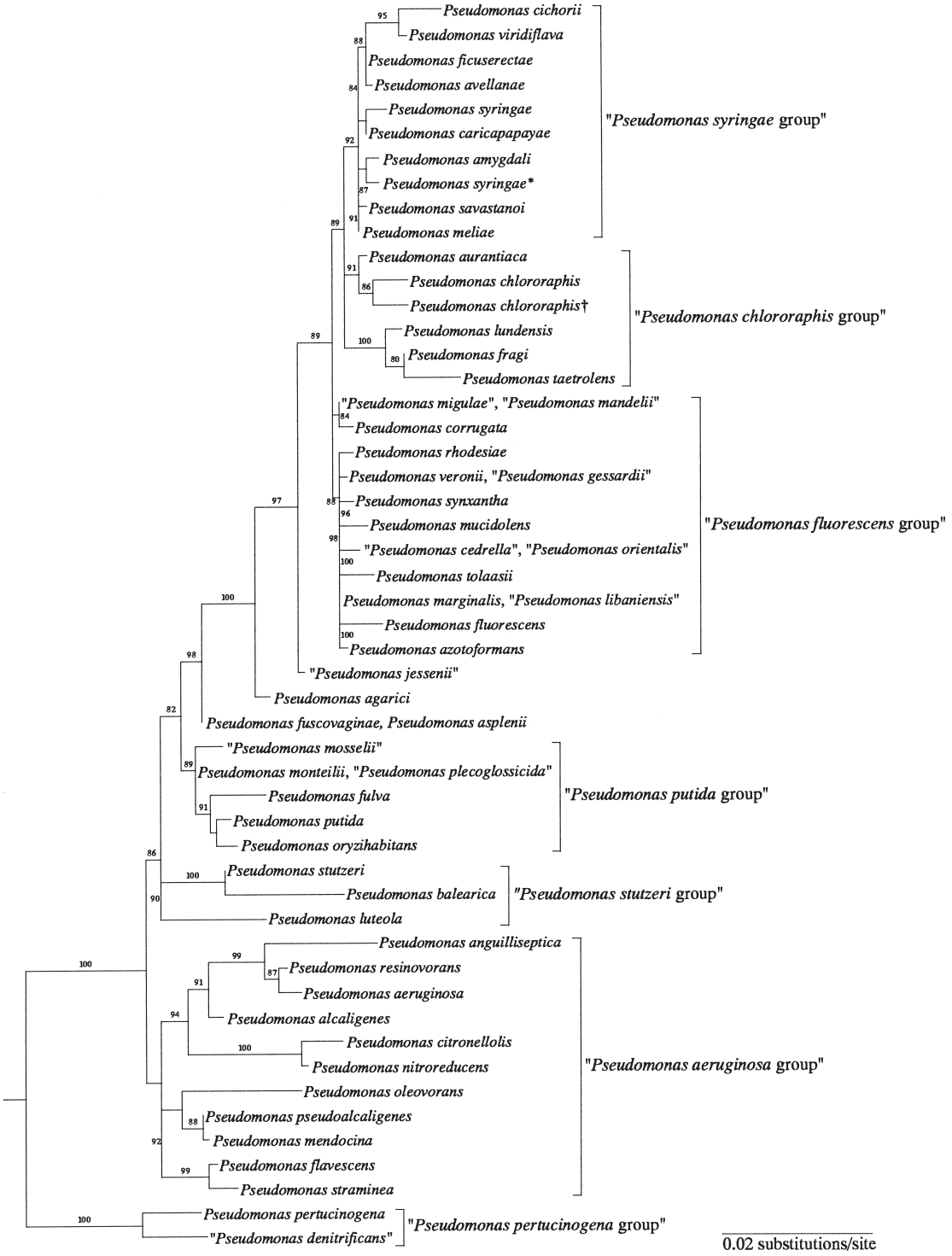
The hybridization methods originally designed for the evaluation of rRNA similarities (Palleroni et al., 1973; De Vos and De Ley, 1983) were eventually replaced by other procedures (oligonucleotide cataloging, rDNA nucleotide sequences). Their use, as expected, confirmed the results of the earlier hybridization experiments. Sequencing the 16S rDNA gene after amplification by PCR is now universally used as the basis for assignment of new species to the genus *Pseudomonas*. As shall be mentioned below, fatty acid analysis can also be effective in differentiating *Pseudomonas* from the other similarity groups.

Pseudomonas sensu stricto (rRNA similarity group I) is still a group of considerable heterogeneity. Internal subgroups can be defined based on plant pathogenicity or pigment production. Among the pigments, the fluorescent siderophores are characteristic of the so-called fluorescent pseudomonads, and production of these compounds is sufficient *per se* to allocate a strain in the genus *Pseudomonas*.

However, the genus also includes nonfluorescent species. It is interesting to note that during the early phenotypic studies carried out at Berkeley, in spite of the differences in pigmentation, a phylogenetic relationship was suspected between some pigmented and nonpigmented species. Obviously, the extensive phenotypic study to which the strains had been subjected implied the expression of a considerable proportion of genes, and it was observed that "the nutritional spectrum of the [alcaligenes group, that is represented by strains that are the least nutritionally versatile of all aerobic pseudomonads] recall[ed] in many respects that of the fluorescent pseudomonads, but it [was] much narrower, notably in the areas of sugars and sugar-acids, and of aromatic compounds" (Stanier et al., 1966). In agreement with the above inference, the numerical taxonomic studies of Sneath et al. (1981), based on part of the published data from the Berkeley group, resulted in a three-dimensional graphical representation, where the so-called fluorescent complex is placed on the same side of the horizontal plane as the nonfluorescent species *P. stutzeri* and *P. alcaligenes*. Although discrete sets of phenotypic properties appear to be inadequate for an absolute differentiation of *Pseudomonas* from other genera of aerobic pseudomonads, the wealth of information contained in extensive nutritional screenings can provide, after appropriate processing, a generally satisfactory graphical representation of the relationships between *Pseudomonas* and other rRNA similarity groups (Sneath et al., 1981). The position of nonfluorescent species is uncertain in graphical representations of values obtained using the Gower or the pattern coefficients, but association of *P. stutzeri* and *P. alcaligenes* is more distinct when using three-dimensional representation by principal coordinate analysis of Euclidean distances (Sneath et al., 1981). Moreover, the phylogenetic representation of Figure 4 shows that the nonfluorescent species *P. alcaligenes* and *P. pseudoalcaligenes*, are placed in the neighborhood of the versatile fluorescent species *Pseudomonas aeruginosa*.

The original descriptions of the various phenotypic groups of *Pseudomonas* species included the inability of species of group I to accumulate PHB granules as carbon reserve material, with the exception of some *P. pseudoalcaligenes* strains that appeared to accumulate small amounts of the polymer. The diagnostic value of this negative characteristic appeared to be rather limited: on the one hand, the species of group V (*Xanthomonas* and *Stenotrophomonas*) are also negative, and on the other, the present treatment includes some species (*P. corrugata, P. amygdali, P. ficuserectae*) that have been reported in the literature to be able to accumulate PHB. However, *P. corrugata* and *P. ficuserectae* actually do not synthesize PHB but instead accumulate PHAs of different monomer composition than PHB, thus buttressing the taxonomic value of inability to accumulate PHB as a generic character (Kessler and Palleroni, 2000). The lack of ability to form PHB is shared by species of group V (*Xanthomonas* and *Stenotrophomonas*), which is interesting in view of the relatively close relationship of this group with group I (*Pseudomonas*). (See also the chapter on *Stenotrophomonas* in this *Manual*.)

FIGURE 4. Phylogenetic tree of species of the genus *Pseudomonas* derived from the 16S rDNA sequences. Bootstrap values of 80% or higher are indicated at the branch points *Escherichia coli* (V00348) was used as the root organism. (Reproduced with permission from Y. Anzai et al., International Journal of Systematic and Evolutionary Bacteriology *50*: 1563–1589, 2000, ©International Union of Microbiological Societies.)



The main body of the present treatment of the genus *Pseudomonas* comprises the species listed in group I of the first edition of the *Manual*. In addition, there are species that were not included in the early experiments, although they were mentioned as probable members of group I, and were later assigned to *Pseudomonas* mainly from additional screenings performed at the University of Gent, Belgium (De Vos and De Ley, 1983; De Vos et al., 1985, 1989). An excellent source of information on the genuine *Pseudomonas* species as well as the present position of many species assigned to *Pseudomonas* but now transferred to rRNA groups other than group I (Palleroni, 1984) is the review article by Kersters et al. (1996). The list of species below will refer with special detail to species whose assignment to *Pseudomonas* has been decided based on phylogenetic criteria.

Taxonomic comments

The proposed division of the aerobic pseudomonads into five RNA similarity groups has received confirmation from numerous contributions by workers in many different laboratories. The investigations have followed various approaches, including investigations on metabolic pathways and their regulatory mechanisms, nucleic acid similarity studies, amino acid sequences of selected proteins, immunological studies, and cell wall composition. The results obtained by application of these approaches were extensively discussed in previous reviews (Palleroni, 1975, 1986, 1992b, b, 1993), and determinative keys have been proposed (Palleroni, 1977; Bergan, 1981; Stolp and Gadkari, 1981). The data add considerable precision to the circumscription of the genus *Pseudomonas*, and some of the methodologies can be of help for determinative purposes.

Interest in the taxonomic intricacies of this genus has not subsided, and of particular interest is a compilation of papers published by members of a consortium of European research groups in vol. 19, pp. 465–568 of *Systematic and Applied Microbiology*. These reports represent to a great extent further explorations into the formidable phenotypic and genomic diversity of the genus *Pseudomonas* as presently circumscribed using a number of experimental approaches.

In addition to the techniques for the determination of special properties already described, this section includes a discussion of other methods or variations of previous methodologies that have been particularly informative in taxonomic analysis of *Pseudomonas* species. Details of the experimental procedures may be found in the respective bibliographic sources.

Nutritional and metabolic studies

Even though the formidable body of phenotypic information collected at Berkeley constituted a very appropriate subject for numerical taxonomic studies, this type of data treatment was reported only for a fraction of the available strains. A considerable amount of work was done in this area, but it remained unpublished, except for a few selected instances (Sands et al., 1970; Palleroni et al., 1972; Champion et al., 1980). Numerical treatment of phenotypic data also is available in original Ph.D. theses from the Department of Bacteriology (Ballard, 1970; Barrett Ralston, 1972).

A more extensive numerical analysis of published nutritional data of the Berkeley collection has given results in very good agreement with the proposed groupings, as indicated by Sneath et al. (1981). Their paper includes an illustrative three-dimensional representation using the system of principal coordinate analysis. A different system of data processing not requiring a complete matrix with all pairwise comparisons also results in useful three-dimensional representations, and it has been applied to published data of the pseudomonads (Hildebrand et al., 1984).

In addition to the extensive phenotypic studies performed at Berkeley on many strains of aerobic pseudomonads following conventional bacteriological techniques, in recent times additional observations have been performed using available commercial kits. Many strains of *Pseudomonas* species were subjected to nutritional studies using API Biotype-100 strips (BioMérieux, La Balme les Grotes, France) for carbon assimilation and the Biolog GN MicroPlate System (Biolog Inc.), which is based on substrate oxidation as detected by the change of color of an indicator (Grimont et al., 1996). Each of these systems included a list of nearly 100 organic compounds, the data obtained were analyzed by computerized systems, and the results were represented graphically in dendrogram form. *P. aeruginosa, P. tolaasii, P. mendocina, P. cichorii, P. viridiflava, P. fragi, P. stutzeri, P. agarici, P. alcaligenes*, and *P. pseudoalcaligenes* appeared as homogeneous phenons. In contrast, some of the fluorescent species (*P. putida, P. fluorescens, P. marginalis*) did not form discrete clusters but were found distributed in several phenons, confirming the heterogeneity previously reported elsewhere.

In general, according to Grimont et al. (1996), there was a remarkable correlation between the Biotype-100 data and the results obtained over 30 years ago using a more conventional methodology (Stanier et al., 1966; Ogawa et al., 1992). This is in agreement with the basic idea behind the two approaches, which rely on the utilization of substrates for growth. The work of Stanier et al. (1966) did not include

testing for the production of acid from carbohydrates, a technique widely used at the time. As discussed elsewhere (Palleroni and Doudoroff, 1972) and in a previous section of this chapter, in spite of their reproducibility the results of the oxidation tests may be redundant; i.e., one enzyme may produce acid from more than one substrate. On the other hand, acid production does not necessarily correlate with utilization of a given substrate for growth. However, acid production from carbohydrates in some instances is useful and provides an additional metabolic test.

A study of strains of *P. putida* and other fluorescent pseudomonads was performed by a determination of the assimilation of carbon compounds of three chemical families (carbohydrates, acids, and amino acids); this was done using the API system (BioMérieux, La Balme les Grotes, France) and following a methodology described elsewhere for a numerical taxonomic study on nonfluorescent species of *Pseudomonas* (Gavini et al., 1989a). This determination was supplemented by an extensive enzymatic study using kits from the same manufacturer for testing 59 peptidase, 10 esterase, and 20 oxidase activities (Elomari et al., 1994). Five main phenotypic clusters were obtained by application of the unweighted pair group average linked method and the Dice similarity coefficient. Of these, one group (II) was identified as *P. putida* biotype A, and could be subdivided into four subclusters, IIa–IId. Strains of cluster II were characterized by ribotyping (see below) using the restriction enzyme *Pvu*II, and seven ribotype subclusters were obtained. Two phenotypic subclusters correlated completely with two ribotype clusters. Based on the results of these studies, the authors urged a revision of *P. putida* and its biovars (of which A represents the "genuine" *P. putida*). The studies indicated the convenience of creating a third biovar, in confirmation of a similar suggestion that emerged from the studies of Barrett et al. (1986), and they serve to illustrate once again the enormous complexity of the species of the fluorescent group. In future studies, the *P. putida* biovars may be circumscribed more precisely as different species (Elomari et al., 1994).

The phenetic taxonomy of a large number of fluorescent pseudomonads isolated from tomato roots was performed using Jaccard similarity coefficients (Stenström et al., 1990). The field strains belong to the *P. fluorescens*/*P. putida* complex, and a suggestion was made to convert the biovars of the first species to the rank of individual species. Once again, the *P. putida* strains included a group that differed from the two known biovars (Stenström et al., 1990).

The usefulness of 36 determinative tests was estimated for 32 pathovars of the phytopathogenic fluorescent species *P. syringae*. The results allowed differentiation of pathovars in some cases, but in others, no distinctive clusters could be detected (Young and Triggs, 1994).

A numerical taxonomic analysis of the nonfluorescent species of RNA similarity group I (*Pseudomonas*), including a large number of strains, suggested that there may be future subdivision in a larger number of species than those recognized at present (Gavini et al., 1989a). Since the analysis was restricted to phenotypic properties, further work using molecular approaches will be necessary to substantiate such suggestion. Other examples illustrate the power of the numerical analysis of extensive phenotypic data for defining clusters that, upon further analysis, may be segregated as biovars or even described as independent species within a population of related strains. One case in point is the circumscription of *P. lundensis*, which, as mentioned in the species description (see below in list of species), had been previously identified as a well-defined cluster within a collection of strains isolated from meat (Molin and Ternström, 1982, 1986). It was similarly defined independently by an analysis of a collection of fluorescent strains from the Berkeley collection (Barrett et al., 1986). The studies resulted in defining four clusters of strains, of which two were identified as *P. fragi*. The other two clusters, of fluorescent pseudomonads, could not be identified as any of the described biovars of *P. fluorescens* or *P. putida*. The authors emphasized the value of substrate utilization tests for elucidating the relationships among *Pseudomonas* strains (Shaw and Latty, 1982).

The numerical analysis of a collection of strains isolated from tomato pith necrosis demonstrated that the strains of *P. corrugata* constituted a single phenon. This study served as the basis for an emended description of this species (Sutra et al., 1997). Numerical taxonomic studies of strains isolated from natural mineral waters led to the discovery of the new species *P. rhodesiae* (Coroler et al., 1996) and *P. veronii* (Elomari et al., 1996).

Mol% G + C studies

The original phenotypic studies by Stanier and collaborators in 1966 were supplemented with base composition determinations of the DNA of the strains (Mandel, 1966), which were later extended for inclusion in the description of new species under study in the same laboratory. In modern species descriptions, mol% G + C values of the DNAs are routinely included, although they are still missing for some of the species assigned to the genus.

DNA–DNA hybridization studies

The phenotypic studies on the aerobic pseudomonads were followed by DNA–DNA hybridization studies, and, by virtue of the wide range of similarity values obtained, the results provided the first clues of the profound genomic differences among the species of this group of organisms. The results eventually suggested the need for similarity studies involving molecules of a more conservative nature. Within the genus *Pseudomonas*, the DNA–DNA hybridization studies included experiments with strains of the *P. stutzeri* group (Wendt-Potthoff et al., 1992), with fluorescent organisms (Palleroni et al., 1972), and with miscellaneous members of other groups. In all these experiments, the hybridizations were done by the competition technique of Johnson and Ordal (1968), but in later experiments the S1 nuclease procedure described by Johnson (1994a) was chosen.

The most important taxonomic application of DNA–DNA hybridization techniques involving the whole genome is in defining relationships at the species level. When applied to strains of aerobic pseudomonads, the hybridization experiments confirmed the relationships among species of the same phenotypic groups; at the same time, as mentioned above, the values were very low or negligible for species of different groups, even among strains of species that unequivocally could be assigned to the same genus. In spite of these observations, in some instances the results of DNA hybridization experiments occasionally have been used to attempt the precise circumscription of genera. One example was the proposal of two new generic names (*Chryseomonas* and *Flavimonas*) (Holmes et al., 1987) for two species previously assigned to the genus *Pseudomonas* (Kodama et al., 1985); the low DNA similarity values obtained in hybridization with DNA of *Pseudomonas* species were taken as the basis for such a proposal. Later studies suggested that the newly created generic names should in fact be considered as junior subjective synonyms of *Pseudomonas* (Anzai et al., 1997), and they are described as such in the present treatment.

Restriction analysis of the whole genome

A useful approach adopted for the characterization of bacterial genomes combines the action of endonucleases that infrequently cut the chromosome with pulsed-field agarose gel electrophoresis for the separation of large DNA fragments. A limited number of cuts per genome is desirable for genomic restriction mapping (McClelland et al., 1987), with clear advantages over frequently cutting endonucleases for the production of fingerprints characteristic of different taxa (Mielenz et al., 1979; Dobritsa, 1985; Sorensen et al., 1985). Application of this principle has resulted in a practical method for constructing and analyzing macrorestriction patterns of 234 strains of different species of aerobic pseudomonads (Grothues and Tümmler, 1991). The restriction nucleases *AsnI, DraI, SpeI, XbaI*, and *PacI* were found to be most appropriate by being specific for AT-rich regions, or for sites including the extremely uncommon tetranucleotide CTAG (McClelland et al., 1987).

The macrorestriction patterns are compared for the number and position of the bands, and use of appropriate equations often indicates a correlation between the estimated similarities of fingerprints and conventional taxonomic groupings. The results obtained by Grothues and Tümmler (1991) in general confirm the classification of pseudomonads based on extensive numbers of phenotypic properties and nucleic acid hybridization studies.

Strains sharing the same mol% G + C contents in the chromosomal DNA, the same codon usage, and similar genome size, give similarity coefficients useful for determinative purposes. The genome patterns also have the practical advantage of helping in epidemiological studies. Differences have been found between *Pseudomonas* strains that are susceptible or multiply resistant to antibiotics (Yamashita et al., 1997).

Differences in restriction fragment size distribution may result from various chromosomal rearrangements and/or mutations at the restriction sites, which suggests that the methodology based on restriction fingerprints should be supplemented with other approaches to determine the degree of homology of the fragments. Large genomic rearrangements have been observed in *P. aeruginosa* strains isolated from clinical samples or from the environment (Schmidt et al., 1996c). The former strains came mostly from cystic fibrosis cases. A 95-kb plasmid was detected in environmental strains and it integrated into the chromosome in cystic fibrosis strains. Exchange of DNA blocks and large DNA inversions led to divergence of clones in this species. The presence of inversions only in cystic fibrosis strains suggests that this niche causes or selects for substantial changes in the genome (Römling et al., 1997).

A study of pathovars of fluorescent plant pathogenic pseudomonads (Grothues and Rudolph, 1991) indicated that two strains belonging to the same pathovar, but of different origins, can give almost identical restriction fingerprints. However, this is not always the case. Pathogens of wide host range give more diverse restriction patterns than those of restricted host range. The methodology was also used for the examination of representative strains of *P. stutzeri*, a species notorious for its heterogeneity (Stanier et al., 1966;

Palleroni et al., 1970). The results show a marked correlation of genome structure with fatty acid composition, and with data of nucleic acid hybridization experiments (Rainey et al., 1994b). A high degree of heterogeneity in macrorestriction patterns that did not correlate with the subdivision of the species in genomovars was also observed by another group of workers, and the marked heterogeneity of *P. stutzeri* was attributed, at least in part, to large chromosomal rearrangements (Ginard et al., 1997). The results obtained by these two groups of workers also indicate that the genome size of *P. stutzeri* ranges from 3.4–4.64 Mb for the strains subjected to their studies.

Rotating field electrophoresis was used to separate fragments of DNA of common and of genetically modified strains of *Pseudomonas* species after digestion with rare-cutting restriction endonucleases. The technique was adapted to the identification of *Pseudomonas* strains deliberately released in the environment. The fingerprints were different for strains of the same species, although they were identical for related strains, and the differences were not affected by the presence of natural or genetically modified plasmids (Claus et al., 1992).

Sequence determination of 16S/23S spacer regions

A study of the genomic organization of the ribosomal RNA genes in the *P. aeruginosa* has been the basis for the hypothesis that this species carries at least four sets of genes, each containing the genes for 16S, 23S, and 5S (Hartmann et al., 1986). Much information is now available for the 16S rRNA genes, in contrast with the other components of the ribosomal RNA complex, and it has been suggested that the spacer regions between the respective genes may be interesting subjects for sequence studies applicable to identification and typing procedures. Working with *Pseudomonas* strains isolated from different types of soils, Gill et al. (1994) amplified the spacer regions by PCR and subjected them to sequencing studies. The results suggest a limited degree of variability among strains identified as *P. putida* and *P. fluorescens*. However, the data may be useful for the recognition of particular *Pseudomonas* environmental strains. A review with information on studies of the spacer regions in a number of bacterial species, including some aerobic pseudomonads, is now available (Gürtler and Stanisich, 1996).

Distribution of repetitive sequences in the genome

These sequences have been taken as the basis for genomic studies based on amplification of the fragments separating the repeated sequences. Work on pseudomonads (Louws et al., 1994) is an extension of studies performed mostly on rhizobia and *Xanthomonas* (Judd et al., 1993), following an original report suggesting the use of the distribution of the repetitive sequences as a tool for the characterization of the genome (Versalovic et al., 1991).

