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CHAPTER 3.1.11

The Genus *Paracoccus*

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Introduction

At the time that Henk van Verseveld and Adriaan Stouthamer (1991) described *Paracoccus* in the second edition of *The Prokaryotes*, only two species were recognized: the type species (*P. denitrificans*) and *P. halodenitrificans* (Kocur, 1984). Of those two, *Paracoccus halodenitrificans* was subsequently excluded because it was shown to be a member of the genus *Halomonas* in the γ-subclass of the Proteobacteria (Dobson and Franzmann, 1996; Miller et al., 1994; Ohara et al., 1990; Urakami et al., 1990). In the past decade, a number of new species of *Paracoccus* have been described and a major critical review of the diverse biotypes of *P. denitrificans* and similar organisms was undertaken, leading to a clearer definition of the type species. At the time of writing, a total of 14 species of *Paracoccus* have been proposed (Table 1). These include two well-studied species of facultatively chemolithoautotrophic sulfur bacteria, *Thiosphaera pantotropha* and *Thiobacillus versutus*, which have been reclassified as species of *P. pantotrophus* and *P. versutus*. *Thiosphaera pantotropha* strain GB17 was initially believed to be a strain of *P. denitrificans* (Ludwig et al., 1993), until it and several biotypes of *P. denitrificans* were reclassified as a separate species, *P. pantotrophus* (Rainey et al., 1999). *Paracoccus denitrificans* and *P. pantotrophus* have continued to be the focus of much study of the molecular biology, respiratory mechanisms, and regulation of carbon and nitrogen dissimilation in *Paracoccus*.

The treatment of *Paracoccus* in the edition second of *The Prokaryotes* (van Verseveld and Stouthamer, 1991) concentrated on *P. denitrificans*, and we refer the reader to that treatment, and other more recent studies of *P. denitrificans*, for in-depth information concerning the physiology and molecular biology of *P. denitrificans* (and in some cases *P. pantotrophus*). More relevant reviews include those by Stouthamer (1992), Stouthamer et al. (1997), Baker et al. (1998) and van Spanning et al. (2000).

History of the Type Species, *Paracoccus denitrificans*

The original isolate by Beijerinck and Minkman (1910) is still extant as the type strain of the genus (Goodhew et al., 1996; Rainey et al., 1999). It was originally named *Micrococcus denitrificans* and shown to grow anaerobically in pure culture in a bouillon medium supplemented with ammonium nitrate (10 g · liter^{-1}), reducing nitrate to dinitrogen (N_2) and nitrous oxide (N_2O) in approximately a 2 : 1 ratio (Beijerinck and Minkman, 1910). Subsequently many workers used derivatives of that strain and another isolated by Koster (Goodhew et al., 1996), and *Paracoccus denitrificans* became a model organism for the study of its cytochrome system and electron transport mechanisms, denitrification, methylotrophy, and various aspects of metabolic and molecular regulation. It became apparent that a number of seemingly distinct biotypes were in use in various laboratories, and the isolation of other species with similar properties (initially named *Thiobacillus versutus* and *Thiosphaera pantotropha*) helped lead to a questioning of the relatedness of these to *P. denitrificans* and between the biotypes of *P. denitrificans*.

As a consequence of studies of their cytochromes and the history of *P. denitrificans* cultures by Goodhew et al. (1996), of their chromosomes by Winterstein and Ludwig (1998), and of their 16S rRNA sequences and DNA:DNA hybridization among *P. denitrificans* strains (Rainey et al., 1999), it became clear that the physiologically similar strains described as *P. denitrificans* actually fell into two distinct groups that differed so significantly that they justified separation into distinct species. These were *P. denitrificans* and *P. pantotrophus* comb. nov (Rainey et al., 1999): the type strain of *P. denitrificans* is ATCC 17741^T (LMD 22.21; Beijerinck and Minkman, 1910; Ludwig et al., 1993). The type strain of *P. pantotrophus* is ATCC 35512^T (LMD 82.5), which is the original isolate of *Thiosphaera pantotropha* (Kuenen and

Table 1. List of the currently recognized species of *Paracoccus*, representative strains available in culture collections, and their accession numbers for 16S rRNA gene sequences.

Species	Type strain numbers	16S rRNA gene sequence accession numbers	References[a]
P. denitrificans[Tb]	ATCC 17741[T], LMD 22.21[T], DSM 413[T]	Y16927, Y16928, Y16929	Rainey et al. (1999) Goodhew et al. (1996)
P. pantotrophus	ATCC 35512[T], LMD 82.5[T]	Y16933, X69159	Rainey et al. (1999) Ludwig et al. (1993)
P. versutus	ATCC 25364[T], DSM 582[T]	Y16932, Y16931	Rainey et al. (1999)
P. aminophilus	JCM 7686[T]	D42239	Katayama et al. (1995)
P. aminovorans	JCM 7685[T]	D32240	Katayama et al. (1995)
P. alcaliphilus	JCM 7364[T]	D32238	Katayama et al. (1995)
P. thiocyanatus	IAM 12816[T]	D32242	Katayama et al. (1995)
P. solventivorans	DSM 6637[T]	Y07705	Siller et al. (1996)
P. kocurii	JCM 7684[T]	D32241	Katayama et al. (1995)
P. marcusii	DSM 11574[T]	Y12703	Harker et al. (1998)
P. alkenifer	DSM 11593[T]	Y13827	Lipski et al. (1998)
P. carotinifaciens	IFO 16121[T]	AB006899	Tsubokura et al. (1999)
P. methylutens	VKM B-2164[T]	AF250334	Doronina et al. (1998)
P. kondratievae	VKM B-2222[PT]	AF250332	Y. A. Trotsenko and T. P. Tourova (personal communications, 2000)

Symbols: [T]indicates type strain of each species; ATCC, American Type Culture Collection; LMD, Delft Collection of Microorganisms; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; JCM, Japan Collection of Microorganisms; IAM, Institute of Applied Microbiology, Tokyo, Japan; IFO, Institute of Fermentation, Osaka, Japan; VKM, All-Russian Collection of Microorganisms.

[a]References are for details of culture methods and sources of cultures.

[b]*P. denitrificans* is the type species of the genus.

Robertson, 1989; Robertson and Kuenen, 1983). Culture collection strains that (by this work) are indicated to be identical to each of these type strains are listed in Table 2. Winterstein and Ludwig (1998) have shown that the genome of *P. denitrificans* ATCC 13453, DSM 413 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 *Thiosphaera pantotropha* LMD 82.5 contain 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 last 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 and Stouthamer, 1991), our work and that of Goodhew et al. (1996) 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. The results show that at some stage some culture collection strains supposedly derived from *P. denitrificans* LMD 22.21 (the original isolate of Beijerinck and Minkman, 1910) and ATCC 17741[T] must have been replaced with other strains (i.e. of *P. pantotrophus*). 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 the type strain, reference to the authentic type strain held by the collection is essential. It is obviously highly desirable that cross-checking by culture collection curators of the authenticity of type strains and the authenticity of cultures reportedly derived from them is undertaken.

Classical Taxonomic and Physiological Characteristics of Species of the *Paracoccus* Genus

Morphologically, all the species are coccoid, between 0.4–0.9 μm in diameter, or coccobacilli, up to 2 μm in length, and occur as single cells, pairs or clusters. All are Gram-negative and most species are non-motile. All species grow aerobically on a wide range of organic substrates and some are capable of anaerobic growth with nitrate or nitrous oxide as the terminal oxidant, producing dinitrogen as the final product. None is known to be able to grow fermentatively. The optimum temperature for growth of all the species is in the range 25–37°C, and the pH for good

Table 2. Partial list of *Paracoccus* strains derived from the original isolate of *P. denitrificans* and of those strains transferred to the new species, *P. pantotrophus* comb. nov.[a]

Paracoccus denitrificans	Paracoccus pantotrophus
P. denitrificans ATCC 17741^T	*T. pantotropha* ATCC 35512^T
P. denitrificans LMD 22.21^T	*T. pantotropha* LMD 82.5^{Tb}
P. denitrificans DSM 413^T	*T. pantotropha* LMD 92.63
P. denitrificans ATCC 19367	*P. denitrificans* DSM 65[c]
P. denitrificans ATCC 13543	*P. denitrificans* LMG 4218
P. denitrificans IFO 13301	*P. denitrificans* LAM 12479
P. denitrificans DSM 1404	*P. denitrificans* JCM 6892
P. denitrificans DSM 1405	*P. denitrificans* DSM 11072
P. denitrificans NCIMB 8944	*P. denitrificans* DSM 11073
P. denitrificans strain Pd 1222[d]	*P. denitrificans* DSM 11104

Symbols: LMG, Ghent Collection of Microorganisms, Ghent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria; other symbols defined in Table 1.

[a]Transfer was based on 16S rRNA gene sequence similarities and DNA-DNA hybridization (Rainey et al., 1999) and cytochrome *c* profiles (Goodhew et al., 1996).

[b]*Thio. pantotropha* strain GB17, as used by Ludwig et al. (1993).

[c]Formerly regarded as the type strain of *P. denitrificans*.

[d]A derivative of *P. denitrificans* DSM 413.

growth ranges between pH 6.5–8.5 for different species, except for *P. alcaliphilus* (which is moderately alkaliphilic with an optimum of pH 8–9 and grows at pH 9.5, but only weakly at pH 7.0; Urakami et al., 1989). All the species examined to date contain ubiquinone-10 as the respiratory quinone, as expected for members of the α-3 subclass of the Proteobacteria, with small amounts of ubiquinone-9 and ubiquinone-11 also being reported for some species. Typically the major fatty acids are 18:1 and 18:0 straight-chain acids, 19cyc, and 10:0(3-OH), 12:1(3-OH), and 14:0(3-OH) hydroxy-acids (Katayama et al., 1995; Lipski et al., 1998). Most, if not all, strains may accumulate poly-β-hydroxybutyrate under carbon-sufficient growth conditions and are generally catalase and oxidase positive. The mol% G+C content of the DNA of the species described to date ranges between 63–71 mol%. When the genus *Paracoccus* was created (Davis et al., 1969), its sole member was the *Micrococcus denitrificans* of Beijerinck and Minkman (1910), a distinguishing feature of which was autotrophic growth on carbon dioxide using hydrogen oxidation as the source of metabolic energy (Kornberg et al., 1960). This property is not universal among the 14 species now named. Indeed, a number of the characters commonly used to define the general features of the genus (Van Verseveld and Stouthamer, 1991; Van Spanning et al., 2000) derive from the properties exhibited by the most-studied strains of *P. denitrificans*, *P. pantotrophus* and *P. versutus* and do not apply to some of the more recently-described species. Thus, the ability to use the oxidation of hydrogen or inorganic sulfur compounds to support autotrophic growth, the ability to grow either autotrophically or methylotrophically on methanol or methylamine, or the ability to grow by anaerobic denitrification do not now appear to be exhibited by all the species and are thus not common taxonomically diagnostic features of all *Paracoccus* isolates (Table 3).

