NOTE

Transfer of *Pseudomonas nautica* to *Marinobacter hydrocarbonoclasticus*

Cathrin Spröer, Elke Lang, Petra Hobeck, Jutta Burghardt,
Erko Stackebrandt and B. J. Tindall

Author for correspondence: B. J. Tindall. Tel: +49 531 2616 224. Fax: +49 531 2616 418.
e-mail: bti@gbf.de

DSMZ–Deutsche Sammlung
von Mikroorganismen und
Zellkulturen GmbH,
Mascheroder Weg 1b,
D-38124 Braunschweig,
Germany

A combination of genotypic and phenotypic properties (a polyphasic taxonomic approach) was used to determine the relatedness between the type strains of *Pseudomonas nautica* Bauman *et al.* 1982 and *Marinobacter hydrocarbonoclasticus* Gauthier *et al.* 1992, which were originally found to be highly related by partial 16S rDNA sequence analysis. Analysis of genotypic properties, such as comparison of the almost complete 16S rDNA sequences, base composition of the total genomic DNA and DNA–DNA hybridization revealed that the two strains were highly similar and should be considered members of the same species. The phenotypic properties, such as the physiology and chemotaxonomic data (i.e. fatty acid composition, polar lipid patterns and respiratory lipoquinone content), confirmed the genotypic evaluation, and has led to the proposal for a unification of the two species, *Pseudomonas nautica* (DSM 50418^T) and *Marinobacter hydrocarbonoclasticus* (DSM 8798^T) as *Marinobacter hydrocarbonoclasticus*.

Keywords: *Marinobacter hydrocarbonoclasticus*, *Pseudomonas nautica*, 16S rRNA sequence, chemotaxonomy, taxonomy

Baumann *et al.* (1972) described aerobic, oxidase-positive, Gram-negative and motile strains which showed a high degree of physiological similarity, but were different from other members of the genus *Pseudomonas* from the marine environment. The delineation of the genus on the basis of the few (key) characters listed above allowed Baumann *et al.* (1972, 1983) to place these strains in a new species in the genus, as *Pseudomonas nautica*. However, over the past 20 years, increasing knowledge of the properties of the members of this genus (both phenotypic and genotypic) has shown them to exhibit a diverse range of properties. Despite the physiological diversity (Stanier *et al.*, 1966) evident in members of this genus, studies on RNA–DNA hybridization (Palleroni *et al.*, 1973) and rRNA cataloguing (Woese *et al.*, 1984) provided concrete evidence for the evolutionary diversity with the members of the genus *Pseudomonas* as defined by *Bergey's Manual of Determinative Bacteriology* (Doudoroff & Palleroni, 1973). Further studies have shown that the evolutionary diversity within this genus is also evident in the physiology and chemical properties of this group, which is extremely heterogeneous and can no longer be restricted to a single genus. As a result of the growing database on the properties of this group, this genus has been restricted

to members of the *Pseudomonas aeruginosa* rRNA branch of the rRNA superfamily I (De Ley, 1978), with the remaining species being transferred to other genera, e.g. *Brevundimonas*, *Herbaspirillum*, *Hydrogenophaga*, *Sphingomonas*, *Aminobacter*, *Methylobacterium* (belonging to the α -subclass of the class *Proteobacteria*), *Acidovorax*, *Burkholderia*, *Comamonas*, *Ralstonia*, *Telluria* and *Vogesella* (β -subclass) as well as *Xanthomonas* and *Stenotrophomonas* (γ -subclass). Although the majority of species clustering with the type species *P. aeruginosa*, based on 16S rDNA sequence analysis, are considered to constitute the genus *Pseudomonas sensu stricto*, the 16S rDNA sequence and chemotaxonomic clustering of the strains may be suggestive of further taxonomic rearrangements (Moore *et al.*, 1996).

Analysis of the database of 16S rDNA sequences contained in the Ribosomal Database Project (RDP) (Maidak *et al.*, 1996) revealed that *P. nautica* clustered very close to *Marinobacter hydrocarbonoclasticus*. Due to the fact that a short stretch of the 16S rDNA had been deposited, the almost complete sequence of the 16S rDNA was determined (Rainey *et al.*, 1996; accession no. Y16735) and compared with sequences deposited in the RDP and EMBL using the

Table 1. Physiological properties of *Marinobacter hydrocarbonoclasticus* DSM 8798^T and *Pseudomonas nautica* DSM 50418^T: comparison of previously published and new data

d/+, Variable within species, type strain positive; NT, not tested; w, weak reaction.

