NOTE

Transfer of [*Pseudomonas*] *lemoignei*, a Gram-negative rod with restricted catabolic capacity, to *Paucimonas* gen. nov. with one species, *Paucimonas lemoignei* comb. nov.

Institut für Mikrobiologie,
Universität Stuttgart,
Allmandring 31, 70550
Stuttgart, Germany

Dieter Jendrossek

Tel: +49 711 685 5483. Fax: +49 711 685 5725. e-mail: dieter.jendrossek@po.uni-stuttgart.de

The phylogenetic positions of the type strain and strain A62 of [*Pseudomonas*] *lemoignei*, as well as those of nine bacterial isolates closely related to [*Pseudomonas*] *lemoignei*, were re-evaluated by analysing morphological, physiological and molecular-biological characteristics. 16S rDNA analysis confirmed that the type strain of [*Pseudomonas*] *lemoignei* and strain A62 belong to the order 'Burkholderiales' of the β -Proteobacteria and represent a new cluster, for which the name *Paucimonas* gen. nov. is proposed. *Paucimonas* contains a single species, *Paucimonas lemoignei* comb. nov.

Keywords: *Pseudomonas lemoignei*, *Paucimonas*, PHB depolymerase, PHV

The genus *Pseudomonas* is one of the most interesting genera among the aerobic Gram-negative bacteria. Since its original description by Migula (1894), the genus *Pseudomonas* has been repeatedly re-evaluated: morphological, physiological and biochemical characteristics were the basis of classification by Stanier *et al.* (1966). Later, DNA–rRNA hybridization analysis led to the identification of five rRNA similarity groups among the pseudomonads (Palleroni *et al.*, 1973). The taxonomic analysis of bacteria, which claims to be based on true phylogenetic relationships, has been improved and facilitated during the last two decades by comparing phylogenetic marker molecules such as 16S rDNA and by the establishment of modern rapid PCR and sequencing methods. A new taxon, the *Proteobacteria*, was created which currently includes the α , β , γ , δ and ϵ subclasses (Stackebrandt *et al.*, 1988; see also De Ley, 1992; Moore *et al.*, 1996). Thirty validly described *Pseudomonas* species *sensu stricto* have been defined by Kersters *et al.* (1996), which belong to the γ -Proteobacteria and include bacteria of the former rRNA similarity group I (*Pseudomonas aeruginosa* and related species).

[*Pseudomonas*] *lemoignei* was isolated and classified 36 years ago by its ability to utilize poly(3-hydroxybutyrate) (PHB) as a carbon source (Delafield *et al.*, 1965). The specific epithet *lemoignei* was chosen in

honour of Maurice Lemoigne, who had discovered PHB in cells of *Bacillus megaterium*. Although [*Pseudomonas*] *lemoignei* fulfilled the criteria of the 'early' pseudomonads (aerobic, Gram-negative, polar-flagellated rods), it was evident that this species differed from most other pseudomonads in that it had only ten known substrates that could be used as a sole carbon source (Delafield *et al.*, 1965; Müller & Jendrossek, 1993).

My colleagues and I previously undertook the analysis of bacteria that are specific for the utilization of poly(3-hydroxyvalerate) (PHV) as a carbon source. Several PHV-degrading bacteria were isolated from soil in an earlier study (Mergaert *et al.*, 1996). Interestingly, all PHV-degrading isolates degraded both PHV and PHB and it was not possible to isolate any bacterium that could utilize PHV as the only polyester. A few actinomycetes and 12 Gram-negative PHV- and PHB-utilizing bacteria were identified. Ten of the 12 Gram-negative isolates showed a very narrow substrate range, comparable to that of [*Pseudomonas*] *lemoignei*; sugars, sugar alcohols, alcohols, amino acids or complex media such as nutrient broth or Luria–Bertani broth did not support growth of any of the isolates. The same ten isolates [A51 (LMG 16484), A52 (LMG 16485), A53 (LMG 16486), A58 (LMG 16487), A60a (LMG 16488), A60b (LMG 16489), A62 (LMG 16480), A64 (LMG 16841), K21 (LMG 16842) and K24 (LMG 16843)] and [*Pseudomonas*] *lemoignei* ATCC 17989^T (= DSM 7445^T = LMG 2207^T) were able to fix nitrogen if they were cultivated under