The chemolithoautotrophic growth on inorganic sulfur compounds (e.g. thiosulfate) of three species of *Paracoccus* has been studied in some detail: *P. versutus*, *P. pantotrophus* and *P. thiocyanatus*. The type species, *P. denitrificans*, is also defined as being able to grow on thiosulfate. This property is in need of further study, as the analyses of Goodhew et al. (1996) and Rainey et al. (1999) showed that numerous strains of *P. denitrificans* were in fact strains of *P. pantotrophus*, and most comprehensive work on thiosulfate oxidation has been carried out with *P. versutus* and *P. pantotrophus* strain GB17 (Kelly et al., 1997; Friedrich, 1998). The type strain of *P. denitrificans* was received from C.B. van Niel by Davis et al. (1969) and became ATCC 17741, which Davis et al. (1969) thought likely to be the same as strains ATCC 19367 and ATCC 13543. We showed that this was true (Rainey et al., 1999) and that these strains were all identical to the original Beijerinck isolate (LMD 22.21). However, some strains supposedly also derived from the van Niel strain (via the strain Stanier 381: DSM 65 and LMG 4218) were in fact *P. pantotrophus* (Goodhew et al., 1996; Rainey et al., 1999). Strains DSM 413 and Pd 1222 are also representatives of the type

Table 3. Distinguishing properties of the 14 species of the genus *Paracoccus*.

	Species[a]													
Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Denitrification	+	–	–	–	–	+	+	+	+	–	+	+	–	+
Pigmentation	–	–	+	+	–	–	na	–	+	–	–	–	–	–
Motility	–	–	–	+	–	–	–	–	–	–	–	+	–	–
Urease	+	+	na	–	–	na	na	–	–	–	–	–	+	+
Growth on:														
$H_2 + CO_2$	+	–	na	na	–	–	na	na	+	–	+	+	+	+
Thiosulfate + CO_2	+	na	na	na	na	na	na	–	w	na	+	+	–	+
Thiocyanate + CO_2	–	na	na	na	na	na	na	na	+	na	na	–	–	na
Methanol	+	+	–	na	–	–	+	–	–	–	–	+	+	+
Methylamine	+	+	–	na	+	+	–	+	–	+	–	+	+	+
Formate	+	–	+	na	–	–	–	+	w	–	–	+	+	na
Trimethylamine	w	–	–	na	+	–	–	+	–	+	–	–	–	–
Xylose	–	+	na	na	+	–	–	–	–	–	na	–	–	–
Fructose	+	+	+	na	–	–	–	–	+	+	+	+	+	+
Sucrose	+	–	+	na	–	–	–	–	–	–	na	+	+	–
Glycerol	+	+	+	na	+	–	–	w	–	+	na	+	+	+
Mannitol	+	+	+	+	–	–	–	–	+	+	na	+	+	+
Inositol	+	+	+	na	–	–	w	–	–	–	na	+	+	+

Symbols: +, present; –, absent; na, data not found in the published literature; w, weak growth.
[a]List of species (numbered in the same sequence as in Table 4): 1. *P. denitrificans*; 2. *P. alcaliphilus*; 3. *P. marcusii*; 4. *P. carotinifaciens*; 5. *P. aminophilus*; 6. *P. solventivorans*; 7. *P. alkenifer*; 8. *P. kocurii*; 9. *P. thiocyanatus*; 10. *P. aminovorans*; 11. *P. pantotrophus*; 12. *P. versutus*; 13. *P. methylutens*; 14. *P. kondratievae*.

strain, *P. denitrificans* LMD 22.21, having been derived through the "Morris strain" (Goodhew et al., 1996). In an early study, Friedrich and Mitrenga (1981) showed that strain Stanier 381 (= *P. pantotrophus*) oxidized and grew on thiosulfate, while the Morris strain (= *P. denitrificans*) did not. The original Beijerinck isolate of the type strain, LMD 22.21, was, however, shown to grow autotrophically on thiosulfate (Robertson and Kuenen, 1983), but no other studies of the use of inorganic sulfur compounds by authentic strains of *P. denitrificans* seem to have been published.

Growth of the type species, *P. denitrificans*, and of *P. versutus* and *P. kondratievae* on methanol or methylamine has been proved to be autotrophic, using the complete oxidation of the one-carbon compounds to carbon dioxide to provide metabolic energy and the fixation of carbon dioxide by the Calvin cycle for biosynthesis (Bamforth and Quayle, 1978; Van Verseveld and Stouthamer, 1978; Kelly et al., 1979; Kelly and Wood, 1982; Van Verseveld and Thauer, 1987; Doronina et al., 2001). The type strain of *P. pantotrophus* (formerly *Thiosphaera pantotropha*; Robertson and Kuenen, 1983) does not grow on methanol or methylamine, but spontaneous mutational events during prolonged incubation with methanol resulted in the appearance of a strain able to grow on methanol, using ribulose bisphosphate carboxylase to fix carbon dioxide (Egert et al., 1993). In contrast, three *Paracoccus* strains isolated by Jordan et al. (1995) and subsequently shown to be strains of *P. pantotrophus* (Jordan et al., 1997; Rainey et al., 1999) were capable of good growth on methanol without prior selective methods.

While a number of other *Paracoccus* species also grow on one-carbon compounds (Table 3) and some (*P. aminovorans*, *P. aminophilus* and *P. kocurii*) use some or all of mono-, di-, and tri-methylamines, trimethylamine-*N*-oxide, formamide, *N*-methylformamide, *N*,*N*-dimethylformamide, and formate as growth substrates, experiments proving that all these are also autotrophic have not been reported. This information is quite important to obtain, as the possibility of the occurrence of alternative assimilatory pathways for one-carbon compounds (such as the serine pathway) has not yet been excluded. The existence of such pathways in any of these species would have considerable taxonomic importance. Of relevance is the observation that significant activities of hydroxypyruvate reductase, a key enzyme of the serine pathway, were found in *P. versutus* grown on formaldehyde (Kelly and Wood, 1984) and in *P. methylutens* grown on methylamine or dichloromethane (Doronina et al., 1998), although the major pathway for carbon fixation was the Calvin cycle in both species.

Phylogenetic Relationships Between the 14 Named Species of *Paracoccus*

16S rRNA gene sequences are available for the type strains of all 14 species of the genus *Paracoccus* and a number of additional strains. Phylogenetic analyses have demonstrated that all the described species of the genus *Paracoccus* form a coherent cluster within the α-3 subclass of the Proteobacteria with the closest relatives being members of the genus *Rhodobacter* (Tsubokura et al., 1999). Comparison of the 16S rRNA gene sequences of the type strains of each of the 14 species of the genus *Paracoccus* described to date shows the 16S rRNA gene sequence similarities within the genus to be in the range 93.5 to 99.8% (Table 4). *P. methylutens* shows the lowest 16S rRNA gene sequence similarity (93.5 to 97.5%) to the other species of the genus while *P. carotinifaciens* and *P. marcusii* share 99.8% sequence similarity. The phylogenetic dendrogram shown in Fig. 1 demonstrates the relationships between the species of the genus *Paracoccus*. The majority of species are found to comprise distinct lineages with long branches not closely related to their next neighbor. The lack of close relationships between the species is also seen through the bootstrap values, which clearly indicate low confidence in most of the branching points within the dendrogram. With the exception of the relationships between (i) *P. marcusii* and *P. carotinifaciens*, (ii) *P. solventivorans* and *P. alkenifer*, and (iii) *P. pantotrophus*, *P. versutus* and *P. methylutens* no other branching points are well supported by the bootstrap analyses. Considering the overall structure of the phylogeny based on the 14 described species, the high degree of divergence between the majority of these species and the fact that some true species share high levels (<99.5%) of 16S rRNA gene sequence similarity, it is clear that there is room to add numerous new species to this genus based on sequence analyses before the phylogenetic structure becomes saturated. Because of the use of different methodologies in studies to determine the degree of DNA reassociation between *Paracoccus* species and strains it is difficult to make any correlation between 16S rRNA gene sequence similarity values and percentage DNA reassociation values. However, from the DNA reassociation data of Rainey et al. (1999), it is clear that strains sharing high 16S rRNA gene sequence similarities can still be distinct species. Hybridization between DNA from *P. pantotrophus* DSM 65 and *P. versutus* DSM 582 showed 54% reassociation while the 16S rRNA gene sequences from these two strains showed <99.0% similarity. This comparison indicates that 16S rRNA gene sequence data are useful in assigning new isolates to the *Paracoccus* genus, but that high similarity values between 16S rRNA sequences do not necessarily indicate species identity. Additional tests such as assessment of DNA-DNA hybridization are also required to establish identity at the species level.

The "ae2" editor (Maidak et al., 1996) was used to align the 16S rRNA gene sequences of *Paracoccus* spp. available from the public databases (accession numbers indicated in parentheses). The programs of the PHYLIP package including "dnadist" and "neighbor" were used for the phylogenetic analyses (Felsenstein, 1993). The tree topology was reanalyzed using 1,000 bootstrapped data sets and the programs "seqboot," dnadist and "consense" of the PHYLIP package (Felsenstein, 1993). Bootstrap values from the analyses of 1,000 data sets (expressed as percentages) are shown at the branching points. The 16S rRNA gene sequence of *Rhodobacter capsulatus* was used as an outgroup in the

Table 4. The 16S rRNA gene sequence similarity values for the species of the genus *Paracoccus*.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*P. denitrificans*	100													
2	*P. alcaliphilus*	97.4	100												
3	*P. marcusii*	96.4	96.7	100											
4	*P. carotinifaciens*	96.2	96.5	99.8	100										
5	*P. aminophilus*	97.8	96.4	96.7	96.6	100									
6	*P. solventivorans*	96.4	95.8	95.4	95.3	96.0	100								
7	*P. alkenifer*	95.5	95.8	95.0	94.9	94.9	98.1	100							
8	*P. kocurii*	95.7	96.0	95.0	94.9	95.1	96.9	95.7	100						
9	*P. thiocyanatus*	97.8	96.7	95.8	95.7	96.8	96.4	96.1	96.4	100					
10	*P. aminovorans*	97.9	97.4	96.6	96.4	97.1	96.0	95.9	95.3	97.4	100				
11	*P. pantotrophus*	98.2	96.4	95.0	94.8	96.7	96.7	95.4	96.5	97.1	96.4	100			
12	*P. versutus*	97.9	96.2	95.0	94.8	96.7	96.7	95.4	96.5	97.1	96.7	99.7	100		
13	*P. methylutens*	96.2	94.7	93.6	93.5	95.0	95.3	94.0	95.4	95.7	95.0	97.5	97.5	100	
14	*P. kondratievae*	97.1	95.2	95.0	94.9	96.0	96.1	94.7	96.3	96.6	97.0	97.5	97.5	96.2	100
	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14

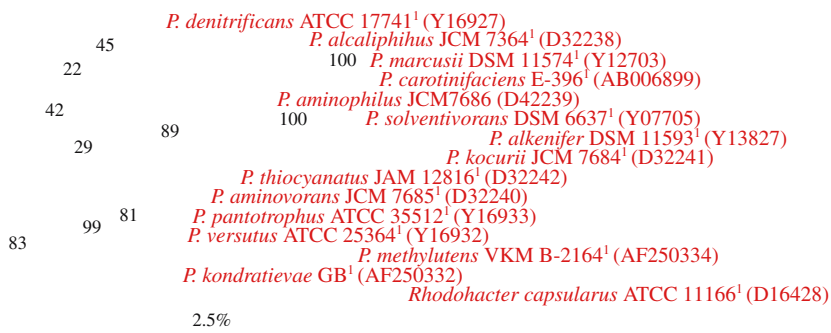

Fig. 1. Phylogenetic dendrogram derived from 16S rRNA gene sequences of all the species of the genus *Paracoccus* described to date.