Property	<i>M. hydrocarbonoclasticus</i>		<i>P. nautica</i>	
	Gauthier <i>et al.</i> (1992); <i>n</i> = 1	DSM 8798 ^T ; <i>n</i> = 1	Baumann <i>et al.</i> (1983); <i>n</i> = 34	DSM 50418 ^T ; <i>n</i> = 1
Oxidase	+	+	+	+
Catalase	+	+	NT	+
API 20NE:*				
Nitrate reduced to N ₂	+	+	d/+	+
Indole from tryptophan	NT	—	NT	—
Arginine dihydrolase	—	—	—	—
Urease	—	—	NT	—
Aesculin hydrolysis	—	—	NT	—
Gelatin hydrolysis	—	—	—	—
Utilization of:				
Glucose, arabinose, mannose, mannitol, <i>N</i> -acetylglucosamine, gluconate	—	—	—	—
Maltose, phenylacetate	NT	—	—	—
Caprate	NT	—	d/+	—
Adipate, citrate	+	+	+	+
Malate	NT	+	+	+
Utilization of:†				
Hexadecane	+	+	d/+	+
Phenanthrene	+	—	NT	—
DL-Alanine	—	w	d/+	+
L-Glutamate	+	+	d/+	+
<i>p</i> -Hydroxybenzoate	+	—	—	—
<i>m</i> -Hydroxybenzoate, L-serine	—	—	—	—
Succinate	+	+	d/+	+

* DSM strains were tested using the API 20NE system in the presence of 3% NaCl.

† DSM strains were tested in mineral medium in the presence of 3% NaCl.

ae2 editor (Maidak *et al.*, 1996). Sequence similarities, calculated for *P. nautica* and related strains, indicated 100% sequence identity with strain CAB (accession no. U61648), and 99.5% with the type strain of *Marinobacter hydrocarbonoclasticus* (accession no. X67022). Critical analysis of the 16S rDNA sequence of the latter strain (Gauthier *et al.*, 1992) indicates deviations from highly conserved base positions and structural properties [i.e. at positions 92, 338, 653, 913, 919, 965, 1449 and 1467 according to *Escherichia coli* nomenclature (Brosius *et al.*, 1978)], the correction of which would result in 100% sequence similarity with the sequence of *P. nautica*. The closest relatives of these three strains are *Microbulbifer hydrolyticus* (90% similarity), members of *Pseudomonas sensu stricto* (88–89% similarity), *Oceanospirillum* species (89–90% similarity), as well as *Marinomonas* and *Marinobacterium* species (88–89% similarity). In previous studies, Pot *et al.* (1989) and De Vos *et al.* (1989)

showed that *P. nautica* was not a member of the genus *Pseudomonas sensu stricto* and was specifically associated with the group of organisms comprising members of the genera *Oceanospirillum* and *Marinomonas*. However, at that time neither *Marinobacter hydrocarbonoclasticus* nor *Microbulbifer hydrolyticus* had been described, and the current data expands on the previous work.

DNA was extracted by the method of Cashion *et al.* (1977) and used for the determination of the G+C content of the DNA as well as for DNA–DNA hybridization experiments. Determination of the DNA G+C contents by HPLC (Mesbah *et al.*, 1989) for the type strains of *P. nautica* and *Marinobacter hydrocarbonoclasticus* gave values of 57.7 and 57.3 mol%, respectively. The latter values differed from that in the literature, determined by the thermal denaturation method, by 4.6 mol% (Gauthier *et al.*, 1992). DNA–

Fig. 1. Polar lipid patterns of (a) *Pseudomonas nautica* DSM 50418^T and (b) *Marinobacter hydrocarbonoclasticus* DSM 8798^T. PG, Phosphatidyl glycerol; PE, phosphatidyl ethanolamine; DPG, diphosphatidyl glycerol; PL, unidentified phospholipids.

DNA hybridization as determined by the renaturation method (Escara & Hutton, 1980; Huß *et al.*, 1983) and evaluated using the BASIC computer program of Jahnke (1992) gave 100.8% binding between the type strains of the two species. The genetic similarity extended to the epigenetic (physiological and chemotaxonomic) level. At the physiological level a comparison of the literature data (Baumann *et al.*, 1972, 1983; Gauthier *et al.*, 1992) revealed a total match in those compounds and reactions tested in both studies. To confirm these findings both strains were subjected in parallel to about 30 physiological tests, which were also compared to the literature values (Table 1). The only differences from the published data were the failure to utilize phenanthrene and *p*-hydroxybenzoate by *Marinobacter hydrocarbonoclasticus*, where the reactions were recorded positive by Gauthier *et al.* (1992).

Chemotaxonomic studies included examination of the polar lipid (Tindall, 1990a, b), respiratory lipoquinone (Tindall, 1990a, b) and fatty acid (B. J. Tindall, unpublished) composition. The high degree of similarity between the two strains of *P. nautica* and *Marinobacter hydrocarbonoclasticus* species was also reflected in common chemotaxonomic properties, e.g. composition of the fatty acids (Table 2) and polar lipids (Fig. 1) as well as in the presence of ubiquinone-9 (Q-9) as the major respiratory lipoquinone (menaquinones were not detected). The chemical composition also supported the placement of the two species in the same phyletic group.