Identification of special genetic markers

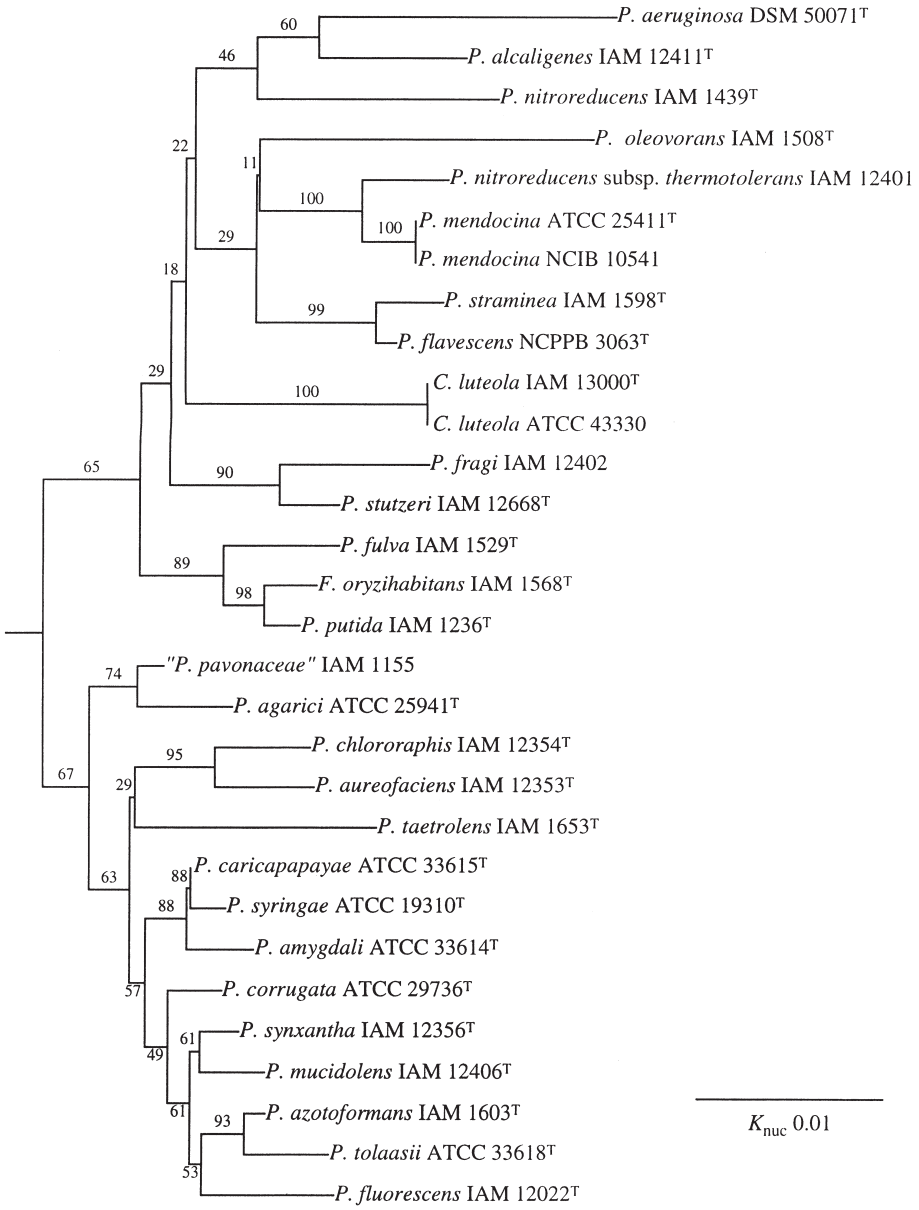
Saint-Onge et al. (1992) succeeded in cloning and sequencing the lipoprotein I gene (*oprl*) of *P. aeruginosa* PAO. The sequence showed similarity to published sequences from other sources, and oligonucleotide primers were designed to amplify the gene by PCR. The amplified gene or the oligonucleotide primers are convenient identification tools in Southern blot analyses. DNA obtained from small amounts of material taken from colonies could be used for amplification, and when bands of the expected mobility were obtained, they could be further identified by the use of a probe prepared with the amplified gene from *P. aeruginosa*. The gene was identified in species of *Pseudomonas* (rRNA similarity group I) and in strains of the genera *Chryseomonas* and *Flavimonas*, whose genomic DNAs reacted with the probe in Southern blots. Saint-Onge and collaborators concluded that the *oprI* gene was found primarily in strains of group I, but they wisely added that it would be of interest to search for other indications leading to a reclassification of the organisms assigned to these newly created genera. Evidence supporting this prediction was eventually found, and, as mentioned above in the section on DNA–DNA hybridization, supported the conclusion that the genera *Chryseomonas* and *Flavimonas* should be considered as synonyms of *Pseudomonas* (Anzai et al., 1997). The main conclusions of the work of Anzai and collaborators are summarized graphically in Figure 5. For the time being, the two genera *Chryseomonas* and *Flavimonas* should be considered as junior subjective synonyms of *Pseudomonas*.

Additional confirmation of the relationship among species of *Pseudomonas* came from work demonstrating that four alginate production genes were conserved within the group (Fialho et al., 1990). Conservation also was observed in the *oprP* gene in various species of similarity group I (Siehnel et al., 1990).

rRNA–DNA hybridization

This technique has had fundamental importance in *Pseudomonas* taxonomic and phylogenetic studies because its application resulted in the demonstration of the complexity of the aerobic pseudomonads as a bacterial group (Palleroni et al., 1973). Because of these studies, *Pseudomonas* is now

FIGURE 5. Phylogenetic tree for different species of the genus *Pseudomonas* derived from 16S rDNA sequence similarities to show the relative position of *Chryseomonas* and *Flavimonas*. Bootstrap values are indicated at the branch points. The confidence limits for the position of the branches has been estimated using the bootstrap analysis. *Escherichia coli* was chosen as root organism. (Reproduced with permission from Y. Anzai et al., International Journal of Systematic Bacteriology *47*: 249–251, 1997, ©International Union of Microbiological Societies.)



restricted to one of five rRNA similarity groups. In these early studies, the competition technique was used (see Kilpper-Bälz, 1991, for a review of the methodology). Later, modifications of the hybridization technique were used by other workers, confirming and extending the original results (De Vos and De Ley, 1983; De Vos et al., 1985, 1989). Since the development of practical methods for sequencing the components of the rRNA operon, rRNA–DNA hybridization methods are seldom being used for taxonomic purposes.

rRNA gene sequencing

Following the initial rRNA–DNA hybridization studies on the aerobic pseudomonads, oligonucleotide catalogs (Uchida et al., 1974) were used for phylogenetic characterization

of bacterial species. Comparisons among catalogs were expressed numerically by means of association coefficients (S_{AB}), and the values could be represented graphically in dendrograms (Fox et al., 1977). Cataloging eventually gave way to a more comprehensive approach directed to the determination of the total sequence of ribosomal RNA components. Initially this was achieved by the isolation of rRNA and DNA synthesis by transcriptase reaction, but the advent of the PCR technique has greatly simplified the experimental methodologies by avoiding RNA isolation, and it is widely used today. An article by Moore et al. (1996) specifically refers to determination and comparison of the 16S rRNA sequences of *Pseudomonas* species. A European compilation of pseudomonad sequences is available (van de Peer et al., 1996).

The results of the contribution by Moore et al. (1996) have been summarized in the creation of "lineages", which are grouped into two "clusters", the *P. aeruginosa* intrageneric cluster and the *P. fluorescens* intrageneric cluster. Within the first cluster there are four lineages: the *P. aeruginosa* lineage (*P. aeruginosa, P. citronellolis, P. alcaligenes, P. stutzeri*), the *P. resinovorans* lineage (*P. resinovorans, P. balearica*), the *P. mendocina* lineage (*P. mendocina, P. pseudoalcaligenes, P. oleovorans*), and the *P. flavescens* lineage (only *P. flavescens*). Within the *P. fluorescens* cluster there are located five lineages: the *P. fluorescens* lineage (*P. fluorescens, P. marginalis, P. tolaasii, P. chlororaphis, P. aureofaciens, P. viridiflava*), the *P. syringae* lineage (*P. syringae, P. amygdali*, "*P. coronafaciens*", *P. ficuserectae*), the *P. cichorii* lineage (*P. cichorii*), the *P. putida* lineage (*P. putida, P. asplenii*), and the *P. agarici* lineage (*P. agarici*) (Moore et al., 1996). It is, however, hard to draw any solid conclusions on the proposed classification. In the first place, since only type strains have been included in the published sequences, it is impossible to know the intraspecific variations, and although several strains of some of the species have been included, the selection has not taken into account the degree of heterogeneity in both DNA similarities and in phenotypic features characteristic of some of these species. Thus, while *P. aeruginosa* and *P. mendocina* are notoriously homogeneous in their phenotypic and genotypic properties, they are represented by a total of 12 strains. On the other hand, a very heterogeneous species in both phenotypic features and in DNA similarity such as *P. stutzeri* is represented by only one strain. The authors did not find obvious correlations of their molecular data with the results of standard phenotypic criteria. This is actually information from papers published by other groups; data corresponding to the strains subjected to the 16S rRNA sequence analysis are not reported.

The above-mentioned study included a comparison of sequences of the 16S rRNA variable region (*E. coli* positions 453–479) for the various lineages and for the species within each lineage. With respect to this approach, it may be interesting to mention here the findings on the *P. putida* biovars reported by Yamamoto and Harayama (1998). In a study of fluorescent pseudomonads done on the basis of the sequences of 16S rRNA, gyrase B subunit (*gyrB*) and RNA polymerase σ70 factor (*rpoD*), they found that in *P. putida* B the pairwise distances estimated from the variable regions of the 16S rRNA gene correlated rather poorly with the synonymous distances estimated from the *gyrB* and *rpoD* genes. On the other hand, the correlation of the non-variable regions of these genes was highly significant, and a recommendation is formulated that only the nonvariable regions should be used for phylogenetic analysis.

Ribotyping

The methodology allows comparisons of restriction patterns of ribosomal RNA genes (Stull et al., 1988), and it became widely used for epidemiological purposes because of its capacity for detection of prokaryotic diversity at the infraspecific level. When applied to the aerobic pseudomonads (*Pseudomonas sensu lato*), using the restriction enzymes *Sma*I and *Hind*II, followed by hybridization with 16 and 23S rRNA, the method was capable of resolving 169 and 159 unique ribotyping patterns, respectively (Brosch et al., 1996). Of the two enzymes, *Sma*I was the most discriminative, except for some of the species. An analysis of *P. putida* biovar A strains in combination with a phenotypic analysis supported earlier observations, and suggested that a revision of the taxonomic status and internal subdivision of this species might be in order (Elomari et al., 1994). These considerations are mentioned in some detail in the section on taxonomic comments for this species. The methodology has limitations from the phylogenetic point of view, particularly because of significant differences that may be created by single mutations. However, aside from being a useful typing tool (Grimont and Grimont, 1986), it also appears to have taxonomic implications, and some of the observations by Brosch and collaborators confirm the marked heterogeneity of some of the species, particularly *P. fluorescens*.

Nucleic acid probes

Based on the capacity of nucleic acid hybridization for the elucidation of genomic relatedness among bacteria, the development of nucleic acid probes allows for high-resolution, rapid, automated identification of specific

microorganisms when they are mixed with other members of natural communities. The preparation and use of probes have been described in detail (Stahl and Amann, 1991; Schleifer et al., 1993).

Schleifer et al. (1992) have described a very effective use of the nucleic acid probes for the specific identification of pseudomonads in the environment. More recently, Amann et al. (1996a) have described the application of such probes to the detection of certain *Pseudomonas* species and pseudomonad species now assigned to other genera. In this report, the main goal was to identify the target organisms as either "former" or "genuine" pseudomonads. Unfortunately, the title of the paper carries this distinction, which is most confusing, since all pseudomonads, no matter what genera they have been assigned to, are equally "genuine".

A simplified cell blot technique combined with the use of 16S rRNA-directed probes was used for the identification of environmental isolates. The sequence of a group probe identifying *P. aeruginosa, P. mendocina, P. fluorescens, Comamonas acidovorans*, and "*Flavobacterium lutescens*" is described (Braun-Howland et al., 1993).

Low molecular weight (LMW) RNA profiles

As an important addition to phylogenetic methodologies, Höfle (1991) has proposed a "rapid genotypic approach that bridges the gap between the solid systematics of type strains and the everyday need for relating new isolates and old reference strains". His technique is based on the examination of profiles of low-molecular-weight (LMW) RNA components in *Pseudomonas* species using high-resolution gel electrophoresis (Höfle, 1988). The reference strains of this genus, according to the author, showed very specific band patterns with discriminative power at the genus and species level (Höfle, 1990).

Whole-cell protein fingerprinting

Protein banding patterns obtained by polyacrylamide gel electrophoresis, particularly when performed under denaturing conditions, are highly characteristic at the strain level, and have been used for many years for the purpose of typing and classification. An interesting application is the verification of strain authenticity, a sensitive and rapid control method that bypasses lengthy and tedious procedures for rechecking exchanged cultures (Kersters et al., 1994). At present, objective quantification of the band patterns, particularly when a substantial number of strains are analyzed, is being done by computer-assisted methods. Among the variants on the basic theme of protein separation in an electric field, electrophoresis in the presence of a denaturing agent such as sodium dodecyl ("lauryl") sulfate (SDS) (Laemmli, 1970) is the technique of choice. SDS polyacrylamide gel electrophoresis (SDS-PAGE) combines high resolution and good reproducibility, which from a practical point of view compensate for the fact that no specific identification of the bands is usually performed. A description of various methodologies and their evaluation has been conveniently summarized by Jackman (1985, 1987).

Of the various references available for descriptions of whole-cell protein fingerprinting methods, a recent publication of a one-dimensional electrophoretic analysis applied to *Pseudomonas* species is a convenient source of technical and bibliographic information (Vancanneyt et al., 1996a). Over 200 reference strains of various species were included. Aside from the few species for which only the type strain has been analyzed, uniform and distinct patterns were observed for many species of fluorescent and nonfluorescent organisms (*P. aeruginosa, P. agarici, P. alcaligenes, P. amygdali, P. caricapapayae, P. chlororaphis, P. chichorii*, "*P. coronafaciens*", *P. corrugata, P. ficuserectae, P. fragi, P. mendocina, P. pertucinogena, P. tolaasii*, and *P. viridiflava*). In contrast, *P. fluorescens, P. marginalis, P. pseudoalcaligenes, P. putida, P. stanieri*, and *P. stutzeri* were found to be particularly heterogeneous, in confirmation with earlier data on their phenotypic characteristics.

Ribosomal proteins

These proteins, similarly to the ribonucleic acids with which they are associated in the ribosome, are of a highly conservative nature. Obviously, this should be reflected in a comparison of their sequences. In fact, partial amino acid sequences of preparations of protein L30 from species of various ribosomal RNA similarity groups give clusters similar to those that had originally been defined in rRNA–DNA hybridization studies (Ochi, 1995). Despite different levels of conservatism, other ribosomal proteins (S20, S21, L27, L20, L31, L32, and L33 protein families) give comparable results.

Other membrane proteins

The discussion on this point will be limited to the identification of lipoprotein I in species of *Pseudomonas*. Work performed on the specificity of the *P. aeruginosa* lipoprotein I gene used as a probe for rapid identification purposes was an extension of the experiments of Saint-Onge et al. (1992). The work included more strains of species of RNA group I (*Pseudomonas*), as well as the related "*Azotomonas*" and *Azomonas* species and other species of Gram-negative organisms. All of

the species of *Pseudomonas* that were tested gave positive reactions of variable intensities in PCR and dot blot tests using monoclonal antibodies for the lipoprotein I, whereas species of other similarity groups and of other Gram-negative genera gave negative reactions. These data, still considered to be of a preliminary nature, suggest that *P. aeruginosa* can be differentiated from the other species of the same genus that have been tested, with the exception of *P. mendocina, P. pseudoalcaligenes*, and *P. oleovorans*, which have similar restriction patterns in their lipoprotein I gene (De Vos et al., 1993).

Fatty acid analysis

Fatty acid composition and the quinone system have been used for groupings of *Pseudomonas* species (Ikemoto et al., 1978; Yamada et al., 1982; Oyaizu and Komagata, 1983). A study of the fatty acid composition of 50 strains of various *Pseudomonas* species revealed the presence of the straight-chain saturated acid of $C_{16:0}$ and straight-chain unsaturated acids $C_{16:1}$ and $C_{18:1}$ in all strains (Ikemoto et al., 1978). Distribution of hydroxy acids, cyclopropane acids, and branched-chain acids follows quite closely the accepted subdivision of the aerobic pseudomonads into RNA similarity groups. These cellular components are very useful for identification purposes.

The results of fatty acid and quinones analyses, performed on a collection of 75 strains that included phytopathogenic pseudomonads, have been reported (Oyaizu and Komagata, 1983). The survey gave a basis for the classification of the collection into groups with particular reference to the presence of 3-OH fatty acids, and some of the groups coincided with the RNA similarity groups already defined (Palleroni et al., 1973).

The analysis of a large number of fatty acid profiles obtained from a collection of 340 strains of pseudomonads that includes plant pathogenic species has been published (Stead, 1992). The results on the distribution of 2-OH and 3-OH fatty acids could serve as one of the bases for a subdivision of the collection into six groups warranting independent generic designations.

The above comments on fatty acid composition studies refer to *Pseudomonas sensu lato* and coincide in supporting the division of these organisms into the various genera that later were to be proposed in various communications from different laboratories. A summary of the results of the above sources is presented in Table 3.

In a more recent communication (Vancanneyt et al., 1996b) the fatty acid composition of whole-cell hydrolysates and phospholipid fractions are reported. The results confirm some of the conclusions of the above-cited references. In general, *Pseudomonas* species are characterized by the presence of $C_{10:0\ 3OH}$ and $C_{12:0\ 3OH}$ acids. When the fatty acid contents of phospholipids were taken into account, no clear separation could be found between *Pseudomonas* RNA group I organisms and RNA similarity groups II and III.

TABLE 3. Fatty acid and ubiquinone composition of *Pseudomonas* and other rRNA similarity groups[a]

Compound	Ribosomal RNA similarity groups				
	I	II	III	IV	V
Fatty acids:					
$C_{10:0\ 3OH}$	+		+		+
$C_{11:0\ 3OH}$					+
$C_{11:0\ iso\ 3OH}$					+
$C_{12:0\ 3OH}$	+			+	+
$C_{12:0\ iso\ 3OH}$					+
$C_{13:0\ iso\ 3OH}$					+
$C_{14:0\ 3OH}$		+		+	
$C_{16:1\ 3OH}$		+			
$C_{12:0\ 2OH}$	(+)				
$C_{16:0\ 2OH}$		(+)			
$C_{16:1\ 2OH}$		(+)			
$C_{18\text{-}1\ 2OH}$		+			
Ubiquinones	Q-9	Q-8	Q-8	Q-10	Q-8

[a]Data taken from Oyaizu and Komagata (1983) and Stead (1992). Data in parentheses: not all strains of the group are positive.

Polyamine composition

Polyamine patterns appear to be good chemotaxonomic markers for the Gram-negative bacteria in the phylum *Proteobacteria*, allowing ready differentiation of some of the pseudomonad groups (Busse and Auling, 1988). In addition to quinone analysis, polyamines are useful for rapid identification at the genus level (Busse et al., 1989). This last contribution includes a discussion of various other approaches applicable to species and strain identification.

In an attempt to relieve the dearth of simple and reliable tests for the classification of newly isolated strains of the genus *Xanthomonas*, a determination of polyamine composition of strains of this genus and of phytopathogenic *Pseudomonas* species was introduced as a rapid chemotaxonomic identification tool (Auling et al., 1991). Spermidine was found to be the main polyamine of *Xanthomonas*, whereas strains of

Pseudomonas RNA group I were characterized by the presence of putrescine. The polyamine pattern of *Azotobacter* and *Azomonas* was the same as that of the fluorescent pseudomonads, which are placed in the same branch of *Proteobacteria*. The results obtained by Auling et al. (1991) were confirmed in a more recent study by Goris et al. (1998). Aside from the presence of putrescine and spermidine mentioned above, the species of *Pseudomonas* could be divided into two sub-lineages, of which only one contained cadaverine. *P. aeruginosa* (as well as *Azotobacter vinelandii*) is in the sublineage that contains this polyamine.

List of species of the genus *Pseudomonas*

The following list includes those species whose assignment to the genus *Pseudomonas* has been definitely established. Part of the information for some of the species has been condensed in the form of tables representing updated versions of those of the first edition of this *Manual*.

The order is alphabetical, thus avoiding the problem of ordering the species on the basis of properties such as pathogenicity, pigmentation, or certain physiological peculiarities, which only seldom correlate with the order of groups based on 16S rRNA sequence similarities (Figures 4 and 5). *Pseudomonas* can be subdivided into two big groups, the fluorescent and the nonfluorescent species and, with respect to plant pathogenicity, a subdivision of the genus into saprophytes and plant pathogenic species is possible. These subdivisions do not correlate with each other. Some correlations, however, are apparent. In Figure 4 the plant pathogens congregate mostly in the so-called *P. syringae* group.

The members of some pairs of species (*P. migulae–P. mandelii*; *P. veronii–P. gessardii*; *P. cedrella–P. orientalis*; *P. marginalis–P. libanensis*; *P. fuscovaginae–P. asplenii*, and *P. monteilii–*"*P. ayucida*") were found to have identical 16S rRNA sequences (see Figure 4) (Anzai et al., 1997). It is to be hoped that the precise taxonomic allocation of these taxa may be further investigated. Some comments are included in the corresponding descriptions below.

A number of species assigned to *Pseudomonas* will not be treated here. As mentioned by Kersters et al. (1996), the phylogenetic position of the following species has not yet been determined. In the list that follows, references to the original descriptions are given in parentheses, and the number in italics refers to the page where the species is described in the first edition of this *Manual* (Palleroni, 1984) : *P. antimicrobica* (Attafuah and Bradbury, 1989), *P. cissicola* (Goto and Makino, 1977), *P. flectens* (Johnson, 1956), *P. gelidicola* (Kadota, 1951), *P. halophila* (Fendrich, 1988), *P. indigofera* (Elazari-Volcani, 1939), *P. iners* (Iizuka and Komagata, 1964b), *P. lanceolata* (Leifson, 1962a), *P. mephitica* (Haynes and Burkholder, 1957), and *P. spinosa* (Leifson, 1962b). Since the descriptions of *P. antimicrobica, P. flectens*, and *P. halophila* are not given in the first edition of the *Manual*, they will be added at the end of the list of species, under the subtitle Other Species.

Table 4 lists a number of species previously assigned to *Pseudomonas sensu lato*, which have been transferred to various other genera or groups. The information has been taken mainly from Kersters et al. (1996), and also Behrendt et al. (1999) and Anzai et al. (1997). The paper by Kersters et al. (1996) includes a handy table with the current classification of the species that have been assigned to *Pseudomonas* prior to the subdivision of the genus into rRNA similarity groups, their present assignments, and references to the studies deciding phylogenetic allocations and/or revised taxonomic status for each species.

Recently, three new species have been added to the list of those requiring further confirmation—*Pseudomonas abietaniphila, P. multiresinivorans*, and *P. vancouverensis*, which are isolated from resin acids (Mohn et al., 1999a, b). Even though the reported catabolic activities of these organisms are unique, DNA–DNA hybridization data to confirm their genomic relationships vs. related organisms have not been reported. For this reason, the description of these species will be found at the end, in the section Other Species.

Finally, many new species of *Pseudomonas* have been described in the *International Journal of Systematic and Evolutionary Microbiology* since mid-2001, the cut-off point for inclusion of new genera and species in this edition of the *Manual*.

Pseudomonas aeruginosa (Schroeter 1872) Migula 1900, 884[AL] (*Bacterium aeruginosum* Schroeter 1872, 126.)

ae.ru.gi.no'sa. L. fem. adj. *aeruginosa* full of copper rust or verdigris, hence green.

