

A re-evaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the combination *Paracoccus pantotrophus* comb. nov.

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Comparison of both 16S rRNA coding sequences and DNA–DNA hybridization of ten strains of α -subclass of *Proteobacteria* currently classified as strains of *Paracoccus denitrificans* has shown that they fall into two groups which are distinct from each other at the species level. Comparison with published data on the cytochrome *c* profiles and other 16S rRNA coding sequences in the literature has confirmed these observations and enabled several other strains also to be assigned to these two groups. Group A comprises strains ATCC 17741^T (the type strain of *P. denitrificans*), LMD 22.21^T, DSM 413^T, ATCC 19367, ATCC 13543, DSM 1404, DSM 1405, Pd 1222 (a genetic modification of DSM 413^T) and NCIMB 8944. Group B comprises ATCC 35512^T (the original type strain of *Thiosphaera pantotropha*), LMD 82.5^T, LMD 92.63, DSM 65, LMG 4218, IAM 12479, JCM 6892, DSM 11072, DSM 11073 and DSM 11104. In light of these findings, it is proposed that: (1) strains of group A are retained as *P. denitrificans*, with ATCC 17741^T as the type strain of the type species; and (2) all strains of group B are assigned to the new species combination *Paracoccus pantotrophus* comb. nov., with strain ATCC 35512^T as the type strain. Comparative 16S rRNA sequence analysis and DNA–DNA hybridization of strains of *Paracoccus versutus* confirm that this species is distinct from both *P. denitrificans* and *P. pantotrophus*, but that its nearest phylogenetic neighbour is *P. pantotrophus*.

Keywords: *Paracoccus denitrificans*, *Paracoccus pantotrophus*, *Thiosphaera*, 16S rRNA gene sequencing, DNA–DNA hybridization

INTRODUCTION

The genus *Paracoccus* and its type species *Paracoccus denitrificans* (Beijerinck & Minkman, 1910; formerly '*Micrococcus denitrificans*' Beijerinck 1910) were defined by Davis *et al.* (1969) in their assignment of 'hydrogen bacteria' to a variety of new and existing genera. The type strain was established as ATCC (American Type Culture Collection, Manassas, VA, USA) 17741^T (formerly '*Micrococcus denitrificans*'

Beijerinck 1910), with other extant examples of the original isolate being LMD (Delft Collection of Microorganisms, Delft, The Netherlands) 22.21^T, ATCC 19367 and ATCC 13543 (Davis *et al.*, 1969). Numerous similar isolates were subsequently assigned to *P. denitrificans*, which became recognized as a heterogeneous species comprising several biotypes (Jordan *et al.*, 1997; Van Verseveld & Stouthamer, 1992). A related single-species genus, *Thiosphaera pantotropha* (Robertson & Kuenen, 1983), was also assigned to *P. denitrificans* on the basis of analysis of the base sequence of the 16S rRNA gene (Ludwig *et al.*, 1993).

Recently, the history of the strains in culture

The EMBL accession numbers for the 16S rRNA gene sequences reported in this paper are given in Table 1.

Table 1. Strains of *Paracoccus* and *Thiosphaera* compared in this study: sources and accession numbers for their 16S rRNA gene sequences

ATCC, American Type Culture Collection, Manassas, VA, USA; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; LMD, Delft Collection of Microorganisms, Delft, The Netherlands; LMG, Ghent Collection of Microorganisms, Ghent, Belgium.

