Phylogenetic diversity, polyamine pattern and DNA base composition of members of the order *Planctomycetales*

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The 16S rDNA sequences of 20 novel isolates of members of the order *Planctomycetales* were compared to those of the type strains of described planctomycete species and 22 planctomycete isolates for which the 16S rDNA sequences had been previously determined. The novel isolates could be assigned to several phylogenetically broad groups, four of which are defined by the genera *Gemmata*, *Isosphaera*, *Planctomyces* and *Pirellula*. To evaluate polyamines as a chemotaxonomic marker within this order, the polyamine pool was determined for six planctomycete reference species and for 20 planctomycete isolates. All analysed members of the order *Planctomycetales* contained significant amounts of polyamines. *sym*-Homospermidine (HSPD) is present in all strains except *Planctomyces limnophilus* and related strains, which had high amounts of putrescine (PUT) as the dominant polyamine component. The distribution of PUT, HSPD and spermidine reflects the phylogenetic diversity within the *Planctomycetales* as closely related representatives of the phylogenetic groups defined by described species and novel isolates exhibit similar polyamine patterns. Determination of the DNA base composition revealed G+C contents of >60 mol% for members of *Gemmata* and *Isosphaera* whereas, except for two isolates, strains which are phylogenetically associated with *Planctomyces* and *Pirellula* had G+C contents of 51–57 mol%.

Keywords: *Planctomycetales*, 16S rDNA sequence analyses, chemotaxonomy, polyamines, DNA base composition

INTRODUCTION

The order *Planctomycetales* represents one of the major lines of descent of the domain *Bacteria* as defined on the basis of 16S rRNA sequence analysis (Woese, 1987; Ward *et al.*, 1995). Members of this order differ in several characteristics from other bacterial taxa: (i) they multiply by forming buds directly from the spherical, ovoid or pear-shaped

mother cell; (ii) the cell wall is stabilized by a proteinaceous layer rather than a peptidoglycan layer (König *et al.*, 1984; Liesack *et al.*, 1986); (iii) the cell surface is scattered with 'crateriform structures' (Schlesner & Stackebrandt, 1986; Staley *et al.*, 1992) which, outside the planctomycetes proper, has only been reported for *Porphyrobacter neustonensis* (Fuerst *et al.*, 1993); (iv) the 5S rRNA contains only 109–111 bases (Bomar *et al.*, 1988); and (v) certain idiosyncrasies have been detected at the genome level (Liesack & Stackebrandt, 1989; Ward-Rainey *et al.*, 1996).

The order *Planctomycetales* contains the family *Planctomycetaceae* (Schlesner & Stackebrandt, 1986) in which four genera have been described mainly on a morphological basis. Members of *Planctomyces* are

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Abbreviations: ASW, artificial seawater; CAD, cadaverine; DAP, diamino-propane; HSPD, *sym*-homospermidine; PUT, putrescine; SPD, spermidine; SPM, spermine.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are AJ231168–AJ231195.

Table 1. Strains investigated in this study, their culture conditions and G + C content (mol %) of their rRNA genes

Strains are arranged according to their phylogenetic position. Unless otherwise indicated, all strains were isolated in northern Germany. See Methods for growth media references.