Characteristics of the species are summarized in Tables 1, 5, 6, and 7. Rods, motile by means of a single polar flagellum. Two main colony types can be observed on common solid media. One is large, smooth, with flat edges and elevated center ("fried egg" appearance), and the other is small, rough, convex. On the large colonies, silver-gray metallic shining patches may be observed, and pitting is fairly common. Clinical materials are, in general, good sources of the large colony type, whereas the small type is commonly obtained from natural sources (Véron and Berche, 1976). Variation of the large type to the small is rather common but the reverse variation is extremely rare. A third colony type (mucoid) often can be

TABLE 4. *Pseudomonas sensu lato* species transferred to other taxa

Pseudomonas species	*Previous description* in *Bergey's Manuals*	New allocation	References
P. aminovorans	Palleroni (1984)	*Aminobacter*	Urakami et al. (1992)
P. beijerinckii	Palleroni (1984)	*Halomonas* rRNA lineage	De Vos et al. (1989)
P. beteli	Palleroni (1984)	*Xanthomonas* rRNA lineage	De Vos et al. (1985)
P. boreopolis	Palleroni (1984)	*Xanthomonas* rRNA lineage	De Vos et al. (1989)
P. carboxydohydrogena	Palleroni (1984)	*Bradyrhizobium-Rhodopseudomonas* rRNA lineage	Auling et al. (1988)
"*P. carboxydovorans*"	Palleroni (1984)	*Oligotropha*	Meyer et al. (1993)
"*P. compransoris*"	Palleroni (1984)	*Zavarzinia*	Meyer et al. (1993)
P. doudoroffii	Palleroni (1984)	*Aeromonadaceae*	De Vos et al. (1989)
P. echinoides	Palleroni (1984)	*Sphingomonas* rRNA lineage	De Vos et al. (1989)
P. elongata	Palleroni (1984)	*Oceanospirillum*	De Vos et al. (1989)
"*P. extorquens*"	Haynes and Burkholder (1957)	*Methylobacterium*	Bousfield and Green (1985)
P. geniculata	Haynes and Burkholder (1957)	*Xanthomonas* rRNA lineage	Byng et al. (1983)
P. hibiscicola	Palleroni (1984)	*Xanthomonas* rRNA lineage	De Vos et al. (1985)
P. huttiensis	Palleroni (1984)	*Herbaspirillum*	Kersters et al. (1996)
P. lemoignei	Palleroni (1984)	rRNA group II	De Vos and De Ley (1983), Mergaert et al. (1996)
P. marina	Palleroni (1984)	*Halomonas* rRNA lineage	De Vos et al. (1989)
P. mesophilica	Palleroni (1984)	*Methylobacterium*	Green and Bousfield (1983)
P. mixta		*Telluria*	Bowman et al. (1988, 1993a)
P. nautica	Palleroni (1984)	*Oceanospirillum*	De Vos et al. (1989)
P. paucimobilis	Palleroni (1984)	*Sphingomonas*	De Vos et al. (1989)
P. pictorum	Palleroni (1984)	*Xanthomonas* rRNA lineage	De Vos et al. (1989)
P. radiora	Palleroni (1984)	*Methylobacterium*	Green and Bousfield (1983)
P. rhodos	Palleroni (1984)	*Methylobacterium*	Green and Bousfield (1983)
"*P. riboflavina*"	Haynes and Burkholder (1957)	*Devosia*	Nakagawa et al. (1996)
"*P. rosea*"		*Methylobacterium*	Bousfield and Green (1985)
P. rubrisubalbicans	Palleroni (1984)	*Herbaspirillum*	Baldani et al. (1996)
P. saccharophila	Palleroni (1984)	*Comamonadaceae*	Palleroni et al. (1973), Willems et al. (1992)
P. stanieri		*Marinomonas*	Y. Ansai, personal communication

TABLE 5. General phenotypic characteristics of some fluorescent *Pseudomonas* species[a]

Characteristics	*P. aeruginosa*	*P. chlororaphis* subsp. *chlororaphis*	*P. chlororaphis* subsp. *aurefaciens*	*P. flavescens*	*P. fluorescens* bv. I	*P. fluorescens* bv. II	*P. fluorescens* bv. III	*P. fluorescens* bv. IV	*P. fluorescens* bv. V	*P. lundensis*	*P. monteilii*	*P. putida* bioyar A	*P. putida* bioyar B	*P. rhodesiae*	*P. veronii*
Number of flagella	1	>1	>1	1	>1	>1	>1	>1	>1	1		>1	>1	1	1
Nonfluorescent pigment, color:															
Green	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Orange	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Yellow	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Blue	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Gelatin liquefaction	+	+	+	−	+	+	+	+	+	+	−	−	−	−	+
Denitrification	+	+	−	−	−	+	+	+	−	−	−	−	−	−	+
Levan formation	−	+	+	−	+	+	−	+	−	−	−	−	−	+	
Lecithinase	−	+	+		+	d	+	+	d		−	−	−	+	−
Lipase	+	+	+	−	+	−	d	d	d	−	−	−	−	−	−
Growth at 4°C	−	+	+	−	+	+	+	+	+	+	−	d	+	+	+
Growth at 41°C	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Mol% G + C of the DNA	67	63	64	63	60	60	60	60	60	60	60	62	61	59	61

[a]For symbols see standard definitions. Data from Palleroni (1984), Molin et al. (1986), Hildebrand et al. (1994), Elomari et al. (1996, 1997), and Coroler et al. (1996).

obtained from respiratory and urinary tract secretions and was first observed by Sonnenshein (1927). Mucoid mutants can be divided into two groups according to the capacity of forming mucus (alginate) in chemically defined media (Fyfe and Govan, 1980). Aside from pyoverdine and pyocyanin, other pigments may be produced by some strains, including a dark red pigment (see above section on pigments).

Denitrification and gelatin liquefaction is present in the great majority of strains. The hydrolysis of Tween 80 is weak and the egg-yolk reaction is negative. Optimum temperature, 37°C. *P. aeruginosa* is probably the most widespread of all bacterial species. It can be isolated from soil and water, particularly from enrichment cultures for denitrifying bacteria. Commonly isolated from clinical specimens (wound, burn, and urinary tract infections). Causative agent of "blue pus", which accounts for the origin of the synonym pyocyaneus. Occasionally pathogenic for plants. Strains isolated from leaf spot of tobacco, identical with or similar to *P. aeruginosa* have been named "*P. polycolor*" (Clara, 1930). The species can be internally divided into a number of subgroups (types) useful for epidemiological purposes.

The mol% G + C of the DNA is: 67.2 (Bd).

Type strain: ATCC 10145, DSM 50071, NCIB 8395, NCTC 10332.

GenBank accession number (*16S rRNA*): X06684, Z76651.

Pseudomonas agarici Young 1970, 985[AL]

a.gar'i.ci. M.L. n. *Agaricus* a genus of fungi; M.L. gen. n. *agarici* of *Agaricus*.

The following description is a summary of the one presented by Young (1970).

Short rods (no dimensions given), motile by one or, rarely, two polar flagella. Green diffusible pigment with weak fluorescence under ultraviolet light of unspecified wavelength (in our experience, the maximum intensity of fluorescence is observed at low wavelength UV, ~257 nm). Acid is produced from arabinose, glucose, and mannitol; little acid from fructose, galactose, and ribose, and no acid from rhamnose, xylose, mannose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, raffinose, starch, inulin, dextrin, glycogen, adonitol, sorbitol, inositol, and salicin.

Acetate, benzoate, citrate, formate, fumarate, gluconate, lactate, propionate, and succinate are utilized. Galacturonate, oxalate, and tartrate are not. Oxidase and catalase reactions positive; nitrate reduction, pectate liquefaction, starch hydrolysis, esculin hydrolysis, levan production, and growth factor requirements are all negative. Further extensive phenotypic studies on strains of this species in comparison with other fluorescent organisms have been published (Fahy, 1981). Some of the reported properties are summarized

TABLE 6. Nutritional characteristics of some fluorescent *Pseudomonas* species[a]

Substrates	*P. aeruginosa*	*P. chlororaphis* supsp. *chlororaphis*	*P. chlororaphis* supsp. *aurefaciens*	*P. flavescens*	*P. fluorescens* bv. I	*P. fluorescens* bv. II	*P. fluorescens* bv. III	*P. fluorescens* bv. IV	*P. fluorescens* bv. V	*P. lundensis*	*P. monteilii*[b]	*P. putida* biovar A	*P. putida* biovar B	*P. rhodesiae*[c]	*P. veronii*
Acetamide	+	–	–	–	–	–	–	–	–	–	–	d	d	–	–
Acetate, L-alanine[d], γ-aminobutyrate, L-asparagine e, L-aspartate, betaine, caprate, caprylate, citrate, fumarate, L-glutamate[d], glutarate, glycerol, heptanoate, β-hydroxybutyrate, DL-lactate, L-malate[d], pelargonate[e], L-proline, putrescine, pyruvate[d], succinate[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
cis-Aconitate	+	d	+	+	+	+	d	+	d	–	+	+	+	+	+
Adipate	+	–	–	–	–	–	d	–	–	–	–	–	d	–	d
Adonitol	–	–	–	–	+	–	d	–	d		–	–	–	–	–
β-Alanine, L-arginine, spermine, L-tyrosine	+	+	+	–	+	+	+	+	+	+	+	+	+		+
D-Alanine	+	+	–	+	+	+	+	+	–	–	+	+	+	+	+
α-Aminobutyrate	–	–	–	–	–	–	–	–	–	–	–	d	–	+	+
α-Aminovalerate	–	–	–	–	–	–	d	–	–			d	–		
D-Aminovalerate	+	d	d	–	d	+	+	+	+		+	+	+	+	
α-Amylamine	–	–	+	–	–	d	d	–	d	–	+	d	+	–	–
Anthranilate	+	+	+	–	d	d	d	–	d	–	–	–	+	–	d
D-Arabinose	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
L-Arabinose	–	–	+	+	+	+	d	+	d	+	+	d	+	+	+
Azelate	+	–	–	–	–	–	d	–	–	–	–	–	–	–	–
Benzoate	+	+	d	–	d	d	d	+	d	+	+	d	+	–	–
Benzoylformate	+	+	+	–	–	–	–	–	–	–		d	d		
Benzylamine	–	–	–	–	–	–	d	–	d		+	d	+	–	–
Butanol	+	d	–	–	d	d	d	+	d			+	+		
Butylamine	–	–	d	–	–	–	–	–	d	–	+	+	+	–	–
2,3-Butylene glycol	+	d	d	+	d	+	d	+	d	–		d	d		
Butyrate	+	+	d	–	–	d	d	+	d	+	+	+	+	–	+
Caproate	+	d	+	+	+	d	+	+	+	+	+	+	+	+	+
Cellobiose, ethylene glycol[e], D-fucose, inulin[f], isopropanol[e,f], lactose, maleate, maltose, methanol[e,f], oxalate, phthalate, poly-β-hydroxybutyrate[e,f], salicin[f], starch, L-threonine[e,f]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Citraconate	–	–	–	–	d	d	d	+	d	–	–	–	d	–	–
L-Citrulline	d	d	d	+	d	d	d	–	d	–		d	d	–	d
Creatine	–	–	–	+	–	–	d	–	–	+	+	d	d	–	–
Dodecane, hexadecane	d	–	–	–	–	–	–	–	–			–	–		
Erythritol	–	–	–	–	d	d	+	–	d	–	–	–	–	–	+
Ethanol, propanol	+	d	–	–	–	+	d	–	d	–		d	d		
Ethanolamine	d	d	+	+	+	d	d	+	d	+	+	d	d	+	+
D-Fructose	+	d	d		+	d	+	+	+	+	+	+	+	+	+
D-Galactose	–	d	+	+	+	+	d	+	d	–	–	–	d	+	+
Geraniol	+	–	–	–	–	–	–	–	–	–		–	–		
D-Glucose	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+
Gluconate	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+
Glycerate	+	+	+	+	+	+	d	+	d		+	d	+	+	+
Glycine	d	–	–	–	–	–	d	–	d	–	–	d	+	–	–
Glycolate	–	–	–	+	–	–	–	–	–	–	–	d	–	–	d
Hippurate	–	–	–	–	–	–	d	–	–	+		d	d		
Histamine	+	d	d	–	d	–	d	–	d	–	d	d	+	–	–
L-Histidine	+	+	+	+	+	d	+	+	d	+	+	+	+	+	d
o-Hydroxybenzoate	–	d	–	–	–	–	–	–	–	–	–	d	d	–	–
m-Hydroxybenzoate	–	d	d	–	–	–	–	–	–	–	–	d	d	–	–
p-Hydroxybenzoate	+	+	+	+	+	+	d	+	d	–	+	+	+	+	+
Hydroxymethylglutarate	–	–	–	+	d	d	–	–	d	–		–	–		
m-Inositol	–	+	+	–	d	+	d	+	d	–	+	–	–	+	+
Isobutanol	+	–	–	–	–	d	d	–	d	–		d	d		
Isobutyrate	+	–	d	–	–	d	d	–	d		+	d	d	–	+
L-Isoleucine, L-valine	d	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Isovalerate	+	+	+	–	d	d	d	–	d	–	+	+	+	d	–
Itaconate, mesaconate	+	+	+	+	+	d	d	–	d	–	–	d	–	–	

TABLE 6. (*Continued*)

Substrates	*P. aeruginosa*	*P. chlororaphis* subsp. *chlororaphis*	*P. chlororaphis* subsp. *aureofaciens*	*P. flavescens*	*P. fluorescens* bv. I	*P. fluorescens* bv. II	*P. fluorescens* bv. III	*P. fluorescens* bv. IV	*P. fluorescens* bv. V	*P. lundensis*	*P. monteilii*[b]	*P. putida* bioyar A	*P. putida* bioyar B	*P. rhodesiae*[c]	*P. veronii*
2-Ketogluconate	+	+	d	−	+	+	+	d	+		+	d	+	+	+
α-Ketoglutarate	+	+	+	+	+	+	+	+	+	+		+	+		
L-Kynurenine	+	+	+	−	d	d	d	−	d	−	−	−	+	−	−
Kynurenate	d	d	d	−	d	−	d	−	−	−		−	d		
L-Leucine	+	+	+	+	+	d	+	+	+	+	+	+	+	+	d
Levulinate	+	d	d	−	d	−	d	−	d	−	−	d	d	−	d
L-Lysine	+	d	d	−	+	d	d	+	d	+	+	+	d	−	−
D-Malate	d	d	−	+	−	d	d	+	d		d	d	d	−	−
Malonate	+	+	+	+	+	+	d	+	d	−	d	d	+	+	+
D-Mandelate	−	−	−	−	−	−	−	−	−	−	−	d	d	−	−
L-Mandelate	+	−	−	−	−	−	−	−	d	−	−	−	d	−	−
Mannitol	+	+	+		+	+	d	+	d	−	−	d	d	+	+
D-Mannose	−	+	+	+	+	+	+	+	d	+	−	d	d	+	+
Mucate	−	+	+	+	+	+	d	+	+	−	−	d	+	+	d
Naphthalene	−	−	−	−	−	−	−	−	−	−	−	−	d		
Nicotinate	−	−	−	−	−	−	−	−	d	−		d	+		
DL-Norleucine	−	−	−	−	−	−	−	−	−	−	+	−	−	−	
L-Ornithine	+	d	+	−	+	d	d	d	d	+	+	+	+	−	d
Pantothenate	−	−	−	−	−	−	d	−	−			−	−		
Phenol	−	−	−	−	−	−	d	−	−	−		d	d		
Phenylacetate	−	d	+	−	−	−	d	−	d	−	+	d	+	−	−
L-Phenylalanine	d	d	+	−	d	d	d	+	d	+		+	+	+	+
Phenylethanediol	−	−	−	−	−	−	−	−	−	−	−	d	−		
Pimelate, suberate	d	−	−	−	−	−	d	−	−	−	−	−	−	−	−
Propionate	+	+	+	−	+	+	d	+	+	+	+	+	+	+	+
Propylene glycol	d	−	−	−	−	−	d	−	−	−	−	−	−		
Quinate	d	+	+	−	+	+	+	+	d	−		+	+		
L-Rhamnose	−	−	−	−	−	d	d	−	d	−	−	−	−	−	−
D-Ribose	+	+	+	+	+	+	d	+	d	+	+	d	d	+	+
Saccharate	−	+	+	+	+	+	d	+	d	−		+	+		
Sarcosine	d	d	+		+	d	d	+	d	+	+	+	+	+	+
Sebacate	+	−	−	−	−	−	d	−	−	−	−	−	d	−	−
L-Serine	d	d	+	+	+	d	+	+	d	−	d	d	d	+	+
Sorbitol	−	−	−	−	+	+	d	+	d	−	−	−	d	+	+
Sucrose	−	+	d	+	+	+	−	+	d	−	−	−	d		+
D(−)-Tartrate	−	−	−	−	−	d	−	−	d	−	−	d	d	−	−
L(+)-Tartrate	−	d	−	−	−	−	−	+	−	−	−	d	d	−	−
m-Tartrate	−	−	−	+	−	−	d	−	d	−	−	d	−	−	−
Testosterone	−	−	−	−	−	−	−	−	−	−		−	+		
Trehalose	−	+	d	+	+	+	d	+	d		−	−	−	+	d
Trigonelline	−	−	−	+	d	d	d	−	d	−	d	d	+	−	+
Tryptamine	−	−	−		−	d	d	−	−	−	−	d	+	−	−
D-Tryptophan	−	−	−	−	−	−	−	−	−	−	−	−	d	−	−
L-Tryptophan	d	+	+	−	+	d	d	−	d		−	−	+	−	+
Valerate	+	+	+	−	d	d	d	−	d	−	+	+	+	+	+
D-Xylose	−	−	−	+	+	d	d	d	d	−	−	d	d	+	+

[a]For symbols see standard definitions. Data from Palleroni (1984), Molin et al. (1986), Hildebrand et al. (1994), Elomari et al. (1996, 1997), and Coroler et al. (1996).

[b]*P. monteilii* was also reported to be unable to use the following carbon sources: *N*-acetylglucosamine, esculin, *m*-aminobenzoate, *p*-aminobenzoate, 3-aminobutyrate, amygdalin, D-arabitol, L-arabitol, arbutin, L-cysteine, dulcitol, ethylamine, L-fucose, β-gentiobiose, glucosamine, glycogen, isophthalate, 5-ketogluconate, D-lyxose, L-lyxose, melezitose, melibiose, L-methionine, α-methylglucoside, α-methyl-D-mannoside, α-methyl-xyloside, raffinose, L-sorbose, terephthalate, raffinose, tagatose, D-tryptophan, D-turanose, xylitol, and L-xylose.

[c]*P. rhodesiae* was also found to use acetylglucosamine and D-arabitol, and was unable to grow on esculin, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, 3-aminobutyrate, amygdalin, L-arabitol, arbutin, L-cysteine, dulcitol, ethylamine, L-fucose, gentiobiose, glucosamine, glycogen, norvaline, raffinose, salicin, sorbose, D-tagatose, terephthalate, D-turanose, urea, xylitol, and L-xylose.

[d]Positive for *P. veronii*; results on other substrates not reported.

[e]*P. monteilii* not tested.

[f]No information on *P. veronii*.

TABLE 7. Characteristics differentiating *Pseudomonas aeruginosa, P. balearica, P. stutzeri*, and *P. putida*[a]

Characteristics	*P. aeruginosa*	*P. balearica*	*P. putida*	*P. stutzeri*
Type of colony:				
Smooth	+		+	
Wrinkled		+		+
Number of flagella	1	1	>1	1
Hydrolysis of:				
Gelatin	+	–	–	–
Starch	–	+	–	+
Utilization of:				
Maltose	–	+	d	+
Xylose	–	+	d	–
γ-Aminobutyrate	–	–	d	d
Malate	d	+	–	+
Suberate	d	–	–	d
Mannitol	+	–	–	d
Ethylene glycol	–	–	–	+
Denitrification	+	+	–	+
Growth at:				
42°C	+	+	–	d
46°C	–	+	–	d
Growth in media with 8.5% NaCl	–	+	–	–
Fatty acid content (%):				
$C_{17:0\ cyclo}$	0.8	4.71	>5	0.28–1.72
$C_{19:0\ cyclo}$	1.2	3.8	Traces	0.32-1.45
Mol% G + C of the DNA	67	64.1–64.4	60.7–62.5	60.9–64.9

[a]For symbols see standard definitions. Data from Bennasar et al. (1996) and Stanier et al. (1966).

in Table 8. Details of its fatty acid composition are known (Stead, 1992).

The organism causes drippy gill of mushrooms, and one of the main differences with another mushroom pathogen, *P. tolaasii*, is in the utilization of benzoate. Catechol is oxidized to a black pigment diffusing into the medium, a character also present in *P. agarici*. The species was tentatively assigned to RNA group I by Byng et al. (1980), and this position was further confirmed by rRNA–DNA hybridization (De Vos et al., 1985) and by rDNA sequencing (Moore et al., 1996).

The mol% G + C of the DNA is: 58.8–61.1 (T_m).

Type strain: ATCC 25941, DSM 11810, LMG 2289.

GenBank accession number (*16S rRNA*): Z76652.

Pseudomonas alcaligenes Monias 1928, 332[AL]

al.ca.li'ge.nes. M.L. adj. *alcaligenes* alkali-producing.

Characteristics useful to differentiate the species from other *Pseudomonas* species are given in Tables 9 and 10. For further descriptive information see Ralston-Barrett et al. (1976) and Stanier et al. (1966). The nutritional spectrum is very narrow, resembling that of highly mutated fluorescent organisms. The gelatinase reaction is negative. The type strain was isolated from swimming pool water (Hugh and Ikari, 1964).

The mol% G + C of the DNA is: 64–68 (Bd).

TABLE 8. Characters distinguishing some fluorescent *Pseudomonas* species associated with mushroom culture[a]

Characteristics	*P. agarici*	*P. cichorii*	*P. fluorescens* biovar II	*P. tolaasii*
Levan formation from sucrose	–	–	+	–
Arginine dihydrolase	–	–	+	+
Denitrification	–	–	+	–
Gelatin hydrolysis	–	–	+	+
Egg yolk reaction	–	+	–	+
Growth at the expense of:				
Trehalose	–	–	+	d
2-Ketogluconate	–	–	+	d
meso-Inositol	–	+	+	+
L-Valine	d	–	+	+
β-Alanine	+	–	+	+
L-Arabinose	–	+	+	d
Sucrose	–	+	+	–
Sorbitol	–	–	+	+
Adonitol	–	–	d	d
Ethanol	–	–	+	d
meso-Tartrate	–	+	–	+
Nicotinate	–	–	–	+
Staining of mushroom caps	d			+
Pitting of mushroom caps	–			+

[a]For symbols see standard definitions. Data from Fahy (1981) and Stanier et al. (1966).

Type strain: Stanier 142, ATCC 14909, LMG 1224NCIB 9945, NCTC 10367.

GenBank accession number (*16S rRNA*): Z76653.

Pseudomonas amygdali Psallidas and Panagopoulos 1975, 105[AL]

a.myg'da.li. L. n. *amygdalum* almond; L. gen. n. *amygdali* of the almond.

The following description is taken from Psallidas and Panagopoulos (1975).

Rods, 0.7 × 1.7 μm or much longer (filaments can be 10–15 times the length of normal cells). Motile by means of one to six polar flagella. No PHB accumulated. Grows better in potato-dextrose medium than in nutrient agar. Growth range, 3–32°C. No growth below pH 5. No fluorescent pigment produced. Acid is formed from D-ribose, L-arabinose, glucose, mannose, galactose, fructose, sucrose, mannitol, and sorbitol. No utilization of xylose, L-rhamnose, L-sorbose, cellobiose, lactose, maltose, melibiose, trehalose, raffinose, inulin, esculin, amygdalin, arbutin, salicin, dulcitol, erythritol, glycerol, inositol, dextrin and α-methyl-D-glucoside. Malate, citrate, succinate, and fumarate are utilized. Gluconate is slowly assimilated. Acetate, propionate, oxalate, maleate, malonate, tartrate, lactate, sulfanilic acid, picrate, hippurate, and benzoate are not utilized. Among the natural amino acids, serine, aspartate, glutamate, arginine, asparagine, proline, and histidine are utilized. Not used as carbon and/or nitrogen sources are glycine, β-alanine, leucine, isoleucine, valine, lysine, ornithine, tyrosine, phenylalanine, tryptophan, cystine, cysteine, methionine, and creatine.

Some isolates are urease positive. Tween 80 and tributyrin are rapidly hydrolyzed. Lechithinase and arginine dihydrolase negative. Gelatin, casein, esculin, arbutin, and starch are not hydrolyzed. Nitrates are not reduced. Rotting of potato slices does not occur, but the organism is positive in the hypersensitivity test on tobacco leaves. Further details

TABLE 9. General characteristics of some nonfluorescent *Pseudomonas* species[a]

Characteristics	*P. alcaligenes*	*P. corrugata*	*P. luteola*	*P. mendocina*	*P. oryzihabitans*	*P. pseudoalcaligenes*	*P. stutzeri*
Cell diameter, μm	0.5		0.8	0.7–0.8	0.8	0.7–0.8	0.7–0.8
Cell length, μm	2.0–3.0		2.5	1.4–2.8	2	1.2–2.5	1.4–2.8
Number of flagella	1	>1	>1	1b	1	1	1[b]
Yellow or orange pigments	d	+	+	+	+	–	–
Oxidase reaction	+	+	–	+	–	+	+
PHB accumulation	–	+	+	–	–	d	–
Gelatin liquefaction	d	d	+	–	–	d	–
Starch hydrolysis	–	–	–	–	–	–	+
Lecithinase	–	+		–		–	–
Lipase	d	d	–	+	–	–	+
Growth at 41°C	+	–	+	+	+	+	+
Denitrification	+		–	+	–	d	+
Arginine dihydrolase	+	d	+	+	–	d	–
Mol% G + C of the DNA	64–68	58–61	55.4	63–64	65.1	62–64	61–66

[a]For symbols see standard definitions. Data from Palleroni (1984), Kodama et al. (1985), and Sutra et al. (1997).
[b]Lateral flagella of short wavelength may be produced under certain conditions.

are given in the original paper. Assignment to rRNA group I has been decided based on rRNA–DNA hybridization studies (De Vos et al., 1985), and later confirmed by rDNA sequence analysis (Moore et al., 1996; Anzai et al., 1997). The fatty acid composition places the species in one of the subgroups of group I (Stead, 1992). Pathogenic for the almond tree (*Prunus dulcis*, family Rosaceae), in which it produces a hyperplasic bacterial canker. Not pathogenic for other fruit trees.

The mol% G + C of the DNA is: 57.7–58.5 (T_m); strain NCPPB 2610, 52.2.

Type strain: ATCC 33614, DSM 7298, LMG 2123, NCPPB 2607.

GenBank accession number (*16S rRNA*): D84007, Z76654.

Additional Remarks: The DNA similarity studies of Gardan et al. (1999) have included the type strains of this species as well as those of *P. ficuserectae, P. meliae*, and *P. savastanoi*. They indicate that these names should be considered as synonymous, and that *P. amygdali* is the correct one. Unfortunately, no decision on this point seems advisable in view of the similarity in known phenotypic properties among these organisms.

Pseudomonas anguilliseptica Wakabayashi and Egusa 1972, 584[AL]

an.guil.li.sep'ti.ca. L. n. *anguilla* eel; Gr. adj. *septica* putrefactive; M.L. adj. *anguilliseptica* pertaining to diseased eels.

The description below is taken from the original paper.