Organism	Strain no.	Source	Accession no.	Reference
<i>Paracoccus denitrificans</i>	ATCC 17741 ^T	C. Goodhew	Y16927	This study
<i>Paracoccus denitrificans</i>	ATCC 19367	ATCC	Y16930	This study
<i>Paracoccus denitrificans</i>	LMD 22.21 ^T	C. Goodhew	Y16928	This study
<i>Paracoccus denitrificans</i>	DSM 413 ^T	DSMZ	Y16929	This study
<i>Paracoccus denitrificans</i>	LMG 4218	LMG	X69159	Ludwig <i>et al.</i> (1993)
<i>Paracoccus denitrificans</i>	LMG 4218	C. Goodhew	Y16936	This study
<i>Paracoccus denitrificans</i>	DSM 65	DSMZ	Y16935	This study
<i>Paracoccus denitrificans</i>	IAM 12479	IAM	Y17512	This study
<i>Paracoccus denitrificans</i>	JCM 6892	JCM	Y17511	This study
<i>Paracoccus denitrificans</i> KL1	DSM 11073	DSMZ	U58017	Jordan <i>et al.</i> (1997)
<i>Paracoccus denitrificans</i> KS1	DSM 11072	DSMZ	U58015	Jordan <i>et al.</i> (1997)
<i>Paracoccus denitrificans</i> KS2	DSM 11104	DSMZ	U58016	Jordan <i>et al.</i> (1997)
<i>Thiosphaera pantotropha</i>	ATCC 35512 ^T	ATCC	Y16933	This study
<i>Thiosphaera pantotropha</i>	LMD 92.63	S. Baker	Y16934	This study
<i>Thiosphaera pantotropha</i> GB17 ^T	LMD 82.5 ^T	L. A. Robertson	X69159	Ludwig <i>et al.</i> (1993)
<i>Paracoccus versutus</i>	IAM 12814	IAM	D32243	Katayama <i>et al.</i> (1995)
<i>Paracoccus versutus</i>	ATCC 25364 ^T	ATCC	Y16932	This study
<i>Paracoccus versutus</i>	DSM 582	DSMZ	Y16931	This study
<i>Paracoccus thiocyanatus</i>	IAM 12816 ^T	IAM	D32242	Katayama <i>et al.</i> (1995)
<i>Paracoccus kocurii</i>	JCM 7684 ^T	JCM	D32241	Katayama <i>et al.</i> (1995)
<i>Paracoccus aminophilus</i>	JCM 7686 ^T	JCM	D42239	Katayama <i>et al.</i> (1995)
<i>Paracoccus alcaliphilus</i>	JCM 7364 ^T	JCM	D32238	Katayama <i>et al.</i> (1995)
<i>Paracoccus aminovorans</i>	JCM 7685 ^T	JCM	D32240	Katayama <i>et al.</i> (1995)
<i>Paracoccus solventivorans</i>	DSM 6637 ^T	DSMZ	Y07705	Siller <i>et al.</i> (1996)

collections in relation to the sources of the original isolates was examined by comparative analysis of their *c*-type cytochrome and protein profiles, and the case for the reclassification of these strains into more than one species was considered (Goodhew *et al.*, 1996). Using 16S rRNA sequence analysis, three new isolates of carbon-disulfide-using facultative chemolithotrophs were assigned to *P. denitrificans* (Jordan *et al.*, 1995, 1997). This classification, like those used by Ludwig *et al.* (1993) in classifying *T. pantotropha*, as well as for our and Urakami's descriptions of several new *Paracoccus* species (Katayama *et al.*, 1995; Ohara *et al.*, 1990; Urakami *et al.*, 1989, 1990), relied on the 16S rRNA sequence reported by Ludwig *et al.* (1993) for *P. denitrificans* LMG (Ghent Collection of Microorganisms, Ghent, Belgium) 4218, supposedly a derivative of the type strain. The study of Goodhew *et al.* (1996) cast doubt on the authenticity of strain LMG 4218 as being representative of the type strain and indicated that this strain and *T. pantotropha* were different at the species level from the *P. denitrificans* type strain, ATCC 17741^T. Consequently, a comparative analysis of the 16S rRNA sequences was undertaken, complemented by examination of DNA-

DNA hybridization, to establish the relationship between the key culture collection strains of *P. denitrificans*, *T. pantotropha* and the other strains recently designated as *P. denitrificans*. The taxonomic position of *Paracoccus versutus*, which is closely related to *P. denitrificans* and *T. pantotropha*, has also been reinvestigated to establish whether it can remain as a valid species distinct from other *Paracoccus* species.