Strain	Source	Growth medium	Culture temp. (°C)	G + C content (mol%)
<i>Gemmata obscuriglobus</i> DSM 5831 ^T		31PY	30	56.9
633† (IFAM 3609)	Leakage water from compost heap	1aPY	20	57.6
<i>Isoosphaera pallida</i> DSM 9630 ^T		DSM 765*		62.0
394 (IFAM 3265)	Pitcher of <i>Nepenthes</i> sp.	31PY	30	60.6
640 (IFAM 3595)†	Leakage water from compost heap	31PY	30	61.5
666 (IFAM 3634)†	Leakage water from compost heap	1aPY	30	61.6
532 (IFAM 3443)	'Cattle manure'‡	1aPY	30	61.9
563 (IFAM 3384)	Sewage treatment sludge	31PY	30	62.3
657 (IFAM 3637)†	Leakage from garbage pile	31PY	30	60.1
<i>Planctomyces maris</i> DSM 8797 ^T		13a	30	54.2
130 (IFAM 1317)†	Kiel Fjord	13a	30	54.6
<i>Planctomyces brasiliensis</i> DSM 5305 ^T		13a	30	55.7
269 (IFAM 3050)†	Schrevenpark Pond, Kiel	31PY	20	56.7
423 (IFAM 3293)	Waste water pond of sugar processing plant	31	20	56.7
658 (IFAM 3678)†	Leakage water from industrial compost heap	1aPY	30	57.3
664 (IFAM 3625)†	Leakage water from industrial compost heap	1aPY	30	57.8
668 (IFAM 3626)†	Leakage water from industrial compost heap	1aPY	30	56.5
248 (IFAM 2297)	Campus Pond, University Kiel	22	30	54.2
<i>Planctomyces limnophilus</i> DSM 3776 ^T ‡		31PY	20	56.2
282 (IFAM 3016)	Schrevenpark, Kiel	13(3x)	20	56.0
287 (IFAM 3011)	Lake Mondsee, Kiel	31PY	20	56.0
599 (IFAM 3521)	'Cattle manure'‡	30	30	57.2
639 (IFAM 3594)	Leakage water from industrial compost heap	1	30	56.1
642 (IFAM 3596)†	Leakage water from compost heap	1aPY	30	55.9
638 (IFAM 3610)†	Leakage water from industrial compost heap	1	30	55.9
<i>Pirellula staleyii</i> DSM 6068 ^T		31PY	20	54.5
670 (IFAM 3638)†	'Cattle manure'‡	31PY	20	55.1
678 (IFAM 3665)†	Lake Fuhlensee, near Kiel	1aPY	20	54.8
292 (IFAM 3017)	Lake Fuhlensee, near Kiel	13a	20	54.7
<i>Pirellula marina</i> DSM 3645 ^T §		13a	30	55.3
611 (IFAM 3511)	Keauhou Beach, Kona, Hawaii	13a	30	55.0
391 (IFAM 3241)	Aquarium	13a	20	54.7
116 (IFAM 1432)	Kiel Fjord	13a	30	54.7
302 (IFAM 3198)†	Chalk mine	13(3x)	30	55.4
467 (IFAM 3480)	Waste water pond of sugar processing plant	30	20	54.6
555 (IFAM 3335)	'Sewage sludge'‡	1a	20	54.7
683	Surface water of garbage dump	1aPY	30	55.0
535 (IFAM 3385)	'Sewage sludge'‡	1a	20	54.9
449 (IFAM 3304)	Chalk mine	1	30	54.6
382 (IFAM 3183)†	Aquarium	30aPY	20	55.5
1 (IFAM 1310)†	Kiel Fjord	13a	30	54.5
608 (IFAM 3508)	Beach near Kailua Kona, Hawaii	13a	30	54.9
140 (IFAM 1319)†	Kiel Fjord	13a	30	54.4
384 (IFAM 3190)†	Aquarium	13a	20	54.0
529 (IFAM 3414)	Methane seeps, Kattegatt	30PY	20	54.4
610 (IFAM 3510)	Keauhou Beach, Kona, Hawaii	30aPY	30	55.2
158 (IFAM 1452)†	Kiel Fjord	13a	20	55.5
139 (IFAM 1318)†	Kiel Fjord	33a	20	56.8
337 (IFAM 3363)	Chalk mine	30PY	20	55.6
567 (IFAM 3405)	Lake Heidensee, near Plön	1a	20	56.0
516 (IFAM 3412)†	'Sewage sludge'‡	1a	20	55.9
543 (IFAM 3592)	'Sewage sludge'‡	1	20	56.0

* See DSMZ Catalogue of Strains, 6th edn, 1998.

† 16S rDNA sequences have been published by Ward *et al.* (1995).

‡ 'Cattle manure' and 'sewage sludge' are pilot projects for converting manure and sludge to fertilizer.