Abbreviations: PHB, poly(3-hydroxybutyrate); PHV, poly(3-hydroxyvalerate).

microaerobic conditions in 15 ml tubes filled with 10 ml ammonium-free mineral salts solution supplemented with 0.4% 3-hydroxybutyrate and 0.2% agar. These results indicated a high degree of relatedness between the novel isolates and [*Pseudomonas*] *lemoignei* and this was confirmed for one representative isolate (strain A62) by determination of the 16S rDNA sequence (Mergaert *et al.*, 1996). The sequence was 99.1% identical to that of the type strain of [*Pseudomonas*] *lemoignei* and revealed significant similarities to other species within the β -Proteobacteria.

Comparison of the 16S rDNA sequences of both strains with databases in July 2000 revealed the highest degree of sequence similarity to species of the genus *Herbaspirillum* [*Herbaspirillum seropedicae*, 95.6–95.9% similarity; *Herbaspirillum frisingense*, 95.4–95.8% similarity (Kirchhof *et al.*, 2001), *Herbaspirillum rubrisubalbicans*, 95.0–95.7% similarity] and to strains of *Oxalobacter formigenes* (92.9–94.0% similarity). The 16S rDNA-based relationship between [*Pseudomonas*] *lemoignei* and species of the related genera *Herbaspirillum*, *Oxalobacter*, *Telluria*, *Janthinobacterium* and *Zoogloea* and representative species of the β -Proteobacteria is shown in Fig. 1. The mean 16S rDNA similarity of [*Pseudomonas*] *lemoignei* and of isolate A62 to members of the genus *Burkholderia* was 90.7%.

Differentiation from *Herbaspirillum*

The 16S rDNA sequences of [*Pseudomonas*] *lemoignei* and isolate A62 showed the highest, although only moderate, similarity to those of *Herbaspirillum* (Baldani *et al.*, 1986, 1996; Kirchhof *et al.*, 2001). This moderate relationship is supported by phenotypic differences. [*Pseudomonas*] *lemoignei* and related PHV-degrading isolates described by Mergaert *et al.* (1996) obviously should not be grouped to this genus because: (i) they are rod-shaped; (ii) they do not utilize amino acids, sugars such as glucose and fructose, polyols such as mannitol, sorbitol and glycerol or other carbon sources such as dicarboxylates (except succinate) and tricarboxylates (the latter are the preferred substrates of *Herbaspirillum* species); (iii) they are apparently not associated with plants; and (iv) they have a significantly lower G + C content (59 ± 2 mol%) than that of *Herbaspirillum* species (60–65 mol%).

Differentiation from *Oxalobacter*

The even more distant phylogenetic status of the genus *Oxalobacter* (*O. formigenes*; Allison *et al.*, 1985) is supported by a strictly anaerobic metabolism. *O. formigenes* is restricted to the utilization of only one known compound, oxalate, and therefore is unlikely to be closely related to the strains of this study. Other characteristics of *O. formigenes* that differ from those of [*Pseudomonas*] *lemoignei* are: (i) non-motility; (ii) growth at 45 °C; (iii) low G + C content (~ 50 mol%); and (iv) fatty acid profile (Allison *et al.*, 1985).

