

Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence

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The broad and vague phenotypic definition allowed the genus *Pseudomonas* to become a dumping ground for incompletely characterized polarly flagellated, Gram-negative, rod-shaped, aerobic bacteria, and a large number of species have been accommodated in the genus *Pseudomonas*. The 16S rRNA sequences of 128 valid and invalid *Pseudomonas* species, which included almost valid species of the genus *Pseudomonas* listed in the Approved Lists of Bacterial Names, were obtained: sequences of 59 species were determined and those of 69 species were obtained from the GenBank/EMBL/DDBJ databases. These sequences were compared with the sequences of other species of the *Proteobacteria*. Fifty-seven valid or invalid species including *Pseudomonas aeruginosa* (type species of the genus *Pseudomonas* Migula 1894) belonged to the genus *Pseudomonas* (*sensu stricto*). Seven subclusters were formed in the cluster of the genus *Pseudomonas* (*sensu stricto*), and the resulting clusters conformed well to the rRNA–DNA hybridization study by Palleroni (1984). The other species did not belong to the genus *Pseudomonas* (*sensu stricto*) and were related to other genera, which were placed in four subclasses of the *Proteobacteria* (α , β , γ and γ - β subclasses). Twenty-six examined species, which were not included in the cluster of the *Pseudomonas* (*sensu stricto*) and have not been transferred to other genera as yet, are listed alphabetically: '*Pseudomonas abikonensis*', *Pseudomonas antimicrobica*, *Pseudomonas beijeinckii*, *Pseudomonas beteli*, *Pseudomonas boreopolis*, '*Pseudomonas butanovora*', *Pseudomonas carboxydohydrogena*, *Pseudomonas cissicola*, *Pseudomonas doudoroffii*, *Pseudomonas echinoides*, *Pseudomonas elongata*, *Pseudomonas flectens*, *Pseudomonas geniculata*, *Pseudomonas halophila*, *Pseudomonas hibiscicola*, *Pseudomonas huttiensis*, *Pseudomonas iners*, *Pseudomonas lanceolata*, *Pseudomonas lemoignei*, *Pseudomonas mephitica*, *Pseudomonas pictorum*, *Pseudomonas saccharophila*, *Pseudomonas spinosa*, *Pseudomonas stanier*, *Pseudomonas syzygii* and *Pseudomonas woodsii*. The phylogenetic affiliations of these 26 pseudomonads species are shown.

Keywords: *Pseudomonas*, pseudomonads, phylogenetic analysis, 16S rRNA

INTRODUCTION

The genus *Pseudomonas* Migula 1894 was described so that it included polarly flagellated strictly aerobic rods with a respiratory type of metabolism in which oxygen is used. Defined in this way, the genus was very heterogeneous, and a large number of species of the

genus was reported. In 1984, over 100 species of the genus were listed, and the characteristics of these species were described in *Bergey's Manual of Systematic Bacteriology* (Palleroni, 1984). At the time of writing (December 1998), 118 species and subspecies of the genus *Pseudomonas* were validated on the Approved Lists of Bacterial Names (Bacterial Nomenclature Up-to-Date, available on the WorldWide Web at URL: <http://www.dsmz.de/bactnom/bactname.htm>).

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Table 1. Current classification or phylogenetic affiliation of the pseudomonads, and accession no. of 16S rRNA gene sequence used

Proteobacteria subclass*	Previous name	Current classification or phylogenetic affiliation	Reference	Strain no.	Accession no.	
α	* <i>Pseudomonas abikonensis</i>	<i>Sphingomonas</i> rRNA lineage	This study	IAM 12404 ^T	AB021416‡	
	<i>Pseudomonas aminovorans</i>	<i>Aminobacter aminovorans</i>	Kerstens <i>et al.</i> (1996)			
	<i>Pseudomonas carboxydohydrogena</i>	<i>Bradyrhizobium</i> group rRNA lineage	This study	DSM 1083 ^T	AB021393‡	
	* <i>Pseudomonas carboxydovorans</i>	<i>Oligotropha carboxydovorans</i>	Kerstens <i>et al.</i> (1996)			
	* <i>Pseudomonas compransoris</i>	<i>Zavarzinia compransoris</i>	Kerstens <i>et al.</i> (1996)			
	<i>Pseudomonas diminuta</i>	<i>Brevundimonas diminuta</i>	Kerstens <i>et al.</i> (1996)	IAM 12691 ^T	AB021415‡	
	<i>Pseudomonas echinoides</i>	<i>Sphingomonas</i> rRNA lineage	Kerstens <i>et al.</i> (1996)	ATCC 14820 ^T	AB021370‡	
	* <i>Pseudomonas extorquens</i> and * <i>Pseudomonas rosea</i>	<i>Methylobacterium extorquens</i>	Kerstens <i>et al.</i> (1996)	JCM2802 ^T	D32224	
	<i>Pseudomonas mesophilica</i>	<i>Methylobacterium mesophilicum</i>	Kerstens <i>et al.</i> (1996)	JCM 2829 ^T	D32225	
	<i>Pseudomonas paucimobilis</i>	<i>Sphingomonas paucimobilis</i>	Kerstens <i>et al.</i> (1996)	GIFU 2395 ^T	D16144	
	<i>Pseudomonas radiora</i>	<i>Methylobacterium radiotolerans</i>	Kerstens <i>et al.</i> (1996)	JCM 2831 ^T	D32227	
	<i>Pseudomonas rhodos</i>	<i>Methylobacterium rhodinum</i>	Kerstens <i>et al.</i> (1996)	JCM 2811 ^T	D32229	
	* <i>Pseudomonas riboflavina</i>	<i>Devosia riboflavina</i>	Kerstens <i>et al.</i> (1996)	IFO 13584 ^T	D49423	
	<i>Pseudomonas vesicularis</i>	<i>Brevundimonas vesicularis</i>	Kerstens <i>et al.</i> (1996)	IAM 12105 ^T	AB021414‡	
	β	<i>Pseudomonas acidovorans</i>	<i>Comamonas acidovorans</i>	Kerstens <i>et al.</i> (1996)	IAM 12409 ^T	AB021417‡
		<i>Pseudomonas andropogonis</i>	<i>Burkholderia andropogonis</i>	Kerstens <i>et al.</i> (1996)	ATCC 23061 ^T	X67037
		<i>Pseudomonas antimicrobica</i>	<i>Burkholderia</i> rRNA lineage	This study	NCIMB 9898 ^T	AB021384‡
		<i>Pseudomonas avenae</i> subsp. <i>avenae</i>	<i>Acidovorax avenae</i> subsp. <i>avenae</i>	Kerstens <i>et al.</i> (1996)	ATCC 19860 ^T	AF078759
		* <i>Pseudomonas butanovora</i>	<i>Thaueria</i> rRNA lineage	This study	IAM 12574 ^T	AB021377‡
		<i>Pseudomonas caryophylli</i>	<i>Burkholderia caryophylli</i>	Kerstens <i>et al.</i> (1996)	ATCC 25341 ^T	AB021423‡
		<i>Pseudomonas cattleyae</i>	<i>Acidovorax avenae</i> subsp. <i>cattleyae</i>	Kerstens <i>et al.</i> (1996)	NCPPB 961 ^T	AF078762
		<i>Pseudomonas cepacia</i>	<i>Burkholderia cepacia</i>	Kerstens <i>et al.</i> (1996)	ATCC 25416 ^T	M22518
		<i>Pseudomonas cocovenenans</i>	<i>Burkholderia cocovenenans</i>	Kerstens <i>et al.</i> (1996)	ATCC 33664 ^T	AB021389‡
		<i>Pseudomonas delafieldii</i>	<i>Acidovorax delafieldii</i>	Kerstens <i>et al.</i> (1996)	ATCC 17505 ^T	AF078764
		<i>Pseudomonas facilis</i>	<i>Acidovorax facilis</i>	Kerstens <i>et al.</i> (1996)	CCUG 2113 ^T	AF078765
		<i>Pseudomonas flava</i>	<i>Hydrogenophaga flava</i>	Kerstens <i>et al.</i> (1996)	DSM 619 ^T	AB021420‡
		<i>Pseudomonas gladioli</i>	<i>Burkholderia gladioli</i>	Kerstens <i>et al.</i> (1996)	ATCC 10248 ^T	X67038
<i>Pseudomonas glathei</i>		<i>Burkholderia glathei</i>	Vandamme <i>et al.</i> (1997), Viillard <i>et al.</i> (1998)	ATCC 29195 ^T	AB021374‡	
<i>Pseudomonas glumae</i>		<i>Burkholderia glumae</i>	Kerstens <i>et al.</i> (1996)	LMG 2196 ^T	U96931	
<i>Pseudomonas huttiensis</i>		<i>Herbaspirillum</i> rRNA lineage	This study	ATCC 14670 ^T	AB021366‡	
<i>Pseudomonas indigofera</i>		<i>Vogesella indigofera</i>	Grimes <i>et al.</i> (1997)	ATCC 19706 ^T	AB021385‡	
<i>Pseudomonas lanceolata</i>		<i>Comamonadaceae</i> rRNA lineage	This study	ATCC 14669 ^T	AB021390‡	
<i>Pseudomonas lemoignei</i>		<i>Burkholderia</i> group rRNA lineage	This study	ATCC 17989 ^T	AB021375‡	
<i>Pseudomonas mallei</i>		<i>Burkholderia mallei</i>	Kerstens <i>et al.</i> (1996)	ATCC 23344 ^T	1680504	
<i>Pseudomonas mephitica</i>		<i>Janthinobacterium</i> rRNA lineage	This study	ATCC 33665 ^T	AB021388‡	
<i>Pseudomonas mixta</i>		<i>Telluria mixta</i>	Kerstens <i>et al.</i> (1996)	ACM17	X65589	
<i>Pseudomonas palleronii</i>		<i>Hydrogenophaga palleronii</i>	Kerstens <i>et al.</i> (1996)	DSM 63 ^T	AF019073	
<i>Pseudomonas phenazinium</i>		<i>Burkholderia phenazinium</i>	Viillard <i>et al.</i> (1998)	ATCC 33666 ^T	AB021394‡	
<i>Pseudomonas pickettii</i>		<i>Ralstonia pickettii</i>	Kerstens <i>et al.</i> (1996)	ATCC 27511 ^T	1680507	
<i>Pseudomonas plantarii</i>		<i>Burkholderia plantarii</i>	Kerstens <i>et al.</i> (1996)	LMG 9035 ^T	U96933	
<i>Pseudomonas pseudoalcaligenes</i> subsp. <i>citulli</i>		<i>Acidovorax avenae</i> subsp. <i>citulli</i>	Kerstens <i>et al.</i> (1996)	ATCC 29625 ^T	AF078761	
<i>Pseudomonas pseudoalcaligenes</i> subsp. <i>konjaci</i>	<i>Acidovorax konjaci</i>	Kerstens <i>et al.</i> (1996)	ATCC 33996 ^T	AF078760		
<i>Pseudomonas pseudoftava</i>	<i>Hydrogenophaga pseudoftava</i>	Kerstens <i>et al.</i> (1996)	ATCC 33668 ^T	AF078770		
<i>Pseudomonas pseudomallei</i>	<i>Burkholderia pseudomallei</i>	Kerstens <i>et al.</i> (1996)	1026b	U91839		
<i>Pseudomonas pyrrocinia</i>	<i>Burkholderia pyrrocinia</i>	Vandamme <i>et al.</i> (1997), Viillard <i>et al.</i> (1998)	ATCC 15958 ^T	AB021369‡		
<i>Pseudomonas rubrilineans</i>	<i>Acidovorax avenae</i> subsp. <i>avenae</i>	Kerstens <i>et al.</i> (1996)	ATCC 19307 ^T	AB021421‡		
<i>Pseudomonas rubrisubalbicans</i>	<i>Herbaspirillum rubrisubalbicans</i>	Kerstens <i>et al.</i> (1996)	ATCC 19308 ^T	AB021424‡		
<i>Pseudomonas saccharophila</i>	* <i>Matsuebacter</i> -Proteobacterium rRNA lineage	This study	DSM 654 ^T	AB021407‡		
<i>Pseudomonas solanacearum</i>	<i>Ralstonia solanacearum</i>	Kerstens <i>et al.</i> (1996)	ATCC 11696 ^T	X67036		
<i>Pseudomonas spinosa</i>	<i>Hydrogenophaga</i> rRNA lineage	This study	ATCC 14606 ^T	AB021387‡		
<i>Pseudomonas syzygii</i>	<i>Ralstonia</i> rRNA lineage	This study	ATCC 49543 ^T	AB021403‡		
<i>Pseudomonas taeniospiralis</i>	<i>Hydrogenophaga taeniospiralis</i>	Kerstens <i>et al.</i> (1996)	ATCC 49743 ^T	AF078768		
* <i>Pseudomonas terrigena</i>	<i>Comamonas terrigena</i>	Kerstens <i>et al.</i> (1996)	IAM 12052 ^T	AB021418‡		
<i>Pseudomonas testosteronei</i>	<i>Comamonas testosteronei</i>	Kerstens <i>et al.</i> (1996)	ATCC 11996 ^T	M11224		
<i>Pseudomonas woodsii</i>	<i>Burkholderia</i> rRNA lineage	This study	ATCC 19311 ^T	AB021422‡		
γ-β	<i>Pseudomonas beteli</i>	<i>Stenotrophomonas</i> rRNA lineage	This study	ATCC 19861 ^T	AB021406‡	
	<i>Pseudomonas boreopolis</i>	<i>Xanthomonas-Xylella</i> rRNA lineage	This study	ATCC 33662 ^T	AB021391‡	
	<i>Pseudomonas cissicola</i>	<i>Xanthomonas</i> rRNA lineage	This study	ATCC 33616 ^T	AB021399‡	
	<i>Pseudomonas geniculata</i>	<i>Stenotrophomonas</i> rRNA lineage	This study	ATCC 19374 ^T	AB021404‡	
	<i>Pseudomonas hibiscicola</i>	<i>Stenotrophomonas</i> rRNA lineage	This study	ATCC 19867 ^T	AB021405‡	
	<i>Pseudomonas maltophilia</i>	<i>Stenotrophomonas maltophilia</i>	Kerstens <i>et al.</i> (1996)	ATCC 13637 ^T	AB008509	
	<i>Pseudomonas pictorum</i>	<i>Stenotrophomonas-Xanthomonas</i> rRNA lineage	This study	ATCC 23328 ^T	AB021392‡	
γ	<i>Pseudomonas beijerinckii</i>	<i>Chromohalobacter</i> rRNA lineage	This study	ATCC 19372 ^T	AB021386‡	
	<i>Pseudomonas doudoroffii</i>	<i>Aeromonas</i> group- <i>Vibrionaceae</i> rRNA lineage	This study	ATCC 27123 ^T	AB021371‡	
	<i>Pseudomonas elongata</i>	<i>Microbulbifer</i> rRNA lineage	This study	ATCC 10144 ^T	AB021368‡	
	<i>Pseudomonas flectens</i>	<i>Enterobacteriaceae</i> rRNA lineage	This study	ATCC 12775 ^T	AB021400‡	
	* <i>Pseudomonas halodurans</i>	<i>Halomonas halodurans</i>	Hebert & Vreeland (1987)	DSM 5160 ^T	L42619	
	<i>Pseudomonas halophila</i>	<i>Marinobacter-Alcanivorax</i> rRNA lineage	This study	DSM 3050 ^T	AB021383‡	
	<i>Pseudomonas iners</i>	<i>Marinobacterium</i> rRNA lineage	This study	IAM 1419 ^T	AB021408‡	
	<i>Pseudomonas marina</i>	<i>Halomonadaceae</i> rRNA lineage	This study	ATCC 25374 ^T	M93354	
	<i>Pseudomonas nautica</i>	<i>Marinobacter hydrocarbonoclasticus</i>	Spröer <i>et al.</i> (1998)	ATCC 27132 ^T	AB021372‡	
	* <i>Pseudomonas nigrifaciens</i>	<i>Pseudoalteromonas nigrifaciens</i>	Gauthier <i>et al.</i> (1995)	NCIMB 8614 ^T	X82146	

Table 1 (cont.)