Rods, 0.4 × 2.0 μm, with a tendency to become filamentous. Motile by a single polar flagellum; motility is better at 15°C than at 25°C. Catalase and oxidase reactions positive. Gelatin is liquefied. Nitrate reduction, urease, fluorescent pigment production, and starch hydrolysis are all negative. There is no acid production from arabinose, xylose, rhamnose, fructose, galactose, glucose, mannose, sorbose, lactose, maltose, raffinose, sucrose, starch, dextrin, salicin, glycerol, mannitol, or inositol. rDNA sequencing studies place this species close to *P. aeruginosa* (see Figure 4). Isolated from diseased pond-cultured eels (*Anguilla japonica*).

The mol% G + C of the DNA is: unknown.

Type strain: ATCC 33660, DSM 12111, NCMB 1949.

GenBank accession number (*16S rRNA*): X99540.

Pseudomonas asplenii (Ark and Tompkins 1946) Săvulescu 1947, 11[AL] (*Phytomonas asplenii* Ark and Tompkins 1946, 760.)

a.sple'ni.i. M.L. neut. n. *Asplenium* genus of ferns, spleenworts; M.L. gen. n. *asplenii* of *Asplenium*.

A description is given by Haynes and Burkholder (1957) in the seventh edition of the *Manual*.

The species was tentatively placed in RNA group I by Byng et al. (1980), an assignment confirmed by nucleic acid hybridization studies (De Vos et al., 1985; Kersters et al.,

TABLE 10. Nutritional characteristics of some nonfluorescent species of *Pseudomonas*[a]

Substrates[b]	*P. alcaligenes*	*P. corrugata*[c]	*P. mendocina*	*P. pseudoalcaligenes*	*P. stutzeri*
Acetate, L-alanine, caprate, caprylate, fumarate, L-glutamate, α-ketoglutarate, lactate, L-proline, succinate	+	+	+	+	+
Aconitate, caproate, heptanoate, L-tyrosine, valerate	d	+	+	d	d
Adipate	–	–	–	–	d
β-Alanine	d		+	d	–
D-Alanine, citrate	d	+	+	d	+
α-Aminobutyrate	–	–	+	–	d
γ-Aminobutyrate	+	+	–	+	–
L-Arabinose, D-galactose, *m*-inositol, D-mannose, D-ribose, sucrose, trehalose, trigonelline, D-xylose	–	+	–	–	–
δ-Aminovalerate	d	+	–	d	d
α-Amylamine	d		–	–	–
L-Arginine	+	+	+	+	–
L-Aspartate, L-isoleucine, malonate, L-valine	–	+	+	–	d
Azelate, maltose, sebacate, starch	–	–	–	–	+
Benzoate	–	–	d	–	d
Betaine	–	+	+	+	–
Butanol, propanol, putrescine	d		+	+	d
Butylamine	d	–	–	–	–
2,3-Butylene glycol	–		–	–	d
Butyrate	d	d	+	+	+
Isobutyrate, citraconate	–	d	d	–	–
Creatine, sorbitol	–	–	–	d	–
Dodecane	d		–	–	–
Ethanolamine	–	d	–	+	d
Ethylene glycol	–		+	d	+
D-Fructose	–	+	d	+	d
Gluconate, glutarate, L-serine	–	+	+	d	d
D-Glucose	–	+	+	–	+
Glycerate	–	d	+	+	d
Glycerol	–	+	d	d	+
Glycine, D-malate	–	–	d	d	d
Glycolate	–	–	+	–	+
Histamine	d	d	–	d	–
L-Histidine	d	+	+	d	–
p-Hydroxybenzoate, mannitol	–	+	–	–	d

TABLE 10. (*Continued*)

Substrates[b]	*P. alcaligenes*	*P. corrugata*[c]	*P. mendocina*	*P. pseudoalcaligenes*	*P. stutzeri*
β-Hydroxybutyrate	–	+	+	+	+
Hydroxymethylglutarate	–		d	–	d
Isobutanol	d		+	–	d
Isovalerate, mucate, saccharate	–		+	–	d
Itaconate	–	–	+	d	+
L-Leucine	+	+	+	d	+
Levulinate	–	–	+	–	–
L-Lysine, DL-norleucine, L-ornithine, D(–)-tartrate, m-tartrate	–	d	–	–	–
L-Malate	+	+	–	+	+
Mesaconate	–	–	+	+	+
Pelargonate, propionate, spermine	+	+	+	+	d
L-Phenylalanine	–	+	d	d	d
Pyruvate	+	d	d	+	+
Sarcosine	–	+	+	d	–
L(+)-Tartrate, tryptamine	–	–	d	–	–

[a]For symbols see standard definitions. Data from Palleroni (1984) and Sutra et al. (1997).
[b]The following compounds were not used by any of the species: adonitol, anthranilate[d], D-arabinose, benzoyl-formate[d], benzylamine, cellobiose, citrulline, erythritol, hexadecane[d], hippurate[d], *o*-hydroxybenzoate, *m*-hydroxybenzoate, isopropanol, 2-ketogluconate, kynurenate[d], lactose, maleate, mandelate, D- and L-mandelate, naphthalene[d], nicotinate[d], oxalate, panthothenate[d], phenol[d], phenyl-acetate, phenyl-ethanediol[d], phthalate, pimelate, L-rhamnose, salicin, suberate, testosterone[d], and L-threonine.
[c]*N*-acetylglucosamine, D-arabitol, diaminobutane are all positive for *P. corrugata* (other species untested). D-lyxose was variable for *P. corrugata* (other species untested). Substrates not used by *P. corrugata* (other species not tested) are adonitol, 2, 3, and 4-aminobenzoates, amygdalin, L-arabitol, arbutin, creatine, cysteine, dulcitol, ethylamine, D- and L-fucose, gentiobiose, glycogen, inulin, 5-ketogluconate, melezitose, melibiose, methionine, methylglucoside, methylmannoside, methylxyloside, norvaline, isophthalate, terephthalate, raffinose, tagatose, turanose, urea, and xylitol.
[d]Compounds not been tested for *P. corrugata*.

1996). Pathogenic for the bird's-nest fern (*Asplenium nidus*), which has been the source of isolation.

The mol% G + C of the DNA is: unknown.

Type strain: ATCC 23835, LMG 2137.

GenBank accession number (*16S rRNA*): Z76655.

Pseudomonas aurantiaca Nakhimovskaya 1948, 64[AL]

au.ran.ti'a.ca. M.L. adj. *aurantiaca* orange colored.

The description is taken from the original paper.

Rods, 0.3–0.5 × 0.8–2.0 μm; motile, lophotrichous, with four to six flagella. Two main diffusible pigments, green and orange, are produced. The colony may remain orange, while the medium is stained green and fluoresces. Colorless colonies are also produced, presumably by mutation. Gelatin liquefaction is rapid. Starch is not hydrolyzed. Growth is good in complex and synthetic media. Nitrogen sources assimilated include ammonia, nitrate, amino acids, and peptone. The organism has been described as "oligonitrophilic", that is, its nitrogen requirements are satisfied by the traces of nitrogenous compounds that are contained in the "nitrogen-free" medium used in the experiments (Nakhimovskaya, 1948). Optimum temperature 25°C. Capable of growth and acid formation from arabinose, xylose, glucose, galactose, sucrose, raffinose, glycerol, and mannitol. Lactose, maltose, and starch are not utilized.

Studies on the type strain of this species have shown that it actually corresponds to *P. chlororaphis* subsp. *aureofaciens* (Kiprianova et al., 1985). These authors propose to retain the name *P. aurantiaca* for pigmented strains of biovar II of *P. fluorescens*.

The mol% G + C of the DNA is: unknown.

Type strain: NCIB 10068.

Pseudomonas avellanae
Janse, Rossi, Angelucci, Scortichini, Derks, Akkermans, De Vrijer and Psallidas 1997, 601[VP]
(Effective publication: Janse, Rossi, Angelucci, Scortichini, Derks, Akkermans, De Vrijer and Psallidas 1996, 594.)

a'vel.la.nae. L. adj. *avellana* the species name of the host, *Corylus avellana*.

The following description is taken from Janse et al. (1996), who transcribed the information from the original paper by Psallidas (1993).

Rods occurring single or in pairs. Motile by means of one to four polar flagella. PHB is not accumulated. Slow growth in nutrient agar. On sucrose-containing media they form circular, dome-shaped, glistening, semitranslucent, butyrous, radially striated, cream-white or pearl-white colonies, 2–2.5 mm and 3.5–4 mm in diameter after 3 and 7 d, respectively. A levan is formed on nutrient medium plus 5% sucrose. Weak, blue-green fluorescent diffusible pigment formed in King B medium. Pantothenate is required for growth. 4% NaCl in the medium is tolerated. Catalase and lipase (Tween 80 hydrolysis) are positive. Urease, oxidase reaction, tyrosinase, arginine and ornithine dihydrolase, amylase, gelatin liquefaction, hydrolysis of casein, and protopectinase reaction (rotting of potato slices) are all negative. Nitrates are not reduced. Hydrolysis of esculin and arbutin are negative. H_2S is not produced from peptone, cysteine, or thiosulfate. Ammonia and 2-ketogluconate are not produced.

The following carbon compounds are used for growth: L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-galactose, D-mannose, sucrose, glycerol, mannitol, *meso*-inositol, citrate, formate, fumarate, maleate, malate, malonate, mucate, nonanoate, propionate, and succinate. Some strains use D-alanine, L-alanine, L- and DL-aspartate, L-glutamate, glutamine, L-proline, and L- and DL-serine. Organic compounds not used for growth include adonitol, esculin, amygdalin, arbutin, cellobiose, dulcitol, inulin, lactose, maltose, melezitose, melibiose, *meso*-erythritol, α-methylglucoside, L-rhamnose, salicin, L-sorbose, sorbitol, trehalose, adipate, benzoate, cinnamate, hippurate, itaconate, lactate, oxalate, picrate, sulfanilate, D(−)-tartrate, L(+)-tartrate, DL-tartrate, L-aspartate, β-alanine, L-arginine, L- or DL-asparagine, L-citrulline, L-cysteine, L-cystine, glycine, L-histidine, DL-homoserine, L-hydroxyproline, L-leucine, L-lysine, L-methionine, DL-ornithine, DL-phenylalanine, L-sarcosine, DL-tyrosine, L-threonine, DL-tryptophan, and L-valine. Further details are mentioned in the original paper (Janse et al., 1996). Optimum growth temperature, 23–25°C. Maximum, 30°C. Strains contain plasmids in the range of 33–100 MDa. It causes hypersensitive reaction in tobacco leaves. Isolated from hazelnut trees (*Corylus avellana* L.), where it causes cankers and dieback on stems and branches.

The mol% G + C of the DNA is: unknown.

Type strain: Fl1, DSM 11809, ICMP 9746, NCPPB 3487.

Additional Remarks: Interesting additional information on this species is found in the literature (Janse et al., 1996). Fourteen strains were analyzed for plasmid DNA, fatty acids of whole cells, whole-cell proteins, and restriction fragment patterns of 16S rRNA. In comparison with other fluorescent species of the genus *Pseudomonas, P. avellanae* strains are homogeneous in their properties, and the molecular approaches indicate that the species, until recently considered to be a pathovar of *P. syringae* (*Pseudomonas syringae* pathovar *avellanae*, Psallidas, 1993), warrants independent species allocation.

Pseudomonas azotoformans
Iizuka and Komagata 1963b, 137[AL]

a.zo.to.for'mans. Fr. n. *azote* nitrogen; L. v. *formo* to fashion, form; M.L. part. adj. *azotoformans* nitrogen forming (by denitrification).

The description is taken from the paper by Iizuka and Komagata (1963b).

Rods, 0.6–0.8 × 1.4–2.0 μm, motile with polar flagella. Fluorescent pigment is produced. Oxidase, gelatin liquefaction, nitrate reduction, and denitrification are all positive. H_2S is not produced and starch is not hydrolyzed. Acid is produced from glucose, glycerol, xylose, sucrose, but not from lactose or starch. Glucose, gluconate, 2-ketogluconate, citrate, succinate, ethanol, *p*-hydroxybenzoate, protocatechuate, and anthranilate are assimilate, but not phenol, benzoate, salicylate, *m*-hydroxybenzoate, gentisate, *p*-aminobenzoate, or 5-ketogluconate. Additional details can be found in the original paper. Optimum growth temperature, 25–30°C. No growth at 37°C. The description suggests that this species is similar to some of the denitrifying biovars of *P. fluorescens*, but no comparative studies have been performed. Assignment to the genus *Pseudomonas* has been confirmed by 16S rDNA sequence analysis studies (Anzai et al., 1997). Isolated from Japanese rice paddies.

The mol% G + C of the DNA is: unknown.

Type strain: IAM 1603.

GenBank accession number (*16S rRNA*): D84009.

Pseudomonas balearica
Bennasar, Rosselló-Mora, Lalucat and Moore 1996, 204[VP]

ba.le.a'ri.ca. L. fem. adj. *balearica* of the Balearic Islands, where the organism was isolated.

Description taken from the paper by Bennasar et al. (1996).

Short, straight rods, 0.3–0.5 × 1.5–3.0 μm. Motile by means of a single polar flagellum. It grows aerobically, or under anaerobic conditions in the presence of nitrate. Vigorous denitrifier, liberating copious amounts of nitrogen gas from nitrate. Nonpigmented. It shares with *P. stutzeri* some of the phenotypic and morphological characteristics, including starch hydrolysis, growth with maltose, negative arginine dihydrolase, and gelatinase reactions, and a typical wrinkled colony morphology when freshly isolated. Differences with *P. stutzeri* include the ability to grow at 46°C and to tolerate 8.5% NaCl in the medium. It is also able to grow on xylose, but it does not utilize ethylene glycol, mannitol, 4-aminobutyrate, and suberate. Table 7 summarizes differences with other species of *Pseudomonas*. Some characteristics that differentiate the species from other denitrifying pseudomonads are presented in the chapter on *Burkholderia*. The two strains that have been isolated are able to degrade 2-methylnaphthalene at 40°C in wastewater or marine sediment samples.

The mol% G + C of the DNA is: 64.1–64.4 (HPLC).

Type strain: SP1402, DSM 6083.

GenBank accession number (*16S rRNA*): U26418.

Pseudomonas caricapapayae
Robbs 1956, 74[AL]

ca.ri.ca.pa'pay.ae. M.L. gen. n. *caricapapayae* of *Carica papaya*, pawpaw.

The description is taken from Robbs (1956).

Straight or slightly curved rods, 0.9–1.1 × 1.3–3.0 μm. Motile by three to six polar flagella. Green fluorescent pigment produced. Gelatin is liquefied. Starch is not hydrolyzed. Nitrates are not reduced. Acid is produced from glucose, mannose, sucrose, glycerol, and mannitol, but not from lactose, maltose, salicin, or starch. Citrate and tartrate are utilized; lactate is not. rRNA–DNA hybridization studies have confirmed the allocation of this species among the fluorescent members of the genus *Pseudomonas* (De Vos et al., 1985). Optimum growth temperature: 23–29°C; no growth below 7°C and above 45°C. Isolated from water-soaked, angular spots on leaves of pawpaw.

The mol% G + C of the DNA is: 58.9–59.4 (T_m).

Type strain: ATCC 33615, NCPPB 1873.

GenBank accession number (*16S rRNA*): D84010.

"*Pseudomonas cedrella*"
Dabboussi, Hamze, Elomari, Verhille, Baida, Izard and Leclerc 1999b, 303

ced.rel'la. M.L. fem. gen. n. *cedrella* of the cedar tree.

Rods, motile by means of a single polar flagellum. Colonies on nutrient agar are circular and smooth. On blood agar there are no signs of hemolysis. Fluorescent pigment is produced on King B agar. Oxidase, catalase, and arginine dihydrolase reactions are all negative. Some strains (2/7) liquefy gelatin. Levan is produced from sucrose. Two out of seven strains liquefied gelatin. Growth occurs at 4°C but not at 41°C. No growth occurs with 5% NaCl in the medium. Various enzymatic reactions are described in the paper (Dabboussi et al., 1999b), but gelatinase is not reported. Denitrification is a variable character. Growth at the expense of various carbon sources, particularly carbohydrates and derivatives, and various enzymatic activities are reported in the description. A comparison of growth characteristics and some general phenotypic characters, with those of other fluorescent species, is presented in the paper.

The results of DNA–DNA hybridization experiments suggest that perhaps this species should be considered as a genomovar of some of the biovars of *P. fluorescens*. Isolated from Lebanese spring water.

The mol% G + C of the DNA is: 59–60.

Type strain: CFML 96–198; CIP 105541.

GenBank accession number (*16S rRNA*): AF064461.

Pseudomonas chlororaphis
(Guignard and Sauvageau 1894) Bergey, Harrison, Breed, Hammer and Hun toon 1930, 166[AL] (*Bacillus chlororaphis* Guignard and Sauvageau 1894, 841.)

chlo.ro.ra'phis. Gr. adj. *chlorus* green; Gr. n. *raphis* a needle; M.L. fem. n. *chlororaphis* a green needle.

Fluorescent pigment is produced. Even though the species name refers to the production of the green insoluble phenazine pigment chlororaphin, which often is excreted and crystallizes around the colonies, *P. chlororaphis* has been found to be closely related to *P. aureofaciens* Kluyver (which does not produce this pigment, but instead makes another phenazine pigment that is orange and freely diffuses into the medium. The relationship between the two species was demonstrated by a high DNA similarity as determined by DNA–DNA hybridization (Palleroni et al., 1972; Johnson and Palleroni, 1989). For this reason, *P. chlororaphis* may include

two subspecies: subsp. *chlororaphis*, producing chlororaphin, and subsp. *aureofaciens*, which produces the water-soluble phenazine monocarboxylic acid. Other phenotypic properties that differentiate the two subspecies are the utilization of L-arabinose and α-amylamine by subsp. *aureofaciens*, and of D-alanine by subsp. *chlororaphis*. Optimum temperature, about 30°C. The type strain of *P. chlororaphis* subsp. *chlororaphis* was isolated from dead larvae of the cockchafer (a large European beetle). Strains of this subspecies and of subsp. *aureofaciens* have been isolated from various sources, including water. The first strain of subsp. *aureofaciens* (ATCC 13985, NCIB 9030) was isolated from clay suspended in kerosene for 3 weeks (Kluyver, 1956).

The mol% G + C of the DNA is: 63.5 (Bd).

Type strain: ATCC 9446, DSM 50083, IFO 3904, NRRL B-560, NCIB 9392.

GenBank accession number (*16S rRNA*): D86004, Z76657.

Pseudomonas cichorii (Swingle 1925) Stapp 1928, 291[AL] (*Phytomonas cichorii* Swingle 1925, 730.)

ci.cho'ri.i. Gr. pl. *cichora* succory, chicory; L. n. *cichorium* chicory; M.L. gen. n. *cichorii* of chicory.

Selected characteristics of the species are given in Tables 8 and 11. A fluorescent pigment is produced. The oxidase reaction is weak and slow and the gelatinase reaction is negative. Optimum temperature, ~30°C. Isolated from *Cichorium intybus* and *C. endivia*, for which it is pathogenic.

The mol% G + C of the DNA is: 59 (Bd).

Type strain: ATCC 10857, DSM 50259, LMG 2162, NCPPB 943, PDDCC 5707.

GenBank accession number (*16S rRNA*): Z76658.

Pseudomonas citronellolis Seubert 1960, 428[AL]

cit.ro.nel'lo.lis. M.L. gen. n. *citronellolis* of citronellol.

The following description is summarized from the original paper by Seubert (1960).

Rods, 0.5 × 1.0–1.5 μm. Motile by means of a single polar flagellum. Optimum growth temperature, approximately 31°C. Gelatin is not liquefied. Nitrates are reduced to nitrites. Growth can occur anaerobically in the presence of nitrate. H_2S is not produced. Besides citronellol, glucose, acetate, farnesol, and ionone can support growth. No growth was observed with squalene or camphoric acid. Ammonium salts, nitrate, peptone, or yeast extract can be used as nitrogen sources. Acid is produced from glycerol, but not from glucose, galactose, arabinose, fructose, sucrose, maltose, lactose, dulcitol, inositol, mannitol, inulin, or dextrin.

16S rDNA sequence analysis allocates this species in the genus *Pseudomonas* (Moore et al., 1996). Isolated from soil by enrichment with citronellol as carbon source.

The mol% G + C of the DNA is: unknown.

Type strain: ATCC 13674, DSM 50332.

GenBank accession number (*16S rRNA*): Z76659.

Pseudomonas corrugata Roberts and Scarlett 1981, 216[VP] (Effective publication: Roberts and Scarlett *in* Scarlett, Fletcher, Roberts and Lelliott 1978, 109) emend. Sutra, Siverio, Lopez, Hunault, Bollet and Gardan 1997, 1032.

cor.ru'ga.ta. L. v. *corrugare* to wrinkle up; part adj. *corrugatus* wrinkled up.

The following description is summarized from the original paper by Scarlett et al. (1978).

Rods motile by multitrichous polar flagella. According to the original description, the cells are reported to accumulate PHB as carbon reserve material; however, the granules are in fact poly-hydroxyalkanoates of different monomer composition (Kessler and Palleroni, 2000. The colonies are wrinkled (the reason for the species name), yellowish, sometimes with a green center. With age, the color may change to khaki or fawn, depending on the medium. A yellow to yellow-green diffusible, nonfluorescent pigment is produced. Gelatin is hydrolyzed, but starch is not. Egg yolk (lecithinase) reaction positive. Levan production negative. There is growth at 37°C but not at 41°C. Among the characteristics that differentiate this species from *Burkholderia cepacia* and *B. gladioli* ("*Pseudomonas alliicola*") are the absence of pectate hydrolysis and rot of onion slices, and the lack of utilization of D-arabinose, cellobiose, adipate, *meso*-tartrate, and citraconate. All these characteristics were positive for the strains of the last two species included in the study.

The above description has been emended recently (Sutra et al., 1997). This detailed study included a large number of strains of the species as well as strains of fluorescent pseudomonads also isolated from tomato pith necrosis, and the results of the analysis were studied by numerical methods, which showed that *P. corrugata* constituted a single phenon. The general phenotypic characters and nutritional properties of the strains, taken from this comprehensive report, are summarized in Tables 9 and 10. The cellular fatty acid composition places this species in a subgroup within the genus (Stead, 1992). The results of rRNA–DNA hybridization experiments allocate this species as a member of the genus *Pseudomonas* (De Vos et al., 1985), which was later confirmed by 16S rDNA

TABLE 11. Nutritional properties of some of the fluorescent phytopathogenic *Pseudomonas* species[a]

Characteristics	*P. cichorii*[b]	*P. syringae* pathovars[c]	*P. viridiflava*[d]	*P. viridiflava*[e]
Utilization of[f] :				
Glucose, mucate, succinate, glycerol, L-aspartate, L-glutamate, L-glutamine, γ-aminobutyrate	+	+	+	+
D-Ribose, D-xylose, acetate, propionate, β-hydroxybutyrate	+	d	d	d
L-Arabinose, gluconate, L-malate, citrate, aconitate	+	d	d	+
D-Mannose, D-galactose, caproate, L-arginine, betaine	+	d	+	d
D-Fructose, caprylate, pelargonate, lactate, mannitol, *m*-inositol, *p*-hydroxybenzoate, quinate, L-serine, L-proline	+	d	+	+
Raffinose	d	d	–	–
Fumarate	+	+	+	–
Sucrose, glutarate	+	d	+	–
Saccharate	+	d	d	+
Valerate	d	d	d	–
Caprate	d	+	+	d
Malonate, *m*-tartrate	+	d	d	
D-Malate, glycerate, trigonelline	+	d	+	
D(–)-Tartrate	–	–	+	+
L(+)-Tartrate, α-ketoglutarate	+	d	–	–
Hydroxymethylglutarate	–	d	d	
Pyruvate	+	+	+	d
Erythritol, sorbitol	–	d	+	+
L-Alanine	+	+	d	+
D-Alanine	d	d	+	
L-Leucine	–	–	–	+
L-Histidine	d	d	+	d
L-Tyrosine	+	–	d	–
L-Tryptophan	–	–	–	d
Putrescine	–	d	d	–
Sarcosine	d	d	–	d
Laurate				d
L-Sorbose, melezitose, amygdalin, dextrin, formate, dulcitol, isophthalate, L-methionine, *m*-aminobenzoate, *p*-aminobenzoate, methylamine				–

TABLE 11. (*Continued*)

Characteristics	*P. cichorii*[b]	*P. syringae* pathovars[c]	*P. viridiflava*[d]	*P. viridiflava*[e]
Linolenate	d	d	+	
Triacetin	+	d	+	
Tripropionin	+	d	+	
Tricaproin	d	d	+	
Ascorbate	–	d	–	
Isoascorbate	–	d	+	
Lecithin	+	d	d	
L-Asparagine	+	d	+	

[a]For symbols see standard definitions.
[b]Data from Sands et al., 1970.
[c]Data from Sands et al., 1970.
[d]Data from Sands et al., 1970.
[e]Data from Billing, 1970b.
[f]The following compounds were not utilized by any strains of the species in the table: D-arabinose, D-fucose[g], L-rhamnose, trehalose, maltose, cellobiose, lactose, melibiose, methylglucoside[g], starch, inulin, 2-ketogluconate[g], salicin[g], *N*-acetylglucosamine[g], isobutyrate, isovalerate, linoleate[g], laurylsulfate[g], tannate[g], oxalate, maleate, adipate, pimelate, suberate, azelate, sebacate, glycolate, thioglycolate[g], levulinate, citraconate, itaconate, mesaconate, 3-phosphoglycerate[g], hydroxymethylbutyrate[g],adonitol,ethylene glycol, propylene glycol, 2,3-butyleneglycol, methanol[g], ethanol, n-propanol, isopropanol, n-butanol, isobutanol, geraniol[g], D-mandelate, L-mandelate[g], benzoylformate[g], benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, phthalate, phenylacetate, phenylethanediol[g], eicosenedioate[g], naphthalene, phenol, testosterone, glycine, β-alanine, L-threonine, L-isoleucine, L-norleucine, L-valine, L-lysine, L-ornithine, L-citrulline, α-aminobutyrate, δ-aminovalerate[g], L-phenylalanine, L-hydroxyproline[g], D-tryptophan[g], indoleacetic acid[g], L-kynurenine[g], kynurenate[g], anthranilate, methylamine, ethanolamine, benzylamine, spermine, histamine[g], tryptamine[g], butylamine, α-amylamine, creatine, choline[g], hippurate, urate[g], pantothenate, acetamide, nicotinate, dodecane, hexadecane, poly-β-hydroxybutyrate[g], pectate[g], chlorogenate[g], and uridine[g].
[g]Compounds not been tried by Billing, 1970b.

sequence analysis (Anzai et al., 1997). Isolated from tomato pith necrosis.