METHODS

Bacterial strains. The strains used directly in this study and those referred to in the Discussion are listed in Table 1. *Paracoccus halodenitrificans* [DSM (DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) 735, CCM (Czech Collection of Microorganisms, J. E. Juryne University, Brno, Czech Republic) 286; Van Verseveld & Stouthamer, 1992] is excluded from consideration because it was subsequently shown to be a member of the genus *Halomonas* in the γ -subclass of the *Proteobacteria* (Dobson & Franzmann, 1996; Miller *et al.*, 1994; Ohara *et al.*, 1990; Urakami *et al.*, 1990). Two separate sources of the type strain of *P. versutus* were used: (1) the original isolate deposited by D. S. Hoare with the ATCC as ATCC 25364^T [this was initially called *Thiobacillus* strain A2

(Taylor & Hoare, 1969), subsequently renamed *Thiobacillus versutus* (Harrison, 1983), then reassigned to *Paracoccus* (Katayama *et al.*, 1995)]; and (2) the culture supplied by D. S. Hoare to D. P. Kelly in 1970 and deposited by D. P. Kelly with the DSMZ as DSM 582.

DNA isolation and determination of DNA–DNA hybridization. The DNA was isolated as described by Cashion *et al.* (1977). DNA hybridization was carried out according to De Ley *et al.* (1970) with modifications as described by Escara & Hutton (1980) and Huß *et al.* (1983) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. Renaturation rates were computed by the program TRANSFER.BAS (Jahnke, 1992).

Isolation of genomic DNA and determination of 16S rRNA gene sequences. Genomic DNA was extracted and subjected to PCR-mediated amplification of the 16S rRNA coding sequences as described by Rainey *et al.* (1996). The purified PCR products were directly sequenced using described protocols (Rainey *et al.*, 1996) and the sequence reaction mixtures were electrophoresed using an Applied Biosystems model 310 Genetic Analyzer. The 16S rRNA coding sequences of strains IAM (Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan) 12479 and JCM (Japan Collection of Microorganisms, Saitama, Japan) 6892 were determined both by direct sequencing and by a combination of cloning and cycle sequencing (Hiraishi, 1992; Hiraishi *et al.*, 1994, 1995). The 16S rRNA gene sequences were manually aligned with members of the alpha-2 subclass of the *Proteobacteria* using the ae2 editor (Maidak *et al.*, 1994). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were reconstructed using tree algorithms contained in the PHYLIP package (Felsenstein, 1993).

RESULTS AND DISCUSSION

Implications of studies on the c-type cytochrome profiles and the structure of cytochrome c-550 of strains of *P. denitrificans*

The work carried out by Goodhew *et al.* (1996) showed that the ten strains studied fell into two distinct groups, whose origins could each be traced to distinct original isolates of organisms, subsequently classified as *Paracoccus denitrificans*. These were the type strain isolated by Beijerinck in 1909 (Beijerinck & Minkman, 1910) and a strain isolated by Koster (Goodhew *et al.*, 1996). These were deposited in the Delft Culture Collection as LMD 22.21^T and LMD 52.44 in 1922 and 1952, respectively. One group, representing the original Beijerinck type strain, was shown to contain culture collection strains ATCC 17741^T, ATCC 13543, ATCC 19367, DSM 413^T, LMD 22.21^T, NCIMB (National Collection of Marine and Industrial Bacteria, Aberdeen, UK) 8944 and *P. denitrificans* Pd 1222 (a genetically modified strain of DSM 413^T; Goodhew *et al.*, 1996). The other group, informally proposed to be a distinct species, comprised culture collection strains DSM 65, LMD 52.44 and LMG 4218. Representative examples from each group, and other isolates including two strains of *Thiosphaera pantotropha*, were subjected to comparative analysis by 16S rRNA sequence analysis and DNA–DNA hybridization.