Proteobacteria subclass*	Previous name	Current classification or phylogenetic affiliation	Reference	Strain no.	Accession no.
(Authentic <i>Pseudomonas</i>)	' <i>Pseudomonas piscicida</i> ' <i>Pseudomonas stanieri</i>	<i>Pseudoalteromonas piscicida</i> <i>Marinobacterium</i> rRNA lineage	Gauthier <i>et al.</i> (1995) This study	ATCC 15057 ^T ATCC 27130 ^T	X82215 AB021367†
	<i>Pseudomonas aeruginosa</i> group				
	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	Kerstens <i>et al.</i> (1996)	LMG 1242 ^T	Z76651
	<i>Pseudomonas alcaligenes</i>	<i>Pseudomonas alcaligenes</i>	Kerstens <i>et al.</i> (1996)	IAM 12411 ^T	D84006
	<i>Pseudomonas anguilliseptica</i>	<i>Pseudomonas anguilliseptica</i>	Kerstens <i>et al.</i> (1996)	NCMB 1949 ^T	AB021376†
	<i>Pseudomonas citronellolis</i>	<i>Pseudomonas citronellolis</i>	Kerstens <i>et al.</i> (1996)	ATCC 13674 ^T	AB021396†
	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i>	Kerstens <i>et al.</i> (1996)	B62 ^T	U01916
	<i>Pseudomonas mendocina</i>	<i>Pseudomonas mendocina</i>	Kerstens <i>et al.</i> (1996)	ATCC 25411 ^T	M59154
	<i>Pseudomonas nitroreducens</i>	<i>Pseudomonas nitroreducens</i>	Anzai <i>et al.</i> (1997)	IAM 1439 ^T	D84021
	<i>Pseudomonas oleovorans</i>	<i>Pseudomonas oleovorans</i>	Kerstens <i>et al.</i> (1996)	IAM 1508 ^T	D84018
	<i>Pseudomonas pseudoalcaligenes</i>	<i>Pseudomonas pseudoalcaligenes</i>	Kerstens <i>et al.</i> (1996)	JCM 5968 ^T	AB021379†
	<i>Pseudomonas resinovorans</i>	<i>Pseudomonas resinovorans</i>	Kerstens <i>et al.</i> (1996)	ATCC 14235 ^T	AB021373†
	<i>Pseudomonas straminea</i>	<i>Pseudomonas straminea</i>	Anzai <i>et al.</i> (1997)	IAM 1598 ^T	D84023
	<i>Pseudomonas chlororaphis</i> group				
	<i>Pseudomonas aurantiaca</i>	<i>Pseudomonas aurantiaca</i>	This study	ATCC 33663 ^T	AB021412†
	<i>Pseudomonas aureofaciens</i>	<i>Pseudomonas chlororaphis</i>	Kerstens <i>et al.</i> (1996)	IAM 12353 ^T	D84008
	<i>Pseudomonas chlororaphis</i>	<i>Pseudomonas chlororaphis</i>	Kerstens <i>et al.</i> (1996)	IAM 12354 ^T	D84011
	<i>Pseudomonas fragi</i>	<i>Pseudomonas fragi</i>	Kerstens <i>et al.</i> (1996)	IFO 3458 ^T	AB021413‡
	<i>Pseudomonas lundensis</i>	<i>Pseudomonas lundensis</i>	Kerstens <i>et al.</i> (1996)	ATCC 49968 ^T	AB021395†
	<i>Pseudomonas taetrolens</i>	<i>Pseudomonas taetrolens</i>	Kerstens <i>et al.</i> (1996)	IAM 1653 ^T	D84027
	<i>Pseudomonas fluorescens</i> group				
	<i>Pseudomonas azotoformans</i>	<i>Pseudomonas azotoformans</i>	Anzai <i>et al.</i> (1997)	IAM 1603 ^T	D84009
	' <i>Pseudomonas cedrella</i> '	' <i>Pseudomonas cedrella</i> '	This study	CFML 96-198	AF064461
	<i>Pseudomonas corrugata</i>	<i>Pseudomonas corrugata</i>	Kerstens <i>et al.</i> (1996)	ATCC 29736 ^T	D84012
	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i>	Kerstens <i>et al.</i> (1996)	IAM 12022 ^T	D84013
	' <i>Pseudomonas gessardii</i> '	' <i>Pseudomonas gessardii</i> '	This study	CIP 105469	AF074384
	' <i>Pseudomonas libaniensis</i> '	' <i>Pseudomonas libaniensis</i> '	This study	CIP 105460	AF057645
	' <i>Pseudomonas mandelii</i> '	' <i>Pseudomonas mandelii</i> '	This study	CIP 105273	AF058286
	<i>Pseudomonas marginalis</i>	<i>Pseudomonas marginalis</i>	Kerstens <i>et al.</i> (1996)	ATCC 10844 ^T	AB021401†
	' <i>Pseudomonas migulae</i> '	' <i>Pseudomonas migulae</i> '	This study	CIP 105470	AF074383
	<i>Pseudomonas mucidolens</i>	<i>Pseudomonas mucidolens</i>	Kerstens <i>et al.</i> (1996)	IAM 12406 ^T	D84017
	' <i>Pseudomonas orientalis</i> '	' <i>Pseudomonas orientalis</i> '	This study	CFML 96-170	AF064457
	<i>Pseudomonas rhodesiae</i>	<i>Pseudomonas rhodesiae</i>	This study	CIP 104664 ^T	AB021410†
	<i>Pseudomonas synxantha</i>	<i>Pseudomonas synxantha</i>	Kerstens <i>et al.</i> (1996)	IAM 12356 ^T	D84025
	<i>Pseudomonas tolaasii</i>	<i>Pseudomonas tolaasii</i>	Kerstens <i>et al.</i> (1996)	ATCC 33618 ^T	D84028
	<i>Pseudomonas veronii</i>	<i>Pseudomonas veronii</i>	This study	CIP 104663 ^T	AB021411†
	<i>Pseudomonas pertucinogena</i> group				
	' <i>Pseudomonas denitrificans</i> '	' <i>Pseudomonas denitrificans</i> '	This study	IAM 12023 ^T	AB021419‡
	<i>Pseudomonas pertucinogena</i>	<i>Pseudomonas pertucinogena</i>	This study	IFO 14163 ^T	AB021380†
	<i>Pseudomonas putida</i> group				
	' <i>Pseudomonas mosselii</i> '	' <i>Pseudomonas mosselii</i> '	This study	CIP 105259	AF072688
	<i>Pseudomonas fulva</i>	<i>Pseudomonas fulva</i>	Anzai <i>et al.</i> (1997)	IAM 1529 ^T	D84015
	<i>Pseudomonas monteilii</i>	<i>Pseudomonas monteilii</i>	This study	CIP 104883 ^T	AB021409†
	<i>Pseudomonas oryzae</i>	<i>Pseudomonas oryzae</i>	Anzai <i>et al.</i> (1997)	IAM 1568 ^T	D84004
	' <i>Pseudomonas plecoglossicida</i> '	' <i>Pseudomonas plecoglossicida</i> '	This study	FPC951	AB009457
	<i>Pseudomonas putida</i>	<i>Pseudomonas putida</i>	Kerstens <i>et al.</i> (1996)	IAM 1236 ^T	D84020
	<i>Pseudomonas stutzeri</i> group				
	<i>Pseudomonas balearica</i>	<i>Pseudomonas balearica</i>	Kerstens <i>et al.</i> (1996)	SP 1402 ^T	U26418
	<i>Pseudomonas luteola</i>	<i>Pseudomonas luteola</i>	Anzai <i>et al.</i> (1997)	IAM 13000 ^T	D84002
	<i>Pseudomonas stutzeri</i>	<i>Pseudomonas stutzeri</i>	Kerstens <i>et al.</i> (1996)	CCUG 11256 ^T	U26262
	<i>Pseudomonas syringae</i> group				
	<i>Pseudomonas amygdali</i>	<i>Pseudomonas amygdali</i>	Kerstens <i>et al.</i> (1996)	ATCC 33614 ^T	D84007
	<i>Pseudomonas avellanae</i>	<i>Pseudomonas avellanae</i>	Kerstens <i>et al.</i> (1996)	P90	U49384
	<i>Pseudomonas caricapapayae</i>	<i>Pseudomonas caricapapayae</i>	Kerstens <i>et al.</i> (1996)	ATCC 33615 ^T	D84010
	<i>Pseudomonas cichorii</i>	<i>Pseudomonas cichorii</i>	Kerstens <i>et al.</i> (1996)	ATCC 10857 ^T	AB021398†
	<i>Pseudomonas coronafaciens</i>	<i>Pseudomonas syringae</i>	Kerstens <i>et al.</i> (1996)	LMG 13190 ^T	Z76660
	<i>Pseudomonas ficuserectae</i>	<i>Pseudomonas ficuserectae</i>	Kerstens <i>et al.</i> (1996)	JCM 2400 ^T	AB021378†
	<i>Pseudomonas meliae</i>	<i>Pseudomonas meliae</i>	Kerstens <i>et al.</i> (1996)	MAFF 301463 ^T	AB021382†
	<i>Pseudomonas savastanoi</i>	<i>Pseudomonas savastanoi</i>	Kerstens <i>et al.</i> (1996)	ATCC 13522 ^T	AB021402†
	<i>Pseudomonas syringae</i>	<i>Pseudomonas syringae</i>	Kerstens <i>et al.</i> (1996)	ATCC 19310 ^T	D84026
	<i>Pseudomonas viridiflava</i>	<i>Pseudomonas viridiflava</i>	Kerstens <i>et al.</i> (1996)	LGM 2352 ^T	Z76671
	Ungrouped				
	<i>Pseudomonas agarici</i>	<i>Pseudomonas agarici</i>	Kerstens <i>et al.</i> (1996)	ATCC 25941 ^T	D84005
	<i>Pseudomonas asplenii</i>	<i>Pseudomonas asplenii</i>	Kerstens <i>et al.</i> (1996)	ATCC 23835 ^T	AB021397†
	<i>Pseudomonas fuscovaginae</i>	<i>Pseudomonas fuscovaginae</i>	Kerstens <i>et al.</i> (1996)	MAFF 301177 ^T	AB021381†
	' <i>Pseudomonas jessenii</i> '	' <i>Pseudomonas jessenii</i> '	This study	CIP 105274	AF068259

* Subclasses of *Proteobacteria* according to Woese (1987), Stackebrandt *et al.* (1988) and Kersters *et al.* (1996).

† Sequence determined by direct sequencing method in this study.

‡ Sequence determined by cloning sequencing method in this study.

In the past two decades, polyphasic taxonomic studies, especially using methods for analysing micro-organisms at the molecular level, have played a crucial role in improving the classification of the pseudo-

monads. Many of the organisms originally described as species of the genus *Pseudomonas* have been reclassified to the genera *Acidovorax*, *Aminobacter*, *Brevundimonas*, *Burkholderia*, *Comamonas*, *Halo-*

monas, *Herbaspirillum*, *Hydrogenophaga*, *Marinobacter*, *Methylobacterium*, *Oligotropha*, *Pseudoalteromonas*, *Ralstonia*, *Sphingomonas*, *Stenotrophomonas*, *Telluria*, *Vogesella* and *Zavarzinia* (Baldani *et al.*, 1996; Baumann *et al.*, 1983a; Bowman *et al.*, 1993; Gauthier *et al.*, 1995; Gillis *et al.*, 1995; Green & Bousfield, 1983; Grimes *et al.*, 1997; Hebert & Vreeland, 1987; Meyer *et al.*, 1993; Palleroni & Bradbury, 1993; Segers *et al.*, 1994; Spröer *et al.*, 1998; Tamaoka *et al.*, 1987; Urakami *et al.*, 1992, 1994; Vandamme *et al.*, 1997; Viillard *et al.*, 1998; Willems *et al.*, 1989, 1990, 1992; Yabuuchi *et al.*, 1990, 1992, 1995). Forty-six species and subspecies of the genus *Pseudomonas* described in the Approved List of Bacterial Names (December 1998) have been transferred to those other genera.

An overview of the past and current taxonomic organization of *Pseudomonas* has been presented by Kersters *et al.* (1996), who described validly named *Pseudomonas* species which, on the basis of the rRNA relationship, do not belong to the genus *Pseudomonas* (*sensu stricto*) and which can therefore be expected to be transferred to existing or new genera in the future are *Pseudomonas beijerinckii*, *Pseudomonas beteli*, *Pseudomonas boreopolis*, *Pseudomonas carboxydohydrogena*, *Pseudomonas doudoroffii*, *Pseudomonas* (now *Sphingomonas*) *echinoides*, *Pseudomonas elongata*, *Pseudomonas geniculata*, *Pseudomonas* (now *Burkholderia*) *glathiei*, *Pseudomonas hibiscicola*, *Pseudomonas huttiensis*, *Pseudomonas lemoignei*, *Pseudomonas nautica* (now *Marinobacter hydrocarbonoclasticus*), *Pseudomonas pertucinogena*, *Pseudomonas* (now *Burkholderia*) *phenazinium*, *Pseudomonas pictorum*, *Pseudomonas pyrrocinia*, *Pseudomonas saccharophila* and *Pseudomonas syzygii*, and validly described *Pseudomonas* species, whose phylogenetic positions were undetermined, are *Pseudomonas antimicrobica*, *Pseudomonas aurantiaca*, *Pseudomonas azotoformans*, *Pseudomonas cissicola*, *Pseudomonas flectens*, *Pseudomonas fulva*, *Pseudomonas gelidicola*, *Pseudomonas halophila*, *Pseudomonas* (now *Vogesella*) *indigofera*, *Pseudomonas iners*, *Pseudomonas lanceolata*, *Pseudomonas mephitica*, *Pseudomonas nitroreducens*, *Pseudomonas spinosa* and *Pseudomonas straminea*. Recently, *P. glathiei*, *P. phenazinium* and *P. pyrrocinia* were transferred to the genus *Burkholderia* by Vandamme *et al.* (1997) and Viillard *et al.* (1998). *P. indigofera* was reclassified to the genus *Vogesella* by Grimes *et al.* (1997). More recently still, *P. nautica* was transferred to *Marinobacter hydrocarbonoclasticus* by Spröer *et al.* (1998). On 16S rRNA sequence analysis, it was shown that *P. azotoformans*, *P. nitroreducens* and *P. straminea* belonged to the authentic *Pseudomonas* (Anzai *et al.*, 1997). The establishment of the genera *Chryseomonas* and *Flavimonas* by Holmes *et al.* was based upon low values of DNA–DNA homology between the strains of *Chryseomonas* and *Flavimonas* and some strains of the genus *Pseudomonas* (Holmes *et al.*, 1986, 1987). We described previously that *Chryseomonas luteola* and *Flavimonas oryzihabitans* were

found in the cluster of the authentic *Pseudomonas* strains, and the genera *Chryseomonas* and *Flavimonas* are junior subjective synonyms of *Pseudomonas* (Anzai *et al.*, 1997).

Seventy-eight species of *Pseudomonas* have been described in the genus *Pseudomonas* on the Approved List of Bacterial Names (December 1998). Thirty-nine *Pseudomonas* species were described as species of the authentic *Pseudomonas* by Kersters *et al.* (1996). However, the phylogenetic positions of several species have not been described. In the present study, almost complete 16S rRNA sequences of 59 species of validly or invalidly described *Pseudomonas* were determined. The phylogenetic affiliation of most *Pseudomonas* species was shown using 16S rRNA sequences.

METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study were purchased from: ATCC, American Type Culture Collection, Manassas, VA, USA; CIP, Collection des Bacteries de l'Institut Pasteur, Paris, France; DSM, DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IAM, The University of Tokyo, Tokyo, Japan; IFO, Institute of Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Japan; MAFF, National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fishery, Tsukuba, Japan; and NCIMB, National Collection of Industrial and Marine Bacteria Ltd, Aberdeen, UK. *Pseudomonas anguilliseptica* NCMB 1949^T was used as the original strain described by Wakabayashi & Egusa (1972). For the sequencing study, these strains were cultured on agar plates, which were recommended by the culture collection for culture of each strain, or in nutrient broth (Difco).