§ 16S rDNA sequences have been published by Liesack *et al.* (1992).

|| Isolated and kindly provided by J. T. Staley, Seattle, WA, USA.

characterized by a non-cellular stalk terminating in a holdfast opposite the reproductive cell pole. Cells of members of *Pirellula* excrete holdfast substance directly from the cell pole opposite the reproductive pole. In both genera, rosette formation is common. The daughter cells are non-motile or possess a single flagellum (Schlesner & Hirsch, 1984; Staley *et al.*, 1992) whereas the swarmer cells of *Gemmata obscuriglobus* (the only described species of the genus) have bundles of flagella (Franzmann & Skerman, 1984). *Isosphaera pallida* (the only described species of the genus) has spherical cells which form gliding filaments in which daughter cells are formed within the chain of cells (Giovannoni *et al.*, 1987).

Ten planctomycete species are currently described, of which only seven have been cultured. Considering the large phylogenetic depth separating these cultured strains (Liesack *et al.*, 1992), it is likely that the majority of planctomycete strains have not yet been recovered from the environment. The recent development of selective media containing *N*-acetylglucosamine as carbon and nitrogen source as well as antibiotics, i.e. ampicillin and cycloheximide, added for suppression of growth of other micro-organisms, has allowed the isolation of numerous strains from diverse aquatic habitats with a wide range of salinity (fresh to hypersaline water), pH (4.2–11.6) and pollution (oligotrophic vs eutrophic) (Schlesner, 1994). Phylogenetic studies on a large number of these strains have expanded the phylogenetic structure at the inter- and intrageneric level (Ward *et al.*, 1995). Whether or not the emerging structure can be used to describe novel taxa depends upon the availability of phenotypic properties, such as phospholipids and fatty acids (Sittig & Schlesner, 1993), as well as physiological properties.

In this study, the 16S rDNA sequences were determined for 20 novel planctomycete isolates, and the DNA base composition and polyamine patterns, the latter of which have been found useful in the characterization of methanogenic archaea (Scherer & Kneifel, 1983), members of the *Vibrionaceae* (Yamamoto *et al.*, 1983) and the *Proteobacteria* (Busse & Auling, 1988; Auling, 1992) and certain Gram-positive bacteria (Altenburger *et al.*, 1997), were analysed.

METHODS

Bacterial strains. *Pirellula marina* IFAM 1313^T (=DSM 3645^T), *Pirellula staleyii* IFAM 1189^T (=DSM 6068^T), *Planctomyces limnophilus* IFAM 1008^T (=DSM 3776^T), *Pirellula brasiliensis* IFAM 1448^T (=DSM 5305^T) and *Pirellula maris* IFAM 1190^T (=DSM 8797^T) were taken from the IFAM (Institut für Allgemeine Mikrobiologie, University of Kiel, Germany) departmental culture collection. *G. obscuriglobus* DSM 5831^T (=UQM 2246^T) was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Planctomycete strains originated from various aquatic habitats (Table 1) and were obtained from IFAM. Described planctomycete species were obtained from the DSMZ for

16S rDNA analysis. Methods of sampling, enrichment and isolation have been recently described (Schlesner, 1994).

Media. Media differing in carbon and nitrogen sources and in salinity were as described previously (Schlesner, 1986, 1994). Additionally, M33a was used, which consisted of: gelatin, 2.0 g; yeast extract, 0.25 g; glucose, 0.25 g; Hutner's basal salts medium (HBM; Cohen-Bazire *et al.*, 1957), 20 ml; vitamin solution no. 6 (Staley, 1968), 10 ml; 100 mM Tris/HCl buffer (pH 7.5), 50 ml; artificial seawater (ASW; Lyman & Fleming, 1940), 500 ml; double-distilled water, 420 ml; and agar, 18 g.

Cultivation of bacteria. Cell material for DNA base composition determination was grown on heavily inoculated Petri dishes with the appropriate solid medium (Table 1). Cells were harvested with a rubber spatula, lyophilized and stored at –20 °C. For polyamine analysis, strains were grown in liquid cultures. Cells were harvested near the end of the exponential growth phase by centrifugation, washed with distilled water, lyophilized and stored at –20 °C.

Determination of DNA base ratio. DNA was extracted from 0.02 g freeze-dried cells in 1.5 ml Eppendorf reaction caps following the procedure of Marmur (1961). Analysis of the G+C content of DNA by HPLC was performed according to Mesbah *et al.* (1989).