Differentiation from *Telluria*, *Janthinobacterium* and *Zoogloea*

The only moderate 16S rDNA sequence similarity of [*Pseudomonas*] *lemoignei* to species of the genera *Telluria*, *Janthinobacterium* and *Zoogloea* correlates with differences in physiological markers such as: utilization of carbohydrates, polysaccharides and polypeptides (*Telluria*; Bowman *et al.*, 1993); utilization of carbohydrates, peptone, relative low temperature maximum of 32 °C and violacein production (*Janthinobacterium*; De Ley *et al.*, 1978); and utilization of amino acids, complex media, alcohols and certain aromatic compounds (benzoate, toluate) and zoogloea production (*Zoogloea*; Itzigsohn, 1868; Unz, 1984).

Proposal of *Paucimonas* gen. nov.

Based on the properties summarized above, it is concluded that the type strain of [*Pseudomonas*] *lemoignei* (ATCC 17989^T = DSM 7445^T = LMG 2207^T) and the other PHV-degrading bacteria [strains A51 (LMG 16484), A52 (LMG 16485), A53 (LMG 16486), A58 (LMG 16487), A60a (LMG 16488), A60b (LMG 16489), A62 (LMG 16480), A64 (LMG 16841), K21 (LMG 16842) and K24 (LMG 16843)] (Mergaert *et al.*, 1996) are members of the β -Proteobacteria and are related to the *Burkholderia*–*Ralstonia* sublineage. However, their phylogenetic positions do not allow them to be attributed to a known genus within the ‘*Burkholderiales*’. Moreover, the 16S rDNA sequences of [*Pseudomonas*] *lemoignei* and strain A62 form a separate cluster within the β -Proteobacteria. Therefore, a new genus, *Paucimonas*, is proposed and it is suggested that the type strain of [*Pseudomonas*] *lemoignei*, strain A62 and the other nine strains mentioned above should be grouped in this genus. *Paucimonas* currently contains only one species, *Paucimonas lemoignei* comb. nov. (see below).

Description of *Paucimonas* gen. nov.

Paucimonas [Pau.ci.mo'nas. L. adj. *paucus* little, few; Gr. fem. *monas* unit, cell; *Paucimonas* bacterium with restricted (few) catabolic capacities].

Gram-negative bacterium with chemoorganotrophic and strictly respiratory metabolism. Catalase- and oxidase-positive. Preferred carbon sources are organic acids. Most sugars (e.g. glucose, fructose, xylose), sugar acids (e.g. gluconate), polyalcohols (e.g. sorbitol, glycerol), alcohols (e.g. ethanol, butanediol, phenol), amino acids, polypeptides (e.g. nutrient broth, gelatin, Luria–Bertani broth) or polysaccharides (e.g. starch, cellulose) do not support growth. PHB and related homopolymers and/or co-polymers of hydroxyalkanoic acids can be accumulated intracellularly. Chemolithoautotrophic or phototrophic growth has not been observed. Type species of the genus is *Paucimonas lemoignei*.

Fig. 1. 16S rDNA-based tree reflecting the relationships of *Paucimonas lemoignei* (type strain and strain A62), its closest relatives and selected reference species of the β -Proteobacteria. Only type strains are shown (except *Paucimonas lemoignei* A62 and *Zoogloea ramigera* ATCC 25935). Bar, 5% estimated base divergence. The tree topology is based upon results of distance matrix analyses of all available almost complete 16S rDNA sequences from β -Proteobacteria and homologous primary structures from selected representatives of the major bacterial and archaeal phyla. Only sequence positions sharing identical nucleotides in at least 50% of all available almost complete 16S rDNA sequences from the phylogenetic group comprising *Burkholderia*, *Ralstonia* and relatives were included for calculation. The tree topology was evaluated and corrected according to the results of maximum-parsimony and maximum-likelihood analyses of all available (about 22000) sequences or a selection of small-subunit rDNA sequences from representatives of the β -Proteobacteria. Multifurcations indicate that a relative branching order could not be determined unambiguously or was not supported by applying alternative treeing methods. The various calculations were performed by applying the ARB program package and the ARB rRNA databases (Ludwig & Strunk, 1996).