Sequencing of 16S rRNA. Total DNA was extracted from cells cultured on agar plates by the benzyl chloride method according to Zhu *et al.* (1993) or by the phenol method from cells cultured in liquid medium by SDS followed by RNase treatment. Amplification of the 16S rRNA coding region of the DNA and sequencing of the 16S rDNA were performed as described by Anzai *et al.* (1997). The 16S rDNA sequences were determined by two methods, cloning and direct sequencing methods. Sequencing gel electrophoresis was performed, and nucleotide sequences were automatically obtained using a model 373A or 310 DNA sequencer (Applied Biosystems), or a model DSQ-1000L sequencer (Shimadzu) and the protocol and software recommended by the manufacturer. For the 373A or 310 DNA sequencer, M13RP1 and –21M13 dye-labelled primers were purchased from Applied Biosystems, and the following dye-labelled primers, five nucleotides (5'-CAGGA-OH-3') which were added to the 5' end of the oligonucleotides to be suitable for the system of the autosequencer, were obtained from Takara Shuzo 520F (5'-CAGGAGTGCCAGCAGCCGCGG-OH-3'; same as positions 515–530, in the *Escherichia coli* numbering system), 520R (5'-CAGGAACCGCGGCTGCTGGC-OH-3'; complementary to positions 531–517), 800F (5'-CAGGAATTAGATACCCCTGGTA-OH-3'; same as positions 787–802), 800R (5'-CAGGACTACCAGGGTATCTAAT-OH-3'; complementary to positions 803–787), 1100F (5'-CAGGAGCAACGAGCGCAACCC-OH-3'; same as positions 1099–1114) and 1100R (5'-CAGGAA-

Table 2. 16S rRNA sequences excluding the pseudomonads obtained from the GenBank/EMBL/DDBJ databases

Species	Strain no.	Accession no.
<i>Acetobacter aceti</i>	NCIB 8621 ^T	X74066
<i>Acidiphilium cryptum</i>	ATCC 33463 ^T	D30773
<i>Acidomonas methanolica</i>	IMET 10945 ^T	D30770
<i>Acidovorax temperans</i>	CCUG 11779 ^T	AF078766
<i>Acinetobacter calcoaceticus</i>	DSM 30006 ^T	X81661
<i>Actinobacillus lignieresii</i>	NCTC 4189 ^T	M75068
<i>Aeromonas caviae</i>	ATCC 15468 ^T	X74674
<i>Aeromonas eucrenophila</i>	ATCC 23309 ^T	X74675
<i>Aeromonas hydrophila</i>	ATCC 7966 ^T	X74677
<i>Aeromonas jandaei</i>	ATCC 49568 ^T	X74678
<i>Aeromonas media</i>	ATCC 33907 ^T	X74679
<i>Aeromonas salmonicida</i>	ATCC 33658 ^T	X74681
<i>Aeromonas schubertii</i>	ATCC 43700 ^T	X74682
<i>Aeromonas sobria</i>	ATCC 43979 ^T	X74683
<i>Aeromonas veronii</i>	ATCC 35624 ^T	X74684
<i>Afipia broomeae</i>	F186	U87759
<i>Afipia clevelandensis</i>	ND	M69186
<i>Afipia felis</i>	AfTA-1	AF003937
<i>Agrobacterium tumefaciens</i>	NCPPB 2437 ^T	D14500
<i>Alcaligenes faecalis</i>	IAM 12369 ^T	D88008
<i>Alcaligenes xylosoxidans</i>	IAM 12684	D88005
<i>Alcanivorax borkumii</i>	Sk2 ^T	Y12579
<i>Alteromonas macleodii</i>	IAM 12920 ^T	X82145
<i>Amaricoccus kaplicensis</i>	BEN 101 ^T	U88041
<i>Anaplasma marginale</i>	ND	M60313
<i>Ancylobacter aquaticus</i>	ATCC 25396 ^T	M62790
<i>Arhodomonas aquaeolei</i>	ATCC 49307 ^T	M26631
<i>Arsenophonus nasoniae</i>	ATCC 49151 ^T	M90801
<i>Asticcacaulis excentricus</i>	ATCC 15261 ^T	AB016610
<i>Azoarcus evansii</i>	KB 740 ^T	X77679
<i>Azoarcus indigens</i>	VB32 ^T	AF011345
<i>Azorhizobium caulinodans</i>	ORS 571 ^T	D11342
<i>Azospirillum lipoferum</i>	ATCC 29707 ^T	M59061
<i>Bartonella bacilliformis</i>	ND	M65249
<i>Beggiatoa alba</i>	B15LD	L40994
<i>Beijerinckia indica</i>	ATCC 9039 ^T	M59060
<i>Blastobacter capsulatus</i>	ND	X73042
<i>Blastobacter denitrificans</i>	LMC 8443 ^T	S46917
<i>Blastochloris viridis</i>	DSM 133 ^T	AF084495
<i>Bordetella pertussis</i>	ATCC 9797 ^T	U04950
<i>Brachymonas denitrificans</i>	AS-P1 ^T	D14320
<i>Bradyrhizobium elkanii</i>	USDA 76 ^T	U35000
<i>Bradyrhizobium japonicum</i>	DSM 30131 ^T	X87272
<i>Brenneria nigrifluens</i>	ATCC 13028 ^T	U80203
<i>Buchnera aphidicola</i>	ND	M63246
<i>Burkholderia graminis</i>	C4D1M ^T	U96939
<i>Burkholderia thailandensis</i>	E264 ^T	U91838
<i>Burkholderia vietnamiensis</i>	TVV75 ^T	U96928
<i>Cardiobacterium hominis</i>	ATCC 16826	M35014
<i>Caulobacter bacteroides</i> †	CB7	M83796
<i>Caulobacter fusiformis</i>	ATCC 15257 ^T	AJ007803
<i>Caulobacter henricii</i>	ATCC 15253 ^T	AJ007805
<i>Caulobacter intermedius</i> †	ATCC 15262 ^T	AJ007802

Table 2 (cont.)

Species	Strain no.	Accession no.
<i>Caulobacter subvibrioides</i> †	ATCC 15264 ^T	X94470
<i>Caulobacter variabilis</i> †	ATCC 15255 ^T	AJ007808
<i>Chromohalobacter marismortui</i>	ATCC 17056 ^T	X87219
<i>Citrobacter freundii</i>	CDC 621-64	AF025365
<i>Colwellia psychroerythrea</i>	ATCC 27364 ^T	AB011364
<i>Cowdria ruminantium</i>	Mara 87/7	AF069758
<i>Craurococcus roseus</i>	NS130 ^T	D85828
<i>Ehrlichia canis</i>	Florida	M73226
<i>Eikenella corrodens</i>	ATCC 23834 ^T	M22512
<i>Enterobacter aerogenes</i>	JCM 1235 ^T	AB004750
<i>Enterobacter amnigenus</i>	JCM 1237 ^T	AB004749
<i>Enterobacter gergoviae</i>	JCM 1234 ^T	AB004748
<i>Erwinia amylovora</i>	ATCC 15580 ^T	U80195
<i>Erwinia carotovora</i>	LMG 2466	Z96091
<i>Erythrobacter longus</i>	OCh 101 ^T	L01786
<i>Erythromicrobium ramosum</i>	DSM 8510 ^T	AB013355
<i>Escherichia coli</i>	ND	V00348
<i>Ferrimonas balearica</i>	PAT ^T	X93021
<i>Francisella tularensis</i>	ATCC 6223 ^T	Z21931
<i>Frateuria aurantia</i>	IFO 3245 ^T	AJ010481
<i>Gallionella ferruginea</i>	ND	L07897
<i>Gluconobacter oxydans</i>	DSM 3503 ^T	X73820
<i>Haemophilus influenzae</i>	ATCC 33391 ^T	M35019
<i>Hafnia alvei</i>	ATCC 13337 ^T	M59155
<i>Halomonas aquamarina</i>	ATCC 14400 ^T	M93352
<i>Halomonas cupida</i>	DSM 4740 ^T	L42615
<i>Halomonas desiderata</i>	FB2 ^T	X92417
<i>Halomonas elongata</i>	ATCC 33173 ^T	X67023
<i>Halomonas eurihalina</i>	ATCC 49336 ^T	X87218
<i>Halomonas halmophila</i>	ATCC 19717 ^T	M59153
<i>Halomonas meridiana</i>	DSM 5425 ^T	M93356
<i>Halomonas pacifica</i>	DSM 4742 ^T	L42616
<i>Halomonas pantelleriensis</i>	AAP ^T	X93493
<i>Halomonas salina</i>	ATCC 49509 ^T	X87217
<i>Halomonas subglaciescola</i>	DSM 4683 ^T	M93358
<i>Herbaspirillum seropedicae</i>	DSM 6445 ^T	Y10146
<i>Hyphomonas jannaschiana</i>	VP-2 ^T	AF082789
<i>Ideonella dechloratans</i>	ND	X72724
<i>Iodobacter fluviatilis</i>	ATCC 33051 ^T	M22511
<i>Janthinobacterium lividum</i>	DSM 1522 ^T	Y08846
<i>Kingella kingae</i>	ATCC 23330 ^T	M22517
<i>Klebsiella ornithinolytica</i>	JCM 7251	AB004756
<i>Klebsiella pneumoniae</i>	JCM 1662 ^T	AB004753
<i>Klebsiella terrigena</i>	ATCC 33257 ^T	Y17658
<i>Leptothrix cholodnii</i>	CCM 1827	X97070
<i>Leptothrix discophora</i>	SS-1 ^T	L33975
<i>Leptothrix mobilis</i>	Feox-1 ^T	X97071
<i>Listonella anguillarum</i>	ATCC 12964 ^T	X16895
<i>Listonella pelagia</i>	ATCC 25916 ^T	X74722
<i>Lonopinella koalarum</i>	ACM 3666 ^T	Y17189
<i>Magnetospirillum gryphiswaldense</i>	DSM 6361 ^T	Y10109
<i>Marinobacter aquaeolei</i>	VT8 ^T	AJ000726
<i>Marinobacter hydrocarbonoclasticus</i>	ATCC 49840 ^T	X67022
<i>Marinobacterium georgiense</i>	KW-40 ^T	U58339

Table 2 (cont.)

Species	Strain no.	Accession no.
<i>Marinomonas vaga</i>	ATCC 27119 ^T	X67025
' <i>Matsuebacter chitosanotabidus</i> '	ND	AB006851
<i>Mesorhizobium loti</i>	ATCC 33669 ^T	D14514
<i>Methylobacter luteus</i>	ACM 3304	X72772
<i>Methylobacterium organophilum</i>	JCM 2833 ^T	D32226
<i>Methylobacterium rhodesianum</i>	JCM 2810 ^T	D32228
<i>Methylobacterium zatmanii</i>	JCM 2819 ^T	D32230
<i>Methylococcus capsulatus</i>	ATCC 19069 ^T	X72770
<i>Methylomicrobium agile</i>	ACM 3308 ^T	X72767
<i>Methylosphaera hansonii</i>	AM6 ^T	U67929
<i>Microbulbifer hydrolyticus</i>	IRE-31 ^T	U58338
<i>Microvirgula aerodenitrificans</i>	Sgly2 ^T	U89333
<i>Moraxella catarrhalis</i>	ATCC 25238 ^T	AF005185
<i>Neisseria gonorrhoeae</i>	2013	M34130
' <i>Nitrobacter hamburgensis</i> '	Nb14	L35502
<i>Nitrobacter winogradskyi</i>	W	L11661
<i>Nitrococcus mobilis</i>	ATCC 25380 ^T	L35510
<i>Nitrospira briensis</i>	C-128	M96396
<i>Nitrospira multififormis</i>	ATCC 25196 ^T	L35509
<i>Oceanospirillum linum</i>	ATCC 11336 ^T	M22365
<i>Octadecabacter arcticus</i>	238 ^T	U73725
<i>Oxalobacter formigenes</i>	OXCR	U49754
<i>Pantoea agglomerans</i>	JCM 1236 ^T	AB004691
<i>Pantoea ananatis</i>	JCM 6986	AB004758
<i>Paracaraurococcus ruber</i>	NS89 ^T	D85827
<i>Paracoccus denitrificans</i>	LMG 4218 ^T	X69159
<i>Pasteurella multocida</i>	NCTC 10322 ^T	M35018
<i>Pectobacterium cacticida</i>	LMG 17936 ^T	AJ223409
<i>Photobacterium angustum</i>	ATCC 25915 ^T	X74685
<i>Photobacterium histaminum</i>	C-8 ^T	D25308
<i>Photobacterium leiognathi</i>	ATCC 25521 ^T	X74686
<i>Photobacterium phosphoreum</i>	ATCC 11040 ^T	X74687
<i>Photobacterium profundum</i>	SS9	AB003191
<i>Photorhabdus luminescens</i>	DSM 3368 ^T	X82248
<i>Plesiomonas shigelloides</i>	ATCC 14029 ^T	X74688
<i>Polaromonas vacuolata</i>	34-P ^T	U14585
<i>Polynucleobacter necessarius</i>	ATCC 30859 ^T	X93019
Proteobacterium*	DSM 11813	AB003623
Proteobacterium*	DSM 11814	AB003625
<i>Proteus vulgaris</i>	ND	J01874
<i>Pseudoalteromonas haloplanktis</i>	ATCC 14393 ^T	X67024
<i>Psychrobacter immobilis</i>	ATCC 43116 ^T	U39399
' <i>Ralstonia metalotorelans</i> '	CT14	D88001
' <i>Ralstonia silverii</i> '	DSM 2839	D87999
' <i>Ralstonia tsushimaensis</i> '	CT12	D88009
<i>Ralstonia eutropha</i>	ATCC 17697 ^T	M32021
<i>Rhabdochromatium marinum</i>	DSM 5261 ^T	X84316
<i>Rhizobium leguminosarum</i>	IAM 12609 ^T	D14513
<i>Rhizobium lupini</i>	DSM 30140 ^T	X87273
<i>Rhodobacter capsulatus</i>	ATCC 11166 ^T	D16428
<i>Rhodobium orientis</i>	MB312 ^T	D30792
<i>Rhodocyclus purpureus</i>	6770	M34132
<i>Rhodoferax fermentans</i>	FR2 ^T	D16211
<i>Rhodopila globiformis</i>	DSM 161 ^T	D86513

Table 2 (cont.)