Polyamine analysis. The polyamines were extracted and analysed by gradient HPLC and fluorescence detection according to Busse & Auling (1988).

16S rDNA sequence analysis. The extraction of genomic DNA and the amplification of 16S rDNA were performed as described previously (Rainey *et al.*, 1996a). Cloning of PCR products was performed using the pCR-Script kit (Stratagene) as described previously (Rainey *et al.*, 1996b). The PCR products were purified by using the Prep-A-Gene kit (Bio-Rad) as described by the manufacturer. The DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) was used to directly sequence the PCR products as described by the manufacturer. The sequence reactions were electrophoresed on the Applied Biosystems 373A DNA Sequencer. Accession numbers of isolates published previously (Ward *et al.*, 1995) are X81938–X81960.

Data analyses. The sequences were aligned manually against available sequences for members of the order *Planctomycetales*. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969). Parsimony analysis, maximum-likelihood and neighbour-joining were carried out using the programs of the PHYLIP package (Felsenstein, 1993). The 16S rDNA sequence of *Chlamydia psittaci* (EMBL accession no. M13769) was used to root the tree. Bootstrap analyses was used to determine the statistical significance of the branching patterns.

Nucleotide accession numbers. Accession numbers for sequences are as follows: AJ231168, strain 608; AJ231169, strain 529; AJ231170, strain 610; AJ231171, strain 337; AJ231172, strain 567; AJ231173, strain 543; AJ231174, strain 467; AJ231175, strain 683; AJ231176, strain 535; AJ231177, strain 449; AJ231178, strain 555; AJ231179, strain 611; AJ231180, strain 391; AJ231181, strain 116; AJ231182, strain 292; AJ231183, *Pirellula staleyii* DSM 6068^T; AJ231184, *Planctomyces maris* DSM 8797^T; AJ231185, strain 423; AJ231186, strain 248; AJ231187, strain 287; AJ231188, strain 282; AJ231189, strain 599; AJ231190, *Planctomyces brasiliensis* DSM 5305^T; AJ231191, *G. obscuriglobus* DSM 5831^T; AJ231192, strain 394;

AJ231193, strain 563; AJ231194, strain 532; AJ231195, *I. pallida* DSM 9630^T.

RESULTS AND DISCUSSION

Phylogenetic analysis of isolates

Twenty novel planctomycete isolates from aquatic habitats in the state of Schleswig-Holstein, Germany (Schlesner, 1994) were analysed with respect to the primary structure of the 16S rRNA gene. *Planctomyces brasiliensis* DSM 5305^T, which was not investigated in a previous phylogenetic study of members of the four planctomycete genera (Liesack *et al.*, 1992), was also included in this study. The 16S rDNA sequence of *Planctomyces maris* DSM 8797^T was redetermined, because the available sequence (EMBL accession no. X62910) was not complete. New sequence data were obtained for *G. obscuriglobus* DSM 5831^T, *I. pallida* DSM 9630^T and *Pirellula staleyi* DSM 6068^T because the available sequences (X56305 and X54522 for *G. obscuriglobus*, X64372 for *I. pallida*, no deposited 16S rDNA sequence for *Pirellula staleyi*) contained some ambiguous nucleotide positions. The almost complete sequences, covering regions from position 40 through position 1519 (according to *Escherichia coli* nomenclature; Brosius *et al.*, 1978) were aligned to the homologous sequences of described planctomycete species (Liesack *et al.*, 1992) and isolates that have been investigated recently (Ward *et al.*, 1995). Direct sequencing of the 5' region of the 16S rDNA PCR products of *Planctomyces brasiliensis*, *Planctomyces maris* and *G. obscuriglobus* generated sequence data that were not readable. The 16S rDNA PCR products from these strains were subsequently cloned and the clones were sequenced in order to determine whether the mixed sequence was due to sequence heterogeneity contained within multiple *rrn* operons in these strains. Sequence analysis of ten cloned 16S rDNA genes from each strain revealed only a single sequence for each strain. This makes it unlikely that the unreadable sequence data obtained by direct sequencing of the 16S rDNA PCR products from these strains was due to 16S rDNA sequence heterogeneity.