As a result of the study of a combination of phenotypic and genotypic properties (polyphasic approach) of the two species, including 16S rDNA sequence analysis (from which the phylogeny was inferred), DNA-DNA hybridization data, chemotaxonomic, phenotypic and morphological data, we consider the type strains of *P. nautica* and *Marinobacter hydrocarbonoclasticus* to be members of the same species. According to Rule 44 of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), the generic name *Marinobacter* has priority since *Marinobacter hydrocarbonoclasticus* is the oldest named type species, while *P. nautica* is not

Table 2. Percentage composition of the fatty acids of *Pseudomonas nautica* DSM 50418^T and DSM 6419 and *Marinobacter hydrocarbonoclasticus* DSM 8798^T

Fatty acids are listed in their order of elution.

Fatty acid	DSM 50418 ^T	DSM 6419	DSM 8798 ^T
10:0	0.76	0.83	0.74
12:0	5.36	4.31	3.96
3-OH 12:0*	3.61	2.09	3.00
Unknown 1	1.54	1.13	1.27
14:0	2.86	3.32	2.34
15:0	1.72	1.57	1.28
16:1 ω 9 cis	15.20	15.31	11.41
16:1 ω 7 cis	4.19	5.29	5.57
16:0	26.29	26.95	25.19
Unknown 2	0.57	0.91	Trace
Cyclo 17:0	3.14	3.20	2.37
17:0	2.81	2.41	2.43
18:1 ω 9 cis	28.89	29.47	36.41
18:1 ω 7 cis	1.08	1.25	1.80
18:0	1.99	1.97	2.23

*The 3-OH 12:0 fatty acid was not ester-linked, suggesting it was amide-linked, possibly in the LPS.

the type species of the genus *Pseudomonas*. Rule 15 requires that the species epithet *hydrocarbonoclasticus* be used, since the nomenclatural type is 'that element of the taxon with which the name (here the genus *Marinobacter*) is permanently associated'. Thus, in the present case Rule 15 overrules Rule 42, according to which the oldest legitimate epithet must be retained when taxa of equal rank are united (*Pseudomonas nautica* was described in 1972, while *Marinobacter hydrocarbonoclasticus* was described in 1992).

Additional properties of *Marinobacter hydrocarbonoclasticus*

In addition to the properties given by Gauthier *et al.* (1992), Baumann *et al.* (1972) and Baumann *et al.* (1983), the following chemotaxonomic properties can be added to the description: major fatty acids are straight-chain saturated 16:0, 16:1 ω 9 cis and 18:1 ω 9 cis fatty acids, small amounts (> 2%) of 12:0, 14:0, 17:0, 3-OH 12:0, 16:1 ω 7 cis and cyclo 17:0 are also present. The major polar lipids are phosphatidyl glycerol, phosphatidyl ethanolamine and phosphatidyl glycerol. The major ubiquinone is Q-9. The DNA G + C of the type strain ATCC 49840^T (= DSM 8798^T) is 57.5 mol%.

Note added in proof

Marinobacter sp. strain CAB (= DSM 11784) has also been shown by DNA-DNA hybridization to be a strain of *Marinobacter hydrocarbonoclasticus*.

References

- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.
- Baumann, P., Bowditch, R. D., Baumann, L. & Beaman, B. (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffii*. *Int J Syst Bacteriol* **33**, 857–865.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- De Ley, J. (1978). Modern molecular methods in bacterial taxonomy: evaluation, application, prospects. In *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, vol. 1, pp. 347–357. Tours, France: Gilbert-Clarey.
- De Vos, P., van Landschoot, A., Segers, P. & 9 other authors (1989). Genotypic relationships and taxonomic localization of unclassified *Pseudomonas* and *Pseudomonas*-like strains by deoxyribonucleic acid:ribosomal ribonucleic acid hybridizations. *Int J Syst Bacteriol* **39**, 35–49.
- Doudoroff, M. & Palleroni, N. J. (1974). Genus I *Pseudomonas*. In *Bergey's Manual of Determinative Bacteriology*, pp. 217–243. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of renaturation rate. *Biopolymers* **19**, 1315–1327.
- Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992). BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD SYSTEM 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria (1990 Revision)*. Bacteriological Code. Washington, DC: American Society for Microbiology.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project. *Nucleic Acids Res* **24**, 82–85.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- 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 rDNA gene sequences of species of the genus *Pseudomonas* (sensu stricto) and estimation of the natural intrageneric relationships. *Syst Appl Microbiol* **19**, 478–492.
- Palleroni, N. J., Kunisawa, R., Contopoulou, D. & Doudoroff, M. (1973). Nucleic acid homologies in the genus *Pseudomonas*. *Int J Syst Bacteriol* **23**, 333–339.
- Pot, B., Gillis, M., Hoste, B., van de Velde, A., Bekaert, F., Kersters, K. & De Ley, J. (1989). Intra- and intergeneric relationships of the genus *Oceanospirillum*. *Int J Syst Bacteriol* **39**, 23–34.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Woese, C. R., Blanz, P. & Hahn, C. M. (1984). What isn't a pseudomonad: the importance of nomenclature in bacterial classification. *Syst Appl Microbiol* **5**, 179–195.