Species	Strain no.	Accession no.
<i>Rhodoplanes roseus</i>	941 ^T	D25313
<i>Rhodopseudomonas palustris</i>	ATCC 17001 ^T	D25312
<i>Rhodospira trueperi</i>	ND	X99671
<i>Rhodospirillum rubrum</i>	ATCC 11170 ^T	D30778
<i>Rhodovulum sulfidophilum</i>	DSM 1374 ^T	D16423
<i>Rickettsia prowazekii</i>	Brienl	M21789
<i>Roseobacter litoralis</i>	ATCC 49566 ^T	X78312
<i>Roseococcus thiosulfatophilus</i>	RB-3 ^T	X72908
<i>Rubrivivax gelatinosus</i>	ATCC 17011 ^T	D16213
<i>Ruminobacter amylophilus</i>	DSM 1361 ^T	Y15992
<i>Sagittula stellata</i>	ND	U58356
<i>Salinivibrio costicola</i>	NCIMB 701 ^T	X95527
<i>Salmonella typhi</i>	ATCC 19430 ^T	Z47544
<i>Salmonella typhimurium</i>	ATCC 13311 ^T	X80681
<i>Sandaracinobacter sibiricus</i>	RB16-17 ^T	Y10678
<i>Serratia ficaria</i>	JCM 1241 ^T	AB004745
<i>Serratia marcescens</i>	ATCC 13880 ^T	M59160
<i>Serratia rubidaea</i>	JCM 1240 ^T	AB004751
<i>Shewanella putrefaciens</i>	ATCC 8071 ^T	X82133
<i>Shigella dysenteriae</i>	ATCC 13313 ^T	X96966
<i>Sinorhizobium fredii</i>	ATCC 35423 ^T	D14516
<i>Sphingomonas adhaesiva</i>	GIFU 11458 ^T	D16146
<i>Sphingomonas aromaticivorans</i>	SMCC F199 ^T	U20756
<i>Sphingomonas asaccharolytica</i>	IFO 10564 ^T	Y09639
<i>Sphingomonas capsulata</i>	GIFU 11526 ^T	D16147
<i>Sphingomonas chlorophenolica</i>	ATCC 33790 ^T	X87161
<i>Sphingomonas macrogoltabidus</i>	IFO 15033 ^T	D13723
<i>Sphingomonas mali</i>	IFO 10550 ^T	Y09638
<i>Sphingomonas parapaucimobilis</i>	IFO 15100 ^T	D13724
<i>Sphingomonas pruni</i>	IFO 15498 ^T	Y09637
<i>Sphingomonas sanguinis</i>	IFO 13937 ^T	D13726
<i>Sphingomonas subarctica</i>	KF1 ^T	X94102
<i>Sphingomonas suberifaciens</i>	IFO 15211 ^T	D13737
<i>Sphingomonas subterraneae</i>	SMCC B0478 ^T	U20773
<i>Sphingomonas terrae</i>	IFO 15098 ^T	D13727
<i>Sphingomonas trueperi</i>	LMG 2142 ^T	X97776
<i>Sphingomonas ursincola</i>	KR-99 ^T	Y10677
<i>Sphingomonas yanoikuyae</i>	GIFU 9882 ^T	D16145
<i>Spirillum volutans</i>	ATCC 19554 ^T	M34131
<i>Stenotrophomonas africana</i>	MGB ^T	U62646
<i>Sulfitobacter pontiacus</i>	ChLG-10 ^T	Y13155
<i>Suttonella indologenes</i>	ATCC 25869 ^T	M35015
<i>Taylorella equigenitalis</i>	NCTC 11184 ^T	X68645
<i>Thauera aromatica</i>	K 172 ^T	X77118
<i>Thauera linaloolentis</i>	47Lol ^T	AJ005816
<i>Thauera terpenica</i>	58Eu ^T	AJ005817
<i>Thiocystis violacea</i>	DSM 207 ^T	Y11315
<i>Thiolamprovum pedioforme</i>	DSM 3802 ^T	Y12297
<i>Thiomicrospira pelophila</i>	DSM 1534 ^T	L40809
<i>Thiorhodococcus minor</i>	CE2203 ^T	Y11316
<i>Thiorhodovibrio winogradskyi</i>	MBIC2776 ^T	AB016986
<i>Variovorax paradoxus</i>	IAM 12373 ^T	D88006
<i>Vibrio aestuarianus</i>	ATCC 35048 ^T	X74689
<i>Vibrio alginolyticus</i>	ATCC 17749 ^T	X56576

Table 2 (cont.)

Species	Strain no.	Accession no.
<i>Vibrio campbellii</i>	ATCC 25920 ^T	X56575
<i>Vibrio cholerae</i>	ATCC 14035 ^T	X74695
<i>Vibrio diazotrophicus</i>	ATCC 33466 ^T	X56577
<i>Vibrio hollisae</i>	ATCC 33564 ^T	X74707
<i>Vibrio vulnificus</i>	ATCC 27562 ^T	X56582
<i>Xanthobacter autotrophicus</i>	JW33	X94203
<i>Xanthomonas albilineans</i>	LMG 494 ^T	X95918
<i>Xanthomonas arboricola</i>	LMG 747 ^T	Y10757
<i>Xanthomonas axonopodis</i>	LMG 538 ^T	X95919
<i>Xanthomonas bromi</i>	LMG 947 ^T	Y10764
<i>Xanthomonas campestris</i>	LMG 568 ^T	X95917
<i>Xanthomonas cassavae</i>	LMG 673 ^T	Y10762
<i>Xanthomonas codiae</i>	LMG 8678 ^T	Y10765
<i>Xanthomonas cucurbitae</i>	LMG ^T	Y10760
<i>Xanthomonas fragariae</i>	LMG 708 ^T	X95920
<i>Xanthomonas hortorum</i>	LMG 733 ^T	Y10759
<i>Xanthomonas hyacinthi</i>	LMG 739 ^T	Y10754
<i>Xanthomonas melonis</i>	LMG 8670 ^T	Y10756
<i>Xanthomonas oryzae</i>	LMG 5047 ^T	X95921
<i>Xanthomonas pisi</i>	LMG 847 ^T	Y10758
<i>Xanthomonas populi</i>	LMG 5743 ^T	X95922
<i>Xanthomonas sacchari</i>	LMG 471 ^T	Y10766
<i>Xanthomonas theicola</i>	LMG 8684 ^T	Y10763
<i>Xanthomonas translucens</i>	LMG 876 ^T	X99299
<i>Xanthomonas vasicola</i>	LMG 736 ^T	Y10755
<i>Xanthomonas vesicatoria</i>	LMG 911 ^T	Y10761
<i>Xenorhabdus nematophilus</i>	DSM 3370 ^T	X82251
<i>Xylella fastidiosa</i>	ATCC 35880	M26601
<i>Xylophilus ampelinus</i>	ATCC 33914 ^T	AF078758
<i>Yersinia enterocolitica</i>	ATCC 9610 ^T	M59292
<i>Yersinia pestis</i>	ND	L37604
<i>Zoogloea ramigera</i>	TCC 25935	X74914
<i>Zymobacter palmae</i>	T109 ^T	D14555
<i>Zymomonas mobilis</i>	ZM4	U63733

ND, Not described.

* Species names of these strains were not described in the original paper (Suyama *et al.*, 1998).

† Transferred to *Brevundimonas* (Abraham *et al.*, 1999).

GGGTTGCGCTCGTTG-OH-3'; complementary to positions 1115–1100). For DSQ-1000L sequencer, the following 5' FITC-labelled primers were obtained from Amersham Pharmacia Biotech; 360R (5'-CTGCTGCCTCCCGTA-OH-3'; complementary to positions 357–343), 520R (5'-ACCGCGGCTGCTGGC-OH-3'; complementary to positions 531–517), 920F (5'-ACTCAAAGGAATTGACGGG-OH-3'; same as positions 909–928), 920R (5'-CCCCGTCAATTCCTTTGAGT-OH-3'; complementary to positions 928–909) and 1400R (5'-ACGGGCGGTGTGTAC-OH-3'; complementary to positions 1406–1392). The sequencing method used is indicated in Table 1.

Analysis of sequence data. The 16S rRNA sequences of the *Proteobacteria* were obtained from the GenBank/EMBL/DBJ databases for comparison (Table 2). The sequences determined over 1400 bp were adopted in this phylogenetic

analysis except for the sequences of the pseudomonads. Maximum-likelihood analysis (ML) was carried out using the program package MOLPHY (version 2.3b3, Adachi & Hasegawa, 1996). The maximum-likelihood distance matrix was calculated using NucML, and the initial neighbour-joining tree was reconstructed by NJdist in the MOLPHY. The maximum-likelihood tree was finally obtained using NucML with the R (local rearrangement search) option based on the HKY model (Hasegawa *et al.*, 1985). Local bootstrap probabilities (LBPs) were estimated by the resampling of the estimated log-likelihood (RELL) method (Hasegawa & Kishino, 1994; Kishino *et al.*, 1990). Deleted and unknown positions were eliminated for the comparison of sequences. Positions (in the *E. coli* numbering system) 70–100, 181–219, 447–487, 1004–1036, 1133–1141 and 1446–1456 were eliminated from the comparison because the secondary structures

Table 3. Homology and number of nucleotide differences in the 16S rDNA sequences of *Telluria*, *Zoogloea*, *Janthinobacterium*, *Herbaspirillum*, *Oxalobacter*, *Ralstonia* and related *Pseudomonas* species

Values on the lower left are percentage homology, and those on the upper right are the number of nucleotide differences.

Species	1	2	3	4	5	6	7	8	9	10
1. <i>Telluria mixta</i>		100	94	88	121	121	124	144	118	164
2. <i>Zoogloea ramigera</i>	93.1		50	48	77	76	70	118	80	135
3. <i>Pseudomonas mephitica</i>	93.5	96.6		5	55	59	64	121	77	133
4. <i>Janthinobacterium lividum</i>	93.9	96.7	99.7		54	59	61	109	77	140
5. <i>Pseudomonas huttiensis</i>	91.6	94.8	96.3	96.3		19	18	92	67	118
6. <i>Herbaspirillum rubrisubalbicans</i>	91.5	94.8	95.9	95.9	98.7		17	87	69	114
7. <i>Herbaspirillum seropedicae</i>	91.5	95.4	95.6	95.9	98.8	98.8		86	62	111
8. <i>Oxalobacter formigenes</i>	90.1	92.2	91.9	92.6	93.8	94.0	94.3		105	128
9. <i>Pseudomonas lemoignei</i>	91.9	94.7	94.8	94.7	95.5	95.2	95.8	93.0		113
10. <i>Ralstonia solanacearum</i>	88.5	90.6	90.6	90.2	91.8	92.0	92.3	91.1	92.1	

of these regions differed between strains. The level of homology and the number of nucleotide differences were calculated using only unambiguously determined nucleotide positions. The BLAST search (Madden *et al.*, 1996, available on the WorldWide Web at URL <http://www.ncbi.nlm.nih.gov/BLAST/>) was used to find nearly identical sequences for the 16S rRNA sequences determined.

Sequence accession numbers. The 16S rRNA gene sequences which we determined have been deposited in the DDBJ, and these sequences are available from GenBank, EMBL and DDBJ under the accession numbers shown in Table 1.

RESULTS

Almost complete 16S rRNA sequences of 59 species of validly or invalidly described *Pseudomonas* were determined. The 16S rRNA sequences of all *Pseudomonas* species validated in the Approved Lists of Bacterial Names have been deposited in the database without *Pseudomonas gelidicola*. The type strain of *P. gelidicola* was *P. gelidicola* IAM 1127, but we could not obtain this type strain because *P. gelidicola* IAM 1127 was not recorded in the catalogue of IAM culture collections. The 16S rRNA sequences of nine disapproved *Pseudomonas* species, '*Pseudomonas cedrella*', '*Pseudomonas gessardii*', '*Pseudomonas jesenii*', '*Pseudomonas libaniensis*', '*Pseudomonas mandelii*', '*Pseudomonas migulae*', '*Pseudomonas mosselii*', '*Pseudomonas orientalis*' and '*Pseudomonas plecoglossicida*' have been deposited recently in the GenBank/EMBL/DDBJ databases, too, and these sequences were included in this study.

A phylogenetic analysis for all of the *Pseudomonas* species was carried out. A phylogenetic tree constructed by the ML method had four main clusters as the α , β , γ and γ - β subclasses of the *Proteobacteria*, and 11, 41, 69 and seven species were located in the α , β , γ and γ - β clusters, respectively (data not shown). Related subclasses of the *Proteobacteria* were found for each of the determined 16S rRNA sequences by the BLAST

search (data not shown), and phylogenetic analyses were performed for each subclass.

α subclass

A phylogenetic analysis of 90 species of the α *Proteobacteria* was carried out, and the resulting phylogenetic tree is shown in Fig. 1. The total number of nucleotides compared was 1086. Eleven species of *Pseudomonas* described were included in the resulting phylogenetic tree. *P. echinoides* and '*Pseudomonas abikonensis*' were included in the cluster of the genus *Sphingomonas*. The level of homology between *Sphingomonas mali* and *P. echinoides* and between *Sphingomonas pruni* and *P. echinoides* was 96.1 and 96.0%, respectively. The level of homology between *Sphingomonas yanoikuyae* and '*P. abikonensis*' and between *Sphingomonas chlorophenolica* and '*P. abikonensis*' was 96.2 and 96.0%, respectively.

P. carboxydohydrogena was included in the cluster of the *Bradyrhizobium* group, which included the genera *Afipia*, *Blastobacter*, *Bradyrhizobium*, *Nitrobacter* and *Rhodopseudomonas*. The level of homology between *P. carboxydohydrogena* and two species of the genus *Afipia* (*Afipia felis* and *Afipia clevelandensis*) was 99.1 and 98.3%, respectively. The level of homology between *P. carboxydohydrogena* and other species, which constituted the cluster of the *Bradyrhizobium* group, was not as high (95.5–97.7%).

Brevundimonas diminuta and *Brevundimonas vesicularis* constituted a cluster of the *Caulobacter* group with six species of the genus *Caulobacter* and with *Asticcacaulis excentricus*. This cluster had two main clusters excluding *Asticcacaulis excentricus*, although the bootstrap values for the two branches were not high. The first cluster contained two species of the genus *Brevundimonas* and three species of the genus *Caulobacter* (*Caulobacter intermedius*, *Caulobacter subvibrioides* and *Caulobacter variabilis*), and the level

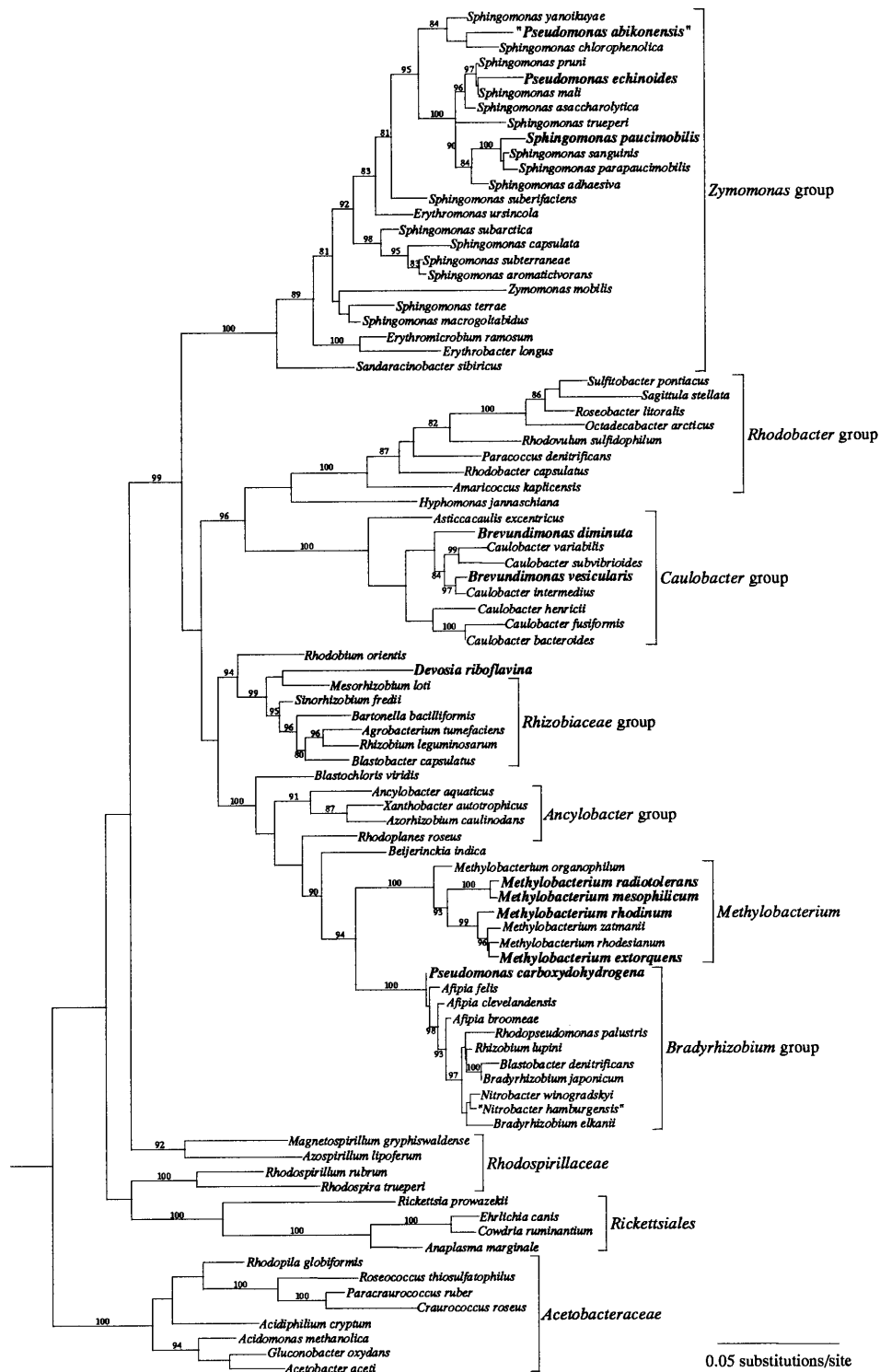


Fig. 1. Phylogenetic tree of the α subclass of the Proteobacteria derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *P. aeruginosa* LMG 1242^T (Z76651) is used as the root organism. Species of the pseudomonads are shown in boldface. Strain and accession numbers are shown in Tables 1 and 2.

of homology among these five species was 94.6–98.9%. The second cluster contained *Caulobacter bacteroides*, *Caulobacter fusiformis* and *Caulobacter henricii*, and

the level of homology between these three species of the genus *Caulobacter* and two species of the genus *Brevundimonas* was 91.9–93.5%.