The addition of sequences of the novel isolates to the database did not significantly change the topology of the previously published phylogenetic dendrogram based on the analysis of 32 planctomycete strains (Ward *et al.*, 1995). Some of the novel strains clustered with strains previously affiliated to *I. pallida* (strains 394, 532 and 563), *Planctomyces limnophilus* (strains 282, 287 and, more distantly, 599), as well as *Pirellula marina* (strains 116, 391 and 611). Other strains formed novel discrete clusters with previously analysed strains. The range of intra- and intercluster 16S rDNA similarity values (not shown) and the sequence idiosyncrasies, described to characterize certain species of the four planctomycete genera (Ward *et al.*, 1995), did not change by the inclusion of the novel isolates. Members of the *Isosphaera* cluster were characterized by a G + C content (mol %) of the rRNA gene that was

Fig. 1. 16S rDNA dendrogram indicating the phylogenetic position between described species of the family Planctomycetaceae and isolates from various sites in northern Germany and from Hawaii. The root was determined by using the 16S rDNA sequence of *Chlamydia psittaci* as an outgroup reference. The dendrogram was generated by the neighbour-joining algorithm (Felsenstein, 1993). Bar, 10% sequence divergence. Numbers refer to bootstrap values.

about 3–4% higher than those found in members of the genera *Gemmata*, *Planctomyces*, *Pirellula* and the novel associated rDNA clusters (Table 1).

The emerging phylogenetic clusters are, in most cases, as unrelated among themselves as they are related to the type strains of described species, i.e. in the order of only 85–88% 16S rDNA sequence similarities. Even some of the intracluster similarities are as low as 87–92%, such as strain 599 and *Planctomyces limnophilus*, strains 678 and 292, the three major clusters of the *Pirellula marina* branch, and certain strains that cluster with *I. pallida* (Fig. 1). When

Table 2. Effect of increasing salinity and nutrient concentration on the polyamine pool [$\mu\text{mol (g dry weight)}^{-1}$] in *Planctomyces limnophilus* DSM 3776^T and strain 1

Strain	Growth condition	PUT	CAD	SPD	HSPD
Strain 1	13 (1/4ASW; S=8.5‰)*	19.5	15.3		19.6
	13a (1/2ASW; S=17‰)*	13.9	7.7		11.1
	13b (ASW; S=34‰)*	0.8	0		2.7
	13	19.5	15.3		19.6
	13(3x)†	21.3	17.2		21.8
	13(5x)†	19.8	16.0		17.4
<i>Planctomyces limnophilus</i> DSM 3776 ^T	31PY	67.7		5.3	
	31PY(1/4x)‡	78.3		2.0	
	31PY(1/2x)‡	71.9		5.4	

* Medium 13 made up with quarter-strength (1/4ASW), half-strength (1/2ASW) or full-strength ASW; S, salinity.

† Medium 13 made up with threefold (3X) or fivefold (5X) concentration of peptone, yeast extract and glucose.

‡ Medium 31PY made up with quarter-strength (1/4X) or half-strength (1/2X) concentration of *N*-acetylglucosamine, peptone and yeast extract.

maximum-likelihood, neighbour-joining and parsimony analysis methods were applied to the same data-set used to produce Fig. 1, only some minor rearrangements of branching points within strain clusters were found (data not shown). Hence, from a phylogenetic point, many of the novel isolates investigated previously (Ward *et al.*, 1995) and in this study represent novel species which cannot be described because of the lack of phenotypic and chemotaxonomic properties. To broaden the basis for future taxonomic description the type strains of described species and representative strains of some of the novel clusters were subjected to analysis of the polyamine pattern and DNA G+C content.

Polyamine analysis

The intended use of polyamines as biomarkers required examination of the influence of growth conditions on the composition of polyamines, for which *Planctomyces limnophilus* DSM 3776^T and strain 1 were selected. Since initial studies revealed that aged planctomycete cultures have a low polyamine content, the data presented here were from exponentially growing cultures. The polyamine pattern was not significantly influenced by diluting or enriching the organic compounds in the medium (Table 2) or simulating oligotrophic and eutrophic conditions, respectively. However, the polyamine pool of strain 1 was affected dramatically by modifying the salinity of the growth medium in that the pool of total polyamines was reduced with increased concentration of artificial sea water. A similar effect has been reported to occur in *Escherichia coli* (Munro *et al.*, 1972), strains of *Vibrio* (Yamamoto *et al.*, 1986) and *Deleya marina* (Auling *et al.*, 1991).