Description of *Paucimonas lemoignei* comb. nov.

Basonym: *Pseudomonas lemoignei* Delafield *et al.* 1965.

Rod-shaped bacterium (0.6–0.8 × 1.5–3.0 µm), rounded ends, single, motile by a single polar flagellum. Circular colonies, small, white to beige, adherent. No secretion of fluorescent pigments but blackening of colonies and agar occurs upon incubation on tyrosine-containing solid media. Endospores not found. G + C content 59 ± 2 mol%. Abilities to produce several extracellular polyhydroxyalkanoate depolymerase isoenzymes and to utilize PHB, PHV, related oligomers and/or polymers for growth (Schöber *et al.*, 2000) are characteristic for *Paucimonas lemoignei*. Utilization of polyesters is visible by clearing zone formation of opaque polyhydroxyalkanoate-containing solid media (Jendrossek *et al.*, 1996). Preferred water-soluble carbon sources are acetate, pyruvate, succinate, butyrate, 3-hydroxybutyrate, valerate and 3-hydroxyvalerate; poor or no growth observed on propionate, malate, 2-oxoglutarate and citrate. Grows between pH ~ 5.5 and ~ 9.0 depending on the substrate but at pH 7.5, growth on succinate is impaired due to restricted uptake of succinate (for details see Terpe *et al.*, 1999). The temperature optimum is near 30 °C; no growth observed above 41 °C. Most strains are able to fix nitrogen and some strains (A52, A53, A60a and A60b) produce gas from nitrate. The main constituents of the cellular fatty acid are hexadecenoic acid (16:1 ω 7*cis*), hexadecanoic acid (16:0), octadecenoic acids and

dodecanoic acid (12:0), as well as several hydroxy fatty acids (3-OH 10:0, 3-OH 12:0 and 2-OH-14:0) (Mergaert *et al.*, 1996). Other characteristics of the species are as for the genus. Type strain of the species is *Paucimonas lemoignei* ATCC 17989^T (= DSM 7445^T = LMG 2207^T), isolated by Delafield *et al.* (1965) from soil of a garden in Berkeley, CA, USA. Strains A62 and A52, as well as the other strains mentioned above, were isolated from a meadow (A-strains) or from compost (K-strains) near Göttingen, Germany. The DNA–DNA binding ratios are 100% for strains A62 versus A52 and 60 and 61% for the type strain versus A52 and A62, respectively (Mergaert *et al.*, 1996). Strains A62, A52 and the type strain can be distinguished from each other by the presence of a megaplasmid of about 200 kbp in strains A62 and A52.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. I thank W. Ludwig for performing 16S rDNA comparisons. The advice of H. G. Trüper is gratefully acknowledged.

References

- Allison, M. J., Dawson, K. A., Mayberry, W. R. & Foss, J. G. (1985). *Oxalobacter formigenes* gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch Microbiol* **141**, 1–7.