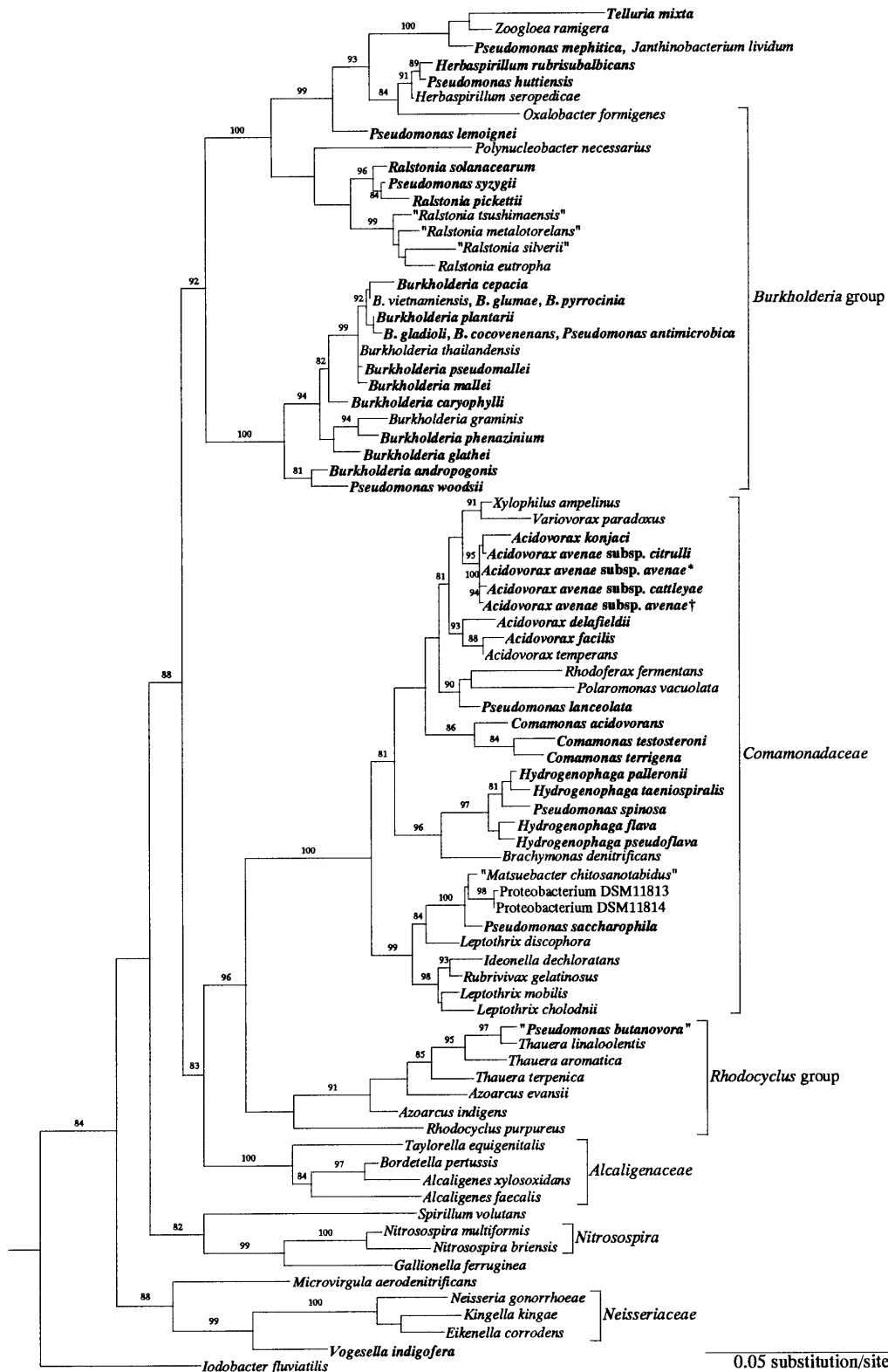


Fig. 2. Phylogenetic tree of the β subclass of the Proteobacteria derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *P. aeruginosa* LMG 1242^T (Z76651) is used as the root organism. Species of the pseudomonads are shown in boldface. Strain and accession numbers are shown in Tables 1 and 2. Symbols: *previously *P. rubrilineans*; †previously *P. avenae* subsp. *avenae*.

β subclass

A phylogenetic analysis of 86 species of the β *Proteobacteria* was carried out, and the resulting phylogenetic tree is shown in Fig. 2. The total number of nucleotides compared was 990. Forty-one species of *Pseudomonas* described were included in the resulting phylogenetic tree. *P. woodsii* and *P. antimicrobica* were included in the cluster of the genus *Burkholderia*. *Burkholderia gladioli*, *Burkholderia cocovenenans* and *P. antimicrobica* had the same sequences in the 990 nucleotides compared. The number of nucleotide differences between *Burkholderia gladioli* and *Burkholderia cocovenenans*, between *Burkholderia cocovenenans* and *P. antimicrobica*, and between *Burkholderia gladioli* and *P. antimicrobica* was four, eight and nine, and the level of homology was 99.7, 99.5 and 99.4%, respectively, when the whole sequences of these three species were compared. *Burkholderia vietnamiensis*, *Burkholderia glumae* and *Burkholderia pyrrocinia* had the same sequences in the 990 nucleotides compared. *P. woodsii* constituted a cluster with *Burkholderia andropogonis*. The bootstrap value for these two species was 81%, and the level of homology was 98.9%.

The cluster of the genus *Ralstonia* had two main clusters, and the bootstrap values for two branches were 96 and 99%. The first cluster contained *Ralstonia pickettii* (the type species of the genus *Ralstonia*), and the second cluster contained *Ralstonia eutrophus*. *P. syzygii* was included in the first cluster of the genus *Ralstonia* with *Ralstonia solanacearum*. The level of homology between *P. syzygii* and *R. pickettii*, and between *P. syzygii* and *R. solanacearum*, was 98.1 and 99.0%, respectively.

P. mephitica, *P. huttiensis* and *P. lemoignei* constituted a cluster with *Telluria mixta*, *Janthinobacterium lividum*, *Herbaspirillum rubrisubalbicans*, *Herbaspirillum seropedicae* and *Oxalobacter formigenes*. The level of homology and number of nucleotide differences among these species are shown in Table 3. *P. mephitica* and *J. lividum* had the same sequences in the 990 nucleotides, which were compared in the phylogenetic analysis. The number of nucleotide differences between *P. mephitica* and *J. lividum* was five, and the level of homology was 99.7%. *P. huttiensis* constituted a cluster with two species of the genus *Herbaspirillum*. The level of homology among these three species was from 98.7 to 98.8%. The level of homology between *P. lemoignei* and other species, which are compared in Table 3, was not so high (91.9–95.2%). On the BLAST search, the highest score was shown between *P. lemoignei* and *H. seropedicae*.

The cluster of the family *Comamonadaceae* included three main clusters. The first cluster contained the genera *Acidovorax* and *Comamonas*, the second cluster contained the genera *Hydrogenophaga* and *Brachymonas*, and the third cluster contained the genera *Ideonella*, *Leptothrix* and *Rubrivivax*. *P. lanceolata* was included in the first cluster and was related to

Rhodiferax fermentans and *Polaromonas vacuolata*, but the level of homology between *P. lanceolata* and *R. fermentans*, and between *P. lanceolata* and *P. vacuolata* was not high (95.6 and 94.1%, respectively). The highest level of homology for *P. lanceolata* was shown with *Xylophilus ampelinus* and *Variovorax paradoxus* (96.1 and 96.2%, respectively). *P. spinosa* was included in the second cluster and was related to four species of the genus *Hydrogenophaga*. The level of homology between *P. spinosa* and four species of the genus *Hydrogenophaga* was 96.6–97.5%. *P. saccharophila* showed the highest scores with the 16S rRNA gene sequences of '*Matsuebacter chitosanotabidus*' (AB006851), *Proteobacterium* DSM 11813 (AB003623), and *Proteobacterium* DSM 11814 (AB003625) by the BLAST search, and *P. saccharophila* constituted a cluster with '*M. chitosanotabidus*', *Proteobacterium* DSM 11813 and *Proteobacterium* DSM 11814 in the third cluster of the family *Comamonadaceae*. The level of homology between *P. saccharophila* and '*M. chitosanotabidus*', between *P. saccharophila* and *Proteobacterium* DSM 11813, and between *P. saccharophila* and *Proteobacterium* DSM 11814 was 97.9, 97.5 and 97.5%, respectively.

'*Pseudomonas butanovora*' was included in the cluster of the *Rhodocyclus* group. The highest level of homology was shown between '*P. butanovora*' and *Thauera linaloolentis*, between '*P. butanovora*' and *Thauera aromatica* and between '*P. butanovora*' and *Thaurea terpenica* (98.7, 97.4 and 96.2%, respectively).

 γ and γ - β subclasses

γ subclass. A phylogenetic analysis of 75 species of the γ and the γ - β *Proteobacteria* was carried out, and the resulting phylogenetic tree is shown in Fig. 3. The total number of nucleotides compared was 864. The representative species of the genus *Pseudomonas* (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas syringae*) and 21 species of *Pseudomonas* were included in the phylogenetic tree. *P. elongata*, *P. halophila* and *Marinobacter hydrocarbonoclasticus* ATCC 27132 (formerly *P. nautica*) constituted a cluster with the genus *Pseudomonas* (*sensu stricto*), two species of the genus *Marinobacter*, *Alcanivorax borkumii* and *Microbulbifer hydrolyticus*. The level of homology and number of nucleotide differences among these species are shown in Table 4. *P. elongata* constituted a cluster with *M. hydrolyticus*, and the level of homology among these species was 97.7%. On the other hand, the level of homology between *P. halophila* and compared related species was relatively low (85.7–90.4%). *Marinobacter hydrocarbonoclasticus* ATCC 27132 and *Marinobacter aquaeolei* had the same sequences in the 864 nucleotides, which were compared in the phylogenetic analysis. The number of nucleotide differences in the whole sequence between *M. hydrocarbonoclasticus* ATCC 27132 and *M. aquaeolei* was two, and the level of homology was 99.9%. On the other hand, the number of differences between *Marinobacter hydrocarbonoclasticus* ATCC

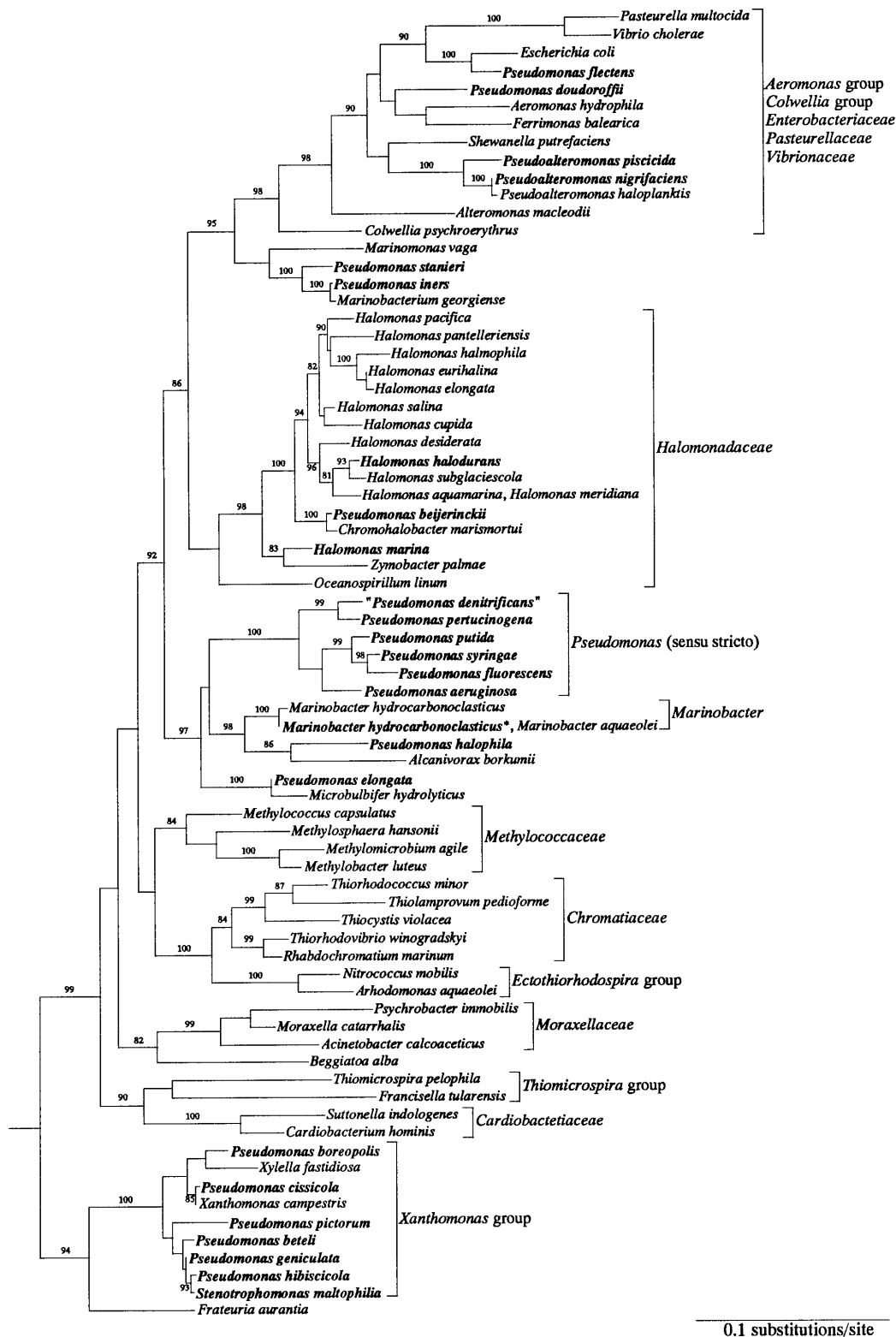


Fig. 3. Phylogenetic tree of the γ and the γ - β subclasses of the *Proteobacteria* derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *Comamonas terrigena* IAM 12052^T (AB021418) is used as the root organism. Species of the pseudomonads are shown in boldface. Strain and accession numbers are shown in Tables 1 and 2. Symbol: *previously *P. nautica*.

Table 4. Homology and number of nucleotide differences in the 16S rDNA sequences of *Marinobacter*, *Alcanivorax*, *Microbulbifer* and related *Pseudomonas* species

Values on the lower left are percentage homology, and those on the upper right are the number of nucleotide differences.

Species	1	2	3	4	5	6	7	8
1. <i>Pseudomonas aeruginosa</i>		157	157	165	213	189	139	126
2. <i>Marinobacter aquaeolei</i>	89.5		2	10	144	147	127	113
3. <i>Marinobacter hydrocarbonoclasticus</i> *	89.5	99.9		10	144	145	125	114
4. <i>Marinobacter hydrocarbonoclasticus</i> †	88.5	99.3	99.3		151	152	134	119
5. <i>Pseudomonas halophila</i>	85.7	90.4	90.3	89.5		159	176	174
6. <i>Alcanivorax borkmensis</i>	87.3	90.1	90.3	89.6	89.3		162	145
7. <i>Pseudomonas elongata</i>	90.6	91.5	91.7	90.7	88.1	89.1		31
8. <i>Microbulbifer hydrolyticus</i>	90.9	91.9	91.8	91.2	87.2	89.6	97.7	

* Previous name is *Pseudomonas nautica*.