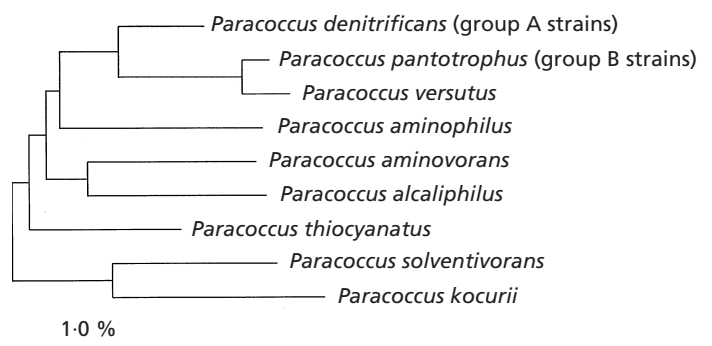


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence data analysis of members of the genus *Paracoccus*. The scale bar represents 1 inferred nucleotide change per 100 nucleotides.

Comparison of 16S rRNA sequences for strains of *P. denitrificans* and *T. pantotropha*

Nearly complete 16S rRNA nucleotide coding sequences (>95% of the *Escherichia coli* sequence) were determined for ten strains held in culture collections as *P. denitrificans* or *T. pantotropha*, as well as two strains of *P. versutus*. The sequences determined for two *P. denitrificans* strains (IAM 12479 and JCM 6892) comprised 1421 nt corresponding to positions 28–1524 of *E. coli* 16S rRNA (Brosius *et al.*, 1978). The sequences for the other six *P. denitrificans* strains and two *T. pantotropha* strains comprised 1391 nt between *E. coli* positions 38 and 1511, whereas the sequences of the two *P. versutus* strains comprised 1391 nt between the same *E. coli* positions. These sequences, along with those available from the public databases (Table 1), were aligned and 1369 unambiguous positions between *E. coli* positions 38 and 1489 were used in the phylogenetic analyses and calculation of 16S rRNA gene sequence similarity values. The results of these analyses showed that the *P. denitrificans* and *T. pantotropha* strains fell into two distinct groups (Fig. 1). Group A comprised *P. denitrificans* strains ATCC 17741^T, ATCC 19367, DSM 413^T and LMD 22.21^T, whose 16S rRNA sequences were 100% identical to each other. Group B comprised *P. denitrificans* strains DSM 65, LMG 4218, IAM 12479 and JCM 6892, and *T. pantotropha* strains ATCC 35512^T and LMD 92.63. The 16S rRNA gene sequences of all of these strains were 100% identical to each other. Strain DSM 65 is currently listed in the DSMZ catalogue of strains as the type strain of *P. denitrificans*, and was reportedly originally received as ‘strain 381’ from R. Y. Stanier. As strain DSM 65 is not phylogenetically identical to strain ATCC 17741^T, it should not be regarded as the type strain. This conclusion was also drawn by Goodhew *et al.* (1996), who pointed out that the ‘Stanier strain 381’ cannot have given rise to both ATCC 17741^T and DSM 65, as stated in the respective culture collection catalogues. The group B sequence was also identical to that reported for *P. denitrificans* strain LMG 4218 (sequence X69159) and for *T.*

pantotropha strain GB17^T (Ludwig *et al.*, 1993), and showed >99% similarity to the three carbon-disulfide-using strains recently classified as *P. denitrificans* DSM 11072, DSM 11073 and DSM 11104 (Jordan *et al.*, 1997). The similarity between members of groups A and B was 98.1% in all cases.

These results are absolutely consistent with the cytochrome-based deductions of Goodhew *et al.* (1996). On the basis of strain ATCC 17741^T being the formally accepted type strain (Davis *et al.*, 1969; Van Verseveld & Stouthamer, 1992), 16S rRNA sequence analysis indicated that the following strains were all *P. denitrificans*: ATCC 17741^T, LMD 22.21^T, DSM 413^T and ATCC 19367. The following strains are not *P. denitrificans* and all contain the 16S rRNA sequence originally found for the type strain of *T. pantotropha*: *P. denitrificans* strains DSM 65 and LMG 4218, and *T. pantotropha* strains ATCC 35512^T and LMD 92.63. The 100% identity of the 16S rRNA sequences of ATCC 35512^T and LMD 82.5^T (an original deposition of the type strain of *T. pantotropha*) also confirms the identity of these two strains. In addition, strains IAM 12479 and JCM 6892 from the Japanese culture collections, supposedly derived from the type strain ATCC 17741^T, were found to fall into the *Thiosphaera* group. The carbon-disulfide-using strains (Jordan *et al.*, 1997) are also members of this group.