Table 5. Sources from which species of *Paracoccus* have been isolated.

Species	Sources of isolates
P. denitrificans	Garden soil, arable soil, field soil, sewage sludge, meadow soil, horse manure, cow dung, canal and well mud, unspecified soils
P. pantotrophus	Denitrifying, sulfide-oxidizing effluent treatment pilot plant (Gist-Brocades, The Netherlands), *Quercus* tree soil (Kew Gardens, England)
P. marcusii	Orange-pigmented contaminant (presumed airborne) on a nutrient agar plate
P. thiocyanatus	Activated sludge enriched with thiocyanate (isolated by H. Murooka; Katayama-Fujimura et al., 1983)
P. solventivorans	Soil from 1-m depth at a defunct natural gas company
P. alkenifer	Biofilters treating waste gas from an animal rendering plant
P. carotinifaciens	Orange-pigmented isolate (on nutrient agar) from soils in the Kanagawa prefecture (Japan)
P. versutus	Mud (used in enrichment culture for *Thiobacillus denitrificans*; Taylor and Hoare, 1969; Taylor et al., 1971)
P. kocurii	Pilot activated sludge system used to treat waste water from a semiconductor manufacturing process
P. alcaliphilus	Not reported (Urakami et al., 1989)
P. aminophilus	Soil (Japan)
P. aminovorans	Soil (Japan)
P. methylutens	Groundwater
P. kondratievae	Maize rhizosphere

analyses. The scale bar represents 2.5 nucleotide substitutions per 100 nucleotides.

Ecological Aspects and Enrichment Culture for Isolation of the Species of *Paracoccus*

Earlier reviews of *Paracoccus* have been devoted almost exclusively to *P. denitrificans* and showed it to occur in many terrestrial situations (Van Verseveld and Stouthamer, 1991). The sources from which 13 of the 14 species discussed here were isolated are given in Table 5 and serve to illustrate the ubiquitous distribution of *Paracoccus* species in terrestrial environments. While the enrichment media used for the isolation of these species were diverse (Table 6), none of these could be regarded as exclusively selective for the species recovered, especially as two species were first obtained on nutrient agar, so it is concluded that there is an element of serendipity in the successful isolation of novel or existing species of *Paracoccus*. To date, no strains seem to have been reported from the marine environment, although the tolerance of 3% (w/v) NaCl by some species (*P. alkenifer* and *P. methylutens*) indicates they would survive in sea water.

All the species of *Paracoccus* can grow heterotrophically, with most species being able to use a very wide range of simple and complex organic substrates. Some species exhibit distinctive and potentially exploitable novel metabolic traits. For example, some species can grow in one or more of the following modes: as methylotrophs on one-carbon compounds, as autotrophs at the expense of oxidizing hydrogen or inorganic sulfur compounds, as autotrophs on thiocyanate or carbon disulfide, and as mixotrophs on compounds such as methylated sulfides (when both methylotrophic and chemolithotrophic energy mechanisms operate). The type species and some others are facultative denitrifiers. This metabolic diversity can enable enrichment culture to be directed towards the selection of specific metabolic types.

The procedures advocated for the enrichment culture of a number of *Paracoccus* species (Van Spanning et al., 2000) depend on selection either of the autotrophic/methylotrophic species in oxic media with hydrogen, methanol or methylamine

Table 6. Substrates used for the enrichment and isolation of the *Paracoccus* species listed in Table 5.

Species	Substrates used for the original pure culture isolations of the strains	References
P. denitrificans	Tartrate and nitrate, anaerobic with denitrification	Beijerinck and Minkman, 1910
P. pantotrophus	Mixotrophic, anaerobic chemostat with 10mM thiosulfate, 10mM acetate and 32mM nitrate	Robertson and Kuenen, 1983
	Aerobic batch enrichment with carbon disulfide	Jordan et al., 1995
P. marcusii	Nutrient agar, aerobic	Harker et al., 1998
P. thiocyanatus	Thiocyanate, autotrophic growth conditions, aerobic	Katayama et al., 1995
P. solventivorans	Acetone, aerobic	Siller et al., 1996
P. alkenifer	Not indicated	Lipski et al., 1992, 1998
P. carotinifaciens	Nutrient agar, aerobic	Tsubokura et al., 1999
P. versutus	Thiosulfate, aerobic organism	Taylor and Hoare, 1969 Taylor et al., 1971
P. kocurii	Tetramethylammonium chloride, with yeast extract, aerobic	Ohara et al., 1990
P. alcaliphilus	Not reported	Urakami et al., 1989
P. aminophilus	*N*,*N*-dimethylformamide, aerobic	Urakami et al., 1990
P. aminovorans	*N*,*N*-dimethylformamide, aerobic	Urakami et al., 1990
P. methylutens	Dichloromethane, aerobic	Doronina et al., 1998
P. kondratievae	Methanol	

(in the presence of carbon dioxide) or of denitrifying species under anoxic conditions with nitrate as the respiratory oxidant and organic compounds as carbon and energy sources. The former procedure could be applied to the enrichment of all species except *P. marcusii*, *P. thiocyanatus*, wild type *P. pantotrophus*, and possibly *P. carotinifaciens*. Growth coupled to heterotrophic denitrification is proved to be exhibited only by eight of the 14 species here described (Table 3). These include *P. thiocyanatus* and *P. pantotrophus*, thus providing a route for selection of these species. *P. marcusii* and *P. carotinifaciens* are incapable of denitrification and might not be enriched by either route.

The type culture isolated by Beijerinck (Beijerinck and Minkman, 1910) was obtained from an anaerobic culture provided with sodium potassium tartrate (Seignettesalz; Rochelle salt) and potassium nitrate (10 $g \cdot l^{-1}$ of each). Nitrous oxide (N_2O) is both an intermediate and a product of nitrate reduction by *P. denitrificans* and can be used as a sole respiratory oxidant by *P. versutus* (Wood and Kelly, 1983) and some other species (Van Spanning et al., 2000). Successful enrichment of some *Paracoccus* species can be achieved anoxically in liquid media supplied with tartrate or succinate and incubated under a nitrous oxide atmosphere, but such enrichments will obviously not be exclusively selective for *Paracoccus* species (Pichinoty et al., 1977a; Pichinoty et al., 1977b).

Growth of most mesophilic facultatively autotrophic hydrogen-oxidizing bacteria is slow or impossible under anaerobic denitrifying culture conditions (Van Verseveld and Stouthamer, 1991). Thus the ability of *P. denitrificans* to grow autotrophically using hydrogen oxidation coupled to nitrate reduction provides a means of enriching for this type of organism. Of the 14 species described, to date only four have been shown capable of both hydrogen oxidation and denitrification (although not necessarily at the same time: *P. denitrificans*, *P. thiocyanatus*, *P. pantotrophus*, *P. versutus*; Table 3), meaning that such a selective regime would select for a maximum of only four of the 14 *Paracoccus* species. Some species are also capable of good autotrophic growth on formate (Table 3), and of those, three are also denitrifiers (*P. denitrificans*, *P. versutus* and *P. kocurii*). Anaerobic enrichment with formate, carbon dioxide and nitrate (or nitrous oxide) could thus provide a selective route for the enrichment at least of *P. denitrificans* and *P. versutus* (Wood and Kelly, 1983). *P. denitrificans* is also capable of (slow) autotrophic growth on methanol under denitrifying conditions (Bamforth and Quayle, 1978), providing a further route for its selective isolation from environments also containing (strictly aerobic) facultative methylotrophs such as *Methylobacterium*.

A number of *Paracoccus* species can grow autotrophically using thiosulfate oxidation to provide energy, while at least some strains of *P. pantotrophus* can use carbon disulfide and methylated sulfur compounds (Jordan et al., 1997), and *P. thiocyanatus* can grow as an aerobic autotroph on thiocyanate (Katayama et al., 1995). These substrates have served to produce successful enrichment cultures from which novel species of *Paracoccus* have been isolated. Since such aerobic enrichment conditions are also elective for some autotrophic chemolithotrophs such as *Thiobacillus thioparus*, further screening to discriminate target species would be required: to date, no *Paracoccus* species have been found

which can use the oxidation of tetrathionate to support growth, whereas this compound is a substrate for most "thiobacilli" (Kelly et al., 2000).

Media for the Isolation and Culture of *Paracoccus* Species

It has been made clear in the preceding sections that media absolutely specific for the isolation of each of the Paracoccus species do not exist, as most strains are versatile heterotrophs with some sharing methylotrophic and lithotrophic properties with other unrelated genera. We give below the composition of some media which will enrich for and serve as growth media for *Paracoccus* species able to grow anaerobically with hydrogen and nitrate, or lithotrophically with thiosulfate, but which will also support growth of other hydrogen bacteria or chemolithotrophs. For routine isolation or culture media, the basal salts mineral media typically used in any microbiology laboratory are likely to be suitable for the growth of *Paracoccus* species on most substrates, subject to the provision of any required vitamin supplement (Table 7). Commercially available rich nutrient media will support the growth of all the known species for routine maintenance and cultivation. When a lithotrophic oxidation substrate such as thiosulfate is used, the principal technical problem is the acidification of the culture by the sulfuric acid produced as a product of oxidation. This acidification can be ameliorated in batch cultures without pH control by the use of strongly buffered media (e.g. that for *P. versutus* described below), subject to determining the tolerance of any given species to the relatively high concentrations of phosphate (or other buffer compounds) employed.

For Routine Culture on Chemoorganotrophic or Methylotrophic Substrates

We suggest a basal salts medium containing the following constituents ($g \cdot l^{-1}$):

K_2HPO_4, 3; KH_2PO_4, 2; NH_4Cl, 2; $MgSO_4 \cdot 7H_2O$, 0.2; sodium molybdate, 0.2; $FeSO_4 \cdot 7H_2O$, 0.2; with KNO_3, 5, for anaerobic cultivation. For autotrophic growth, $NaHCO_3$ ($1\ g \cdot liter^{-1}$) should be added. The molybdate and iron can be replaced with a suitable trace metal solution (e.g. one of those described below), and for strains whose potential vitamin requirement has not been tested, yeast extract ($0.1\ g \cdot l^{-1}$) or a vitamin mixture (see below) should also be added. Known requirements (e.g. thiamine) can typically be provided at $0.2\ mg \cdot l^{-1}$. Growth substrates can be added at the desired concentrations, such as 10–20 mM for sugars, organic acids (as their salts), alcohols, and other heterotrophic substrates and 10–50 mM for substrates such as formate, methanol, methylamines, or thiosulfate. Toxic substrates need testing initially at lower concentrations, such as 0.5–5 mM for dimethylsulfide, carbon disulfide, carbonyl sulfide, sodium sulfide, or formaldehyde and 5–30 mM for thiocyanate when tested as an autotrophic substrate.