The mol% G + C of the DNA is: 58.4–60.8 (T_m).

Type strain: ATCC 29736, DSM 7228, NCPPB 2445.

GenBank accession number (*16S rRNA*): D84012.

Pseudomonas ficuserectae Goto 1983a, 547[VP]

fi.cus.e.rec'tae. L. n. *ficuserectae* of *Ficus erecta*, the name of the host species.

The following description is taken from the paper by Goto (1983a)

Nonencapsulated straight rods, 0.5 × 2.0 μm. Motile by means of one to five polar flagella. The original description reports the accumulation of PHB granules by the cells. However, an examination of the type strain has shown that these granules are of poly-β-hydroxyalkanoates other than PHB (Kessler and Palleroni, 2000). Colonies on yeast extract-peptone agar at 28°C are white, transparent, circular, convex, 0.2–3.0 mm in diameter after 6 d. Growth on yeast extract-peptone agar slants becomes very viscid after several days. Denitrification, nitrate reduction, growth factor requirement, arginine dihydrolase reaction, starch hydrolysis, decarboxylase reaction with lysine and ornithine, phenylalanine deaminase, growth in 5% NaCl, gelatin liquefaction, hydrolysis of Tween 80, urease, and growth at 41°C are all negative. The strains grow with L-arabinose, glucose, mannose, sucrose, raffinose, glycerol, and malonate, but do not grow at the expense of galactose, maltose, trehalose, erythritol, dulcitol, xylan, inulin, glycogen, salicin, carboxymethyl cellulose, α-methylglucoside, esculin, oxalate, glycolate, butyrate, sebacate, hippurate, arginine, betaine, valine, or ethylene glycol. Additional characteristics can be found in the original paper by Goto (1983a).

A numerical analysis that included strains of this species as well as many others of aerobic pseudomonads gave results

showing that *P. ficuserectae* and *P. meliae* formed a single cluster, and suggested that they may be pathovars of *P. syringae* (Hu et al. 1991). This comment hinges on the observation that the strains under study did not accumulate PHB. For the moment, the position of *P. ficuserectae* remains unsettled, and more authentic strains will be needed for examination to confirm the correct taxonomic status. However, rDNA sequence analysis confirms its allocation to *Pseudomonas* (Moore et al., 1996). See also Additional Remarks at the end of the description of *P. amygdali* in this list. Pathogenic for *Ficus erecta*, in which it produces leaf spots that develop beside the thick veins.

The mol% G + C of the DNA is: 59 (T_m).

Type strain: ATCC 35104, DSM 6929, LMG 5694, PDDCC 7848.

GenBank accession number (*16S rRNA*): Z76661.

Pseudomonas flavescens Hildebrand, Palleroni, Hendson, Toth and Johnson 1994, 413[VP]

fla.ves'cens. L. v. *flavesco* to become golden yellow; L. part. adj. *flavescens* becoming golden yellow.

The information for the following description is taken from Hildebrand et al. (1994).

Characteristics useful for differentiation from some of *Pseudomonas* species are given in Tables 5 and 6. Slightly curved rods, 0.6–0.7 × 1.6–2.3 µm. Motile by means of a single polar flagellum. No PHB accumulated. The following characteristics are negative: arginine dihydrolase, ice nucleation, hypersensitivity reaction, denitrification, and hydrolysis of gelatin, starch, or Tween 80. It grows well in nutrient agar and other common complex media. The colonies are yellow on King medium B and other complex media. In King B, a diffusible fluorescent pigment is produced. The original report gives a list of more than 50 carbon compounds that can be used individually as carbon and energy sources, and a list of more than 80 compounds that do not support growth. Optimum temperature 28°C. No growth occurs at 37°C. Isolated from walnut blight cankers.

The mol% G + C of the DNA is: 63 (T_m).

Type strain: B62, DSM 12071, NCPPB 3063.

GenBank accession number (*16S rRNA*): U01916.

Pseudomonas fluorescens (Trevisan 1889b) Migula 1895a, 29[AL] (*Bacillus fluorescens* Trevisan 1889b, 18.)

flu.o.res'cens. L. n. *fluor* a flux; M.L. v. *fluoresce*, M.L. part. adj. *fluorescens* fluorescing.

Characteristics of the species are given in Tables 1, 5, 6, 8, and 12. Optimum temperature, 25–30°C. Found in soil and water, from which it can be isolated after enrichment in media containing various carbon sources, incubated aerobically; strains of the denitrifying biovars can be enriched in similar media containing nitrate, incubated under anaerobic conditions. Commonly associated with spoilage of foods (eggs, cured meats, fish, and milk). Often isolated from clinical specimens. Some strains assigned to this species (biovar II) have been isolated from diseased plants (e.g., lettuce), and identified as *Pseudomonas marginalis* (Brown, 1918) Stevens 1925[AL]. For more details on this species see the description below. Biovar I (biotype A of Stanier et al., 1966) is considered to by typical of *P. fluorescens*, and the type strain of the species belongs to this group. Aside from *P. marginalis* strains, biovar II (previously biotype B) also includes saprophytic organisms. In their studies on the species *P. aurantiaca* Nakhimovskaya 1948, Kiprianova et al. (1985) found that the type strain was actually *P. aureofaciens* (now a subspecies of *P. chlororaphis*), but they propose to retain the name *P. aurantiaca* for the pigmented strains of biovar II of *P. fluorescens*. Within biovar III (biotype C of Stanier et al., 1966) at least two subgroups, which differ from each other in their capacity for utilization of dicarboxylic acids, can be defined. Biovar IV (biotype F of Stanier et al., 1966) contains the type strain of "*P. lemonnieri*" (Lasseur 1913) Breed 1948, 178. Several strains are known at present, and these can be grouped into at least two clusters.

The group of miscellaneous strains assigned to *P. fluorescens* biotype G by Stanier et al. (1966) constitutes the last (V) biovar of the species. The biovar is very heterogeneous in its nutritional properties, and it may consist of strains that have lost one or more of the properties considered to be of diagnostic importance in differentiating among the better characterized biovars. Among the non-authentic strains assigned to this biovar are strains labeled "*P. schuylkilliensis*" and *P. geniculata* (Wright 1895) Chester 1901, 313[AL] (see Stanier et al., 1966). The group of strains isolated from meat that eventually was described as a new species under the name *P. lundensis* (Molin et al., 1986) corresponded to a cluster identified within biotype G (Barrett et al., 1986) (see below). Strains of biovar V are common in various materials, and have been isolated from soils (Sands and Rovira, 1971). It is quite possible that isolation of more strains of each of the different biovars may result in the future in the proposal of new species with the general properties of *P. fluorescens*, but circumscribed based on genomic differences.

The mol% G + C of the DNA is: 59.4–61.3 (Bd).

Type strain: ATCC 13525, DSM 50090, NCIB 9046, NCTC 10038.

GenBank accession number (*16S rRNA*): Z76662.

TABLE 12. Characteristics of *Pseudomonas chlororaphis* and *P. fluorescens* biovars[a]

Characteristics	*P. chlororaphis* subsp. *chlororaphis*	*P. chlororaphis* subsp. *aureofaciens*	*P. fluorescens* bv. I	*P. fluorescens* bv. II	*P. fluorescens* bv. III	*P. fluorescens* bv. IV	*P. fluorescens* bv. V
P. fluorescens biovars as designated by Stanier et al. (1966)	D	E	A	B	C	F	G
Nonfluorescent pigments, color:							
Green (chlororaphin)	+	–	–	–	–	–	–
Orange (phenazine-I-carboxylate)	–	+	–	–	–	–	–
Blue, nondiflusible	–	–	–	–	–	+	–
Levan formation from sucrose	+	+	+	+	–	+	–
Denitrification	+	d	–	+	+	+	–
Substrates used for growth:							
L-Arabinose	–	+	+	+	d	+	d
Sucrose	+	d	+	+	–	+	d
Saccharate	+	+	+	+	d	+	d
Propionate	+	+	+	–	d	+	+
Butyrate	+	d	–	d	d	+	d
Sorbitol	–	–	+	+	d	+	d
Adonitol	–	–	+	–	d	–	d
Propylene glycol	–	–	–	+	d	–	d
Ethanol	d	–	–	+	d	–	d

[a]For symbols see standard definitions. Data from Palleroni (1984).

Pseudomonas fragi (Eicholz 1902) Gruber 1905, 122[AL] (*Bacterium fragi* Eicholz 1902, 425.)

fra'gi. L. neut. n. *fragum* strawberry; L. gen. n. *fragi* of the strawberry.

The description of this species is given in the seventh edition of the *Manual* (Haynes and Burkholder, 1957).

Rods motile with a polar flagellum. The gelatinase reaction is positive. Nitrate is not reduced to nitrite. Ammonia is produced from peptone. Description of growth in various media and acid production from some sugars and sugar-alcohols are given by Haynes and Burkholder (1957). Growth occurs from 10–30°C. No growth at 37°C. The organism is very sensitive to heat.

This species was tentatively placed in rRNA group I by Byng et al. (1980), a conclusion supported by rRNA–DNA hybridization studies (De Vos et al., 1989) and by 16S rDNA sequence analysis (Moore et al., 1996; Anzai et al., 1997). Isolated from milk, dairy products, water.

The mol% G + C of the DNA is: 60.6 (T_m).

Type strain: ATCC 4973, DSM 3456, LMG 2191.

Pseudomonas fulva Iizuka and Komagata 1963b, 138[AL]

ful'va. L. adj. *fulva* tawny, yellowish brown.

Rods, 0.6–0.8 × 1.2–1.8 µm. Motile with one to three polar flagella. Fluorescent pigment is produced. Oxidase reaction feebly positive. Gelatin is not liquefied. Nitrate not reduced to nitrite or denitrified. H_2S not produced. Starch is not hydrolyzed. Acid is produced from glucose. No acid from glycerol, xylose, sucrose, lactose, or starch. Glucose, gluconate, 2-ketogluconate, citrate, succinate, ethanol, *p*-hydroxybenzoate, and protocatechuate are assimilated. Phenol, benzoate, *m*-hydroxybenzoate, gentisate, *p*-aminobenzoate, and 5-ketogluconate are not assimilated.

Optimum temperature for growth, 25–30°C. Poor growth at 37°C and no growth at 42°C. Allocation to the genus *Pseudomonas* has been confirmed by rRNA sequence analysis (Anzai et al., 1997). Isolated from Japanese rice paddies.

The mol% G + C of the DNA is: 60.6 (T_m).

Type strain: IAM 1529.

GenBank accession number (*16S rRNA*): D84015.

Pseudomonas fuscovaginae (ex Tanii, Miyajima and Akita 1976) Miyajima, Tanii and Akita 1983, 656[VP]

fus.co.va.gi'nae. L. adj. *fuscus* fuscous; L. fem. n. *vagina* vagina, sheath; M.L. fem. n. *fuscovaginae* of a fuscous vagina.

The following description is taken from the original paper.

Rods with round ends, 0.5–0.8 × 2.0–3.5 µm. Cells occur singly or in pairs. Motile by one to four polar flagella. Moderate growth on nutrient agar after 4 or 5 d at 28°C; white to light brown colonies, smooth, glistening, raised, translucent, circular, butyrous. A green fluorescent, diffusible pigment is produced on King's medium B. No slime is produced on nutrient agar plus 5% sucrose. Catalase and Kovác's oxidase reaction are positive. Denitrification and nitrate reduction are negative. Hydrolysis of Tween 80 and of gelatin is positive; esculin and arbutin are not hydrolyzed. Arginine dihydrolase reaction positive, but phenylalanine deaminase and formation of 2-ketogluconate and H_2S are negative. No organic growth factors are required. Citrate, malonate, succinate, urate, acetate, β-alanine, L-valine, and L-lysine are utilized for growth, but not tartrate, hippurate, 2-ketogluconate, or polygalacturonic acid. Optimal growth temperature is approximately 28°C. No growth occurs at 37°C. Additional characteristics may be found in the paper by Miyajima et al. (1983).

Pathogenic for rice (*Oryza sativa*) and various other plants of the family Gramineae. In rice it produces first water-soaked, dark green spots, which later become brown to dark brown. Characteristics differentiating this species from other fluorescent pseudomonads are mentioned in the original paper. Assignment to the genus *Pseudomonas* has been confirmed by rRNA–DNA hybridization studies (De Vos et al., 1985). Isolated from diseased leaf sheaths of *O. saliva* in Japan.

The mol% G + C of the DNA is: unknown.

Type strain: 6801, DSM 7231, NCPPB 3085, PDDCC 5940.

Pseudomonas gessardii Verhille, Baïda, Dabboussi, Hamze, Izard and Leclerc 1999a, 1566[VP]

ges.sar'di.i. gessardii of C. Gessard, French chemist who isolated "*Bacterium aeruginosum*" for the first time in 1882 and studied its pigment.

The following description is taken from the original paper (Verhille et al., 1999a).

Rods, motile by means of a single flagellum. Fluorescent pigment is produced on King B; no phenazine pigments are observed on King A. Oxidase, catalase, and production of levan from sucrose are all positive. Many compounds were tested as carbon and energy sources for growth and a number of enzymatic reactions were determined with the cultures. The results are listed in the original paper. According to the

authors, the utilization of L(−)-arabitol, xylitol, *myo*-inositol, adonitol, and *i*-inositol and the absence of growth in the presence of L-arabinose, D-xylose, D-saccharate, *meso*-tartrate, tricarballylate, glucuronate, galacturonate phenylacetate, and histamine, differentiate this species from *P. migulae*, a closely related species. Differentiation from other *Pseudomonas* species is given in tables in the original report. A minority of the strains (1/13) had gelatinase activity. No growth is observed in the presence of 5% NaCl in the medium. The range of growth temperatures goes from 4°C to 35°C, and the optimum is around 30°C. Isolated from natural mineral waters.

The mol% G + C of the DNA is: 58.

Type strain: CIP 105469.

GenBank accession number (*16S rRNA*): AF074384.

Pseudomonas graminis Behrendt, Ulrich, Schumann, Erler, Burghardt and Seyfarth 1999, 306[VP]

gra'mi.nis. L. n. *gramen* grass; *graminis* of grass, the source of the organism.

Rods, 0.5–1.0 × 3.5–5.0 μm, motile by one polar flagellum. Colonies are yellow, glistening, moderately convex, circular, with smooth edges. Fluorescent pigment production, levan production from sucrose, starch hydrolysis, oxidase, nitrate reduction to nitrite, denitrification, arginine dihydrolase, lysine and ornithine decarboxylase, tryptophan deaminase, DNase, urease, β-hemolysis of sheep blood, and β-galactosidase reactions are all negative. Catalase and hydrolysis of Tweens 40 and 80 are positive. Utilization of a number of carbon compounds is listed in the original paper by Behrendt et al. (1999). Unfortunately, few of the substrates tested are the same as the ones that appear in the tables of this chapter, and the species has not been included for comparison. However, the paper gives a selection of properties that allow a differentiation of this species from others of similar characteristics. The strains use monosaccharides but not the disaccharides tested.

A comparison of rRNA sequences with those of other species of *Pseudomonas* and species of other RNA similarity groups indicates that the assignment to the genus *Pseudomonas* is correct. The creation of a new species name for the strains is supported by low DNA similarity with other species of the genus. The paper is an excellent source of taxonomic information on the allocation of this species within the group of fluorescent pseudomonads. The original description includes the presence of ubiquinone Q-9 and the hydroxy fatty acids 3-hydroxydodecanoic and 2-hydroxydodecanoic. Isolated from the phyllosphere of grasses.

The mol% G + C of the DNA is: 60–61.

Type strain: P 294/08, DSM 11363.

GenBank accession number (*16S rRNA*): Y11150.

Pseudomonas jessenii Verhille, Baïda, Dabboussi, Izard and Leclerc 1999b, 54[VP]

jes.sen'ni.i. M.L. masc. gen. n. *jessenii* of Jessen, named after O. Jessen, eminent Danish bacteriologist who contributed substantially to the knowledge of fluorescent pseudomonads.

Rods, motile by means of a single polar flagellum. Colonies on nutrient agar are circular, smooth, nonpigmented. Nonhemolytic when grown on blood agar. Fluorescent pigment is produced on King B agar medium. Catalase, oxidase, and arginine dihydrolase reactions are positive. Denitrification, gelatinase, and lecithinase are negative. Nitrate is reduced to nitrite. The strains grow at 4°C but not at 41°C (range: 4–35°C). Optimal: 30°C. No growth occurs in the presence of 5% NaCl. Other properties as well as the results of nutritional and enzymatic screenings are listed in the original paper. Isolated from mineral water.

The mol% G + C of the DNA is: 57–58.

Type strain: CIP 105274.

GenBank accession number (*16S rRNA*): AF068259.

Pseudomonas libanensis Dabboussi, Hamze, Elomari, Verhille, Baïda, Izard and Leclerc 1999a, 1099[VP]

li.ba.nen'sis. L. n. *Libanus* Lebanon, in southern Syria; L. adj. *libanensis* from or of Lebanon.

The description is taken from the original paper.

Rods, motile by means of a single polar flagellum. No poly-β-hydroxybutyrate is accumulated. Colonies on nutrient agar are smooth, circular, and nonpigmented. No hemolysis is observed on blood agar. A fluorescent pigment is produced on King B medium, and no phenazine pigments are produced on King A medium. Lipase, elastase, and tetrathionate reductase are negative. Arginine dihydrolase, lecithinase, catalase, levan formation from sucrose, and oxidase reaction are all positive. The strains do not denitrify. The majority of the strains (6/7) liquefy gelatin. A number of enzymatic reactions and the results of a nutritional screening on a number of compounds (mostly carbohydrates and derivatives) are reported in the original paper by Dabboussi et al. (1999a). The cultures tolerate 3% but not 7% of NaCl in the medium.

Growth occurs between 4 and 36°C, with optimal growth at 30°C. The strains were isolated from Lebanese spring waters.

The authors of the original description (Dabboussi et al., 1999a) discuss the relationship of this species to other fluorescent organisms. *P. libanensis* seem to be closer to *P. fluorescens* biovar A, as suggested by the results of DNA–DNA hybridization experiments. This similarity extends to the main characteristics of biovar, namely, a negative denitrification test and the capacity of formation of levan from sucrose.

The mol% G + C of the DNA is: 58.

Type strain: CFML 96–195; CIP 105460.

GenBank accession number (*16S rRNA*): AF057645.

Pseudomonas lundensis Molin, Ternström and Ursing 1986, 339[VP]

lund.en'sis. M.L. adj. *lundensis* referring to the city of Lund, Sweden.

The following description is taken from the original paper.

Rods, 0.5–1.0 × 1.0–3.0 µm, motile by means of a single polar flagellum. Colonies on nutrient agar are 1–5 mm in diameter after 3 d at 25°C, circular, and smooth. No organic growth factors are required. Fluorescent pigment is produced in King B medium. Denitrification is negative. PHB is not accumulated in the cells. Arginine dihydrolase, catalase, and oxidase are positive. Gelatin liquefaction is variable. Only one strain has been observed to produce lipase against Tween 80.

All strains produce acid but not gas from L-arabinose, D-galactose, D-glucose, D-mannose, D-ribose, cellobiose, maltose, melibiose, and D-xylose. In assimilation tests, the last four sugars do not serve as carbon and energy sources. The following carbon compounds are assimilated (number of negative strains from a collection of 60 strains of the species, are given in parentheses): acetate, L-alanine, β-alanine, 4-aminobutyrate, 2-aminoethanol (1), 5-aminopantanoate, L-arabinose (1), L-arginine, L-asparagine, L-aspartate, betaine (2), citrate (1), fructose, fumarate, D-gluconate (4), D-glucose, L-glutamate, L-glutamine, glycerate (1), glycerol (1), heptanoate, L-histidine, *meso*-inositol (6), L-lactate (2), L-leucine (1), L-malate (2), L-ornithine, 2-oxoglutarate, pelargonate, L-phenylalanine, L-proline, propionate, putrescine, pyruvate, succinate, DL-tyrosine and DL-valine (1). Less than 90% of the strains can use D-arabinose, benzoate, carnosine, creatine, deoxycholate, D-galactose, DL-hydroxybutyrate, L-isoleucine, D-malate, D-ribose, sarcosine, and taurocholate. Slow utilization of butyrate, caproate, L-lysine, D-mannose, and trehalose. The main characteristics of the species are summarized in Tables 5 and 6. They refer to the type strain.

Growth occurs between 0 and 33°C, and one-third of the strains are able to grow at 37°C. Optimal growth is at 25°C. No growth at pH below 4.5. Isolated from meat. The strains correspond to cluster 2 of a previous numerical taxonomic study of psychrotrophic pseudomonads that had been isolated from meat, soil, and water (Molin and Ternström, 1982, 1986). Interestingly, results of numerical taxonomic studies performed independently at about the same time on the fluorescent pseudomonad collection at Berkeley, which included the biotype G of *P. fluorescens*, identified a tight cluster with the same properties as *P. lundensis* (Barrett et al., 1986).

The mol% G + C of the DNA is: 58–60.

Type strain: ATCC 49968, CCM 3503, DSM 6252.

Pseudomonas luteola Kodama, Kimura and Komagata 1985, 473[VP] (*Chryseomonas luteola* (Kodama, Kimura and Komagata 1985) Holmes, Steigerwalt, Weaver and Brenner 1987, 246.)

lu.te'o.la. L. dim. adj. *luteola* yellowish.

The following description is taken from the original paper.

Rods, 0.8 × 2.5 µm, with rounded ends, occurring singly, rarely in pairs. Motile by means of polar multitrichous flagella. A small number of PHB granules accumulate in the cells. Colonies on 0.5% glucose nutrient agar are smooth or wrinkled, entire or erose, flat or convex, light yellow or pale yellow, 3 mm in diameter after 2 d of growth at 30°C. A pellicle is formed on the surface of 0.5% glucose nutrient broth. A water-insoluble yellow pigment is produced. No water-soluble fluorescent pigment is produced. Catalase positive but oxidase negative. Nitrate is reduced to nitrite, but no denitrification occurs. Production of indole, hydrogen sulfide, and hydrolysis of starch, gelatin, Tween 80, esculin, and ONPG are all negative. No organic growth factors are required. Growth does not occur in a medium with 6.5% NaCl. Growth occurs at 42°C. Cleavage of protocatechuate is of the *ortho* type.

Acid is produced from L-arabinose, D-xylose, D-ribose, D-glucose, D-fructose, D-mannose, D-galactose, L-rhamnose, maltose, trehalose, mannitol, inositol, and salicin, but not from sucrose, lactose, cellobiose, adonitol, sorbitol, or inulin. L-arabinose, D-xylose, D-ribose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, trehalose, mannitol, glycerol, acetate, pyruvate, malonate, DL-β-hydroxybutyrate, fumarate, 2-ketogluconate, gluconate, succinate, *p*-hydroxybenzoate, and glutamate are utilized, but sucrose, lactose, raffinose, inulin, starch, phenol, *o*-hydroxybenzoate,

and *m*-hydroxybenzoate are not. Some properties are summarized in Table 9. The fatty acid and ubiquinone composition is typical of that of the genus *Pseudomonas*. Isolated from clinical specimens.