DNA–DNA hybridization between strains of *P. denitrificans*

Hybridization of DNA from *P. denitrificans* strains DSM 65, DSM 413^T, DSM 11073 and DSM 11104 showed that three of these comprised one group (DSM 65, 11073 and 11104), with DSM 413^T showing significantly lower DNA–DNA hybridization with this group at the species level (Table 2).

These results are consistent with the conclusions of Goodhew *et al.* (1996) and our 16S rRNA sequence data concerning the relationship of DSM 413^T and DSM 65, which are clearly not related at the species level. DSM 413^T is representative of the type species, *P. denitrificans*, but DSM 65, DSM 11073 and DSM 11104 are representative of the *T. pantotropha* group.

Comparison of 16S rRNA gene sequences and DNA–DNA hybridization between *P. versutus* and *P. denitrificans*

The 16S rRNA sequences determined for *P. versutus* strains ATCC 25364^T and DSM 582 were identical to each other and differed in one nucleotide position from the sequence previously reported for other culture collection strains of *P. versutus* (Katayama *et al.*, 1995). Comparison of the *P. versutus* 16S rRNA sequence with the sequences for *P. denitrificans* DSM 65 and DSM 413^T showed 99.3 and 97.4% similarity, respectively. Hybridization between DNA from the *P. versutus* strain DSM 582 and *P. denitrificans* strains DSM 65 and DSM 413^T showed low hybridization

(Table 2). *P. versutus* is thus confirmed as a distinct species, with the DSM 65 group of strains (group B; including *T. pantotropha*) as its closest phylogenetic neighbour.

Comparisons from the literature of 16S rRNA sequences and DNA–DNA hybridization between *Paracoccus* species

All other published 16S rRNA gene sequence comparisons have used the sequence from strain LMG 4218 as representing the type strain of *P. denitrificans* (Katayama *et al.*, 1995; Ludwig *et al.*, 1993). Ludwig *et al.* (1993) previously showed that the sequence from strain LMG 4218 had 100% identity with that of strain GB17^T of *Thiosphaera pantotropha* (obtained as LMD 82.5^T; Chandra & Friedrich, 1986). Their demonstration of 85% DNA–DNA hybridization between *T. pantotropha* LMD 82.5^T and *P. denitrificans* DSM 65 seemed to support their conclusion that *T. pantotropha* was a strain of *P. denitrificans*, but this actually confirms our finding that DNA from DSM 65 (and IAM 12479; Katayama *et al.*, 1995) is homologous at the species level with that of *T. pantotropha*, which is different from that of *P. denitrificans*. In another study, Ohara *et al.* (1990) found only 61% DNA–DNA hybridization between *P. denitrificans* strains IAM 12479 and IFO 13301. Since our results show that IAM 12479 is not representative of the type strain of *P. denitrificans*, but falls into the *T. pantotropha* group, strain IFO 13301 may be properly regarded as a derivative of the type strain of *P. denitrificans*. Strain IFO 13301 was reported to be identical to strains ATCC 19367 and DSM 413^T (Urakami *et al.*, 1989), which have been shown to have the same 16S rRNA sequence as the type strain ATCC 17741^T. It is also noteworthy that DNA–DNA hybridization showed *P. denitrificans* ATCC 17741^T to exhibit 77 and 87% similarity to *P. denitrificans* strains DSM 1404 and DSM 1405, respectively (Urakami *et al.*, 1989), showing that these strains and strain IFO 13301 were correctly classified as strains of *P. denitrificans*.