The Molecular Hydrogen-Nitrate System (Vogt, 1965) for Isolation and Culture of *Paracoccus denitrificans* and Hydrogen-Oxidizing Species of *Paracoccus*

Three separate solutions are prepared, sterilized separately and mixed after cooling to prevent precipitation of phosphates:

Solution 1 ($g \cdot liter^{-1}$): $Na_2HPO_4 \cdot 12H_2O$, 9; KH_2PO_4, 1.5; NH_4Cl, 1; $MgSO_4 \cdot 7H_2O$, 0.2; KNO_3, 1; trace elements solution, 2 ml.

Solution 2 ($mg \cdot 100\ ml^{-1}$): Ferric ammonium citrate, 50; $CaCl_2 \cdot 2H_2O$, 100.

Solution 3 ($g \cdot 100\ ml^{-1}$): $NaHCO_3$, 5.

The trace elements solution, which is not sterilized prior to mixing with Solution 1, contains ($mg \cdot liter^{-1}$): $ZnSO_4 \cdot 7H_2O$, 100; $MnCl_2 \cdot 4H_2O$, 30; H_3BO_3, 300; $CoCl_2 \cdot 6H_2O$, 200; $CuCl \cdot 2H_2O$, 10; $NiCl_2 \cdot 6H_2O$, 20; $Na_2MoO_4 \cdot 2H_2O$, 30.

Solution 2 (10 ml) and solution 3 (10 ml) are added to solution 1 (1 liter). Erlenmeyer flasks containing 10 ml of the combined medium are incubated in an anaerobic jar filled with 85% H_2 and 15% CO_2 and shaken at 30°C. An appropri-

Table 7. Requirement for vitamin supplements exhibited by some species of *Paracoccus* for growth in minimal media.

Organism	Carbon substrate	Vitamin required
P. alcaliphilus	Methanol or glucose	Biotin
P. aminophilus	Various	Thiamine
P. aminovorans	Various	Thiamine
P. kocurii	Methylamines	Thiamine
P. methylutens	One-carbon compounds	B_{12} or yeast autolysate
P. thiocyanatus	Thiocyanate	Thiamine

ate oxygen absorbent may be included in the gas jar. Pure cultures may be maintained on the same medium supplemented with 1.7% (w/v) of a suitable agar.

Enrichment, Isolation and Maintenance Media for Thiosulfate-Oxidizing *Paracoccus* Species (e.g., *P. versutus* and *P. pantotrophus*; Kelly and Wood, 1998)

Four salt solutions are prepared:

Solution 1: (g ·liter $^{-1}$): $Na_2HPO_4 \cdot 2H_2O$, 39.5; KH_2PO_4, 7.5.

Solution 2: 4% (w/v) NH_4Cl.

Solution 3: 4% (w/v) $MgSO_4 \cdot 7H_2O$.

Solution 4: Trace metal solution (g · liter $^{-1}$): EDTA (disodium salt), 50; NaOH, 9; $ZnSO_4 \cdot 7H_2O$, 11; $CaCl_2$, 5; $MnCl_2 \cdot 6H_2O$, 2.5; $CoCl_2 \cdot 6H_2O$, 0.5; ammonium molybdate, 0.5; $FeSO_4 \cdot 7H_2O$, 5.0; $CuSO_4 \cdot 5H_2O$, 0.2. Adjust pH to 6.0 with 1 N NaOH and store, unsterilized, in a dark bottle.

Thiosulfate-agar Medium for Maintenance Slopes or Plates Solution 1, 200 ml; 4% NH_4Cl, 10 ml; 4% $MgSO_4 \cdot 7H_2O$, 2.5 ml; Solution T, 10 ml; 1 N NaOH, 11.5 ml; $Na_2S_2O_3 \cdot 5H_2O$, 5.0 g; agar, 15.0 g; phenol red (saturated solution), 10 ml. Initial pH should be 8.4.

Liquid Culture Medium This is prepared and sterilized in three parts to avoid precipitation of phosphates and any decomposition of growth substrates. The recipe given is for a final volume of 1 liter.

Part I (ml): 4% NH_4Cl, 20; 4% $MgSO_4 \cdot 7H_2O$, 2.5; solution T, 10; distilled water, 550.

Part II (ml): Solution 1, 200; 1 N NaOH, 10. Autoclave separately Parts I and II at 115°C for 10 min, then mix them when they are cool.

Part III (200 ml): An aqueous solution of the growth substrate, which may be thiosulfate (for growth of *P. versutus* and *P. pantotrophus*), carbon disulfide (for growth of *P. pantotrophus*), or other acidigenic substrate or any normal organic substrate.

The pH of this medium may be modified. Omitting the NaOH lowers the pH from 8.4 to 7.3, whereas intermediate pH values are obtained by adding intermediate volumes of NaOH or altering the ratios of the acid and basic phosphate salts employed (Kelly and Wood, 1998).

A number of *Paracoccus* species are known to require vitamins for successful enrichment and culture (Table 7). The following vitamin B mixture may be used generally and might be used to isolate marine species of *Paracoccus*, inasmuch as a vitamin requirement is common among marine bacteria.

Vitamin B mixture (mg ·liter $^{-1}$): thiamine-HCl, 10; nicotinic acid, 20; pyridoxine hydrochloride, 20; *p*-aminobenzoic acid, 10; riboflavin, 20; calcium pantothenate, 20; biotin, 1; cyanocobalamin, 0.5–1.0. Adjust pH to 7.0 by addition of 0.1 M NaOH.

Summary Descriptions of the Species of *Paracoccus*

For sources and original isolation conditions and principal properties of these species, see Tables 3, 4, 5 and 6.

Paracoccus alcaliphilus Type Strain JCM 7364^T

This organism is alkaliphilic and a facultative methanol- and methylamine-user. It grows at pH 7.0–9.5, but not below pH 6.5 or above pH 10. Colonies are white to pale-yellow; granules of poly-β-hydroxybutyric acid are accumulated in the cells. Grows on several hexoses, pentoses and sugar-alcohols but not on sucrose, maltose, lactose and trehalose or on formate, di- and trimethylamine. Nitrate is reduced only to nitrite and neither denitrification nor fermentative growth occurs. Ammonium, nitrate, urea and peptone are used as nitrogen sources. Its major fatty acids are 18:1 and 10:0(3-OH) and 14:0(3-OH). Its % G+C is 64–66.

Paracoccus alkenifer Type Strain DSM 11593^T

This strain will grow over the range pH 6.0–9.0. It denitrifies and will grow on solvents such as acetone, ethanol and methanol, some organic acids and asparagine, but not on carbohydrates or amines. Typical mono-unsaturated fatty acids are 14:1*cis* 7 and 20:1*cis*13, while 12:1*cis*5, 16:0 and 19:0 cyclo11-12 are absent. It also contains the hydroxy-fatty acids 10:0(3-OH) and 14:0(3-OH), of which the first predominates. Its mol% G+C was not reported (Lipski et al., 1998).

Paracoccus aminophilus Type Strain JCM 7686^T

This strain grows on methylamine and *N*,*N*-dimethylformamide, as well as di-and trimethylamine, trimethylamine-*N*-oxide, formamide, and N-methylformamide, but not on methanol or formate. It grows between pH 6.0–9.0 but not at pH 5 or 10, with an optimum of pH 6.5–8.0. It grows at 30°C, but not at 37°C.

Colonies on rich medium (peptone-yeast extract-glucose) are white-pale yellow. Granules of poly-β-hydroxybutyric acid are accumulated. Nitrate is reduced only to nitrite, and no fermentative growth is seen. It grows on various sugars, alcohols and organic acids but not on fructose, disaccharides, sugar-alcohols, citrate, ethanol or butanol. Ammonium and peptone, but not urea or nitrate, are used as nitrogen sources. The major fatty acid is straight-chain unsaturated 18:1 and its main hydroxy-fatty acids are 10:0(3-OH) and 14:0(3-OH). Its mol% G+C is 63.

Paracoccus aminovorans Type Strain JCM 7685^T

The properties of this organism are mainly as given for *P. aminophilus*, except that *P. aminovorans* can grow on fructose, sorbitol, mannose and mannitol, but not on xylose. It grows at both 30 and 37°C, but not at 42°C. Ammonium and peptone, but not urea or nitrate, are used as nitrogen sources. The major fatty acid is straight-chain unsaturated 18:1 and its main hydroxy-fatty acids are 10:0(3-OH) and 14:0(3-OH). Its mol% G+C is 67.

Paracoccus carotinifaciens Type Strain IFO 16121^T

This organism is an orange-pigmented, astaxanthin carotenoid-producing species, which may be a strain of *P. marcusii*. Colonies are orange to red. Growth occurs at 10–33°C, optimum at 28°C, with no growth at 37°C. It grows at pH 6.0–9.0, with an optimum at pH 6.5–7.5. Obligately aerobic. It grows on glucose, mannose, maltose, mannitol and the organic acids gluconate and malate, but not on arabinose, citrate and various other acids. No information on use of one-carbon substrates is available. Ammonium, but not nitrate, is used as a nitrogen source. Its major fatty acid is straight-chain unsaturated 18:1; the major hydroxy-fatty acid is 10:1(3-OH). Its mol% G+C is 67.

Paracoccus denitrificans Type Strain ATCC 17741^T, LMD 22.21^T, DSM 413^T

The type species of the genus, exhibiting metabolic versatility, with respiratory growth aerobically or with denitrification on many substrates, facultative methylotrophy and autotrophy on formate, hydrogen, thiosulfate, methanol, methylamine, and formaldehyde. It produces white or cream-colored colonies with no carotenoid pigments. It stores poly-β-hydroxybutyric acid as an intracellular carbon reserve and does not require vitamins or other organic growth factors. Its optimum temperature is 30–37°C and optimum pH is 7.5–8.0. Numerous sugars, organic acids, amino acids, and alcohols are used as sole energy and carbon sources for growth. These incude ribose, arabinose, glucose, fructose, trehalose, sucrose, mannose, acetate, propionate, malonate, tartrate, lactate, pyruvate, succinate, malate, citrate, gluconate, *p*-hydroxybenzoate, serine, proline, histidine, asparagine, glutamine, ethanol, propanol, butanol, glycerol and sorbitol. It apparently cannot use xylose, rhamnose, lactose, glycogen, cellulose, benzoate, *p*-aminobenzoate, phenol, arginine, threonine, tryptophan, ethanolamine, tetrathionate or thiocyanate for growth. Anaerobic growth can be supported by the respiratory reduction of nitrate, nitrite, or nitrous oxide, with dinitrogen as the end product; nitrous oxide can also be a product of nitrate reduction. Ammonium, nitrate, urea and glutamate are used as nitrogen sources. It is probably desirable for the growth substrate range of an authentic strain of *P. denitrificans* (e.g. ATCC 17741 or LMD 22.21) to be reassessed as it is uncertain how much of the literature data of this kind was actually obtained with strains of *P. denitrificans* rather than strains of *P. pantotrophus*. Its mol% G+C is 64–67.