To allow comparison of data obtained, the majority of strains were grown on media 31PY or 13a (Table 1) which permitted reasonable growth for investigation of polyamines. All strains contained significant amounts of polyamines; in general, the pattern was composed of two or more compounds (Table 3). The concentrations of the polyamine pools in the *Planctomycetales* were comparable to those reported for other Gram-negative bacterial taxa (Busse & Auling, 1988; Hamana & Matsuzaki, 1993). Major compounds were *sym*-homospermidine (HSPD), putrescine (PUT) and spermidine (SPD). Cadaverine (CAD), diamino-propane (DAP) and spermine (SPM) were found as minor compounds only in a few strains.

The qualitative distribution of the polyamine patterns is by and large consistent with the phylogenetic relatedness of closely related strains. The exception is strain 1, which shows significant levels of PUT and CAT that are almost absent in the related strains 608 and 140. At present, there is no explanation for this discrepancy. Except for *Planctomyces limnophilus* and the related strains 282 and 287, HSPD was present in all peptidoglycan-free budding bacteria (Table 3) and was the only major component in strains of *G. obscuriglobus*, *I. pallida* and related strains, as well as in the marine planctomycetes *Planctomyces maris*, the halotolerant *Planctomyces brasiliensis* and strain 130; however, as compared to the other members of the order *Planctomycetales*, these marine strains contained rather low amounts of HSPD. The freshwater planctomycetes possessed PUT as the main compound, sometimes in extremely high amounts, as well as significant amounts of SPD. High amounts of PUT, together with HSPD characterized strains of the strain 269 cluster; *Planctomyces limnophilus* and related

Table 3. Polyamine [$\mu\text{mol (g dry weight)}^{-1}$] pattern and DNA base ratio of budding peptidoglycan-free isolates and reference species belonging to the order *Planctomycetales*

Strain	DAP	PUT	CAD	SPD	HSPD	SPM	G + C (mol%)
<i>Gemmata obscuriglobus</i> DSM 5831 ^T					35.2		64*
633		1.0	t		85.0		70
<i>Isosphaera pallida</i> DSM 9630 ^T							62*
666		1.0			80.1		72
532					12.9		70
<i>Planctomyces maris</i> DSM 8797 ^T		t			5.8		51
130					1.1		53
<i>Planctomyces brasiliensis</i> DSM 5305 ^T				2.7	7.1		56
269		88.3		8.8	25.8		66
668		34.5			8.0		52
<i>Planctomyces limnophilus</i> DSM 3776 ^T		57.8		8.8	t		53
282		77.7		14.6			54
287		73.6		4.4			54
642		80.8	0.8	8.8	0.2		66
<i>Pirellula staleyi</i> DSM 6068 ^T				0.8	50.2		57
<i>Pirellula marina</i> DSM 3645 ^T					11.3		56
611				5.4	28.6		52
302		10.7			19.2		52
382					4.7		57
1		19.5	15.3		19.6		55
608		1.8			7.4		55
140		2.0		0.3	4.3		56
384	1.5	7.2		0.3	4.6		58
529		2.8			5.6		58
158					14.0		54
139	2.6	1.6	1.2		8.5		57
337		21.9			14.9		55

* Result taken from Staley *et al.* (1992).t, Trace amount [$<0.2 \mu\text{mol (g dry weight)}^{-1}$].

strains contained high amounts of PUT and SPD. *Pirellula staleyi* contained high amounts of HSPD as the only predominant polyamine. The amount of polyamines was relatively small and mainly composed of HSPD and PUT in cells of *Pirellula marina* and related strain clusters; some strains possessed small amounts of CAD and DAP. Whether or not the presence of a more diverse pattern of polyamines in these strains is a reflection of their phylogenetic diversity needs to be tested by including more strains from the same phylogenetic cluster in the polyamine studies.