† Type strain of *Marinobacter hydrocarbonoclasticus* (ATCC 49840^T).

49840^T and *M. hydrocarbonoclasticus* ATCC 27132 was 10, and the level of homology was 99.3%.

Pseudomonas stanieri and *P. iners* constituted a cluster with *Marinomonas vaga* and *Marinobacterium georgiense*. The level of homology between *P. stanieri* and *P. iners*, between *P. stanieri* and *M. georgiense*, and between *P. iners* and *M. georgiense*, was 96.1, 95.8 and 99.7%, respectively, but the level of homology between these three species and *M. vaga* was 90.2–91.7%.

The cluster of the family *Halomonadaceae* contained the genera *Halomonas*, *Chromohalobacter*, *Zymobacter* and *Oceanospirillum*. *P. beijerinckii* was included in this cluster and constituted a cluster with *Chromohalobacter marismortui*. The level of homology between *P. beijerinckii* and *C. marismortui* was 98.0%. *Halomonas marina* (formerly *Pseudomonas marina*) was not in the cluster of the genus *Halomonas* and constituted a cluster with *Zymobacter palmae*. The level of homology between *H. marina* and *Zymobacter palmae* and between *H. marina* and *Halomonas elongata* (the type species of the genus *Halomonas*) was 92.4 and 92.6%, respectively.

P. flectens and *P. doudoroffii* were placed in the cluster of the *Aeromonas* group, the *Colwellia* group and the families *Enterobacteriaceae*, *Pasteurellaceae* and *Vibrionaceae* (Fig. 3). A further phylogenetic analysis of 67 species, which were related to the *Enterobacteriaceae* cluster, was carried out, and the resulting phylogenetic tree is shown in Fig. 4. The total number of nucleotides compared was 1059. *P. flectens* was included in the family *Enterobacteriaceae*, and the level of homology between *P. flectens* and *Pantoea ananas* was 96.0%. *P. doudoroffii* was separated from the clusters of the *Aeromonas* group, the *Colwellia* group, and the families *Enterobacteriaceae*, *Pasteurellaceae* and *Vibrionaceae*. The level of homology between *P. doudoroffii* and other related species was less than 93.0%.

γ-β subclass. *P. boreopolis*, *P. cissicola*, *P. beteli*, *P. geniculata*, *P. hibiscicola* and *P. pictorum* were included

in the cluster of the *Xanthomonas* group (Fig. 3). A further phylogenetic analysis of 29 species of the *Xanthomonas* group was carried out. The resulting phylogenetic tree is shown in Fig. 5. The total number of nucleotides compared was 1228. The level of homology and numbers of nucleotide differences among the genera *Xylella*, *Xanthomonas*, *Stenotrophomonas* and *Frateruria*, and the related six species of *Pseudomonas* are shown in Table 5.

P. cissicola was included in the cluster of the genus *Xanthomonas*, and *P. cissicola* and *Xanthomonas vasicola* had the same sequences in the 1228 nucleotides, which were compared in the phylogenetic analysis. The number of nucleotide differences between *P. cissicola* and *X. vasicola* was two, and the level of homology was 99.9%. Moreover, the level of homology between *P. cissicola* and *Xanthomonas campestris* (the type species of the genus *Xanthomonas*) was 99.7%. *P. boreopolis* constituted a cluster with *Xylella fastidiosa*. However, the level of homology between *P. boreopolis* and *Xylella fastidiosa* (95.8%) was lower than those between *P. boreopolis* and three species of the genus *Xanthomonas* (97.3–97.6%).

P. beteli, *P. geniculata* and *P. hibiscicola* were included in the cluster of the genus *Stenotrophomonas*. The level of homology among these three species of *Pseudomonas* and *Stenotrophomonas maltophilia* was 99.2–99.5%, and that between these four species and *Stenotrophomonas africana* was 98.3–98.5%. *P. pictorum* constituted a cluster with *S. africana*, *S. maltophilia*, *P. beteli*, *P. geniculata* and *P. hibiscicola*, but the level of homology between *P. pictorum* and five species constituting the cluster of the genus *Stenotrophomonas* was 96.6–96.9%. The level of homology between *P. pictorum* and three species included in the cluster of the genus *Xanthomonas* was 96.3–96.4%, which was the same as that between *P. pictorum* and five species constituting the cluster of the genus *Stenotrophomonas*.

The authentic *Pseudomonas*. *P. aeruginosa* (the type

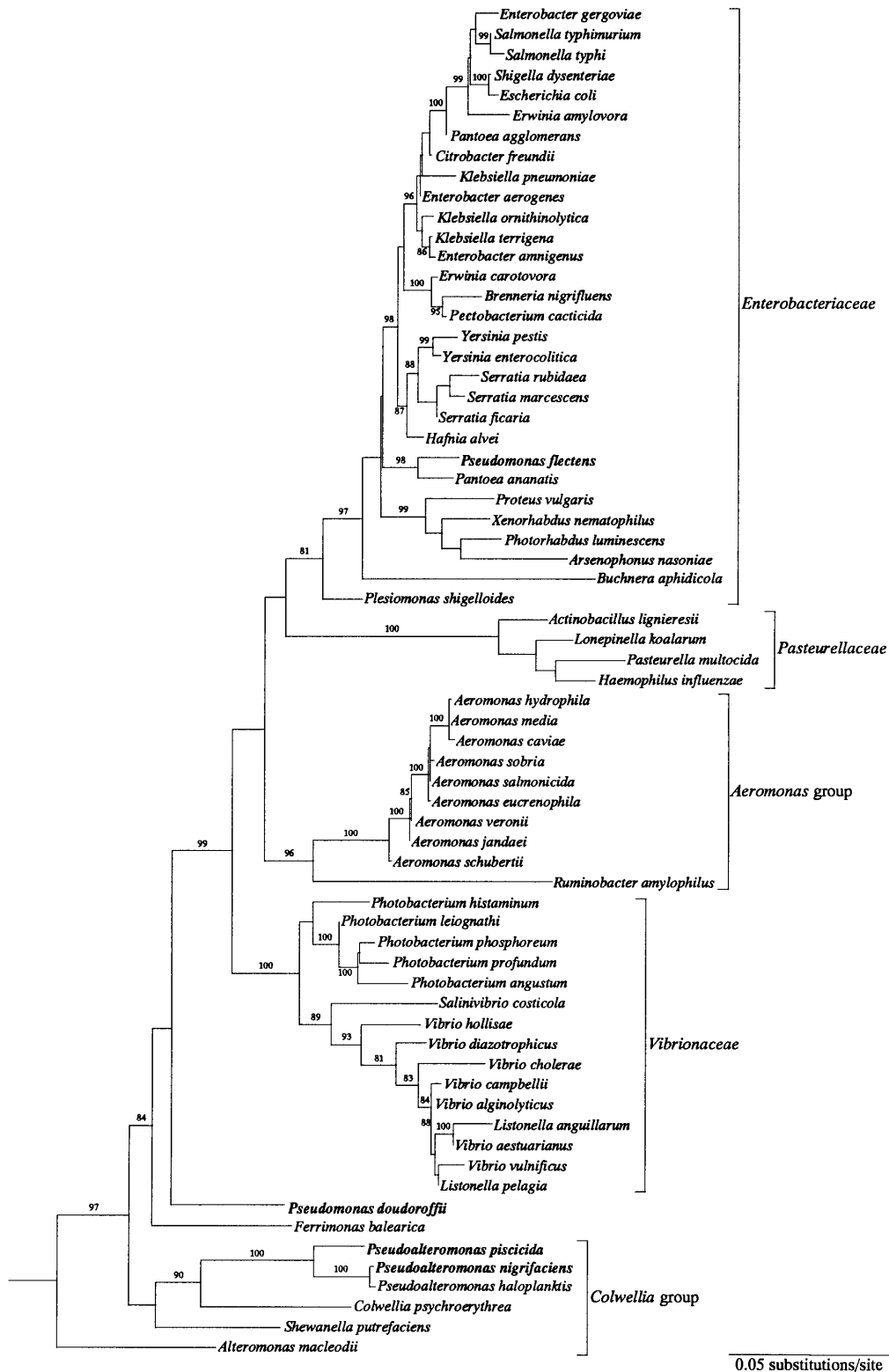


Fig. 4. Phylogenetic tree of the *Aeromonas* and *Colwellia* groups, the families *Enterobacteriaceae*, *Pasteurellaceae* and *Vibrionaceae*, and related species derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *Comamonas terrigena* IAM 12052^T (AB021418) is used as the root organism. Species of the pseudomonads are shown in boldface. Strain and accession numbers are shown in Tables 1 and 2.

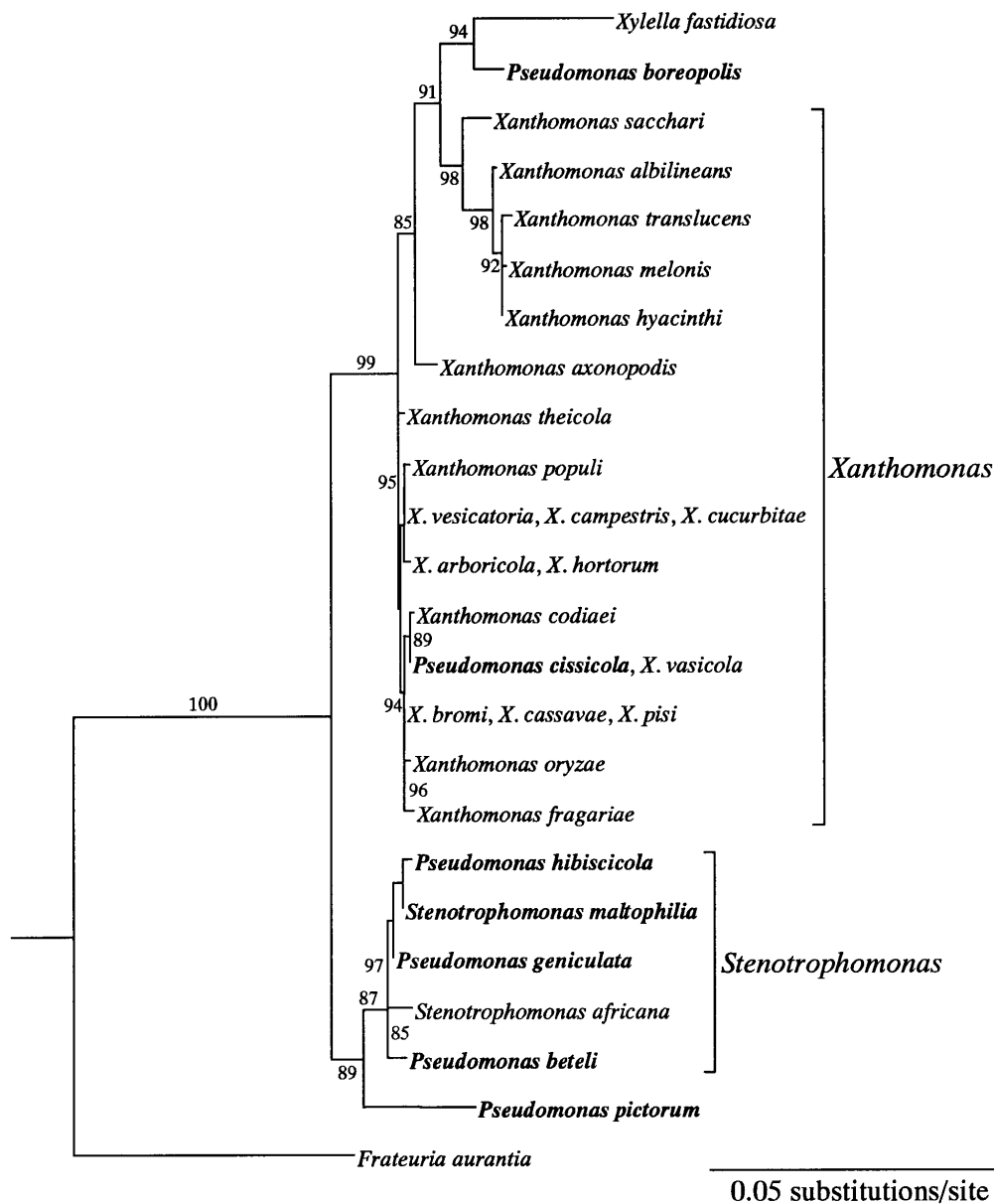


Fig. 5. Phylogenetic tree of the γ - β subclasses of the *Proteobacteria* derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *P. aeruginosa* LMG 1242^T (Z76651) is used as the root organism. Species of the pseudomonads are shown in boldface. Strain and accession numbers are shown in Tables 1 and 2. X. is *Xanthomonas*.

species of the genus *Pseudomonas*) constituted a cluster with 56 other species of *Pseudomonas*, when a phylogenetic analysis for all of the *Pseudomonas* species was carried out (data not shown). The representative four species of the genus *Pseudomonas* constituted a single cluster on the phylogenetic analysis for the γ and the γ - β *Proteobacteria* (Fig. 3). A further phylogenetic analysis of 57 species of *Pseudomonas* was carried out, and the resulting phylogenetic tree is shown in Fig. 6. The total number of nucleotides compared was 1073. Six combinations of species ('*Pseudomonas migulae*' and '*Pseudomonas mandelii*', '*Pseudomonas veronii* and

'*Pseudomonas gessardii*', '*Pseudomonas cedrella*' and '*Pseudomonas orientalis*', '*Pseudomonas marginalis* and '*Pseudomonas libaniensis*', '*Pseudomonas fuscovaginae* and '*Pseudomonas asplenii*', '*Pseudomonas monteillii* and '*Pseudomonas plecoglossicida*') had the same sequences in the 1073 nucleotides, which were compared in the phylogenetic analysis. However, the number of nucleotide differences among these combinations was 21, 18, 14, 18, 1 and 3, respectively. The nucleotide differences in most combinations were in positions 70–100 and 447–487 (in the *E. coli* numbering system), which were eliminated from the comparison.

Table 5. Homology and number of nucleotide differences in the 16S rDNA sequences of *Xylella*, *Xanthomonas*, *Stenotrophomonas*, *Frateuria* and related *Pseudomonas* species

Values on the lower left are percentage homology, and those on the upper right are the number of nucleotide differences.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Xylella fastidiosa</i>		62	74	70	72	96	96	84	97	84	95	159
2. <i>Pseudomonas boreopolis</i>	95.8		40	35	38	67	56	57	52	55	70	144
3. <i>Pseudomonas cissicola</i>	94.9	97.3		4	2	55	52	48	53	50	60	152
4. <i>Xanthomonas campestris</i>	95.2	97.6	99.7		2	53	48	44	49	46	60	157
5. <i>Xanthomonas vasicola</i>	95.0	97.4	99.9	99.9		53	49	45	51	48	62	155
6. <i>Pseudomonas pictorum</i>	93.3	95.5	96.3	96.4	96.4		49	46	51	46	63	158
7. <i>Pseudomonas hibiscicola</i>	94.0	96.2	96.5	96.8	96.7	96.7		7	11	9	23	152
8. <i>Pseudomonas geniculata</i>	94.2	96.1	96.8	97.0	97.0	96.9	99.5		12	10	21	154
9. <i>Pseudomonas beteli</i>	94.1	96.5	96.4	96.7	96.6	96.6	99.3	99.2		8	25	152
10. <i>Stenotrophomonas maltophilia</i>	94.2	96.2	96.9	96.9	96.7	96.8	99.4	99.3	99.5		22	153
11. <i>Stenotrophomonas africana</i>	93.4	95.1	95.9	95.9	95.7	95.6	98.4	98.5	98.3	98.5		161
12. <i>Frateuria aurantia</i>	89.9	90.3	89.8	89.5	89.6	89.4	89.8	89.6	89.8	89.6	88.9	

The phylogenetic tree for the genus *Pseudomonas* (*sensu stricto*) had two main clusters, and the bootstrap values for the two branches were high enough (both 100%). The first cluster was constituted of 56 species of *Pseudomonas* including *P. aeruginosa*. Six clusters were defined within the first cluster and were designated: the '*Pseudomonas syringae* group', the '*Pseudomonas chlororaphis* group', the '*Pseudomonas fluorescens* group', the '*Pseudomonas putida* group', the '*Pseudomonas stutzeri* group', and the '*Pseudomonas aeruginosa* group'. '*Pseudomonas jessenii*', *Pseudomonas agarici*, *Pseudomonas fuscovaginae* and *Pseudomonas asplenii* were not in these six clusters.