Paracoccus kocurii Type Strain JCM 7684^T

This species grows on tetramethylammonium, methylamine, and di- and tri-methylamine, trimethylamine-*N*-oxide, and formate, but not on methanol. Optimum temperature is 25–30°C, with no growth at 20 or 40°C; optimum pH is 6.6–8.2, with no growth below pH 6.1 or above pH 8.8. It grows only a restricted range of other organic compounds, including lactate, acetate, pyruvate, propionate, and butyrate. Ammonium, but not nitrate or glutamate, is used as a nitrogen source. The major fatty acids are 18:1 and 19:0cyc; the major hydroxy-fatty acids are 10:0(3-OH) and 12:0(3-OH). Its mol% G+C is 71.

Paracoccus kondratievae, Proposed Type Strain VKM B-2222^{PT} (STRAIN GB^{PT})

The description of this species has been provided to us prior to publication (Doronina et al., 2001). This species is alkaliphilic and thermotolerant, growing at 30–50°C and pH 7.5–10.5 with optima at 38–42°C and pH 8.0–9.0. It grows aerobically as a facultative chemolithotroph with hydrogen or thiosulfate and as a methylotroph on methanol, methylamine or formaldehyde, assimilating carbon dioxide by the Calvin cycle. It grows anaerobically on methanol with nitrate, shows heterotrophic denitrification, and can ferment glucose. It grows on glucose, fructose, galactose,

ribose, arabinose, adonitol, dulcitol, glycerol, inositol, mannitol, sorbitol, ethanol, acetate, malate, α-ketoglutarate, succinate, fumarate, alanine, aspartate, glutamate, sarcosine, serine, *N*,*N*-dimethylglycine, and betaine. It does not grow on methane, dimethylamine, trimethylamine, chloromethane, dichloromethane, dimethylsulfoxide, lactose, xylose, rhamnose, raffinose, sucrose, trehalose, propionate, citrate, pyruvate, or tartrate. It does not require vitamins but showed growth stimulation by yeast extract (0.01% w/v). Ammonium, nitrate, urea, methylamine and amino acids are used as nitrogen sources for growth. Its major fatty acids are cyclopropane 19cyc and 16:0 and minor fatty acids are 18:0 and 18:1. Its major phospholipids are phosphatidylethanolamine, phosphatidylglycerol, cardiolipin and phosphatidylcholine. DNA-DNA hybridization showed 37–43% similarity of *P. kondratievae* to *P. denitrificans* and *P. methylutens* and 20–30% similarity to *P. alcaliphilus*, *P. alkenifer*, *P. aminophilus*, *P. aminovorans*, *P. marcusii*, *P. pantotrophus*, *P. solventivorans* and *P. thiocyanatus*. Its mol% G+C is 62.5.

Paracoccus marcusii Type Strain DSM 11574^T

This species produces bright-orange colonies caused by the presence of large amounts of carotenoids, including astaxanthin. It is an obligate aerobe and does not reduce nitrate to nitrite. It grows on glucose, fructose, galactose, mannose, arabinose, maltose, cellobiose, lactose, melibiose, sucrose, turanose, trehalose, gentiobiose, gluconate, glucuronate, galacturonic, glycerol, erythritol, mannitol, sorbitol, xylitol, inositol, adonitol, arabitol, propionate, *cis*-aconitate, citrate, lactate, malonate, succinate, formate, malate and alanine. It could not grow on methanol, methylamine, trimethylamine, dimethylformamide, thiosulfate, acetate, trimethylamine or a very wide range of other compounds. The major fatty acid is straight-chain unsaturated 18:1, with some 18:0 and 10:0. Its mol% G+C is 66. The description of this species was validly published in 1998 (Harker et al., 1998) and predated the description of the physiologically and morphologically similar *P. carontinifaciens* (Tsubokura et al., 1999). Subsequent comparison of the 16S rRNA sequences of the two strains showed that *P. carontinifaciens* and *P. marcusii* share 99.8% identity (Table 4), and the two species show only a few differences in growth substrates used. *P. carotinifaciens* was reported as motile by means of peritrichous flagella, whereas *P. marcusii* is reportedly non-motile. Whether or not these are distinct species, as is the case for *P. versutus* and *P. pantotrophus* (cf., Table 4), or are both strains of *P. marcusii* requires investigation, including the assessment of DNA-DNA hybridization between them.

Paracoccus methylutens Type Strain (DM 12) VKM B 2164^T

This species is a facultatively methylotrophic species able to grow on dichloromethane, methanol, methylamine, formate, but not formaldehyde, or di- or tri-methylamine. Nitrate is reduced to nitrite. It grows at 10–37°C, optimally at 25–30°C; pH range is 6.5–9.5 with an optimum at pH 7.0–8.0. Aerobic. It grows on a wide range of organic substrates including glucose, mannose, arabinose, maltose, ethanol, acetate, citrate and Krebs' cycle acids, and propionate, but not acetamide, rhamnose, raffinose or trehalose. Ammonium, nitrate, methylamine and urea are used as nitrogen sources. Its major fatty acid is 18:1w7. Its mol% G+C is 67.

Paracoccus pantotrophus Type Strain ATCC 35512^T, LMD 82.5^T

Coccoid shaped cells may exhibit pleomorphism on very rich media. It is very similar physiologically to both *P. denitrificans* and *P. versutus* but can be distinguished from them by its inability to use citrate and its ability to grow as an anaerobic autotroph by denitrification on sulfide or thiosulfate as sole energy substrate. It grows autotrophically on reduced sulfur compounds and hydrogen and mixotrophically and heterotrophically on a wide range of organic compounds, but the wild type will not grow on one-carbon substrates. Aerobic and anaerobic heterotrophic growth is supported by glucose, fructose, mannose, lactate, pyruvate, acetate, succinate, fumarate, gluconate, glutamate, proline, aspartate, alanine, histidine, leucine, isoleucine, acetone, propan-1,2-diol, and propan-2-ol, but not by arabinose, lactose, methyl acetate, methanol, methyl ethyl ketone, propylene oxide, oxalate or pimelate. It grew aerobically but not anaerobically on benzoate and only anaerobically on propionaldehyde. Ammonium, nitrate, urea and glutamate but not methylamine are used as nitrogen sources. Its mol% G+C is 66.

Paracoccus solventivorans Type Strain DSM 6637^T

This species grows aerobically or with denitrification on acetone, acetoacetate, 2-butanone, 2-propanol, fumarate, gluconate, ribose, pyruvate, and a number of other compounds. Optimum pH is 7.0–8.0 and optimum temperature is 30–37°C.

Ammonium is used as a nitrogen source; other nitrogen compounds are not reported. Characteristic fatty acids are 12:1*cis*5 and 20:1*cis*13. Its mol% G+C is 68.5–70.

Paracoccus thiocyanatus Type Strain IAM 12816^T

As far as is known, this organism is unique among the *Paracoccus* species in being a chemolithoautotroph able to grow aerobically on thiocyanate as its sole energy substrate; it also grows autotrophically on thiosulfate and sulfur. Optimal growth is at pH 7.5–8.5 in rich medium (range pH 6.5–9.5) and pH 7.0–8.0 in thiocyanate medium (range pH 6.0–8.5). Optimum temperature was 30–35°C (range 15–40°C). It grows on a wide range of sugars, alcohols, organic acids and amino acids, but not on citrate, benzoate, maltose, lactose, sucrose, malate and several others. Growth with denitrification occurred only on organic substrates. Ammonium and glutamate, but not nitrate, urea or aspartate, serve as nitrogen sources. It exhibits the fatty acid profile typical of the genus, with 18:1 and 19cyc and 10:0(3-OH). Its mol% G+C is 66.5–67.6.

Paracoccus versutus Type Strain ATCC 25364^T, DSM 5m82^T

This species grows as an autotroph, mixotroph and heterotroph on a very wide range of substrates. Aerobic autotrophic growth occurs on thiosulfate, sulfide, sulfur, methanol, methylamine, formate and formaldehyde, but not on tetrathionate or thiocyanate. It grows anaerobically with denitrification on organic substrates but not with reduced sulfur compounds. It grows on many sugars and organic acids, but not on lactose or cellobiose. It grows optimally at pH 7.5–8.0 in complex media (range pH 6.5–9.5) and optimally at initial pH 8.0–8.8 in thiosulfate medium without pH control; optimum pH is 7.8 on thiosulfate with pH control. Optimum temperature is 30–37°C (range 17–40°C). Ammonium, nitrate, glutamate and aspartate, but not urea, may serve as nitrogen sources. Its mol% G+C is 67–68.

Cytochromes and Electron Transport Systems in *Paracoccus* Species

P. denitrificans and those other species that have been studied in detail show complex electron transport systems both for aerobic respiration and for denitrification (Lu and Kelly, 1984a; Van Verseveld and Stouthamer, 1991; Stouthamer, 1992; Baker et al., 1998), and the respiratory system of *P. denitrificans* has long been regarded as a model for the mitochondrion (Stouthamer, 1992; Baker et al., 1998). A major difference from the mitochondrial system is the presence in *P. denitrificans* of alternative terminal oxidases (ba_3, cbb_3, and aa_3), depending on the oxygen tension in the oxidase environment. The bacteria also exhibit a diversity of branched pathways for electron transport, enabling growth under various conditions of oxygen availability from fully aerobic to anaerobic denitrification. For detailed analysis of the genetics and functioning of these complex systems the reader is referred to Baker et al. (1998). At least 17 *c*-type cytochromes have been identified in *Paracoccus* species (Baker et al., 1998), which are located both in the cytoplasmic membrane and in the periplasmic space depending on the nature of the substrate and the growth conditions, and include those which are components of terminal oxidases, the bc_1 complex and nitrate and nitric oxide reductases. Some *c*-type cytochromes involved in inorganic sulfur oxidation in *P. pantotrophus* and *P. versutus* are unusual in their large size, in their multiple subunits and in containing at least two redox centres, with widely different midpoint potentials (Kelly et al., 1997; Friedrich, 1998). The roles of these separate centres have not yet been fully explained (Lu and Kelly, 1984a; Kelly et al., 1997).

Recently the cytochromes c_{551} and $c_{552.5}$ essential for the thiosulfate-oxidizing system of *Paracoccus versutus* (see following section) have been examined using electron paramagnetic resonance (EPR) spectroscopy (J. K. Shergill, A. C. White, W-P. Lu, D. P. Kelly, A. P. Wood, C. Joannou and R. Cammack, in preparation). Cytochrome c_{551} was estimated to contain approximately six atoms of Fe per mole, and EPR showed the presence of a single type of low-spin heme, with $gz = 3.22$ and $gx \sim 1.17$. The EPR and optical absorption spectra were consistent with methionine-histidine coordination. It was concluded that cytochrome c_{551} is a hexamer of a protein containing heme *c*. Cytochrome $c_{552.5}$ showed a more complex EPR spectrum indicating at least four different types of low-spin hemes (heme-1, $gz = 2.401$, $gy = 2.245$, $gx = 1.914$; heme-2, $gz = 2.516$, $gy = 2.302$, $gx = 1.875$; heme-3, $gz = 2.583$, $gy = 2.395$, and $gx = 1.834$; and heme-4, $gz = 3.5$). These hemes responded differently to changes in pH, reduction by dithionite and the presence of ethylene glycol in the medium, and it was found that heme-2 and heme-3 were interconvertible. A small and variable amount of high-spin heme also was observed. These EPR studies indicated that the cytochrome $c_{552.5}$ of *P. versutus* contained more redox centers than the two centers identified by optical redox measure-

ments, making this cytochrome among the most complex yet characterized.