The mol% G + C of the DNA is: 55.4 (T_m).

Type strain: KS0921, DSM 6975, IAM 13000, JCM 3352.

GenBank accession number (*16S rRNA*): D84002.

Additional Remarks: This species, originally assigned to the genus *Pseudomonas* (Kodama et al., 1985), was subsequently considered a member of a newly proposed genus, *Chryseomonas* (Holmes et al., 1987). Later studies (Anzai et al., 1997) demonstrated that *Pseudomonas* should be its correct generic allocation.

Pseudomonas mandelii Verhille, Baïda, Dabboussi, Izard and Leclerc 1999b, 56[VP]

man.de'li.i. M.L. gen. n. *mandelii* of Mandel, dedicated to M. Mandel, American bacteriologist.

Colonies on nutrient agar are smooth, circular, and non-pigmented. Fluorescent pigment produced on King B agar medium. Catalase, oxidase, denitrification, and levan formation are positive. Gelatin and starch are not hydrolyzed. Sodium chloride inhibits growth at 5% concentration. Growth occurs at 4°C but not at 41°C. Optimal growth at 30°C. Growth at the expense of many organic compounds, as well as the result of many enzymatic reactions, are listed in the original paper (Verhille et al., 1999b).

DNA–DNA hybridization experiments indicate a relative high DNA similarity with that of biovar IV ("*P. lemonnieri*") of *P. fluorescens*. Isolated from mineral water.

The mol% G + C of the DNA is: 57.

Type strain: CFML 95–303, CIP 105273.

GenBank accession number (*16S rRNA*): AF058286.

Pseudomonas marginalis (Brown 1918) Stevens 1925, 30[AL] (*Bacterium marginale* Brown 1918, 386; *Phytomonas marginalis* (Brown 1918) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 182; *Phytomonas intybi* Swingle 1925, 730; *Chlorobacter marginale* (Brown 1918) Patel and Kulkarni 1951b, 80.)

mar.gi.na'lis. L. *margo, marginis* edge, margin; M.L. adj. *marginalis* marginal.

Description taken from Haynes and Burkholder (1957), who cite the original sources.

Rods, motile with one to three polar flagella. Green fluorescent pigment produced in culture. Agar colonies cream to yellowish. Gelatin is liquefied. There is a discrepancy among different authors on nitrate reduction. Feeble hydrolysis of starch. Acid but no gas from glucose, galactose, fructose, mannose, arabinose, xylose, rhamnose, mannitol, and glycerol. Alkali from (indicating utilization of) acetate, citrate, malate, formate, lactate, succinate, and tartrate. Sucrose, maltose, raffinose, and salicin are not degraded. Optimum growth temperature between 25 and 26°C. Minimum, 0°C; maximum, 38°C.

Isolated from marginal lesion on lettuce from Kansas; pathogenic on lettuce and related plants. Strains of this species have been included in biovar II of *P. fluorescens* (Stanier et al., 1966), and it appears under such designation in Tables 5, 6, and 12. Later, the taxon was subdivided by Young et al. (1978) into three pathovars. *P. marginalis* pathovar *marginalis*, which includes the type strain of the species, ATCC 10844; *P. marginalis* pathovar *alfalfa* Shinde and Lukezic 1974b, associated with discolored alfalfa (reference strain PDDCC 5708, NCPPB 2644), and *P. marginalis* pathovar *pastinacae* (Burkholder 1960a), a pathogen of cultivated parsnip (*Pastinaca sativa*) (reference strain ATCC 13889, PDDCC 5709, NCPPB 806). Fatty acid analysis data were taken as the basis for placing this species in a subgroup with other fluorescent phytopathogenic and saprophytic *Pseudomonas* (Stead, 1992). This subgroup also contains the nonfluorescent *P. meliae*.

The mol% G + C of the DNA is: 60 (Bd).

Type strain: ATCC 10844, LMG 2210, NCPPB 667, PDDCC 3553.

GenBank accession number (*16S rRNA*): Z76663.

Pseudomonas meliae Ogimi 1981, 382[VP] (Effective publication: Ogimi 1977, 547.)

me'li.ae. Gr. n. *Melia* Chinaberry tree, *Melia azedarach*; M.L. gen. n. *meliae* of Melia.

The following description is taken from the original paper.

Rods, 0.4–0.5 × 1.4–2.0 μm (average 0.5 × 1.8 μm). Motile by means of one to two polar flagella. Noncapsulated. Do not accumulate PHB as carbon reserve material. Colonies on nutrient agar very small after 48 h, and 0.3–1.0 mm in 72 h. Circular, smooth, convex, translucent, and white when viewed with reflected light. On potato-sucrose agar, the colonies have a rough surface and irregular shape. The rough colonies revert to the smooth type after transfer to nutrient agar. The following properties are negative: nitrate reduction, gelatin liquefaction, H_2S and indole formation, production of pyocyanine, fluorescein, and other pigments,

levan formation on nutrient-sucrose agar, arginine and lysine dihydrase reactions, starch and esculin hydrolysis, production of gluconate, tyrosinase and urease reactions, egg-yolk and lipase (margarine hydrolysis), malonate utilization, phenylalanine deaminase, methyl red, and Voges–Proskauer reactions. The catalase and oxidase reactions are positive. Nicotinate is required as an organic growth factor.

The following carbon compounds are used for growth: ribose, glucose, mannose, galactose, fructose, sucrose, glycerol, citrate, succinate, and malate. No growth is observed at the expense of xylose, rhamnose, arabinose, lactose, maltose, cellobiose, melibiose, trehalose, dextrin, glycogen, starch, inulin, mannitol, sorbitol, inositol, adonitol, dulcitol, salicin, or tartrate. Growth occurs between 4 and 37°C (optimum, 27°C) and between pH 5 and 8 (optimum, pH 7–8). rRNA–DNA hybridization studies have confirmed the allocation of this species in the genus *Pseudomonas* (De Vos et al., 1985). Based on fatty acid analysis, *P. meliae* was placed in the same subgroup constituted by phytopathogenic and saprophytic fluorescent *Pseudomonas* species (Stead, 1992). Isolated from Chinaberry tree (*Melia azedarach* L.) (family Meliaceae), where this pathogen produces the bacterial galls. The galls are formed in the leaf petioles and in branches and trunk.

The mol% G + C of the DNA is: 57.9 (T_m).

Type strain: ATCC 33050, DSM 6759, LMG 2220, NCPPB 3033.

Additional Remarks: See the Additional Remarks at the end of the description of *P. amygdali* in this list.

Pseudomonas mendocina Palleroni *in* Palleroni, Doudoroff, Stanier, Solanes and Mandel 1970, 220[AL]

men.do.ci'na. Sp. fem. n. *mendocina* native of Mendoza (Argentina).

Characteristics of the species useful for differentiation from other species are given in Tables 9 and 10. Properties useful for distinguishing the species from some denitrifying pseudomonads are summarized in the chapter on the genus *Burkholderia*. Colonies are yellowish because of production of carotenoid pigment; not adherent or wrinkled in appearance. Optimum temperature, approximately 35°C. For further descriptive information, see Palleroni et al. (1970). Found in soil and water; isolated by enrichment in media with nitrate under anaerobic conditions, especially at 40°C. Ethanol and L(+)-tartrate can be used as carbon sources in the enrichments. It has also been isolated from clinical specimens (Hugh and Gilardi, 1980).

The mol% G + C of the DNA is: 62.8–64.3 (Bd).

Type strain: ATCC 25411, DSM 50017, LMG 1223.

GenBank accession number (*16S rRNA*): M59154, Z76664.

Pseudomonas migulae Verhille, Baïda, Dabboussi, Hamze, Izard and Leclerc 1999a, 1570[VP]

mi'gu.lae. M.L. gen. n. *migulae* of W. Migula, who created the generic name *Pseudomonas*.

The following description is taken from the original paper.

Rods, motile by means of a single polar flagellum. Colonies on nutrient agar are smooth and circular. Fluorescent pigment is produced on King B agar. No phenazine pigments are synthesized. Starch and gelatin are not hydrolyzed. Levan is produced from sucrose and the arginine dihydrolase system is present in the cells. Low concentrations of NaCl (up to 0.8%) are tolerated, and 5% concentration is inhibitory. Growth occurs at 4°C but not at 41°C. The original paper reports the results of a number of enzymatic tests, as well as a list of compounds that can serve as sources of carbon and energy for growth, and a selection of these characters is used for differentiation from other fluorescent species.

DNA–DNA hybridization experiments suggest that this species may be considered as a genomovar of some of the biovars of *P. fluorescens*. Isolated from natural mineral water.

The mol% G + C of the DNA is: unknown.

Type strain: CFML 95–321, CIP 105470.

GenBank accession number (*16S rRNA*): AF074383.

Pseudomonas monteilii Elomari, Coroler, Verhille, Izard and Leclerc 1997, 849[VP]

mon.tei'li.i. M.L. masc. gen. n. *monteilii* of Monteil, in honor of Henri Monteil, a French microbiologist.

The following description is taken from the original paper.

Motile rods. Colonies on nutrient agar are circular and nonpigmented. Fluorescent pigment is produced. No hemolysis on blood agar. The strains are lipase, elastase, lecithinase, and tetrathionate reductase negative. Arginine dihydrolase, catalase, and cytochrome oxidase are produced. Denitrification, formation of levan from sucrose, gelatinase, starch hydrolysis, hydrolysis of esculin and starch, deamination of phenylalanine, exonuclease activity, lysine and ornithine decarboxylase, and the tributyrin and fibrinolysis tests are all negative. Temperature range for growth is 10–36°C; optimal growth at 30°C. Growth occurs in the presence of 3% NaCl

but not with 5% NaCl. The original paper by Elomari et al. (1997) includes an extensive nutritional and enzymatic characterization of the species. Part of the available information has been summarized in Tables 5 and 6. The strains have been isolated from clinical specimens.

The mol% G + C of the DNA is: 60 (T_m).

Type strain: CFML 90–60, CIP 104883.

GenBank accession number (*16S rRNA*): AF064458.

Pseudomonas mucidolens Levine and Anderson 1932, 344[AL]

mu.ci'do.lens. L. adj. *mucidus* musty; L. v. *oleo* to smell of; L. part. adj. *mucidolens* musty smelling.

The description below is taken from the original paper. Rods, rounded ends, occurring singly and in pairs, actively motile. Filamentous cells are frequently observed. Fluorescent pigment is produced. Gelatin is liquefied. Nitrates reduced to nitrites and gas. Optimum growth temperature 23–25°C; slight growth at 37°C and at 10°C. Acid produced from glucose, rhamnose, arabinose, erythritol, sorbitol, trehalose, galactose, fructose, and mannose. Moderate amount of acid from glycerol, mannitol, dulcitol, glycogen, inulin, maltose, melezitose, pectin, raffinose, salicin, starch, sucrose, and xylan. Lactate, citrate, and urate can be used for growth. Acetate, oxalate, sulfanilate, tartrate, salicylate, and formate do not support growth. A physiologically less active form of this species is described in the same paper under the name "*P. mucidolens* biovar tarda".

P. mucidolens is one of the species causing mustiness in eggs, which were the source for its isolation. The general characteristics as described above roughly conform to those of one of the denitrifying biovars of *P. fluorescens*, but extensive comparative studies of the phenotypic properties have not been done. However, DNA–rRNA hybridization data (De Vos et al., 1989) and rRNA sequence analysis (Anzai et al., 1997) confirm the allocation to the genus *Pseudomonas*.

The mol% G + C of the DNA is: 61.0 (T_m).

Type strain: ATCC 4685, LMG 2223.

Pseudomonas nitroreducens Iizuka and Komagata 1964a, 214[AL]

ni.tro.re.du'cens. L. n. *nitrum* nitate; L. v. *reduco* to draw backwards, bring back to a state or condition; M.L. part. adj. *nitroreducens* nitrate reducing.

The following description is taken from the original paper.

Rods, 0.4–0.6 × 1.4–1.8 μm, occurring singly, rarely in pairs; motile with polar flagella. Fluorescent pigment produced. Gelatin is not liquefied. Nitrates are reduced to nitrites and to gas. Oxidase reaction positive. H_2S not produced. Starch is not hydrolyzed. Acid is produced from glucose. Glucose, gluconate, 2-ketogluconate, citrate, succinate, ethanol, *p*-hydroxybenzoate, and protocatechuate can be utilized as sole carbon sources. 5-Ketogluconate, benzoate, salicylate, gentisate, anthranilate, and *p*-aminobenzoate are not utilized. The fresh isolate could utilize kerosene, but the activity was lost after subcultivation in the laboratory. Optimum growth temperature, 25–30°C. No growth at 37°C. The source of isolation was oil brine in Japan.

The species has two characteristics never found in association in any of the fluorescent species described in RNA group I, namely, the capacity for denitrification and the inability to utilize gelatin. Its assignment to the genus *Pseudomonas* has been confirmed by molecular studies (Anzai et al., 1997).

The mol% G + C of the DNA is: unknown.

Type strain: IAM 1439.

GenBank accession number (*16S rRNA*): D84021.

Pseudomonas oleovorans Lee and Chandler 1941, 377[AL]

o.le.o'vo.rans. L. n. *oleum* oil; L. v. *voro* to destroy, consume; M.L. part. adj. *oleovorans* oil consuming.

Description taken from the original paper.

When grown on agar, the cells are almost coccoid (0.5 × 0.8 μm), but the length increases to about 1.5 μm during the exponential phase in broth. The colonies have a typical fluorescence that is not imparted to the medium. Nitrate is reduced to nitrite. Gelatin not liquefied. Starch is hydrolyzed. Isolated from oil-water emulsions used as lubricants and cooling agents in the cutting and grinding of metals. Apparently the organism lives on some normal constituent of the cutting compound, probably the naphthenic acids, which act as emulsifying agents. Consequently, the name *oleovorans* may not be appropriate. Allocation to the genus *Pseudomonas* was confirmed by DNA–rRNA hybridization studies (De Vos et al., 1989) and by 16S rRNA sequence analysis (Moore et al., 1996; Anzai et al., 1997).

The mol% G + C of the DNA is: 63.5 (T_m).

Type strain: ATCC 8062, DSM 1045.

GenBank accession number (*16S rRNA*): Z76665.

Pseudomonas orientalis Dabboussi, Hamze, Elomari, Verhille, Baida, Izard and Leclerc 1999b, 303[VP]

or.i.en.tal'is. M.L. masc. adj. *orientalis* pertaining to the Orient.

Rods, motile by means of a single polar flagellum. Colonies on nutrient agar are circular, smooth, and nonpigmented. Fluorescent pigment is produced on King B agar.

Catalase, oxidase, gelatinase, esterase-lipase, urease, and arginine dihydrolase reactions are all positive. Levan is produced from sucrose. Reduction of nitrate to nitrite is positive, but there is no denitrification. All strains liquefied gelatin. Various enzymatic reactions are described in the original paper, but not the ability to liquefy gelatin. Assimilation of various organic compounds, particularly carbohydrates, is described.

DNA–DNA hybridization experiments give low reannealing values with other fluorescent species and biovars. Growth occurs at 4°C. Isolated from Lebanese spring water.

The mol% G + C of the DNA is: 60.

Type strain: CFML 96–170, CIP 105540.

GenBank accession number (*16S rRNA*): AF064457.

Pseudomonas oryzihabitans Kodama, Kimura and Komagata 1985, 472[VP] (*Flavimonas oryzihabitans* (Kodama, Kimura and Komagata 1985) Holmes, Steigerwalt, Weaver and Brenner 1987, 245.)

o.ry.zi'ha.bi.tans. L. fem. n. *oryza* rice; L. fem. adj. *habitans* inhabiting; M.L. fem. adj. *oryzihabitans* rice.

The description is taken from the original paper.

Rods, 0.8 × 2.0 μm, with rounded ends, occurring singly, rarely in pairs. Motile by means of a monotrichous flagellum. Granules of PHB do not accumulate. Colonies on 0.5% glucose nutrient agar are smooth or wrinkled, entire or erose, flat to convex, light yellow, 2 mm in diameter after 2 d incubation at 30°C. A pellicle is formed on the surface of 0.5% glucose nutrient broth. A water-insoluble yellow pigment is produced. No water-soluble fluorescent pigment is produced. Catalase is produced; the oxidase reaction is negative. Nitrate reduced to nitrite, but no denitrification occurs. Urease is produced. Production of indole, hydrogen sulfide, and hydrolysis of starch, Tween 80, esculin, and ONPG are all negative.

Growth occurs in medium containing 6.5% NaCl. No organic growth factors are required. Cleavage of protocatechuate is of the *ortho* type. Acid is produced from L-arabinose, D-xylose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, trehalose, mannitol, sorbitol, and inositol, but not from L-rhamnose, sucrose, lactose, cellobiose, adonitol, salicin or inulin. L-arabinose, D-xylose, D-ribose, D-glucose, D-mannose, D-galactose, maltose, trehalose, mannitol, glycerol, acetate, pyruvate, malonate, DL-β-n-hydroxybutyrate, fumarate, gluconate, 2-ketogluconate, succinate, *p*-hydroxybenzoate, and glutamate are utilized, but not sucrose, lactose, raffinose, inulin, starch, phenol, *o*-hydroxybenzoate, or *m*-hydroxybenzoate. Hydrogen is not utilized. Some properties are given in Table 9. The cellular fatty acid and ubiquinone composition is the typical type of the genus *Pseudomonas*. Isolated from a Japanese rice paddy.

The mol% G + C of the DNA is: 65.1 (T_m).

Type strain: KS0036, ATCC 43272, DSM 6835, IAM 1568, JCM 2592.

GenBank accession number (*16S rRNA*): D84004.

Additional Remarks: This species was originally described as a member of the genus *Pseudomonas* (Kodama et al., 1985), but was later assigned to the proposed new genus name *Flavimonas* (Holmes et al., 1987). Further studies (Anzai et al., 1997) demonstrated that the latter should be considered a synonym of *Pseudomonas*.

Pseudomonas pertucinogena Kawai and Yabuuchi 1975, 318[AL]

per.tu.ci.no'ge.na. pertucin (coined word), a bacteriocin active against *Bordetella pertussis*; L. v. *gigno* to produce; M.L. fem. adj. *pertucinogena* intended to mean pertucin producing.

The description is from the original paper, referring to two strains of the species.

Rods, 0.4 × 1.1 μm, motile by means of single polar flagellum. Do not accumulate PHB. Oxidase reaction positive. No pigments are produced. Hydrolysis of gelatin, starch, or Tween 80, and arginine dihydrolase reaction are all negative. Acid production from sugars is in general weak or negative (glucose, D-arabinose, and galactose are positive). Pyruvate, succinate, oxaloacetate, β-hydroxybutyrate, and L-alanine are utilized for growth. A number of amino acids, carbohydrates, and alcohols are not utilized. Further details are given in the original paper. The two known strains produce pertucin, a bacteriocin active against *Bordetella pertussis*, and were kept for many years in the American Type Culture Collection as members of this species.

The mol% G + C of the DNA is: ~60.

Type strain: ATCC 190.

Pseudomonas pseudoalcaligenes Stanier *in* Stanier, Palleroni and Doudoroff 1966, 247[AL]

pseu.do.al.ca.li'ge.nes. Gr. adj. *pseudes* false; M.L. adj. *alcaligenes* alkali-producing; M.L. adj. *pseudoalcaligenes* false alkali-producing.

Characteristics useful for differentiation from other *Pseudomonas* species are given in Tables 9 and 10, and the chapter on the genus *Burkholderia*, which summarizes information on denitrifying pseudomonads. Optimum temperature, 35°C. Further descriptive information can be found in Ralston-Barrett et al. (1976) and Stanier et al. (1966). The

nutritional spectrum is quite narrow (see *P. alcaligenes*). The gelatinase reaction is negative. The species is rather heterogeneous. The collection examined by Stanier et al. (1966) included at least two groups. Strains of one of the groups were capable of PHB accumulation and gave a positive arginine dihydrolase reaction, while the strains of the second group were negative for the two properties and also differed in some nutritional characteristics (Doudoroff and Palleroni, 1974).

Three subspecies were thought to integrate this species, namely, *P. pseudoalcaligenes* subsp. *pseudoalcaligenes* (corresponding to the original description by Stanier et al., 1966), *P. pseudoalcaligenes* subsp. *citrulli* Schaad et al. 1978 (a name given to strains isolated from water soaked lesions on cotyledons of watermelon, *Citrullus lanatus*, and also pathogenic for other plants of the same family) (Schaad et al., 1978), and *P. pseudoalcaligenes* subsp. *konjaci* (the causal agent of bacterial blight of konjac, *Amorphophallus konjaci* Koch) (Goto, 1983b). Nutritional characteristics that were found useful for rapid identification were the utilization of L-arabinose and galactose by the subspecies *citrullus* (subsp. *pseudoalcaligenes* and subsp. *konjaci*, and *P. alcaligenes* are negative), and the utilization of mannitol by subsp. *konjaci* but not by subsp. *pseudoalcaligenes* and subsp. *citrulli*, and *P. alcaligenes* (Goto, 1983b).

The fatty acid composition of *P. pseudoalcaligenes* and its subspecies placed this taxon in one of the subgroups of *Pseudomonas* together with other nonfluorescent species (Stead, 1992). In spite of the above considerations, the results of a numerical analysis supplemented by DNA–DNA reassociation experiments (Hu et al., 1991) were taken as the basis for the conclusion that subsp. *citrulli* and subsp. *konjaci* should be transferred to a different species, *P. avenae*, which had been allocated to rRNA similarity group III (Palleroni, 1984). Consequently, this species should include three subspecies: *P. avenae* subsp. *avenae* Manns 1909 (type strain ICMP 3183), *P. avenae* subsp. *citrulli* (Schaad et al., 1978) comb. nov. (type strain ICMP 7500), and *P. avenae* subsp. *konjaci* (Goto, 1983a) comb. nov. (type strain ICMP 7733) (Hu et al., 1991). Studies by Willems et al. (1992) support the conclusion that these various phytopathogenic pseudomonad taxa should be transferred to the genus *Acidovorax* and the new combinations *Acidovorax avenae* subsp. *citrulli* and *A. avenae* subsp. *cattleyae* as well as the new name *Acidovorax konjaci* have been proposed. *P. pseudoalcaligenes* has been isolated from various natural materials and from clinical specimens.

The mol% G + C of the DNA is: 62.2–63.2 (Bd).

Type strain: ATCC 17440, LMG 1225, NCIB 9946.

GenBank accession number (*16S rRNA*): Z76666.

Pseudomonas putida (Trevisan 1889b) Migula 1895a, 29[AL] (*Bacillus putidus* Trevisan 1889b, 18.)

pu'ti.da. L. fem. adj. *putida* stinking, fetid.

Characteristics of the species (103 strains of biovar A and 9 strains of biovar B) are given in Tables 5 and 6. Other properties of interest are summarized in Tables 1 and 7. The characteristics that are useful for a separation from *P. aeruginosa* and *P. fluorescens* include the inability to liquefy gelatin, to produce any phenazine pigments, to denitrify or to give an egg-yolk reaction, and to grow at 41°C. In this constellation of negative properties, the incapacity to hydrolyze gelatin is the one that has classically defined *P. putida*. Optimum temperature, 25–30°C.

Isolated from soil and water after enrichment in mineral media with various carbon sources. The majority of the strains have been assigned to biovar A (biotype A of Stanier et al., 1966), which is considered typical. Biovar B differs from biovar A only in a few phenotypic properties: all known strains of this biovar utilize L-tryptophan, kynurenine, and anthranilate, and most use D-galactose as carbon sources. None of the strains of biovar B uses nicotinate. Recent studies on many strains of this species, combining a numerical analysis of phenotypic data with rRNA gene restriction patterns (ribotyping), have indicated the convenience of a revision of its present internal subdivision into biovars (Elomari et al., 1994), as previously suggested by Barrett et al. (1986) and by Stenström et al. (1990) (see also the sections on taxonomic comments and on ribotyping, in particular the contribution by Brosch et al., 1996).

The mol% G + C of the DNA is: 62.5 (biovar A; thermal denaturation); 60.7 (biovar B).

Type strain: ATCC 12633, DSM 291, NCIB 9494.

GenBank accession number (*16S rRNA*): D37923.

Pseudomonas resinovorans Delaporte, Raynaud and Daste 1965, 1075[AL]

re.si.no'vor.ans. L. n. *resina* resin; L. v. *voro* to devour, digest; M.L. part. adj. *resinovorans* resin digesting.

The description is taken from the original paper.