- Baldani, J. I., Baldani, V. L. D., Seldin, L. & Döbereiner, J. (1986). Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *Int J Syst Bacteriol* **36**, 86–93.
- Baldani, J. I., Pot, B., Kirchhof, G. & 8 other authors (1996). Emended description of *Herbaspirillum*; inclusion of [*Pseudomonas*] *rubrisubalbicans*, a mild plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3. *Int J Syst Bacteriol* **46**, 802–810.
- Bowman, J. P., Sly, L. I., Hayward, A. C., Spiegel, Y. & Stackebrandt, E. (1993). *Telluria mixta* (*Pseudomonas mixta* Bowman, Sly, and Hayward 1988) gen. nov., comb. nov., and *Telluria chitinolytica* sp. nov., soil-dwelling organisms which actively degrade polysaccharides. *Int J Syst Bacteriol* **43**, 120–124.
- Delafield, F. P., Doudoroff, M., Palleroni, N. J., Lusty, C. J. & Contopoulos, R. (1965). Decomposition of poly- β -hydroxybutyrate by pseudomonads. *J Bacteriol* **90**, 1455–1466.
- De Ley, J. (1992). The Proteobacteria. Ribosomal RNA cistron similarities and bacterial taxonomy. In *The Prokaryotes*, 2nd edn, pp. 2111–2140. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- De Ley, J., Segers, P. & Gillis, M. (1978). Intra- and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. *Int J Syst Bacteriol* **28**, 154–168.
- Itzigsohn, H. (1868). Entwicklungsvorgänge von *Zoogloea*, *Oscillaria*, *Synedra*, *Staurastrum*, *Spirotaenia* und *Chroolepus*. *S B Ges Naturf Fr Berlin* 19 November 1867, pp. 30–31.
- Jendrossek, D., Schirmer, A. & Schlegel, H. G. (1996). Biodegradation of polyhydroxyalkanoic acids. *Appl Microbiol Biotechnol* **49**, 451–463.
- Kerstens, K., Ludwig, W., Vancanneyt, M., De Vos, P., Gillis, M. & Schleifer, K. H. (1996). Recent changes in the classification of the pseudomonads: an overview. *Syst Appl Microbiol* **19**, 465–477.
- Kirchhof, G., Eckert, B., Stoffels, M., Baldani, J. I., Reis, V. M. & Hartmann, A. (2001). *Herbaspirillum frisingense* sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. *Int J Syst Evol Microbiol* **51**, 157–168.
- Ludwig, W. & Strunk, O. (1996). ARB – a software environment for sequence data. <http://www.mikro.biologie.tu-muenchen.de/pub/ARB/documentation/arb.ps>
- Mergaert, J., Schirmer, A., Hauben, L., Mau, M., Hoste, B., Kersters, K., Jendrossek, D. & Swings, J. (1996). Isolation and identification of poly(3-hydroxyvalerate)-degrading strains of *Pseudomonas lemoignei*. *Int J Syst Bacteriol* **46**, 769–773.
- Migula, W. (1894). Über ein neues System der Bakterien. *Arb Bakteriell Inst Karlsruhe* **1**, 235–238.
- Moore, E. R. B., Mau, M., Arnscheidt, A., Böttger, E. C., Hutson, R. A., Collins, M. D., Van De Peer, Y., De Wachter, R. & Timmis, K. N. (1996). The determination and comparison of the 16S rRNA gene sequences of species of the genus *Pseudomonas* (sensu stricto) and estimation of the natural intrageneric relationships. *Syst Appl Microbiol* **19**, 478–492.
- Müller, B. & Jendrossek, D. (1993). Purification and properties of poly(3-hydroxyvaleric acid) depolymerase from *Pseudomonas lemoignei*. *Appl Microbiol Biotechnol* **38**, 487–492.
- Palleroni, N. J., Kunisawa, R., Contopoulos, R. & Doudoroff, M. (1973). Nucleic acid homologies in the genus *Pseudomonas*. *Int J Syst Bacteriol* **23**, 333–339.
- Schöber, U., Thiel, C. & Jendrossek, D. (2000). Poly(3-hydroxyvalerate) depolymerase of *Pseudomonas lemoignei*. *Appl Environ Microbiol* **66**, 1385–1392.
- Stackebrandt, E., Murray, R. G. E. & Trüper, H. G. (1988). *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the ‘purple bacteria and their relatives’. *Int J Syst Bacteriol* **38**, 321–325.
- Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.
- Terpe, K., Kerkhoff, K., Pluta, E. & Jendrossek, D. (1999). Relationship between succinate transport and production of extracellular poly(3-hydroxybutyrate) depolymerase in *Pseudomonas lemoignei*. *Appl Environ Microbiol* **65**, 1703–1709.
- Unz, R. F. (1984). Genus IV. *Zoogloea* Itzigsohn 1868, 30^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 214–219. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.