Sulfur Compound Oxidation by *Paracoccus* Species

The oxidation of thiosulfate, sulfide or thiocyanate supports the autotrophic growth of several species, but only *P. versutus* and *P. pantotrophus* GB17 have been studied in detail (Kelly, 1999):

$$S_2O_3^{2-} + 2O_2 + H_2O = 2SO_4^{2-} + 2H^+$$
$$[\Delta G^\circ = -738.7 kJ \cdot mol^{-1}]$$
$$HS^- + 2O_2 = SO_4^{2-} + H^+$$
$$[\Delta G^\circ = -732.6 kJ \cdot mol^{-1}]$$
$$SCN^- + 2O_2 + 2H_2O = SO_4^{2-} + CO_2 + NH_4^+$$
$$[\Delta G^\circ = -824.5 kJ \cdot mol^{-1}]$$

In these species, thiosulfate is oxidized by a multienzyme system located in the cytoplasm (Lu et al., 1985; Kelly, 1989; Kelly et al., 1997), the mechanism of which is summarized by Kelly et al. (1997), Baker et al. (1998) and Friedrich (1998). Considerable progress has also been made in identifying the genes in *P. pantotrophus* controlling expression of the enzymes of the thiosulfate-oxidizing system (Wodara et al., 1994; Friedrich, 1998).

Phylogenetically, *P. versutus* and *P. pantotrophus* are very similar, although DNA-DNA hybridization showed them to differ at the species level (Fig. 1; Table 4; Rainey et al., 1999). The mechanism and genetic basis of thiosulfate and sulfide oxidation have been studied in depth only in these two species (Lu, 1986; Lu and Kelly, 1983; Lu and Kelly, 1984a; Lu and Kelly, 1984b; Lu et al., 1984c; Lu et al., 1985; Kelly, 1985; Kelly, 1988; Chandra and Friedrich, 1986; Mittenhuber et al., 1991; Wodara et al., 1994; Wodara et al., 1997; Kelly et al., 1997; Friedrich, 1998). It is thus likely that the mechanism and control of inorganic sulfur oxidation are likely to be very similar, if not identical, in the two species, enabling a composite model to be derived from the available data. Such a model may be an oversimplification as differences in detail may exist between the two species (Friedrich, 1998), although these differences may be seen to be less profound as further work is done.

The enzymes and some of the cytochromes of the thiosulfate-oxidizing multienzyme system are located in the periplasm (Lu, 1986; Lu et al., 1985), and periplasmic targeting sequences are seen in at least some of the proteins of the system (Baker et al., 1998; Friedrich, 1998). Schemes interrelating the components and the proposed mechanism of the thiosulfate-oxidizing system are given by Kelly et al. (1997), Baker et al. (1998), and Friedrich (1998).

The cluster of genes involved in coding for the thiosulfate oxidizing system was initially dissected using Tn5-*mob* mutagenesis (Chandra and Friedrich, 1986; Mittenhuber et al., 1991; Friedrich, 1998). To date, a sequence of six genes coding for enzymes and cytochromes involved in thiosulfate and sulfide oxidation have been identified. Collectively these have been defined as the Sox character (ability to oxidize inorganic sulfur) in *Paracoccus* and are coded (in downstream sequence): *soxA* to *soxF* (Friedrich, 1998). The six genes comprise a minimum of 6.3 kb pairs in the *P. pantotrophus* chromosome (Friedrich, 1998), although their location in the genome is uncertain. One strain, deficient in thiosulfate oxidation, was shown to lack the 450 kbp megaplasmid (Chandra and Friedrich, 1986; Baker et al., 1998), and the Sox locus was studied using a mutant containing a full complement of megaplasmids/minichromosomes (Mittenhuber et al., 1991). The genes identified or inferred to date, together with their gene products and the probable biochemical functions of these proteins in the thiosulfate-oxidizing system, are summarized in Table 8.

Dissimilatory Nitrogen Metabolism in *Paracoccus*

Respiratory denitrification has been studied in detail in *P. pantotrophus* and *P. denitrificans* (Berks et al., 1995; Baker et al., 1998) and its genetic regulation has been comprehensively analyzed (Baker et al., 1998). Nitrate reduction to nitrite is catalyzed by two nitrate reductases: one is a membrane-bound enzyme induced in the presence of nitrate under conditions of oxygen limitation; the other is a constitutive periplasmic molybdopterin enzyme with both heme and non-heme iron sites. Nitrite is reduced to nitric oxide (NO) by a periplasmic nitrite reductase, which contains two hemes as prosthetic groups and receives electrons for nitrite reduction from cytochrome *c*550 and a copper-containing pseudoazurin. The hemes are a typical *c*-type and an unusual *d*1 heme. Nitric oxide is reduced to nitrous oxide (N_2O) by a nitric oxide reductase anchored on the periplasmic side of the cytoplasmic membrane. This enzyme contains one large and one small protein subunit, the former binding heme *b* and the latter, heme *c*. Nitrous oxide is reduced to dinitrogen by a periplasmic nitrous oxide reductase which contains two different copper centers.

Table 8. Genes and gene products of the periplasmic thiosulfate-oxidizing multienzyme system of *P. pantotrophus* and *P. versutus*.

Sox gene	Gene product	Properties of mature gene product	Proposed identity of the gene product proteins
soxA	Sox A	29-kDa diheme cytochrome	Electron transport from thiosulfate; possible 29kDa subunit of *P. versutus* cytochrome $c_{552.5}$ (56-kDa dimer)
soxB	Sox B: 60.5kDa (with 16 amino-acid leader peptide)	59-kDa mature protein	Enzyme B (60kDa) of *P. versutus*; oxidation of covalently bound thiosulfate to sulfate
soxC	Sox C: 47.5kDa (with 40 amino-acid signal peptide)	43.7-kDa mature protein	Sulfite:cytochrome *c* oxidoreductase, possibly essential for full activity of the thiosulfate-oxidizing multienzyme system
soxD	Sox D	40-kDa protein	Periplasmic diheme cytochrome *c*; possible 43-kDa subunit of *P. versutus* cytochrome c_{551} (260-kDa hexamer)
soxE	Sox E	26-kDa protein	Periplasmic diheme cytochrome *c*
soxF	Sox F	12-kDa protein	Putative flavocytochrome *c* (possible sulfide dehydrogenase)
"sox Y"	Sox Y	12-kDa peptide	Component of a heterodimer with Sox Y
"soxZ"	Sox Z	16-kDa peptide	Enzyme A (16kDa) of *P. versutus*; probable thiosulfate-binding protein

P. pantotrophus has been reported to be able to carry out denitrification also under aerobic conditions, as well as being able to nitrify under heterotrophic conditions (Robertson et al., 1988; Arts et al., 1995). Periplasmic nitrate reductase was present in such aerobically grown bacteria and was the initiator of nitrate reduction; nitric oxide and nitrous oxide reductases were also active under aerobic conditions (Bell and Ferguson, 1991). The concerted action of the processes of nitrification and denitrification was indicated by the production (by cell cultures) of ^{15}N-labeled dinitrogen and nitrous oxide from ^{15}N-ammonia as well as from ^{15}N-nitrite (Arts et al., 1995). The physiological and ecological significance of these processes is not yet fully understood.

Glucose Dissimilation by *Paracoccus* Species

While central metabolic pathways such as the Krebs' cycle probably occur in all species of *Paracoccus*, different species exhibit a diversity of mechanisms for the aerobic dissimilation of glucose. As well as the Krebs' cycle, Forget and Pichinoty (1965) showed both the Entner-Doudoroff and oxidative pentose phosphate (hexose monophosphate) pathways to operate in *Paracoccus* denitrificans, but a later report that the glycolytic pathway is absent from *P. denitrificans* is not applicable to all species of *Paracoccus* (Slabas and Whatley, 1977; Van Spanning et al., 2000). The most detailed studies of glucose metabolism were conducted with *P. versutus*, in which all three of the Embden-Meyerhof-Parnas, Entner-Doudoroff and pentose phosphate pathways can operate simultaneously in glucose-grown bacteria (Wood et al., 1977; Wood and Kelly, 1978; Wood and Kelly, 1979; Wood and Kelly, 1980). The ratios of these pathways vary according to growth conditions, and during growth on other hexoses, pentoses and disaccharides or on mixtures of glucose and maltose, the Embden-Meyerhof-Parnas pathway is completely repressed, and the Entner-Doudoroff pathway predominates (Wood and Kelly, 1979; Wood and Kelly, 1980; Smith et al., 1980).

Chromosomes and Plasmids in *Paracoccus* Species

Only a few species of *Paracoccus* have been studied with respect to their plasmid complement. The earliest detailed study showed plasmids in three strains described as *P. denitrificans* (Gerstenberg et al., 1982). One was the "Morris" strain which can be assumed to have been correctly described as *P. denitrificans* (Goodhew et al., 1996; Rainey et al., 1999) and contained one megaplasmid of molecular mass exceeding 300 MDa. Another was the "Stanier" strain, which subsequently appeared in culture collections as both ATCC 17741 and DSM 65 (Goodhew et al., 1996). It is now known that ATCC 17741 is the type strain of *P. denitrificans* but that DSM 65 is a strain of *P. pantotrophus* (Rainey et al., 1999). This means that it is uncertain whether the "Stanier" strain used by Gerstenberg et al., 1982) was *P. denitrificans* or

P. pantotrophus. It contained two plasmids of molecular masses around 50 MDa and >300 MDa (Gerstenberg et al., 1982). The "Vogt" strain (DSM 415) contained only one <300 MDa megaplasmid and may thus also be *P. denitrificans* (Gerstenberg et al., 1982). *P. denitrificans* DSM 413 and *P. versutus* were also shown to contain megaplasmids <500 kb (Wlodarczyk and Piechuka, 1995; Jordan et al., 1997). We conclude that the evidence currently available shows the type strain (and its derivatives) of *P. denitrificans* may contain megaplasmids of at least 450 kb in size, but it is uncertain if they can also contain smaller (100 kb) plasmids, as none were present in strains DSM 413 or the "Stanier" strain (Jordan et al., 1997; Rainey et al., 1999). In contrast, some strains of *P. pantotrophus* do contain 85–110 kb plasmids as well as megaplasmids of 450 kb, but one strain (DSM 11072) lacked the 100 kb plasmid (Jordan et al., 1997; Rainey et al., 1999).

The genomic structure of *P. denitrificans* and *P. pantotrophus* is complex in that several very large DNA molecules comprise the genome and are presumed to be chromosomal elements (Baker et al., 1998; Winterstein and Ludwig, 1998). The authentic *P. denitrificans* strains ATCC 13543, DSM 413 and Pd 1222 contain three chromosomal DNA molecules: I, 1.83 Mb; II, 1.16 Mb; and III, 0.67 Mb (Winterstein and Ludwig, 1998), of which at least molecules I and II are linear DNA. The distribution among these molecules of genes coding for respiratory oxidases, cytochrome *c*550, methanol oxidation and *S*-formylglutathione hydrolase appeared "random," indicating that the three replicons together comprise the genome and are probably replicated concurrently (Winterstein and Ludwig, 1998). Two strains of *P. pantotrophus* (LMD 82.5 and DSM 65) contain four DNA molecules: 2.2, 1.5, 0.71 or 0.77, and 0.5 Mb, of which the 0.71 Mb molecule was circular (Winterstein and Ludwig, 1998). In addition, the *P. pantotrophus* strains showed the expected plasmid of molecular size 97 kb (LMD 82.5) or 60 kb (DSM 65).