Rods, 0.6–0.7 × 2.0–2.5 μm. Motile by means of a polar flagellum. Fluorescent pigment is produced. Gelatin is not liquefied. Nitrate reduction is weak, and denitrification is negative. Oxidase reaction positive. Optimum growth temperature, 28–30°C; no growth at 5°C or 42°C. No acid is produced from arabinose, xylose, rhamnose, glucose, fructose, galactose, mannose, lactose, maltose, sucrose, raffinose, inulin, salicin, dextrin, glycerol, mannitol, inositol, or

dulcitol. Starch hydrolysis very weak. Growth occurs at the expense of colophony, Canada balsam, or abietic acid. Phenol, phenanthrene, salicylic acid, *m*-cresol, and naphthalene can also be used as carbon and energy sources for growth. Further information may be found in the original paper by Delaporte et al. (1965). Assignment to the genus *Pseudomonas* is confirmed by results on nucleic acid hybridization (De Vos et al., 1989) and by 16S rDNA sequence analysis (Moore et al., 1996).

The mol% G + C of the DNA is: 63.7 (T_m).

Type strain: ATCC 14235, CCUG 4439, LMG 2274.

GenBank accession number (*16S rRNA*): Z76668.

Pseudomonas rhodesiae Coroler, Elomari, Hoster, Izard and Leclerc 1996, 603^{VP}

rho.de'si.ae. M.L. fem. gen. n. *rhodesiae* of Rhodes, in honor of M.R. Rhodes, an English microbiologist.

Rods, motile by means of single polar flagella. Colonies on nutrient agar are smooth, circular, and nonpigmented. No hemolysis observed on blood agar. Fluorescent pigment is produced in King B medium. Arginine dihydrolase, catalase, cytochrome oxidase, lecithinase, nitrate reduction to nitrite, formation of levan from sucrose, growth on cetrimide agar, lecithinase reaction, and decomposition of L-tyrosine are all positive. PHB is not accumulated. Phenazine pigments are not produced. Gelatin liquefaction, lipase reaction, esculin and starch hydrolysis, deamination of phenylalanine, and exonuclease production are all negative. A large number of enzymatic properties are described in the original paper. A summary of properties of this species is presented in Tables 5 and 6. A number of additional characteristics can be found in the original paper and elsewhere (Elomari et al., 1995). Isolated from natural mineral water.

The mol% G + C of the DNA is: 59 ± 1 (T_m).

Type strain: CIP 104664.

GenBank accession number (*16S rRNA*): AF064459.

Pseudomonas savastanoi (Janse 1982) Gardan, Bollet, Abu Ghorrah, Grimont and Grimont 1992, 611^{VP} (*Pseudomonas syringae* subsp. *savastanoi* Janse 1982, 168.)

sa.vas.ta'no.i. L. gen. n. *savastanoi* of Savastano, the first worker who studied olive knot disease.

The description is taken from the above-mentioned paper.

Rods, 0.4–0.8 × 1.0–3.0 μm. Motile by means of one to four polar flagella. Rather slow growing. Colonies are white or cream, smooth, flat, and glistening with entire or erose margins. Strains produce an hypersensitive reaction on tobacco leaves. Other properties of interest are summarized in Table 2. Metabolism is respiratory. Oxidase negative. Nitrates are not reduced. Blue fluorescent pigment is produced on King B medium and can be observed under UV light. Arginine dihydrolase negative. Esculin, gelatin, and starch are not hydrolyzed. Strains assimilate sucrose, L-arabinose, gluconate, caprylate, fumarate, DL-glycerate, L-malate, pyruvate, citrate, D-alanine, and L-proline. They do not assimilate lactose, L-xylose, adonitol, 2-aminobutyrate, DL-lactate, D-β-hydroxybutyrate, D (−)-tartrate, L-cysteine, L-methionine, and L-valine.

Strains have been isolated for many years from members of the family Oleaceae (olive, oleander). These do not produce levan from sucrose. Strains isolated from another species of the same family (*Fraxinus excelsior* L.), *Phaseolus vulgaris*, and *Glycine max* (Leguminosae) do produce levan. The species is now divided into three pathovars. *P. savastanoi* pathovar *savastanoi* causes knots, galls, and cankers on plants of various genera of the family Oleaceae; *P. savastanoi* pathovar *glycinea* causes bacterial blight of soybean; and *P. savastanoi* pathovar *phaseolicola* causes halo blight of bean.

The mol% G + C of the DNA is: 60 (T_m).

Type strain: ATCC 13522, CFBP 1670, ICMP 4352, NCPPB 639.

Additional Remarks: The paper by Gardan et al. (1992) essentially is a proposal to revive the name *P. savastanoi*, which had been created by Stevens in 1913 in a valid publication. This name was omitted from the 1980 approved list of bacterial species, an omission that had its origin in the fact that the name had been included in a list of pathovars of the species *P. syringae*.

The restoration of *P. syringae* pathovar *savastanoi* to its original status as independent species (*P. savastanoi*) after two demotions (one to the subspecies category, and the other to pathovar), has been formulated based on a phenotypic analysis and DNA–DNA hybridization studies (Gardan et al., 1992). In fact the phenotypic properties that differentiate this taxon from other related fluorescent, oxidase negative plant pathogens, are very few. In addition, the conditions of the nucleic acid hybridization used by Gardan et al. (1992) apparently were those used in the original description of the adopted methodology (Crosa et al., 1973b), even to the point of the incubation at 60°C, which is low for organisms of mol% G + C content higher than *E. coli*. As a consequence, the estimated similarities reported in the paper tend to be high, although the figures can be grouped into blocks that reflect differences between *P. savastanoi* and other *P. syringae*

pathovars, with the exception of the type strains of pathovars *phaseolicola* and *glycinea*. Consequently, the proposal subdivides *P. savastanoi* into pathovar *savastanoi*, pathovar *phaseolicola*, and pathovar *glycinea*. These last two names had also been used until now to designate pathovars of *P. syringae*

Recent studies on DNA similarity by hybridization methods indicate that *P. savastanoi, P. ficuserectae, P. meliae*, and *P. amygdali* should be considered as synonyms, in which case the latter is the correct name of the species (Gardan et al., 1999).

Pseudomonas straminae Iizuka and Komagata 1963b, 139[AL] emend. Uchino, Kosako, Uchimura and Komagata 2000, 1518.

stra.mi'na.e. L. adj. *straminae* made of straw.

Slender rods, 0.3 × 3.0 μm. Motile with single polar flagellum. Colonies are yellow. Fluorescent pigment is produced. Oxidase reaction positive. Gelatin is liquefied. Nitrates are not reduced to nitrites. Denitrification is negative. H_2S is produced. Starch is not hydrolyzed. Acid produced from glucose. Glucose, gluconate, citrate, succinate, and ethanol are assimilated. Phenol, benzoate, salicylate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, protocatechuate, gentisate, anthranilate, *p*-aminobenzoate, 2-ketogluconate, and 5-ketogluconate are not assimilated. Optimum growth temperature, 25–30°C. No growth at 37°C. Assignment to the genus *Pseudomonas* has been confirmed by 16S rDNA sequence analysis (Anzai et al., 1997). The organism has been isolated from Japanese rice paddies.

The mol% G + C of the DNA is: unknown.

Type strain: IAM 1598.

GenBank accession number (*16S rRNA*): D84023.

Pseudomonas stutzeri (Lehmann and Neumann 1896) Sijderius 1946, 115[AL] (*Bacterium stutzeri* Lehmann and Neumann 1896, 237.)

stut'ze.ri. stutzeri Stutzer patronymic, of Stutzer.

Characteristics differentiating the species from other species of *Pseudomonas* are given in Tables 9 and 10. Differentiation from a number of denitrifying pseudomonads can be found in Table 7 and in the chapter on the genus *Burkholderia* appearing in Volume 2 Part C in this *Manual*. Recently isolated colonies are adherent, dark brown, and have a characteristic wrinkled appearance, which usually is lost after repeated transfers in laboratory media. The colonies may eventually become smooth, butyrous, and pale in color. The gelatinase reaction is negative. Among the characteristics useful for its identification are the vigorous denitrification, the appearance of the colonies, and the use of starch as a carbon and energy source. Variations in some of the typical characteristics are observed in some populations, depending of the enrichment conditions. Some strains grow at 43°C. Optimum temperature, ~35°C.

The species is markedly heterogeneous in nutritional properties and in DNA base composition. Further descriptive information: van Niel and Allen (1952), Sijderius (1946), and Palleroni et al. (1970). Found in soil and water, from which it can be isolated after enrichment in media with nitrate under anaerobic conditions at 30°C using various carbon sources. L(+)-Tartrate gives excellent results in the enrichments (van Niel and Allen, 1952) although, paradoxically, strains obtained in this manner may not grow with tartrate in pure culture. Many strains have been isolated from clinical specimens. Recently, the results of studies on the genomic structure and organization, as well as on the genotypic and phenotypic diversity of *P. stutzeri*, have been published (Rosselló et al., 1991, Rosselló–Mora et al., 1994b; Rainey et al., 1994b; Ginard et al., 1997). Seven DNA–DNA similarity groups (genomovars) have been defined in this species (Rosselló-Mora et al., 1994b). This subdivision correlated with clusters obtained by comparison of 16S rDNA gene sequences. One of the genomovars was raised to the category of species, for which the name *P. balearica* was created (see below). The name *P. stanieri* (Mandel 1966) has been proposed for strains with a mol% G + C of ~62. However, this species is not clearly differentiated from *P. stutzeri* based on phenotypic characteristics (Palleroni et al., 1970).

The mol% G + C of the DNA is: from 60.6–66.3 (Bd).

Type strain: AB 201, ATCC 17588, CCUG 11256, DSM 5190.

GenBank accession number (*16S rRNA*): U26262.

Pseudomonas synxantha (Ehrenberg 1840) Holland 1920, 220[AL] (*Vibrio synxanthus* Ehrenberg 1840, 202.)

syn.xan'tha. Gr. pref. *syn* along with, together; Gr. adj. *xanthus* yellow; M.L. adj. *synxanthus* with yellow.

The description of this species is given by Haynes and Burkholder (1957) in the seventh edition of the *Manual*.

Gelatinase positive. Growth characteristics in various solid and liquid media are described by Haynes and Burkholder (1957). A distinctive characteristic is the production of an intense, diffusible, yellow to orange pigment in cream or in the cream layer in milk.

rRNA–DNA hybridization studies indicate that this is a species of the genus *Pseudomonas* (De Vos et al., 1989), which was later confirmed by 16S rDNA sequencing analysis (Anzai et al., 1997). Isolated from bitter milk.

The mol% G + C of the DNA is: 61. (T_m).

Type strain: ATCC 9890, LMG 2190.

Pseudomonas syringae
Van Hall 1902141[AL] (*Phytomonas syringae* (van Hall 1902) Bergey, Harrison, Breed, Hammer and Huntoon 1930, 257; *Pseudomonas barkeri* (Berridge 1924) Clara 1934, 11; *Pseudomonas citrarefaciens* (Lee 1917) Stapp 1928, 190; *Pseudomonas citriputealis* (Smith 1913) Stapp 1928, 190; "*Pseudomonas hibisci*" (Nakada and Takimoto 1923) Stapp 1928; *Pseudomonas prunicola* Wormald 1930, 742; *Pseudomonas punctulans* (Bryan 1933) Săvulescu 1947, 12; *Pseudomonas rimaefaciens* Koning 1938, 11; *Pseudomonas spongiosa* (Leifson 1962b); *Pseudomonas tonelliana* (Ferraris 1926) Burkholder 1948b, 132; *Pseudomonas trifoliorum* (Jones, Williamson, Wolf and McCulloch 1923) Stapp 1928; *Pseudomonas utiformica* Clara 1932, 111; *Pseudomonas vignae* Gardner and Kendrick 1923, 275; *Pseudomonas viridifaciens* Tisdale and Williamson 1923, 150.)

sy.rin'gae. M.L. fem. n. *Syringa* generic name of lilac; M.L. fem. gen. n. *syringae* of the lilac.

Characteristics of the species and of some of its pathovars are described in Tables 11 and 13. The pattern of acid production from sugars will be found in Haynes and Burkholder (1957) and in the original papers describing the nomenspecies here included as pathovars.

Cytochrome *c* is not detectable. Fluorescent pigment is produced. The gelatinase reaction is positive. Rare strains may require organic growth factors. Growth of most strains is slow in mineral media with a single carbon source and is relatively slow in complex media. Nutritional spectrum is less extensive and more heterogeneous than that of the saprophytic fluorescent pseudomonads. Optimum temperature, 25–30°C. The original strain was isolated from lilac (*Syringa vulgaris*, family Oleaceae) but strains conforming to the original description are pathogenic for many unrelated plants.

The mol% G + C of the DNA is: 60.5.

Type strain: ATCC 19310, DSM 6693, LMG 1247, NCPPB 281, PDDCC 3023.

Additional Remarks: The following is a list of pathovars of *P. syringae* and of the respective pathotypes proposed by Dye et al. (1980). The names of the most important host plants and of their botanical families are also included. The list includes pathovar *mori*, although this name has priority over *syringae*. As recommended by Young et al. (1978), *P. syringae* is widely known and it should be conserved over "*P. mori*". Many of the pathovars in the list may not be distinguishable from *P. syringae* except for their host range. Few phenotypic characters are taxonomically useful.

Studies on the internal subdivision of *P. syringae* into pathovars using various molecular techniques, in particular DNA–DNA hybridization, have suggested that some of the pathovars should be elevated to independent species status. At present, however, attempts in this direction have materialized only in a few instances. The difficulty is in finding sets of phenotypic properties that could help in the ready identification of such species without the need of nucleic acid similarity experiments. A recent study centered mainly on this approach and in ribotyping (Gardan et al., 1999) extended early investigations that defined clusters of genomically related strains within *P. syringae* (Pecknold and Grogan, 1973).

The studies of Gardan and collaborators (1999) defined nine genomospecies. The conclusions included the proposal for synonymy of the names *P. savastanoi, P. ficuserectae, P. meliae*, and *P. amygdali*, with *P. amygdali* as the earliest name in this group. In addition, genomospecies 3 and 7 may be named in the future "*P. tomato*" and "*P. tagetis*", respectively, once phenotypic data for differentiation become available. Finally, genomospecies 5 and 9, for which such data are available, are given the species names *P. tremae* and *P. cannabina*, respectively.

The genomospecies 5 (*P. tremae*) is represented by a single strain (Gardan et al., 1999). According to the description given by Gardan and collaborators, *P. tremae* is a non-fluorescent organism, but evaluation of its relationships with other species or pathovars or with nonfluorescent species is not clear at this stage. The description includes a nutritional analysis where the strain appears to be unable to use L-alanine, fructose, glycerol, or gluconate, and it is stated that it did not assimilate the 91 other carbon sources of the Biotype-100 strips (BioMérieux). Since there is only one strain available at this moment, it is not possible to ascertain the intraspecies diversity of the species. Experiments on the DNA relatedness of *P. tremae* and *P. cannabina* to other taxa have been limited mainly to *P. syringae* pathovars but have not included most other species of the genus. Descriptions of *P. tremae* and *P. cannabina*, as given in the paper by Gardan et al. (1999), are transcribed below in the section Other Species.

As a general comment, the taxonomic situation of the members of the conglomerate known under the name *P. syringae* still remains largely unsettled, but it is reasonable to expect that future studies will contribute to reach a satisfactory internal subdivision of the species and a more precise circumscription of pathovars segregated as individual species on the basis of genomic and phenotypic properties.

TABLE 13. Characteristics of some *Pseudomonas syringae* pathovars and of *Pseudomonas savastanoi* and its two biovars, biovar glycinea and biovar phaseolicola[a]

Characteristics	*P. savastanoi*	*P. savastanoi* pathovar *glycinea*	*P. savastanoi* pathovar *phaseolicola*	*P. syringae* pathovar *antirrhini*	*P. syringae* pathovar *aptata*	*P. syringae* pathovar *cannabina*	*P. syringae* pathovar *coronafaciens*	*P. syringae* pathovar *delphinii*	*P. syringae* pathovar *eriobotryae*	*P. syringae* pathovar *lachrymans*	*P. syringae* pathovar *mori*	*P. syringae* pathovar *morsprunorum*	*P. syringae* pathovar *passiflorae*	*P. syringae* pathovar *persicae*	*P. syringae* pathovar *pisi*	*P. syringae* pathovar *sesami*	*P. syringae* pathovar *striafaciens*	*P. syringae* pathovar *syringae*	*P. syringae* pathovar *tabaci*	*P. syringae* pathovar *tomato*
Levan formation	−	+	+	+	+	+	+	d	+	+	+	+	−	+	+	+	+	+	+	+
Pectate gel pitting[b]	4	4	4		−	4	4,8	4	4	4,8		4	4	4	−	4	−	−	4,8	4
β-Glucosidase	−	−	−	+	+	−	+	+	+	+	−	−	+	−	d	−	+	+	+	+
Growth on:																				
Mannitol	+	d	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Betaine	+	−	d	+	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+
Inositol	d	+	−	+	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+
Sorbitol	d	−	−	+	+	−	+	+	+	+	d	+	+	+	+	−	+	+	+	+
Trigonelline	d	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	+
Quinate	−	+	+	+	+	−	+	+	+	+	−	+	+	−	+	+	−	+	+	+
Erythritol	−	−	−	−	+	−	+	+	+	+	−	d	+	−	d	+	+	+	d	−
L-Tartrate	d	−	−	−	−		−	−	+	+	−	+	−	−	−	+	−	−	+	−
D-Tartrate	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	d	−	+
L-Lactate	−	−	−	−	+	−	−	−	−	−	−	−	−	−	d	−	−	+	−	−
Anthranilate	d	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Homoserine	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−

[a]For symbols see standard definitions. Data from Sands et al. (1970) and from Gardan et al. (1992).
[b]Method of Hildebrand (1971); numbers represent pH values at which pitting occurs.

Pseudomonas syringae *pathovar* **syringae** van Hall 1902.

Hosts: lilac (*Syringa vulgaris*, family Oleaceae) and several unrelated plants.

Deposited strain: ATCC 19310, NCPPB 281, PDDCC 3023 (neopathotype strain).

Pseudomonas syringae *pathovar* **aceris** (Ark 1939) Young et al. 1978.

Hosts: *Acer macrophyllum, Acer* spp. (family Aceraceae).

The mol% G + C of the DNA is: 61.1.

Deposited strain: ATCC 10853, NCPPB 958, PDDCC 2802 (Sneath and Skerman, 1966) (neopathotype strain).

Pseudomonas syringae *pathovar* **antirrhini** (Takimoto 1920) Young et al. 1978.

Host: *Antirrhinum majus* (family Scrophulariaceae).

The mol% G + C of the DNA is: 60.4.

Deposited strain: NCPPB 1817, PDDCC 4303 (neopathotype strain).

Pseudomonas syringae *pathovar***apii** (Jagger 1921) Young et al. 1978.

Host: celery (*Apium graveolens*, family Umbelliferae).

The mol% G + C of the DNA is: 60.8.

Deposited strain: ATCC 9654, NCPPB 1626, PDDCC 2814 (neopathotype strain).

Pseudomonas syringae *pathovar* **aptata** (Brown and Jamieson 1913) Young et al. 1978.

Hosts: sugar beet (*Beta vulgaris*, family Chenopodiaceae), *Nasturtium* sp. (family Cruciferae); lettuce (*Lactuca saliva*, family Compositae).

Deposited strain: NCPPB 871, PDDCC 459 (neopathotype strain).

Pseudomonas syringae *pathovar* **atrofaciens** (McCulloch 1920) Young et al. 1978.

Hosts: wheat (*Triticum* sp.) and other plants of the family Gramineae.

Deposited strain: NCPPB 2612, PDDCC 4394 (neopathotype strain).

Pseudomonas syringae *pathovar* **atropurpurea** (Reddy and Godkin 1923) Young et al. 1978.

Hosts: *Bromus inermis* and many other *Bromus* spp., *Agropyron repens* and many other plants of the family Gramineae.

Deposited strain: NCPPB 2397, PDDCC 4457 (neopathotype strain).

Pseudomonas syringae *pathovar* **berberidis** (Thornberry and Anderson 1931a) Young et al. 1978.

Hosts: barberry (*Berberis thunbergii, B. inermis*, family Berberidaceae).

The mol% G + C of the DNA is: 59.7–60.1.

Deposited strain: NCPPB 2724, PDDCC 4116 (neopathotype strain).

Pseudomonas syringae *pathovar* **cannabina** (Šutić and Dowson 1959) Young et al. 1978.

Hosts: *Cannabis sativa* (family Moraceae); *Phaseolus vulgaris, Vicia sativa* (family Leguminosae).

The mol% G + C of the DNA is: 59.3–60.3.

Deposited strain: PDDCC 2823 (NCPPB 1437) (neopathotype strain).

Pseudomonas syringae *pathovar* **ciccaronei** (Ercolani and Caldarola 1972) Young et al. 1978.

Host: carob (*Ceratonia siliqua*, family Leguminosae).

Deposited strain: NCPPB 2355, PDDCC 5710 (pathotype strain).

Pseudomonas syringae *pathovar* **coronafaciens** (Elliott 1920) Young et al. 1978.

Hosts: *Avena sativa, Bromus inermis, Agropyron repens* and various other wild and cultivated plants of the family Gramineae. Description as "*P. coronafaciens*" is transcribed by Haynes and Burkholder (1957) in the seventh edition of *Bergey's Manual of Determinative Bacteriology*.

The mol% G + C of the DNA is: 59.3.

Deposited strain: NCPPB 600, PDDCC 3113 (neopathotype strain).

Pseudomonas syringae *pathovar* **delphinii** (Smith 1904) Young et al. 1978.

Host: *Delphinium* sp. (family Ranunculaceae).

Deposited strain: NCPPB 1879, PDDCC 529 (neopathotype strain).

Pseudomonas syringae *pathovar* **dysoxyli** (Hutchinson 1949) Young et al. 1978.

Host: *Dysoxylum spectabile* (family Meliaceae).

Deposited strain: ATCC 19863, NCPPB 225, PDDCC 545 (neopathotype strain).

Pseudomonas syringae *pathovar* **eriobotryae** (Takimoto 1931) Young et al. 1978.

Host: loquat (*Eriobotrya japonica*, family Rosaceae).

The mol% G + C of the DNA is: 58.3.

Deposited strain: NCPPB 2331, PDDCC 4455 (neopathotype strain).

Pseudomonas syringae *pathovar***garcae** (Amaral, Teixeira and Pinheiro 1956) Young et al. 1978.

Host: coffee (*Coffea arabica*, family Rubiaceae) and many other unrelated plants.

Deposited strain: ATCC 19864, NCPPB 588, PDDCC 4323 (pathotype strain).

Pseudomonas syringae *pathovar* **helianthi** (Kawamura 1934) Young et al. 1978.

Host: sunflower (*Helianthus debilis*, family Compositae).

Deposited strain: NCPPB 2640, PDDCC 4531 (neopathotype strain).

Pseudomonas syringae *pathovar* **japonica** (Mukoo 1955) Dye et al. 1980 (*Pseudomonas striafaciens* pathovar *japonica* Mukoo 1955.)

Hosts: rye (*Secale cereale*), barley (*Hordeum sativum*), wheat (*Triticum vulgare*), rice (*Oryza sativa*) and many other Gramineae of the genera *Setaria, Panicum, Bromus, Lolium, Andropogon, Alopecurus*, etc., as well as plants of other botanical families (Solanaceae, Chenopodiaceae, Oxalidaceae, etc.).

Deposited strain: NCPPB 3093, PDDCC 6305 (neopathotype strain).

Pseudomonas syringae
pathovar **lachrymans** (Smith and Bryan 1915) Young et al. 1978.

Host: cucumber (*Cucumis sativus*, family Cucurbitaceae).

Deposited strain: ATCC 7386, NCPPB 537, PDDCC 3988 (neopathotype strain).

Pseudomonas syringae
pathovar **lapsa** (Ark 1940) Young et al. 1978.

Hosts: corn (*Zea mays*), sugarcane (*Saccharum officinarum*) (family Gramineae).

The mol% G + C of the DNA is: 59.4.

Deposited strain: NCPPB 2096, PDDCC 3947 (neopathotype strain).

Pseudomonas syringae
pathovar **maculicola** (McCulloch 1911) Young et al. 1978.

Hosts: cabbage, cauliflower (*Brassica oleracea*, family Cruciferae).

The mol% G + C of the DNA is: 61.3.

Deposited strain: NCPPB 2039, PDDCC 3935 (neopathotype strain).

Pseudomonas syringae pathovar **mellea** (Johnson 1923) Young et al. 1978.

Host: tobacco (*Nicotiana tabacum*, family Solanaceae).

Deposited strain: NCPPB 2356, PDDCC 5711 (neopathotype strain).

Pseudomonas syringae
pathovar **mori** (Boyer and Lambert 1893) Young et al. 1978.

Host: mulberry (*Morus* spp., family Moraceae).

Deposited strain: ATCC 19873, NCPPB 1034, PDDCC 4331 (neopathotype strain).

Pseudomonas syringae
pathovar **morsprunorum** (Wormald 1931) Young et al. 1978.