The second cluster was constituted of *Pseudomonas pertucinogena* and '*Pseudomonas denitrificans*'. The level of homology between *P. pertucinogena* and *P. aeruginosa* and between '*P. denitrificans*' and *P. aeruginosa* was 94.5 and 94.3%, respectively. The second cluster was designated as the '*Pseudomonas pertucinogena* group'.

DISCUSSION

Taxonomic affiliation of validated *Pseudomonas* species of uncertain nomenclature

The genus *Pseudomonas* was subdivided into five groups based on the rRNA-DNA hybridization studies by Palleroni (1984) (rRNA groups I-V). Later, the five groups were revealed to be related to wide varieties of the *Proteobacteria* (De Vos & De Ley, 1983; De Vos *et al.*, 1985, 1989; De Ley, 1992). In the past two decades, the *Pseudomonas* species belonging to rRNA groups II-V have been transferred to other genera. The species of rRNA group II were transferred to the genera *Burkholderia* or *Ralstonia* (Yabuuchi *et al.*, 1992, 1995), and some species of rRNA group III were transferred to the genera *Acidovorax*, *Coma-*

monas, or *Hydrogenophaga* (Willems *et al.*, 1989, 1990; Tamaoka *et al.*, 1987). These genera belong to the β subclass of the *Proteobacteria*. The species of rRNA group IV were transferred to the genus *Brevundimonas* (Segers *et al.*, 1994), which belong to the α subclass of the *Proteobacteria*. [*Pseudomonas*] *maltophilia*, which was the species of rRNA group V, was combined as the type species of the genus *Stenotrophomonas* (Palleroni & Bradbury, 1993), which belongs to the γ - β subclass of the *Proteobacteria*. At this time, the genus *Pseudomonas* is restricted to rRNA group I, and the genus belongs to the γ subclass of the *Proteobacteria*. In the present study, the phylogenetic analyses based on 16S rRNA sequences were performed for all of *Pseudomonas* species that we could obtain, validated in the Approved Lists of Bacterial Names. The current classification or the phylogenetic affiliation of all examined *Pseudomonas* species are shown in Table 1. We will present the following suggestions concerning the taxonomic treatment of species, for which the current nomenclature was not suitable.

(1) α subclass

Pseudomonas echinoides and '*Pseudomonas abikonensis*'. Kersters *et al.* (1996) described that *P. echinoides* belonged to the *Sphingomonas* rRNA lineage. '*P. abikonensis*' was isolated from soil and is able to metabolize dibenzothiophene to sulfur-containing organic acid compounds (Yamada *et al.*, 1968). *P. echinoides* and '*P. abikonensis*' were included in the *Sphingomonas* cluster (Fig. 1), and the level of homology between these two species and some of the *Sphingomonas* species was more than 96.0%. '*P. abikoensis*' was reported to produce a dull yellow pigment, and this characteristic corresponds with the description of the genus *Sphingomonas*. *P. echinoides* and '*P. abikonensis*' should be transferred to the genus *Sphingomonas* following further taxonomical studies.



Fig. 6. Phylogenetic tree of the authentic *Pseudomonas* derived from the similarities of the 16S rDNA sequence. Bootstrap percentages of 80% or more are indicated at the branch points. *Escherichia coli* (V00348) is used as the root organism. Strain numbers and accession numbers are shown in Table 1. Symbols: *previously *P. coronafaciens*; †previously *P. aureofaciens*.

***Pseudomonas carboxydohydrogena*.** *P. carboxydohydrogena* was described as a species belonging to the *Bradyrhizobium*–*Rhodopseudomonas* rRNA lineage (Kersters *et al.*, 1996). Based on similarity coefficients

(S_{AB} values) obtained from 16S rRNA cataloguing, *P. carboxydohydrogena* constituted a cluster with *Bradyrhizobium lupini* and *Rhodopseudomonas palustris* (Auling *et al.*, 1988). In this study, *P. carboxydo-*

hydrogena was also included in the cluster of the *Bradyrhizobium* group (Fig. 1). The level of homology between *P. carboxydohydrogena* and two species of the genus *Afipia* was higher than 98.0%, and the level of homology between *P. carboxydohydrogena* and the other strains of this cluster was 95.5–97.7%. The genus *Afipia* was established by Brenner *et al.* (1991), with the type species *Afipia felis*, which has been associated with cat-scratch disease. When the 16S rRNA sequences of three species of the genus *Afipia* were determined, phylogenetic analysis was not carried out for related genera (Giladi *et al.*, 1998). *P. carboxydohydrogena* should be transferred to the genus *Afipia* following further taxonomical studies.

(2) β subclass

***Pseudomonas antimicrobica* and *Pseudomonas woodsii*.** *P. antimicrobica*, which was isolated from the mealybug *Planococcoides njalensis* and exhibits antagonism towards a wide range of fungi and bacteria, was proposed as a new species of the genus *Pseudomonas* (Attafuah & Bradbury, 1989). In this proposal, it was described that it was difficult to suggest to which rRNA group of the genus *Pseudomonas* this organism (*P. antimicrobica*) belonged, but it was found to have some affinity for rRNA Group III. Kersters *et al.* (1996) described that the phylogenetic position of *P. antimicrobica* remained to be determined. *P. woodsii* was located in the *R. solanacearum* rRNA branch by rRNA–DNA hybridization (De Vos *et al.*, 1985). After the proposal for *P. woodsii* by Gillis *et al.* (1995), Kersters *et al.* (1996) stated that the names *P. woodsii* and *B. andropogonis* were synonyms. *P. antimicrobica* and *P. woodsii* were included in the cluster of the genus *Burkholderia* (Fig. 2). Therefore, *P. antimicrobica* and *P. woodsii* should be transferred to the genus *Burkholderia* following further taxonomical studies.

***Pseudomonas syzygii*.** DNA hybridization studies showed that *P. syzygii* was closely related to *R. solanacearum* and that this relationship was supported by fatty acid profiles, when the name *P. syzygii* was proposed in 1990 (Roberts *et al.*, 1990). Taghavi *et al.* (1996) showed that *P. syzygii* was closely related to *R. solanacearum* based on 16S rRNA sequence analysis. *P. syzygii* constitutes a cluster with *R. pickettii* and *R. solanacearum* (Fig. 2), and the level of homology among these species is high. Therefore, *P. syzygii* should be transferred to the genus *Ralstonia* following further taxonomical studies.

***Pseudomonas mephitica*.** *P. mephitica* was established by Claydon & Hammer (1939). Kersters *et al.* (1996) stated that the phylogenetic position of *P. mephitica* remained to be determined. The 16S rRNA sequence of *P. mephitica* was very closely related to that of *J. lividum*. *J. lividum* constituted a cluster with *Herbaspirillum rubrisubalbicans* in the dendrogram of the rRNA superfamily III of De Vos and De Ley based on melting temperatures obtained from a rRNA–DNA hybridization study (Willems *et al.*, 1991). The rRNA

superfamily III of De Vos and De Ley corresponded to the β subclass of the *Proteobacteria* (De Ley, 1992). The genus *Janthinobacterium* was established by De Ley *et al.* (1978), and a single species (*J. lividum*) belonged in this genus. Further taxonomical studies should be carried out on the relationship between *P. mephitica* and *J. lividum*.

***Pseudomonas huttiensis*.** *P. huttiensis*, which was isolated from distilled water, was established by Leifson (1962). Kersters *et al.* (1996) described that *P. huttiensis* belonged to the genus *Herbaspirillum* according to unpublished data by M. Gillis. In this study, *P. huttiensis* constituted a cluster with two species of the genus *Herbaspirillum* (Fig. 2), and the level of homology among these three species was very high (98.7–98.8%, Table 3). Therefore, *P. huttiensis* should be transferred to the genus *Herbaspirillum* following further taxonomical studies.

***Pseudomonas lemoignei*.** It was shown by rRNA–DNA hybridization that *P. lemoignei* was phylogenetically related to the rRNA superfamily III of De Vos & De Ley (1983). The 16S rRNA sequences of *P. lemoignei* LGM 2207^T and A62 (accession nos X92554 and X92555, respectively) were determined by Mergaert *et al.*, and *P. lemoignei* LGM 2207^T and A62 exhibited more than 0.91 overall similarity in their 16S rRNA sequences to *Burkholderia solanacearum* and *Alcaligenes eutrophus* and 0.94 overall similarity to *Zoogloea ramigera* ATCC 25935 (Mergaert *et al.*, 1996). Kersters *et al.* (1996) reported that *P. lemoignei* belonged to the *Burkholderia*–*Ralstonia* rRNA sublineages. In this study, *P. lemoignei* was included in a cluster of the genera *Burkholderia* and *Ralstonia*, but this species was not closely related to the two genera (Fig. 2). Any closely related species for *P. lemoignei* could not be found. An extensive study comparing *P. lemoignei* with related genera of the *Burkholderia* group is required for definite taxonomic conclusion.

***Pseudomonas lanceolata*.** *P. lanceolata*, which was isolated from distilled water, was established by Leifson (1962). Kersters *et al.* (1996) stated that the phylogenetic position of *P. lanceolata* remained to be determined. In this study, the strain ATCC 14669 was used, and this strain is the only strain which is maintained at the culture collections. *P. lanceolata* constituted a cluster with *R. fermentans* and *P. vacuolata* in the cluster of the family *Comamonadaceae* (Fig. 2). However, a closely related species for *P. lanceolata* could not be obtained in this study. Further taxonomical studies should be carried out on the relationship between *P. lanceolata* and some genera of the family *Comamonadaceae*, especially the genera *Acidovorax*, *Comamonas*, *Polaromonas*, *Rhodoferax*, *Variovorax* and *Xylophilus*.

***Pseudomonas spinosa*.** *P. spinosa* was established by Leifson (1962). Kersters *et al.* (1996) stated that the phylogenetic position of *P. spinosa* remained to be determined. *P. spinosa* constituted a cluster with four species of the genus *Hydrogenophaga* in the cluster of

the family *Comamonadaceae* (Fig. 2), and the level of homology between *P. spinosa* and four species of the genus *Hydrogenophaga* was high (96.6–97.5%). In this study, the strain ATCC 14606 was used, and this strain is the only strain which is maintained at the culture collections. Therefore, *P. spinosa* should be transferred to the genus *Hydrogenophaga* following further taxonomical studies.

***Pseudomonas saccharophila*.** All species belonging to Palleroni rRNA group III have been transferred to other genera of the β subclass of the *Proteobacteria* except for *P. saccharophila* (Willems *et al.*, 1989, 1992; Tamaoka *et al.*, 1987). *P. saccharophila* was included in the *Leptothrix* and *Rubrivivax* rRNA branch, which was located in the dendrogram of rRNA superfamily III, by rRNA–DNA hybridization, but it was reported that the relationship of *P. saccharophila* to the *Leptothrix* and *Rubrivivax* rRNA branch was ambiguous (Willems *et al.*, 1991). Kersters *et al.* (1996) described that *P. saccharophila* was related to the family *Comamonadaceae*. In this study, *P. saccharophila* constituted a cluster with the genera *Leptothrix* and *Rubrivivax*, which was the third cluster of the family *Comamonadaceae* (Fig. 2). However, the 16S rRNA sequence of *P. saccharophila* was more closely related to that of '*Matsuebacter chitosanotabidus*', Proteobacterium DSM 11813 and Proteobacterium DSM 11814. '*Matsuebacter chitosanotabidus*', which produced chitosanase, was proposed as a new genera and a new species in 1998 (Shimono *et al.*, 1998), but has not been validly published. Proteobacterium DSM 11813 and DSM 11814, which degrade aliphatic polycarbonates, were isolated from river water (Suyama *et al.*, 1998). It was reported that the 16S rRNA sequences of '*Matsuebacter chitosanotabidus*', Proteobacterium DSM 11813 and Proteobacterium DSM 11814 were related to those of the genera *Leptothrix* and *Rubrivivax* (Shimono *et al.*, 1998; Suyama *et al.*, 1998). An extensive study comparing *P. saccharophila* with species, which constitute the third cluster of the family *Comamonadaceae*, especially '*Matsuebacter chitosanotabidus*', Proteobacterium DSM 11813 and Proteobacterium DSM 11814, is required.

'*Pseudomonas butanovora*'. '*P. butanovora*' was isolated from an oil suspension (Takahashi *et al.*, 1980). '*P. butanovora*' was included in the cluster of *Rhodocyclus* group (Fig. 2), and the level of homology between '*P. butanovora*' and three species of the genus *Thauera* was high (96.2–98.7%). Therefore, '*P. butanovora*' should be transferred to the genus *Thauera* following further taxonomical studies.

(3) γ subclass

***Pseudomonas elongata* and *Pseudomonas halophila*.** *P. elongata* was isolated from seawater (Humm, 1946), and *P. halophila*, which predominated among the moderately halophilic population, was isolated from hypersaline surface water of the North Arm of Great Salt Lake, USA (Fendrich, 1988). *P. elongata* was

placed in the *Oceanospirillum* branch in the rRNA–DNA hybridization study (De Vos *et al.*, 1989). However, in this study, this species was not included in the cluster of the family *Halomonadaceae*, which included *Oceanospirillum linum* (the type species of the genus *Oceanospirillum*) (Fig. 3). *P. elongata* constituted a cluster with *M. hydrolyticus* (Fig. 3), and the level of homology between the two species was high (97.7%). Therefore, *P. elongata* should be transferred to the genus *Microbulbifer* following further taxonomical studies. On the other hand, there were no closely related species for *P. halophila* (Fig. 3). Kersters *et al.* (1996) stated that the phylogenetic position of *P. halophila* remained to be determined. In this study, *P. halophila* DSM 3050 was used, and this strain is the only strain which is maintained at the culture collections. An extensive study comparing *P. halophila* with the genera *Alcanivorax*, *Marinobacter* and *Microbulbifer* should be required for definite taxonomic conclusion.

***Pseudomonas iners* and *Pseudomonas stanieri*.** *P. stanieri*, which was isolated from seawater, was proposed in 1983 (Baumann *et al.*, 1983b), and according to this proposal Kersters *et al.* (1996) stated that *P. stanieri* was an authentic *Pseudomonas* species. *P. iners* was isolated from oil brines in Japan (Iizuka & Komagata, 1964). Kersters *et al.* (1996) stated that the phylogenetic position of *P. iners* remained to be determined. In this study, *P. stanieri* and *P. iners* constituted a cluster with *Marinobacterium georgiense* (Fig. 3), and the level of homology between *P. iners* and *Marinobacterium georgiense* was very high (99.7%). The profiles of fatty acid and quinone of *P. iners* were different from those of the authentic *Pseudomonas* (Oyaizu & Komagata, 1983). Further taxonomical studies among these three species (*P. stanieri*, *P. iners* and *Marinobacterium georgiense*) should be done. In this study, *P. iners* IAM 1419^T was used, and this strain is the only strain which is maintained at the culture collections. It is suggested that *P. iners* should be transferred to the genus *Marinobacterium*.