While at least the three large replicons in both *P. denitrificans* and *P. pantotrophus* are likely to represent the chromosomal DNA (with a genome size of around 4 Mb), the genetic importance of the megaplasmids or mini-chromosomes of <450 kb in *P. denitrificans*, *P. pantotrophus* and *P. versutus* is unknown, as is that of the 107 bp pTAV1 plasmid of *P. versutus* (Jagusztyn-Krynicka et al., 1990; Bartosik et al., 1995), although loss of the 450 kb plasmid from *P. pantotrophus* was possibly linked to loss of the ability to carry out thiosulfate oxidation (see section on sulfur compound oxidation in this Chapter; Chandra and Friedrich, 1986).

Biotechnological Potential of *Paracoccus* Species

In common with other facultative denitrifiers, *P. denitrificans* can contribute to the removal of nitrate from wastewaters, and *P. pantotrophus* was isolated from a desulfurizing and denitrifying effluent-treatment system (Robertson and Kuenen, 1983). Experimentally, long-term continuous denitrification was obtained using *P. denitrificans* immobilized with a polyelectrolyte complex (Kokufuta et al., 1987). *Paracoccus* species are probably important components of many wastewater treatment system communities, having been found in denitrifying sand filters and activated sludge systems (Ohara et al., 1990; Katayama et al., 1995; Neef et al., 1996). Others have been isolated from biofilters treating effluent gases and from contaminated soils (Siller et al., 1996; Lipski et al., 1998). The ability of different species to degrade unusual and potentially polluting compounds indicates their potential role in natural or contrived bioremediation systems. Target compounds include methanol, acetone, dichloromethane and other solvents, tetramethylammonium compounds, methylamines, substituted formamides, thiocyanate, sulfides, organic sulfur compounds such as carbon disulfide, carbonyl sulfide and methanethiol, all of which are waste products of diverse commercial processes. As yet, no controlled system inoculated with specific *Paracoccus* strains seems to have been used on a commercial scale.

Exploitation of *Paracoccus* species as potential sources of bio-products has also received little attention to date. A mixture of co-immobilized cells of *P. denitrificans* and Corynebacterium was shown to be effective for the continuous production of L-phenylalanine from acetamidocinnamic acid (Nishida et al., 1987), and the production of relatively large amounts of astaxanthin by *P. marcusii* and *P. carotinifaciens* might enable these species to be exploited for the production of such pigments (Harker et al., 1998; Tsubokura et al., 1999).

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Literature Cited

Arts, P. A. M., L. A. Robertson, and J. G. Kuenen. 1995. Nitrification and denitrification by Thiosphaera pantotropha in aerobic chemostat cultures. FEMS Microbiol. Ecol. 18:305–316.

Baker, S. C., S. J. Ferguson, B. Ludwig, M. D. Page, O.-M. H. Richter, and R. J. M. van Spanning. 1998. Molecular genetics of the genus *Paracoccus*: Metabolically versatile bacteria with bioenergetic flexibility. Microbiol. Molec. Biol. Rev. 62:1046–1078.

Bamforth, C. W., and J. R. Quayle. 1978. Aerobic and anaerobic growth of *Paracoccus* denitrificans on methanol. Arch. Microbiol. 119:91–97.

Bartosik, D., J. Baj, and M. Wlodarczyk. 1995. Construction and characterization of mini-derivatives of the large (107 kb) cryptic plasmid of Thiobacillus versutus. FEMS Microbiol. Lett. 129:169–174.

Beijerinck, M., and D. C. J. Minkman. 1910. Bildung und Verbrauch von Stickoxydul durch Bakterien. Centralblatt f. Bakteriologie, Abt. II 25:30–63.

Bell, L. C., and S. J. Ferguson. 1991. Nitric and nitrous oxide reductases are active under aerobic conditions in cells of Thiosphaera pantotropha. Biochem. J. 273:423–427.

Berks, B. C., S. J. Ferguson, J. W. B. Moir, and D. J. Richardson. 1995. Enzymes and associated electron transport systems that catalyze the respiratory reduction of nitrogen oxides and oxyanions. Biochim. Biophys. Acta 1232:97–173.

Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of the 16S ribosomal RNA gene from Escherichia coli. Proc. Natl. Acad. Sci. USA 75:4801–4805.

Chandra, T. S., and C. G. Friedrich. 1986. Tn5-induced mutations affecting sulfur- oxidizing ability (Sox) of Thiosphaera pantotropha. J. Bacteriol. 166:446–452.

Davis, D. H., M. Doudoroff, and R. Y. Stanier. 1969. Proposal to reject the genus Hydrogenomonas: Taxonomic implications. Int. J. Syst. Bacteriol. 19:375–390.

Dobson, S. J., and P. D. Franzmann. 1996. Unification of the genera Deleya (Baumann et al., 1983), Halomonas (Vreeland et al., 1980), and Halovibrio (Fendrich, 1988) and the species *Paracoccus* halodenitrificans (Robinson & Gibbons, 1952) into a single genus, Halomonas, and placement of the genus Zymobacter in the family Halomonadaceae. Int. J. Syst. Bacteriol. 46:550–558.

Doronina, N. V., Y. A. Trotsenko, V. I. Krausova, and N. E. Suzina. 1998. Paracoccus methylutens sp. nov.—a new aerobic facultatively methylotrophic bacterium utilizing dichloromethane. Syst. Appl. Microbiol. 21:230–236.

Doronina, N. V., Y. A. Trotsenko, B. B. Kuznetsov, and T. P. Tourova. in press. Paracoccus kondratievae sp. nov.—a new thermotolerant and alkaliphilic facultative methylotroph from maize rhizosphere.

Egert, M., A. Hamann, R. Könen, and C. G. Friedrich. 1993. Methanol and methylamine utilization result from mutational events in Thiosphaera pantotropha. Arch. Microbiol. 159:364–371.

Felsenstein, J. 1993. PHYLIP (Phylogenetic Inference Package) version 3.5.1. Department of Genetics, University of Washington. Seattle, WA.

Forget, P., and F. Pichinoty. 1965. Le cycle tricarboxylique chez une bactérie dénitrifiante obligatoire. Ann. Inst. Pasteur 108:364–377.

Friedrich, C. G., and G. Mitrenga. 1981. Oxidation of thiosulfate by *Paracoccus* denitrificans and other hydrogen bacteria. FEMS Microbiol. Lett. 10:209–212.

Friedrich, C. G. 1998. Physiology and genetics of sulfur-oxidizing bacteria. Adv. Microbial Physiol. 39:235–289.

Gerstenberg, C., B. Friedrich, and H. G. Schlegel. 1982. Physical evidence for plasmids in autotrophic, especially hydrogen-oxidizing bacteria. Arch. Microbiol. 133:90–96.

Goodhew, C. F., G. W. Pettigrew, B. Devreese, J. van Beeumen, R. J. M. van Spanning, S. C. Baker, N. Saunders, S. J. Ferguson, and I. P. Thompson. 1996. The cytochromes c-550 of *Paracoccus* denitrificans and Thiosphaera pantotropha: A need for re-evaluation of the history of *Paracoccus* cultures. FEMS Microbiol. Lett. 137:95–101.

Harker, M., J. Hirschberg, and A. Oren. 1998. Paracoccus marcusii sp. nov., an orange Gram-negative coccus. Int. J. Syst. Bacteriol. 48:543–548.

Jagusztyn-Krynicka, E. K., A. Brzescinska-Kujawa, and K. I. Wolska. 1990. Restriction map of Thiobacillus versutus plasmid pTAV1. Acta Microbiol. Polon. 39:85–89.

Jordan, S. L., A. J. Kraczkiewicz-Dowjat, D. P. Kelly, and A. P. Wood. 1995. Novel eubacteria able to grow on carbon disulfide. Arch. Microbiol. 163:131–137.

Jordan, S. L., I. R. McDonald, A. J. Kraczkiewicz-Dowjat, D. P. Kelly, F. A. Rainey, J. C. Murrell, and A. P. Wood. 1997. Autotrophic growth on carbon disulfide is a property of novel strains of *Paracoccus* denitrificans. Arch. Microbiol. 168:225–236.

Katayama, Y., A. Hiraishi, and H. Kuraishi. 1995. Paracoccus thiocyanatus sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of Thiobacillus versutus to the genus *Paracoccus* as *Paracoccus* versutus comb. nov. with emendation of the genus. Microbiology (UK) 141:1469–1477.

Katayama-Fujimura, Y., Y. Enokizona, T. Kaneko, and H. Kuraishi. 1983. Deoxyribonucleic acid homologies among species of the genus Thiobacillus. J. Gen. Appl. Microbiol. 29:287–295.

Kelly, D. P., A. P. Wood, J. C. Gottschal, and J. G. Kuenen. 1979. Autotrophic metabolism of formate by Thiobacillus A2. J. Gen. Microbiol. 114:1–13.

Kelly, D. P., and A. P. Wood. 1982. Autotrophic growth of Thiobacillus A2 on methanol. FEMS Microbiol. Lett. 15:229–233.

Kelly, D. P., and A. P. Wood. 1984. Potential for methylotrophic autotrophy in Thiobacillus versutus (Thiobacillus sp, strain A2). *In:* R. L. Crawford and R. S. Hanson (Eds.) Microbial Growth on C_1 Compounds. American Society for Microbiology. Washington DC, 324–329.

Kelly, D. P. 1985. Physiology of the thiobacilli: Elucidating the sulphur oxidation pathway. Microbiol. Sci. 2:105–109.

Kelly, D. P. 1988. Oxidation of sulphur compounds. Soc. Gen. Microbiol. Symp. 42:65–98.

Kelly, D. P. 1989. Physiology and biochemistry of unicellular sulfur bacteria. *In:* H. G. Schlegel and B. Bowien (Eds.) Biology of Autotrophic Bacteria. Science Tech Publishers. Madison, WI. 193–217.

Kelly, D. P., J. K. Shergill. W.-P. Lu, and A. P. Wood. 1997. Oxidative metabolism of inorganic sulfur compounds by bacteria. Ant. v. Leeuwenhoek 71:95–107.

Kelly, D. P., and A. P. Wood. 1998. Microbes of the sulfur cycle. *In:* R. S. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler (Eds.) Techniques in Microbial Ecology. Oxford University Press. New York, 31–57.

Kelly, D. P. 1999. Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. Arch. Microbiol. 171:219–229.

Kelly, D. P., A. P. Wood, and E. Stackebrandt. in press. Genus Thiobacillus Beijerinck. 1904. *In:* N. R. Krieg, J. T. Staley, and D. Brenner (Eds.) Bergey's Manual of Systematic Bacteriology. 3:Bergey's Manual Trust. MI.

Kocur, M. 1984. Genus *Paracoccus* Davis. *In:* N. R. Krieg (Ed.) Bergey's Manual of Systematic Bacteriology. 1:Williams & Wilkins. Baltimore, MD. 399–402.