Host: *Prunus* spp. (family Rosaceae).

Deposited strain: ATCC 19322, NCPPB 2995, PDDCC 5795 (Sneath and Skerman, 1966) (neopathotype strain).

Pseudomonas syringae
pathovar **panici** (Elliott 1923) Young et al. 1978.

Host: proso or broom-corn millet (*Panicum miliaceum*, family Gramineae).

The mol% G + C of the DNA is: 60.7.

Deposited strain: ATCC 19875, NCPPB 1498, PDDCC 3955 (neopathotype strain).

Pseudomonas syringae
pathovar **papulans** (Rose 1917) Dhanvantari 1977.

Host: apple (*Pyrus malus*, family Rosaceae).

Deposited strain: NCPPB 2848, PDDCC 4048 (neopathotype strain).

Pseudomonas syringae
pathovar **passiflorae** (Reid 1938) Young et al. 1978.

Host: *Passiflora edulis* (family Passifloraceae).

The mol% G + C of the DNA is: 59.4–60.3.

Deposited strain: NCPPB 1387, PDDCC 129 (neopathotype strain).

Pseudomonas syringae
pathovar **persicae** (Prunier et al. 1970) Young et al. 1978.

Host: peach (*Prunus persicae*, family Rosaceae).

Deposited strain: NCPPB 2761, PDDCC 5846 (neopathotype strain).

Pseudomonas syringae
pathovar **pisi** (Sackett 1916) Young et al. 1978.

Host: pea (*Pisum sativum*, family Leguminosae).

Deposited strain: NCPPB 2585, PDDCC 2452 (neopathotype strain).

Pseudomonas syringae
pathovar **primulae** (Ark and Gardner 1936) Young et al. 1978.

Host: *Primula polyantha* (family Primulaceae).

The mol% G + C of the DNA is: 60.4.

Deposited strain: ATCC 19306, NCPPB 133, PDDCC 3956 (neopathotype strain).

Pseudomonas syringae
pathovar **ribicola** (Bohn and Maloit 1946) Young et al. 1978.

Host: golden currant (*Ribes aureum*, family Saxifragaceae).

The mol% G + C of the DNA is: 60.6.

Deposited strain: ATCC 13456, NCPPB 963, PDDCC 3882 (neopathotype strain).

Pseudomonas syringae
pathovar **sesami** (Malkoff 1906) Young et al. 1978.

Host: sesame (*Sesamum indicum*, family Pedaliaceae).

The mol% G + C of the DNA is: 59.4.

Deposited strain: ATCC 19879, NCPPB 1016, PDDCC 763 (neopathotype strain).

Pseudomonas syringae
pathovar **striafaciens** (Elliott 1927) Young et al. 1978.

Hosts: oats (*Avena sativa*), barley (*Hordeum sativum*) (family Gramineae).

The mol% G + C of the DNA is: 59.1.

Deposited strain: ATCC 10730, NCPPB 1898, PDDCC 3961 (pathotype strain); NCPPB 2394, PDDCC 4483 (pathogenic reference strain).

Pseudomonas syringae
pathovar **tabaci** (Wolf and Foster 1917) Young et al. 1978.

Host: tobacco (*Nicotiana tabacum*, family Solanaceae).

Deposited strain: NCPPB 1427, PDDCC 2835 (neopathotype strain).

Pseudomonas syringae
pathovar **theae** (Hori 1915) Young et al. 1978.

Host: tea plant (*Thea sinensis*, family Theaceae).

Deposited strain: NCPPB 2598, PDDCC 3923 (neopathotype strain).

Pseudomonas syringae
pathovar **tomato** (Okabe 1933) Young et al. 1978.

Host: tomato (*Lycopersicum esculentum*, family Solanaceae).

Deposited strain: NCPPB 1106, PDDCC 2844 (neopathotype strain).

Pseudomonas syringae
pathovar **ulmi** (Šutić and Tešić 1958) Young et al. 1978.

Host: elm (*Ulmus* sp., family Ulmaceae).

The mol% G + C of the DNA is: 58.7.

Deposited strain: ATCC 19883, NCPPB 632, PDDCC 3962 (neopathotype strain).

Pseudomonas syringae
pathovar **viburni** (Thornberry and Anderson 1931b) Young et al. 1978.

Host: elm (*Ulmus* sp., family Ulmaceae).

The mol% G + C of the DNA is: 60.1.

Deposited strain: ATCC 13458, NCPPB 1921, PDDCC 3963 (neopathotype strain).

Pseudomonas taetrolens
Haynes 1957, 108[AL]

taet'ro.lens. L. adj. *taeter* offensive; L. part. adj. *olens* having an odor; M.L. part. adj. *taetrolens* foul smelling.

The description of this species is given by Haynes and Burkholder (1957) in the seventh edition of the *Manual*.

The cells are short rods with rounded ends, motile by means of one to five polar flagella. Gelatin and starch are not hydrolyzed. Nitrate is not reduced to nitrite. Acid production from a number of substrates is described by Haynes and Burkholder (1957). A decision to allocate the species in the genus *Pseudomonas* has been made based on rRNA–DNA hybridization studies (De Vos et al., 1989). Isolated from foods that have a musty odor.

The mol% G + C of the DNA is: 59.8 (T_m).

Type strain: ATCC 4683, IAM 1653, LMG 2336.

GenBank accession number (*16S rRNA*): D84027.

Pseudomonas tolaasii
Paine 1919, 210[AL]

to.laa'si.i. Tolaas patronymic; M.L. gen. n. *tolaasii* of Tolaas.

Description is given by Haynes and Burkholder (1957) in the seventh edition of the *Manual*.

Rapid identification tests have been described (Wong and Preece, 1979). Results of a detailed phenotypic study of a collection of strains of this species have been published (Fahy, 1981). Some of the reported characteristics are summarized in Table 8. A gene cluster encoding proteins required for the synthesis of the toxin tolaasin has been identified and characterized (Rainey et al., 1993). Detailed composition of

fatty acids is known (Stead, 1992). rRNA–DNA hybridization studies indicate allocation of this species to the genus *Pseudomonas* (De Vos et al., 1985). Fluorescent pigment is produced. Gelatin is hydrolyzed (Haynes and Burkholder, 1957). Pathogenic for cultivated mushrooms. Isolated from brown-spot of cultivated mushrooms.

The mol% G + C of the DNA is: 60.8–61.3 (T_m).

Type strain: ATCC 33618, LMG 2342, NCPPB 1873.

GenBank accession number (*16S rRNA*): D84028, Z76670.

Pseudomonas veronii Elomari, Coroler, Hoste, Gillis, Izard and Leclerc 1996, 1142.[VP]

ve.ro'ni.i. M.L. masc. gen. n. *veronii* of Véron, in honor of Professor M.M. Véron, a distinguished French microbiologist.

The following description is taken from the original paper.

Rods, motile by means of single polar flagella. PHB is not accumulated by the cells. The cultures produce a fluorescent pigment on King B medium. The colonies on nutrient agar are smooth, circular, and nonpigmented. They are nonhemolytic on blood agar. The oxidase, catalase, and arginine dihydrolase reactions are positive. Most strains liquefy gelatin. All the strains grow on α-aminobutyrate, D-xylose, L-arabinose, D-mannose, D-galactose, sucrose, butyrate, isobutyrate, erythritol, sorbitol, inositol, D-alanine, L-tryptophan, and trigonelline as the sole source of carbon and energy. No strain is able to use isovalerate, sebacate, azelate, L-mandelate, benzoate, L-kynurenine, histamine, or acetamide. The original paper (Elomari et al., 1996) includes extensive information on a variety of enzymatic activities. Additional properties are summarized in Tables 5 and 6 and in a previous communication by the same group of workers (Elomari et al., 1995).

All strains have been isolated from natural mineral waters.

The mol% G + C of the DNA is: 61–62.

Type strain: CFML 92–134, CIP 104663, DSM 11331.

GenBank accession number (*16S rRNA*): AF064460.

Pseudomonas viridiflava (Burkholder 1930) Dowson 1939, 177[AL] (*Phytomonas viridiflava* Burkholder 1930, 63.)

vi.ri.di.fla'va. L. *viridis* green; L. *flavus* yellow; M.L. adj. *viridiflavus* greenish yellow.

Characteristics that are useful for comparison with related taxa are given in Table 11. Further information is given by Haynes and Burkholder (1957), Clara (1934), and Billing (1970b). The following characteristics are mentioned in this last paper. The bacterial mass usually has a yellow tinge in media with 5% sucrose and olive to golden brown in media with yeast extract and glycerol. A blue-green insoluble pigment is produced by some strains. Aside from the characteristics described in the tables, the potato rot and esculin reactions are positive. Fluorescent pigment is produced, and gelatin is liquefied (Haynes and Burkholder, 1957).

From her extensive nutritional screening of strains of the species, Billing (1970b) has concluded that few substrates have diagnostic value. The only ones distinguishing *P. viridiflava* from most other oxidase-negative plant pathogens are the inability to use sucrose and the capacity for use of D(−)-tartrate. This substrate is used by *P. viridiflava*, *P. syringae* pathovar *tomato*, and rarely by other species and pathovars. In addition to these characteristics, Billing (1970b) mentions that the reaction in beans was similar to that produced by *P. syringae* and both were different from the water-soaked lesions produced by "*P. phaseolicola*".

Pathogenic on bean (*Phaseolus vulgaris*).

The mol% G + C of the DNA is: 59.9 (NCPPB 1810).

Type strain: ATCC 13223, DSM 6694, LMG 2352, NCPPB 635, PDDCC 2848.

GenBank accession number (*16S rRNA*): Z76671.

Other species

Some putative *Pseudomonas* species have an uncertain phylogenetic position. The following descriptions are those that are not included in the first edition of the *Manual* (Palleroni, 1984) or in previous editions of *Bergey's Manual of Determinative Bacteriology*.

Pseudomonas abietaniphila Mohn, Wilson, Bicho and Moore 1999b, 935[VP] (Effective publication: Mohn, Wilson, Bicho and Moore 1999a, 76.)

a.bie.ta.ni'phi.la. M.L neut. n. *abietanum* abietane; Gr. adj. *philos* loving, friendly to; M.L. fem. adj. *abietaniphila* abietane-loving.

The following description is taken from the paper by Mohn et al. (1999a).

Rods. When grown on dehydroabietic acid, the cells are 0.7 × 1.2–2.5 μm. Motile. No information is given on the flagellar number and insertion. Colonies are clear, translucent, smooth, circular, and convex. Catalase positive and oxidase negative. Abietic, dehydroabietic, linoleic and pyruvic acids, as well as L-arabinose, D-galactose, D-glucose, D-xylose, and glycerol are used for growth; 12- plus 14–chlorodehydroabietic and palmitic acids are used poorly. Pimaric, isopimaric, and acetic acids are not used. Main cellular fatty acids are $C_{16:1\ \omega7c}$,

$C_{16:0}$, and $C_{18:0}$; minor ones include $C_{10:0\ 3OH}$, $C_{12:0\ 2OH}$, and $C_{12:0\ 3OH}$. Isolated from bleached Kraft pulp mill effluent treatment system near Kamlops, British Columbia, Canada.

The mol% G + C of the DNA is: unknown.

Type strain: Strain BKME-9, ATCC 700689.

Pseudomonas antimicrobica Attafuah and Bradbury 1990, 320[VP] (Effective publication: Attafuah and Bradbury 1989, 571.)

an.ti.mi.cro'bi.ca. Gr. pref. *anti* against; Gr. adj. *micrus* small; Gr. n. *bios* life; *antimicrobica* against microbes (referring to a wide antimicrobial activity).

Description taken from the original paper (Attafuah and Bradbury, 1989).

Rods, 0.5 × 0.8–1.5 µm, occurring singly, in groups, and occasionally in pairs. Motile by means of one or two polar flagella. Two types of colonies were observed on nutrient agar. Those of one variant (NCIB 9897) were circular, raised, creamy-white, and opaque, rugose and butyrous when raised, with microundulate margins; the other type (NCIB 9898) was moist, smooth, glistening, and slightly viscid, with entire margins and occasionally an orange tinge. A yellowish-orange, water-soluble, nonfluorescent pigment was produced in King A and B media, tyrosine medium and others. No pigment was produced on potato dextrose agar. Gelatinase activity, levan formation, accumulation of PHB, starch hydrolysis, pectolytic activity, and rotting of plant tissue were all negative. Growth occurs with NaCl up to 4%, but it decreases to a trace with 6%.

Acid was produced from arabinose, dulcitol, fructose, galactose, glucose, glycerol, lactose, mannitol, *meso*-inositol, sorbitol, and xylose, but not from maltose, raffinose, salicin, or sucrose. Carbon sources for growth were *N*-acetylglucosamine, D-alanine, L-arabinose, caprate, citrate, gluconate, glucose, glutamate, *p*-hydroxybenzoate, *meso*-inositol, α-ketoglutarate, malonate, mannitol, D-mannose, phenylacetate, L-proline, propionate, and raffinose. No growth on adipate, D-iso-ascorbate, malate, *meso*-tartrate, starch, or ethanol. No organic growth factors were required. A wide spectrum of antibiotic activity was present. Good growth occurred between 15 and 37°C; optimum around 30°C. No growth at 41°C. Additional information may be found in the original paper (Attafuah and Bradbury, 1989) and some comments on the relationship of this species to those of other groups of aerobic pseudomonads. Isolated from the mealybug *Planococcoides njalensis*.

The mol% G + C of the DNA is: unknown.

Type strain: DSM 8361, NCIB 9898.

Pseudomonas cannabina (ex Šutić and Dowson 1959) Gardan, Shafik, Belouin, Broch, Grimont and Grimont 1999, 477[VP]

can.na'bi.na. L. fem. adj. *cannabina* pertaining to *Cannabis*, the generic name of the host plant, *Cannabis sativa* L.

Rods, 1.1–3.0 × 3.0–4.0 µm, motile by means of one to four polar flagella. Colonies are gray color and are slightly convex. Production of fluorescent pigment in King B medium. The results of LOPAT (levan production, oxidase test, potato rotting, arginine dihydrolase, and tomato hypersensitivity) are +, –, –, –, and +. Nitrate is not reduced. Negative for hydrolysis of starch, esculin, and gelatin. The strains assimilate D-glucose, glycerol, D-saccharate, mucate, citrate, D-gluconate, L-histidine, L-aspartate, L-glutamate, L-proline, L-alanine, and L-serine, but not the other 83 carbon sources of the Biotype-100 strips (BioMérieux). Pathogenic on *Cannabis sativa* L.

The mol% G + C of the DNA is: 60.2 (T_m).

Type strain: CFBP 2341; ICMP 2823; NCPPB 1437.

Pseudomonas flectens Johnson 1956, 144[AL]

flec'tens. L. v. *flectere* to bend; *flectens* that bends.

The following description is summarized from that in the original paper.

Rods, 0.5–0.75 × 1.4–2.0 µm, with rounded ends. Motile by means of one to two polar flagella. Colonies on meat infusion agar, after 5 d at 27.5°C, circular, 1–1.5 mm in diameter, convex, amorphous, smooth, glistening, with an entire edge, grayish white. No fluorescent pigment is formed. Gelatin liquefaction does not occur. Acid is produced from glucose, mannose, and sucrose, but not from maltose, lactose, starch, glycerol, mannitol, sorbitol, or esculin. Starch hydrolysis is very slight. H_2S is not produced and nitrate is not reduced. The organism is the agent of the pod twist disease of French beans (*Phaseolus vulgaris* L.), where it produces large, diffuse water-soaked lesions on twisted young pods.

The mol% G + C of the DNA is: 31.8 (type strain).

Type strain: ATCC 12775.

Additional Remarks: Strain NCPPB 539, presumed to be the type strain, is not phylogenetically related to the genus *Pseudomonas*, as shown by rRNA–DNA hybridization experiments (De Vos et al., 1989). However, the authenticity of this strain may be questioned because of its unexpectedly low mol% G + C.

Pseudomonas halophila
Fendrich 1989, 205[VP] (Effective publication: Fendrich 1988, 42.)

ha.lo'phi.la. Gr. n. *hals, halos* salt; Gr. v. *philein* to love; M.L. adj. *halophila* salt-loving.

The description is taken from the original paper (Fendrich, 1988).

Rod-shaped cells, 0.8–1.0 × 1.5–5.0 µm, single or in pairs. Motile by means of a polar flagellum. Colonies are circular with entire margins and smooth surfaces, slimy, raised, and reddish-brown colored. Chemoorganotrophic growth on arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, sorbitol, sucrose, trehalose, and xylose with no acid production, and on acetate, caproate, citrate, lactate, pelargonate, propionate, pyruvate, succinate, alanine, glutamine, esculin, ethanol, and glycerol as carbon and energy sources. Catalase and cytochrome oxidase positive. Gelatinase and urease positive. Hydrolysis of starch or cellulose, production of indole or sulfide, arginine dihydrolase reaction, and lysine and ornithine decarboxylase activities are all negative. Strictly aerobic. Growth occurs between 0.02 and 3.3 ml/l NaCl, with optimum at 0.8 mol/l. The pH range is 4.5–9.6; optimum at 7. Temperature range, 4–37°C; optimum at 28°C. Susceptible to ampicillin, chloramphenicol, erythromycin, nalidixic acid, and penicillin G. Isolated from the north arm of Great Salt Lake, Utah, USA.

The mol% G + C of the DNA is: 57.

Type strain: DSM 3050.

Pseudomonas multiresinivorans
Mohn, Wilson, Bicho and Moore 1999b, 935[VP] (Effective publication: Mohn, Wilson, Bicho and Moore 1999a, 77.)

mul.ti.re.si.ni.vo'rans. L. adj. *multi* many; L. fem. n. *resina* resin; L. part. adj. *vorans* devouring; M.L. fem. adj. *multiresinivorans* devouring many resins.

Rods. When grown on isopimaric acid, cells are 1 × 1.5–2 µm, motile. No information is given about insertion and number of flagella. The cells form clumps. Colonies are clear, translucent, smooth, asymmetrical, and flat. Catalase and oxidase reactions are positive. Can live anaerobically in the presence of nitrate. Pimaric, isopimaric, palmitic, linoleic, benzoic, and acetic acids, as well as L-arabinose, D-glucose, citronellol, ethanol, glycerol, and n-hexadecane are used for growth. Poor utilization of abietic, dehydroabietic, and pyruvic acids, 12-plus 14-chlorodehydroabietic acids, D-galactose, or D-xylose. Main cellular fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{16:1\ \omega7c}$; minor ones include $C_{10:0\ 3OH}$, $C_{12:0\ 3OH}$, and $C_{12:0\ 3OH}$. Isolated from a laboratory sequencing batch reactor in Vancouver, British Columbia, Canada.

The mol% G + C of the DNA is: unknown.

Type strain: Strain IpA-1, ATCC 700690.

Pseudomonas tremae
Gardan, Shafik, Belouin, Broch, Grimont and Grimont 1999, 477[VP]

tre'ma.e. M.L. gen. fem. n. *tremae* of *Trema*; generic name of the host plant, *Trema orientalis* BL.

Rods, motile by means of one to four polar flagella. No fluorescent pigment is produced on King B medium. Of the LOPT tests (levan formation from sucrose, oxidase reaction, potato rotting ability, arginine dihydrolase reaction, and tobacco hypersensitive test), the first four are negative. Nitrate is not reduced. Negative for hydrolysis of starch, esculin, gelatin, and Tween 80. The strain assimilates D-saccharate, D- and L-malate, citrate, succinate, fumarate, L-aspartate, and L-glutamate, but not any of the 91 other carbon sources of the Biotype-100 strips (BioMérieux). Pathogenic for *Trema orientalis*.

The mol% G + C of the DNA is: 60.5 (T_m).

Type strain: CFBP3229; ICMP 9151; NCPPB 3465.

Pseudomonas vancouverensis
Mohn, Wilson, Bicho and Moore 1999b, 935[VP] (Effective publication: Mohn, Wilson, Bicho and Moore 1999a, 76.)

van.cou.ver.en'sis. M.L. adj. *vancouverensis* pertaining to the city of Vancouver, Canada.

Rods. When grown on dehydroabietic acid, the cells are 0.9 × 0.9–3.0 µm. Motile. No information is given about number and insertion of flagella. The cells form clumps. Colonies pale yellow, translucent, smooth, circular, and convex. Catalase and oxidase reactions are positive. Abietic, acetic, benzoic, dehydroabietic, linoleic, palmitic, and pyruvic acids, as well as β-citronellol, D-glucose, and glycerol, are used for growth. Acetic, isopimaric, and pimaric acids, and L-arabinose, D-galactose, and D-xylose are not used. Main cellular fatty acids are $C_{16:1\ \omega7c}$, $C_{16:0}$, $C_{17:0\ cyc}$, and $C_{18:1}$; minor ones include $C_{10:0\ 3OH}$, $C_{12:0\ 2OH}$, and $C_{12:0\ 3OH}$. Isolated from forest soil in Vancouver, British Columbia, Canada.

The mol% G + C of the DNA is: unknown.

Type strain: Strain DhA-51, ATCC 700688.

End note

1. [1]*Editorial Note*: The literature search for the chapter on *Pseudomonas* was completed in January, 2000. During the course of unavoidable publication delays, a number of new species were described or reclassified after the chapter was completed. It was not possible to include these species in the text of to include their characteristics in the comparative tables. The reader is encouraged to consult the studies listed in the Further Reading section and the *International Journal of Systematic and Evolutionary Microbiology* (2000–2003).
2. The word "pseudomonad" will be used quite often in this chapter. Although it may be clear to many, the precise meaning is given in the paper "The aerobic pseudomonads: a taxonomic study" by Stanier et al. (1966). It comprises all the species assigned to the genus *Pseudomonas* as classically defined. Ever since this publication, a fairly accurate definition has also been included in the editions of Webster Collegiate Dictionary.
3. Medium B (g/l): protease-peptone (Difco), 20.0; Bacto-agar (Difco), 15.0; glycerol, 10.0; K_2PO_4, 1.5; and $MgSO_4 \cdot 7H_2O$, 1.5. The pH is adjusted to 7.2.
4. Leifson's reagent for flagella staining (Leifson, 1960): 1.2% solution of basic fuchsin in 96% ethanol, 3% solution of tannic acid in water, and 1.5% solution of NaCl in water. Equal parts of the three reagents are mixed before use. The mixture can be left overnight in a refrigerator, where a precipitate may form. The clear stain is applied over the smear, and after about 2–3 min a fine precipitate will form. At this point, the staining is interrupted by washing the slides with water at room temperature. The final point may have to be ascertained by trial and error before optimal conditions are defined. Absolute cleanliness of the slides is essential for good results.
5. Medium of Palleroni and Doudoroff (1972) (g/l of 0.33 M Na-K phosphate buffer, pH 6.8): NH_4Cl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; ferric ammonium citrate, 0.05; and $CaCl_2$, 0.005. The first two ingredients are added to the buffer and sterilized by autoclaving. The ferric ammonium citrate and calcium chloride are added aseptically from a single stock solution that has been sterilized by filtration.
6. Garibaldi's medium: sterile egg white is drawn aseptically from eggs that have been sterilized externally by immersion in 70% ethanol for 5 min, drained and flamed to remove the residual alcohol. The egg white is warmed to 45°C and added aseptically to give a final concentration of 10% (v/v) to any commercial complex solid medium that has been autoclaved and cooled to 45°C. After mixing, the medium is dispensed into Petri dishes. A solution of the protein conalbumin (available commercially) that has been sterilized by filtration can be used instead of egg white at a final concentration of 1.7 mg/ml of medium.
7. Medium of Luisetti et al. (1972) (g/l): vitamin-free Casamino acids, 10.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 1.0; sucrose, 10.0; gelatin, 30.0; and agar, 20.0; pH 7.0.
8. A formula recommended by D.C. Hildebrand (personal communication), who has isolated a number of strains of these organisms, is as follows. Sliced potatoes (250 g) are steeped in 1 liter of water at 65°C for 1 h. The preparation is filtered through three layers of cheesecloth, and 2.0 g of glucose and 20.0 g of agar are added to the solution. The medium is sterilized at 20 psi for 30 min.
9. Thornley's medium (g/l): peptone, 1.0; NaCl, 5.0; K_2HPO_4, 0.3; agar, 3.0; phenol red, 0.01; and L-arginine-HCl, 10.0; pH 7.2.
10. Hugh-Leifson medium (g/l): peptone, 2.0; NaCl, 5.0; K_2HPO_4, 0.3; agar, 3.0; bromothymol blue, 0.03; and carbohydrate, 10.0; pH 7.1.
11. Sierra's medium (g/l): Bacto-peptone (Difco), 10.0; NaCl, 5.0; $CaCl_2 \cdot H_2O$, 0.1; and agar, 17.0. Tween 80 is sterilized separately and added to the medium after autoclaving to give a final concentration of 10.0 g/l.

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