***Pseudomonas beijerinckii*.** *P. beijerinckii* was isolated from salted beans (Hof, 1935). Kersters *et al.* (1996) reported that *P. beijerinckii* belonged to the *Deleya*–*Halomonas* rRNA lineage. *P. beijerinckii* was included in the cluster of the family *Halomonadaceae* (Fig. 3), and *P. beijerinckii* was more closely related to *C. marismortui* than any species of the genus *Halomonas*. *C. marismortui* was established by Ventosa *et al.* (1989) as a moderately halophilic Gram-negative bacteria. Based on a 16S rRNA sequence study, Mellado *et al.* (1995) stated that *C. marismortui* belonged to the family *Halomonadaceae* and had the characteristic 16S rRNA signatures defined for this family, including the distinctive cytosine residue at position 486 (*E. coli* numbering system) found in all members of this family. The cytosine residue was also detected at position 486 (*E. coli* numbering system) of the 16S rRNA sequence of *P. beijerinckii*. *P. beijerinckii* should be transferred

to the genus *Chromohalobacter* following further taxonomical studies.

***Pseudomonas flectens*.** *P. flectens* was reported as a pathogenic bacterium on the pods of the French bean by Johnson (1956). Kersters *et al.* (1996) stated that the phylogenetic position of *P. flectens* remained to be determined. In this study, *P. flectens* ATCC 12775 was used, and this strain is the only strain which is maintained at the culture collections. Because *P. flectens* was included in the cluster of the family *Enterobacteriaceae* (Fig. 4), an extensive study comparing *P. flectens* with the species of the family *Enterobacteriaceae* is required for definite taxonomic conclusion.

***Pseudomonas doudoroffii*.** *P. doudoroffii*, which was isolated from seawater, was proposed as a new species of the genus *Pseudomonas* in 1972 (Baumann *et al.*, 1972). *P. doudoroffii* was located at the lower border of the rRNA branch of the family *Aeromonadaceae* (De Vos *et al.*, 1989), and Kersters *et al.* (1996) described that *P. doudoroffii* was related to the family *Aeromonadaceae*. The 16S rRNA gene sequences of some strains of the family *Aeromonadaceae* and the family *Vibrionaceae* were found to have sequences nearly identical to that of *P. doudoroffii* by the BLAST search. However, there were no species closely related to *P. doudoroffii* in this study. An extensive study comparing *P. doudoroffii* with *F. balearica* and species of the family *Aeromonadaceae*, the family *Vibrionaceae* and the *Colwellia* group is required for definite taxonomic conclusion.

(4) γ - β subclass

***Pseudomonas cissicola*.** *P. cissicola* (formerly *Aplanobacter cissicola*) was reported as a pathogenic bacteria for *Cissus japonica* (family Vitaceae) in 1939 (Taki-moto, 1939). Kersters *et al.* (1996) stated that the phylogenetic position of *P. cissicola* remained to be determined, but at the same time, Hu *et al.* (1997) showed that *P. cissicola* was a previously unreported *Xanthomonas* species based on the fatty acid profile and biochemical test data. *P. cissicola* was included in the cluster of the genus *Xanthomonas* (Fig. 5), and *P. cissicola* was more closely related to *X. vasicola*. Therefore, *P. cissicola* should be transferred to the genus *Xanthomonas* following further taxonomical studies.

***Pseudomonas boreopolis*.** Kersters *et al.* (1996) reported that *P. boreopolis* belonged to the *Xanthomonas* rRNA lineage based on an rRNA–DNA hybridization study by De Vos *et al.* (1989). *P. boreopolis* constituted a cluster with *X. fastidiosa* (Fig. 5). An extensive study comparing *P. boreopolis* with species of the genera *Xanthomonas* and *Xylella* is required for definite taxonomical conclusion.

***Pseudomonas beteli*, *Pseudomonas geniculata* and *Pseudomonas hibiscicola*.** *P. beteli*, *P. geniculata* and *P. hibiscicola* were included in the *Xanthomonas* rRNA branch by rRNA–DNA hybridization (De Vos & De

Ley, 1983; Byng *et al.*, 1983), and Kersters *et al.* (1996) reported that these three species belonged to the *Xanthomonas* rRNA lineage. However, these three species were more closely related to the genus *Stenotrophomonas* than the genus *Xanthomonas* in this study. *P. beteli*, *P. geniculata* and *P. hibiscicola* constituted a cluster of the genus *Stenotrophomonas* (Fig. 5), and the level of homology among these three species and *S. maltophilia* was very high (99.2–99.5%). In consideration of the criticisms of the transfer of *Pseudomonas maltophilia* to the genus plant pathogenic *Xanthomonas* (Swings *et al.*, 1983), the name *Stenotrophomonas* was proposed, which included a single species, *S. maltophilia* (Palleroni & Bradbury, 1993). [*P. maltophilia* belonged to the Palleroni rRNA group V (Palleroni, 1984). Presently, two species were included in the genus *Stenotrophomonas* (*S. maltophilia* and *S. africana*) (Palleroni & Bradbury, 1993; Drancourt *et al.*, 1997). *S. maltophilia* was isolated from several kinds of specimens or environments, e.g. water, milk, frozen food, and the rhizosphere of some cultivated plants (Palleroni, 1984). *P. beteli* and *P. hibiscicola* had plant pathogenicity (Van den Mooter & Swings, 1990), and *P. geniculata* was isolated from water from a river (Haynes & Burkholder, 1957). A close relationship between *S. maltophilia* and *P. hibiscicola* was confirmed by numerical taxonomy and protein gel electrophoregrams (Van Zyl & Steyn, 1990). Because epidemiological differences were found in the species constituting the cluster of the genus *Stenotrophomonas*, extensive studies comparing *P. beteli*, *P. geniculata* and *P. hibiscicola* with species of the genera *Stenotrophomonas* and *Xanthomonas* is required for definite taxonomic conclusion.

***Pseudomonas pictorum*.** *P. pictorum* was included in the *Xanthomonas* rRNA branch by rRNA–DNA hybridization (De Vos & De Ley, 1983), and Kersters *et al.* (1996) reported that *P. pictorum* belonged to the *Xanthomonas* rRNA lineage. In this study, *P. pictorum* showed a moderate relationship between the genera *Stenotrophomonas* and *Xanthomonas*. An extensive study comparing *P. pictorum* with species of the genera *Stenotrophomonas* and *Xanthomonas* is required for definite taxonomic conclusion.

(5) Further comments on other species of the pseudomonads

***Brevundimonas diminuta* and *Brevundimonas vesicularis*.** *B. diminuta* and *B. vesicularis* constituted a cluster with six species of the genus *Caulobacter* (Fig. 1). Previously, it was revealed that some species of the genus *Caulobacter* were closely related to the two species of the genus *Brevundimonas* based on the 16S rRNA sequence and compositions of phospho- and sulfolipids (Stahl *et al.*, 1992; Abraham *et al.*, 1997; Sly *et al.*, 1997). However, two species of the genus *Brevundimonas* were not compared with the genus *Caulobacter* when the name *Brevundimonas* was proposed as a new genus (Segers *et al.*, 1994). The cluster of the genera *Brevundimonas* and *Caulobacter* had two

clusters; the first cluster was constituted of *B. diminuta*, *B. vesicularis*, *C. intermedius*, *C. subvibrioides* and *C. variabilis*, and the second cluster consisted of *C. bacteroides*, *C. fusiformis* and *C. henrici*. Interestingly, the sequences of positions 1258–1278 (in the *E. coli* numbering system) were deleted in the 16S rRNA sequence of all members of *Brevundimonas* cluster. The deletion was found in the sequences of all members of the *Rhodobacter* group cluster, but not in the sequences of *C. bacteroides*, *C. fusiformis* and *C. henrici*. Further taxonomical studies should be carried out on the relationship among species of the genera *Brevundimonas* and *Caulobacter*.

***Halomonas marina*.** *H. marina* was not in the cluster of the genus *Halomonas* (Fig. 3), and the level of homology between *H. marina* and *H. elongata* (the type species of the genus *Halomonas*) was not high (92.6%). It was reported that, using several programs for phylogenetic analysis, *H. marina* was not included in the cluster of the *Halomonas* (Dobson & Franzmann, 1996). The level of homology of *H. marina* and *Zymobacter palmae* was the same as that between *H. marina* and *H. elongata*, although *H. marina* constituted a cluster with *Zymobacter palmae* (Fig. 3). *H. marina* was not distinguished from some of the other *Halomonas* species by the cellular polyamine profile, but the profile of *Zymobacter palmae* was different from those of the genus *Halomonas*: agmatine was additionally detected in *Zymobacter palmae* (Hamana, 1997). An extensive study comparing *H. marina* with species of the family *Holomonadaceae* is required for definite taxonomic conclusion.

***Marinobacter hydrocarbonoclasticus* ATCC 27132.** [*P. nautica*] ATCC 27132, which was isolated from seawater (Baumann *et al.*, 1972), was transferred to *Marinobacter hydrocarbonoclasticus* by Spröer *et al.* (1998). High level DNA–DNA hybridization between the type strains of the two species was shown (Spröer *et al.*, 1998). In this study, *Marinobacter hydrocarbonoclasticus* ATCC 27132 (the previous type strain of *P. nautica*) constituted a cluster with two species of the genus *Marinobacter* (Fig. 3), but 16S rRNA sequence of *Marinobacter hydrocarbonoclasticus* ATCC 27132 was more similar to that of *Marinobacter aquaeolei* than that of *Marinobacter hydrocarbonoclasticus* ATCC 49840^T (Table 4). [*Pseudomonas nautica*] DSM 50418^T and DSM 6419 were compared with only the type strain of *Marinobacter hydrocarbonoclasticus* (DSM 8798^T), but *Marinobacter aquaeolei* was not used for the reference species, when *P. nautica* was transferred to *Marinobacter hydrocarbonoclasticus* (Spröer *et al.*, 1998). Therefore, further taxonomical studies should be carried out on the relationship among these species of the genus *Marinobacter*.

Valid *sensu stricto* species of the genus *Pseudomonas*

The authentic *Pseudomonas* species in Palleroni rRNA group I were classified in three subgroups, including

the *P. aeruginosa* subgroup, the *P. fluorescens* subgroup, and the *P. syringae* subgroup (Palleroni, 1984). We described that the resulting phylogenetic tree by the neighbour-joining method had two main clusters using the 16S rRNA sequences of 29 species of the genus *Pseudomonas (sensu stricto)* (Anzai *et al.*, 1997). Some species of the *P. aeruginosa* subgroup and some of the *P. fluorescens* and the *P. syringae* subgroups were clearly distinguished between these two clusters except for *P. putida*. At the same time, the phylogenetic tree for 24 species of the genus *Pseudomonas (sensu stricto)* was shown by Moore *et al.* (1996) based on the 16S rRNA sequences of these 24 species. The phylogenetic tree gave almost the same result as Palleroni's subgrouping for the 24 studied species of the genus *Pseudomonas (sensu stricto)*. Yamamoto & Harayama (1997) performed phylogenetic analysis of the genus *Pseudomonas (sensu stricto)* using the sequences of the *gyrG* gene that encodes the subunit B protein of DNA gyrase (topoisomerase type II). The phylogenetic tree obtained from sequences of the *gyrG* gene had four main clusters, and their clustering corresponded to the subgrouping of the Palleroni rRNA group I. In this study, the phylogenetic tree for 57 species of the genus *Pseudomonas (sensu stricto)* was generated using the ML method, and this phylogenetic tree had two main clusters (Fig. 6). *P. aurantiaca*, whose phylogenetic position remained to be determined (Kersters *et al.*, 1996), and nine unproven *Pseudomonas* species, whose 16S rRNA sequences have been deposited recently in the GenBank/EMBL/DBJ databases, were included in the first cluster. Six clusters were defined within the first cluster of this phylogenetic tree (Fig. 6). Our results from phylogenetic analysis conformed well to the results of the rRNA–DNA hybridization studies by Palleroni (1984). The 'Pseudomonas syringae group' included *Pseudomonas cichorii*, *P. syringae* and *Pseudomonas viridiflava*, which belonged to the *P. syringae* subgroup of Palleroni rRNA group I. The 'Pseudomonas chlororaphis group' and the 'Pseudomonas fluorescens group' included *Pseudomonas aureofaciens*, *P. chlororaphis* and *P. fluorescens*, which belonged to the *P. fluorescens* subgroup of Palleroni rRNA group I. The 'Pseudomonas stutzeri group' and the 'Pseudomonas aeruginosa group' included *P. aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas mendocina*, *Pseudomonas pseudoalcaligenes* and *P. stutzeri*, which belonged to the *P. aeruginosa* subgroup of Palleroni rRNA group I. The 'Pseudomonas putida group' was located at a moderate position of the first cluster, and this group included *P. putida*, which belonged to the *P. fluorescens* subgroup of Palleroni rRNA group I. *P. agarici* and *P. asplenii* were not in these six groups of the first cluster (Fig. 6). It was only tentative that *P. agarici* and *P. asplenii* belonged to the *P. syringae* subgroup of Palleroni rRNA group I.

The second cluster comprised *P. pertucinogena* and 'P. denitrificans', and this was designated the 'Pseudomonas pertucinogena group' (Fig. 6). *P. pertucinogena* was described as a species belonging to the γ subclass

of the *Proteobacteria* based on unpublished data (Kersters *et al.*, 1996). '*P. denitrificans*' is not an approved *Pseudomonas* species. *Pseudomonas denitrificans* was named by Bergey *et al.* (1923), who attributed the specific epithet to the description by Christensen (1903). Doudoroff *et al.* (1974) described that 18 strains, which were either classified as *P. denitrificans* or might conform to the species description, appeared to belong to several species and to at least two genera based on their taxonomical characteristics, and they recommended that the name *Pseudomonas denitrificans* be abandoned as a *nomen ambiguum*. The 16S rRNA sequence of '*P. denitrificans*' IAM 12023, the neotype of this species, was determined in this study. *P. pertucinogena* and '*P. denitrificans*' constituted an independent cluster from the other authentic *sensu stricto Pseudomonas* species on the phylogenetic tree shown in Fig. 6. However, we concluded that these two species belong to the genus *Pseudomonas (sensu stricto)* according to the level of homology for *P. aeruginosa* (type species of the genus *Pseudomonas*).

The genera *Azotobacter* and *Azomonas* were more closely related to the genus *Pseudomonas (sensu stricto)* based on an rRNA–DNA hybridization study (De Vos *et al.*, 1985). The 16S rRNA sequences of five species of the genera *Azotobacter* and *Azomonas* are available from the GenBank/EMBL/DDBJ databases; *Azomonas macrocytogenes* (AF035212), *Azotobacter chroococcum* (AF035211), *Azotobacter paspali* (AF035210), *Azotobacter salinestrus* (AF035213) and *Azotobacter vinelandii* (L40329). However, these sequences were not adopted in this study, because only 959–999 nucleotides of their rRNA sequences have been sequenced. The level of homology between *P. aeruginosa* and five species of the genera *Azotobacter* and *Azomonas* (95.7–96.8%) were higher than those between *P. pertucinogena* and five species of the genera *Azotobacter* and *Azomonas*, and '*P. denitrificans*' and five species of the genera *Azotobacter* and *Azomonas* (92.8–94.0 and 92.6–93.8%, respectively). Therefore, an extensive study comparing the genus *Pseudomonas (sensu stricto)* with the genera *Azotobacter* and *Azomonas* is required for definite taxonomic conclusion.

In this study, the phylogenetic affiliations of 128 valid and invalid species of the genus *Pseudomonas* were shown by the phylogenetic analyses using the 16S rRNA sequences of these species, and it was recognized that 57 valid or invalid species including *P. aeruginosa* (the type species of the genus *Pseudomonas*) belonged to the genus *Pseudomonas (sensu stricto)*. The other species did not belong to the genus *Pseudomonas (sensu stricto)* and were related to other genera, which were placed in four subclasses of the *Proteobacteria* (α , β , γ and γ - β subclasses). Chemotaxonomical studies of the pseudomonads have been performed by several scientists (Moss *et al.*, 1972; Ikemoto *et al.*, 1978; Oyaizu & Komagata, 1983; Stead, 1992; Vancanneyt *et al.*, 1996), and these results had been incorporated into recent reclassifications of the pseudomonads. The

trend of reclassification is to restrict the genus to *sensu stricto* species. In order to avoid further taxonomic confusion, it is reasonable to change the generic description to restrict the *sensu stricto* species. In the near future, a revised description of the genus *Pseudomonas* would be proposed according to these phylogenetic studies based on 16S rRNA sequences, chemotaxonomical studies and further taxonomical studies.

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