Kokufuta, E., M. Shimohashi, and I. Nakamura. 1987. Continuous column denitrification using polyelectrolyte complex-entrapped P. denitrificans cells. J. Ferment. Technol. 65:359–361.

Kornberg, H. L., J. F. Collins, and D. Bigley. 1960. The influence of growth substrates on metabolic pathways in Micrococcus denitrificans. Biochim. Biophys. Acta 39:9–24.

Kuenen, J. G., and L. A. Robertson. 1989. The genus Thiosphaera. *In:* J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (Eds.) Bergey's Manual of Systematic Bacteriology. 3:Williams & Wilkins. Baltimore, MD. 1861–1862.

Lipski, A., S. Klatte, B. Bendinger, and K. Altendorf. 1992. Differentiation of Gram- negative, nonfermentative bacteria isolated from biofilters on the basis of fatty acid composition, quinone system, and physiological reaction profiles. Appl. Environ. Microbiol. 58:2053–2065.

Lipski, A., K. Reichert, B. Reuter, C. Sprör, and K. Altendorf. 1998. Identification of bacterial isolates from biofilters as *Paracoccus* alkenifer sp. nov. and *Paracoccus* solventivorans with emended description of *Paracoccus* solventivorans. Int. J. Syst. Bacteriol. 48:529–536.

Lu, W.-P., and D. P. Kelly. 1983. Purification and some properties of two principal enzymes of the thiosulphate–oxidizing system from Thiobacillus A2. J. Gen. Microbiol. 129:3549–3564.

Lu, W.-P., and D. P. Kelly. 1984. Purification and characterization of two essential cytochromes of the thiosulphate-oxidizing multi-enzyme system from Thiobacillus versutus (A2). Biochim. Biophys. Acta 765:106–117.

Lu, W.-P., and D. P. Kelly. 1984. Properties and role of sulphite cytochrome c oxido- reductase purified from Thiobacillus versutus. J. Gen. Microbiol. 130:1683–1692.

Lu, W.-P., R. K. Poole, and D. P. Kelly. 1984. Oxidation–reduction potentials and spectral properties of some cytochromes from Thiobacillus versutus. Biochim. Biophys. Acta 767:326–334.

Lu, W.-P., B. E. P. Swoboda, and D. P. Kelly. 1985. Properties of the thiosulphate- oxidizing multi-enzyme system from Thiobacillus versutus. Biochim. Biophys. Acta 828:116–122.

Lu, W.-P. 1986. A periplasmic location for the thiosulphate-oxidizing multi-enzyme system from Thiobacillus versutus. FEMS Microbiol. Lett. 34:313–317.

Ludwig, W., G. Mittenhuber, and C. G. Friedrich. 1993. Transfer of Thiosphaera pantotropha to *Paracoccus* denitrificans. Int. J. Syst. Bacteriol. 43:363–367.

Maidak. 1996. The Ribosomal Database Project (RDP). Nucleic Acids Res. 24:82–85.

Miller, J. M., S. J. Dobson, P. D. Franzmann, and T. A. McKeekin. 1994. Reevaluating the classification of *Paracoccus* halodenitrificans with sequence comparisons of 16S ribosomal DNA. Int. J. Syst. Bacteriol. 44:360–361.

Mittenhuber, G., K. Sonomoto, M. Egert, and C. G. Friedrich. 1991. Identification of the DNA region responsible for sulfur-oxidizing ability of Thiosphaera pantotropha. J. Bacteriol. 173:7340–7344.

Neef, A., A. Zaglauer, H. Meier, R. Amann, H. Lemmer, and K.-H. Schleifer. 1996. Population analysis in a denitrifying sand filter: Conventional and in situ identification of *Paracoccus* spp. in methanol-fed biofilms. Appl. Environ. Microbiol. 62:4329–4339.

Nishida, Y., K. Nakamichi, K. Nabe, and T. Tosa. 1987. Continuous production of L- phenylalanine from acetamidocinnamic acid using co-immobilized cells of a Corynebacterium and *Paracoccus* denitrificans. Int. J. Syst. Bacteriol. 33:26–37.

Ohara, M., Y. Katayama, M. Tsuzaki, S. Nakamoto, and H. Kuraishi. 1990. Paracoccus kocurii sp. nov., a tetramethylammonium-assimilating bacterium. Int. J. Syst. Bacteriol. 40:292–296.

Pichinoty, F., M. Mandel, and J.-L. Garcia. 1977. Étude physiologique et taxonomique de *Paracoccus* denitrificans. Ann. Microbiol. (Institut Pasteur) 128B:243–251.

Pichinoty, F., M. Mandel, B. Greenway, and J.-L. Garcia. 1977. Étude de. 14 bactéries dénitrifiantes appartenant au groupe Pseudomonas stutzerii isolés du sol par culture en présence d'oxyde nitreux. Ann. Microbiol. (Institut Pasteur) 128A:75–89.

Rainey, F. A., D. P. Kelly, E. Stackebrandt, J. Burghardt, A. Hiraishi, Y. Katayama, and A., P. Wood. 1999. A re-evaluation of the taxonomy of *Paracoccus* denitrificans and a proposal for the combination *Paracoccus* pantotrophus comb. nov. Int. J. Syst. Bacteriol. 49:645–651.

Robertson, L. A., and J. G. Kuenen. 1983. Thiosphaera pantotropha gen. nov., sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. J. Gen. Microbiol. 129:2847–2855.

Robertson, L. A., E. W. J. van Niel, R. A. M. Torremans, and J. G. Kuenen. 1988. Simultaneous nitrification and denitrification in chemostat cultures of Thiosphaera pantotropha. Appl. Environ. Microbiol. 54:2812–2818.

Siller, H., F. A. Rainey, E. Stackebrandt, and J. Winter. 1996. Isolation and characterization of a new Gram-negative, acetone-degrading, nitrate-reducing bacterium from soil, *Paracoccus* solventivorans sp. nov. Int. J. Syst. Bacteriol. 46:1125–1130.

Slabas, A. R., and L. R. Whatley. 1977. Metabolic regulation of pyruvate kinase isolated from autotrophically and heterotrophically grown *Paracoccus* denitrificans. Arch. Microbiol. 115:67–71.

Smith, A. L., D. P. Kelly, and A. P. Wood. 1980. Metabolism of Thiobacillus A2 grown under autotrophic, mixotrophic and heterotrophic conditions in chemostat culture. J. Gen. Microbiol. 121:127–138.

Stouthamer, A. H. 1992. Metabolic pathways in *Paracoccus* denitrificans and closely related bacteria in relation to the phylogeny of prokaryotes. Ant. v. Leeuwenhoek 61:1–33.

Stouthamer, S. C., A. P. N. de Boer, J. van der Oost, and R. J. M. van Spanning. 1997. Emerging principles of inorganic nitrogen metabolism in *Paracoccus* denitrificans and related bacteria. Ant. v. Leeuwenhoek 71:33–41.

Taylor, B. F., and D. S. Hoare. 1969. New facultative Thiobacillus and a reevaluation of the heterotrophic potential of Thiobacillus novellus. J. Bacteriol. 100:487–497.

Taylor, B. F., D. S. Hoare, and S. L. Hoare. 1971. Thiobacillus denitrificans as an obligate chemolithotroph: Isolation and growth studies. Arch. Microbiol. 78:193–204.

Tsubokura, A., H. Yoneda, and H. Mizuta. 1999. Paracoccus carotinifaciens sp. nov., a new aerobic Gram-negative astaxanthin-producing bacterium. Int. J. Syst. Bacteriol. 49:277–282.

Urakami, T., J. Tamaoka, K.-I. Suzuki, and K. Komagata. 1989. Paracoccus alcaliphilus sp. nov., an alkaliphilic and facultatively methylotrophic bacterium. Int. J. Syst. Bacteriol. 39:116–121.

Urakami, T., H. Araki, H. Oyanagi, K.-I. Suzuki, and K. Komagata. 1990. Paracoccus aminophilus sp. nov., which utilize N,N-dimethylformamide. Int. J. Syst. Bacteriol. 40:287–291.

Van Spanning, R. J. M., A. H. Stouthamer, S. C. Baker, and H. W. van Verseveld. in press. Genus *Paracoccus* Davis. 1969. *In:* Bergey's Manual of Systematic Bacteriology. Bergey's Manual Trust. MI.

Van Verseveld, H. W., and A. H. Stouthamer. 1978. Growth yields and the efficiency of oxidative phosphorylation during autotrophic growth of *Paracoccus* denitrificans on methanol and formate. Arch. Microbiol. 118:21–26.

Van Verseveld, H. W., and R. K. Thauer. 1987. Energetics of C_1-compound metabolism. *In:* H. W. van Verseveld and J. A. Duine (Eds.) Microbial Growth on C_1 Compounds. Martinus Nijhoff, Kluwer. Dordrecht, 177–185.

Van Verseveld, H. W., and A. H. Stouthamer. 1991. The genus *Paracoccus*. *In:* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (Eds.) The Prokaryotes. 3:Springer-Verlag. New York, NY. 2321–2334.

Vogt, M. 1965. Wachstumphysiologische Untersuchungen an Micrococcus denitrificans Beij. Arch. Mikrobiol. 50:256–281.

Winterstein, C., and B. Ludwig. 1998. Genes coding for respiratory complexes map on all three chromosomes of the *Paracoccus* denitrificans genome. Arch. Microbiol. 169:275–281.

Wlodarczyk, M., and E. Piechuka. 1995. Conjugal transfer of plasmid and chromosomal markers between strains of Thiobacillus versutus. Acta Microbiol. Polon. 43:223–227.

Wodara, C., S. Kostka, M. Egert, D. P. Kelly, and C. G. Friedrich. 1994. Identification and sequence analysis of the soxB gene essential for sulfur oxidation of *Paracoccus* denitrificans GB-17. J. Bacteriol. 176:6188–6191.

Wodara, C., F. Bardichewsky, and C. G. Friedrich. 1997. Cloning and characterization of sulfite dehydrogenase, two c-type cytochromes, and a flavoprotein of *Paracoccus* denitrificans GB17—essential role of sulfite dehydrogenase in lithotrophic sulfur oxidation. J. Bacteriol. 179:5014–5023.

Wood, A. P., D. P. Kelly, and C. F. Thurston. 1977. Simultaneous operation of three catabolic pathways in the metabolism of glucose by Thiobacillus A2. Arch. Microbiol. 113:265–274.

Wood, A. P., and D. P. Kelly. 1978. Triple catabolic pathways for glucose in a fast- growing strain of Thiobacillus A2. Arch. Microbiol. 117:309–310.

Wood, A. P., and D. P. Kelly. 1979. Glucose catabolism by Thiobacillus A2 grown in chemostat culture under carbon or nitrogen limitation. Arch. Microbiol. 122:307–312.

Wood, A. P., and D. P. Kelly. 1980. Carbohydrate degradation pathways in Thiobacillus A2 grown on various sugars. J. Gen. Microbiol. 120:333–345.

Wood, A. P., and D. P. Kelly. 1983. Autotrophic, mixotrophic and heterotrophic growth with denitrification by Thiobacillus A2 under anaerobic conditions. FEMS Microbiol. Lett. 16:363–370.