Katayama *et al.* (1995) showed that the 16S rRNA sequences (D32238–D32242; Table 1) of five species of *Paracoccus* were only 95.5–96.7% similar to that of *P. denitrificans* LMG 4218, whereas those of *P. versutus* (sequences D32243 and D32244) were 99.1% similar. They also reported that *P. denitrificans* strain IAM 12479 showed only 16–56% DNA–DNA hybridization with these six species and whereas the two strains of *P. versutus* (IAM 12814 and 12815) showed 98% hybridization with each other, they had only 18–26% hybridization with four other species. Hybridization of DNA from *P. versutus* IAM 12814 was only 51% with that from *P. denitrificans* IFO 13301 and 49–56% with *P. denitrificans* strain IAM 12479 (Katayama *et al.*, 1995), thereby confirming our conclusion that *P. versutus*, *P. denitrificans* and *T. pantotropha* represent distinct genospecies.

Table 2. DNA–DNA hybridization (%) between strains DSM 65, 413^T, 11073 and 11104 of *P. denitrificans* and with *P. versutus* DSM 582

Strain number	DSM 65	DSM 413 ^T	DSM 11073	DSM 11104
DSM 65	100	58	71	89
DSM 413 ^T	58	100	64	61
DSM 11073	71	64	100	100
DSM 11104	89	61	100	100
DSM 582	54	59	–	–

Table 3. Assignment of *Paracoccus* and *Thiosphaera* strains to *P. denitrificans* (group A on Fig. 1) and *P. pantotrophus* comb. nov. (group B on Fig. 1) on the basis of 16S rRNA gene sequence similarities and DNA–DNA hybridization (this study), and cytochrome c profiles (Goodhew *et al.*, 1996)

<i>Paracoccus denitrificans</i> (group A)	<i>Paracoccus pantotrophus</i> (group B)
<i>P. denitrificans</i> ATCC 17741 ^T	<i>T. pantotropha</i> ATCC 35512 ^T
<i>P. denitrificans</i> LMD 22.21 ^T	<i>T. pantotropha</i> LMD 82.5 ^{T*}
<i>P. denitrificans</i> DSM 413 ^T	<i>T. pantotropha</i> LMD 92.63
<i>P. denitrificans</i> ATCC 19367	<i>P. denitrificans</i> DSM 65†
<i>P. denitrificans</i> ATCC 13543	<i>P. denitrificans</i> LMG 4218
<i>P. denitrificans</i> IFO 13301	<i>P. denitrificans</i> IAM 12479
<i>P. denitrificans</i> DSM 1404	<i>P. denitrificans</i> JCM 6892
<i>P. denitrificans</i> DSM 1405	<i>P. denitrificans</i> DSM 11072
<i>P. denitrificans</i> NCIMB 8944	<i>P. denitrificans</i> DSM 11073
<i>P. denitrificans</i> strain Pd 1222‡	<i>P. denitrificans</i> DSM 11104

* *T. pantotropha* strain GB17^T, as used by Ludwig *et al.* (1993).

† Formerly regarded as the type strain of *P. denitrificans*.

‡ A derivative of *P. denitrificans* DSM 413^T.

Conclusions and a proposal of the new combination *P. pantotrophus* comb. nov. to embrace *T. pantotropha* and some strains currently described as *P. denitrificans*

The earlier studies of Goodhew *et al.* (1996) and Winterstein & Ludwig (1998), along with our 16S rRNA sequence and DNA–DNA hybridization data, clearly demonstrate that the physiologically similar strains currently described as *P. denitrificans* actually fall into two distinct groups that differ so significantly that they justify classification as distinct species. These are here redefined as *P. denitrificans* and *P. pantotrophus* comb. nov. The type strain of *P. denitrificans* is ATCC 17741^T (= LMD 22.21^T; Beijerinck & Minkman, 1910; Ludwig *et al.*, 1993). The type strain of *P. pantotrophus* is ATCC 35512^T (= LMD 82.5^T), which is the original isolate of *T. pantotropha* (Kuenen & Robertson, 1989; Robertson & Kuenen, 1983). Culture collection strains indicated by our work to be identical to each of these type strains are listed in Table 3. In addition, the work of Goodhew *et al.* (1996) and our reassessment of other published work indicates that the following strains are also *P. denitrificans*:

ATCC 13543, NCIMB 8944, strain Pd 1222, DSM 1404, DSM 1405 and IFO 13301. Recently, Winterstein & Ludwig (1998) have shown that the genome of *P. denitrificans* ATCC 13453, DSM 413^T and strain Pd 1222 consists of three chromosomes, whose DNA molecules are approximately 2.1, 1.1 and 0.64 Mb in size. In contrast, *P. denitrificans* DSM 65 and *T. pantotropha* LMD 82.5^T contained four large DNA species that were 2.2, 1.50, 1.71–1.77 and 0.5 Mb in size. This observation is wholly consistent with our confirmation of the first three species as *P. denitrificans* and the assignment of the latter two to the new combination, *P. pantotrophus*.

While it has long been recognized that the strains of *P. denitrificans* comprised several biotypes within a supposedly heterogeneous species (Jordan *et al.*, 1997; Van Verseveld & Stouthamer, 1992), our work, and that of Goodhew *et al.* (1996), has revealed a more significant factor underlying this heterogeneity. This is that the validity of reference strains held by different international culture collections as derivatives of the type strain ATCC 17741^T is uncertain. Our results show that at some stage in the history of some culture

collection strains supposedly derived from *P. denitrificans* LMD 22.21^T (the organism originally isolated by Beijerinck & Minkman, 1910) and ATCC 17741^T switching with other strains (i.e. *P. pantotrophus*) must have occurred. It is clear that where comparison with a type strain is of crucial significance, as in defining a genotype or the identity of a strain with that type strain (in this case, ATCC 17741^T), reference to the authentic type strain held by the collection is essential. In the longer term, it is highly desirable that cross-checking by culture collection curators of the authenticity of all type strains and the authenticity of their putative derivatives is undertaken.

Emended description of *Paracoccus denitrificans* (Beijerinck and Minkman 1910) Davis 1969, 384^{Al}

The formal genus and type species descriptions remain those given by Van Verseveld & Stouthamer (1992) and Katayama *et al.* (1995), together with the emendation of the genus by Ludwig *et al.* (1993); but the emendation of the species description by Ludwig *et al.* (1993) must now be deleted. In addition, some if not all strains contain megaplasmids of at least 450 kb in size, but it is uncertain if they contain smaller (100 kb) plasmids, as none is present in strain DSM 413^T or the 'Stanier strain 381', which was deposited with the ATCC as strain ATCC 17741^T (Gerstenberg *et al.*, 1982; Goodhew *et al.*, 1996; Jordan *et al.*, 1997). The type strain is ATCC 17741^T (= LMD 22.21^T = DSM 413^T).

Description of *Paracoccus pantotrophus* comb. nov. Robertson and Kuenen 1984a, 91^{VP}

The formal description remains that given by Kuenen & Robertson (1989) for *T. pantotropha*, with the following additions (Arts *et al.*, 1995; Goodhew *et al.*, 1996; Jordan *et al.*, 1997; Robertson *et al.*, 1988): (1) some strains are capable of aerobic denitrification (simultaneous reduction of oxygen and nitrate) and heterotrophic nitrification (oxidation of ammonium to nitrite during heterotrophic growth); (2) some strains are capable of aerobic growth on formate, aerobic chemolithoautotrophic growth with carbon disulfide or carbonyl sulfide as energy substrates, methylo-trophic growth on methanol or methylated sulfides, and heterotrophic growth on diethyl sulfide, thio-ethanol, thioacetic acid or substituted thiophenes; (3) some strains can grow anaerobically with denitrification on thiosulfate, carbon disulfide, methanol or formate as energy sources; (4) some strains contain plasmids of 85–110 kb in size and megaplasmids greater than 450 kb in size; (5) distinction from other species of *Paracoccus* can be confirmed by comparison of 16S rRNA gene sequence and DNA hybridization; (6) the amino acid sequence of its cytochrome *c*-550 differs by about 16% from that of *P. denitrificans*; and (7) the G + C content of the DNA is 64–68 mol% (type strain G + C content is 66 mol%). The type strain is ATCC 35512^T (= LMD 82.5^T).

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