Base composition of DNA

The G+C content of the type strains of the three described *Planctomyces* species investigated in this study were within a narrow margin of 51–56 mol% (Table 3). Strains 282 and 287, which are phylogenetically related to and share the same polyamine composition as *Planctomyces limnophilus* DSM 3776

have a very similar DNA G+C content (54 mol%). On the other hand, strain 642 which, phylogenetically, clusters remotely with the type strain of *Planctomyces limnophilus* has a significantly higher DNA G+C content of 66 mol%. *Planctomyces brasiliensis* has a DNA G+C content of 56 mol%. This value is different from those of strains that represent individual branches of a neighbouring cluster, i.e. strain 664 (52 mol%) and strain 269 (66 mol%). The latter two strains also exhibit different polyamine patterns.

The DNA G+C values of type strains of the two *Pirellula* species as well as those of the majority of *Pirellula*-related strains are very similar (54–58 mol%). Exceptions are the moderately related strains 611 and 302; both strains have a G+C value of 52 mol% and exhibit a unique polyamine pattern within the *Pirellula* cluster.

The DNA base ratio of strains related to *G. obscuriglobus* and *I. pallida* are about 6% higher than those of the type strains of these two species which

have been reported by Staley *et al.* (1992). These differences may be due to methods used, as reported frequently in the literature. Nevertheless, the G+C contents of members of these two genera are significantly higher than those of members of *Planctomyces* and *Pirellula*, a finding that supports their phylogenetic and chemotaxonomic distinctness. As mentioned above, members of *Isosphaera* differ markedly from the other strains studied in that their G+C content of the gene(s) encoding 16S rRNA is higher.

In conclusion, phylogenetic analysis of novel strains indicates the dramatic diversity contained within the order *Planctomycetales*. This phylogenetic diversity is confirmed by polyamine composition, the base ratio of DNA and, at least for *I. pallida* and related strains, the base ratio of rRNA. At the genomic and epigenomic level, the diversity of strains of novel planctomycete lineages is as high as that of described planctomycete genera. These novel strain clusters should be subjected to thorough polyphasic taxonomic analyses in order to determine whether they could be described as novel taxa.

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REFERENCES

- Altenburger, P., Kämpfer, P., Akimov, V. N., Lubitz, W. & Busse, H.-J. (1997). Polyamine distribution in actinomycetes with group B peptidoglycan and species of the genera *Brevibacterium*, *Corynebacterium*, and *Tsukamurella*. *Int J Syst Bacteriol* **47**, 270–277.
- Auling, G. (1992). Polyamines, biomarker for taxonomy and ecology of phytopathogenic and other bacteria belonging to the *Proteobacteria*. *Belg J Bot* **125**, 203–209.
- Auling, G., Busse, H.-J., Pilz, F., Webb, L., Kneifel, H. & Claus, D. (1991). Rapid differentiation, by polyamine analysis, of *Xanthomonas* strains from phytopathogenic pseudomonads and other members of the class *Proteobacteria* interacting with plants. *Int J Syst Bacteriol* **41**, 223–228.
- Bomar, D., Giovannoni, S. & Stackebrandt, E. (1988). A unique eubacterial 5S rRNA in members of the order *Planctomycetales*. *J Mol Evol* **27**, 121–125.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Busse, H.-J. & Auling, G. (1988). Polyamine patterns as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulphur purple bacteria. *J Cell Comp Physiol* **49**, 25–68.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5.1. Department of Genetics, University of Washington, Seattle, WA, USA.
- Franzmann, P. D. & Skerman, V. B. D. (1984). *Gemmata obscuriglobus*, a new genus and species of the budding bacteria. *Antonie Leeuwenhoek* **50**, 261–268.
- Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.
- Giovannoni, S. J., Schabtach, E. & Castenholz, R. W. (1987). *Isosphaera pallida* gen. nov., comb. nov., a gliding, budding eubacterium from hot springs. *Arch Microbiol* **147**, 276–284.
- Hamana, K. & Matsuzaki, S. (1993). Polyamine distribution patterns serve as a phenotypic marker in the chemotaxonomy of the *Proteobacteria*. *Can J Microbiol* **39**, 304–310.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- König, H., Schlesner, H. & Hirsch, P. (1984). Cell wall studies on budding bacteria of the *Planctomyces/Pasteuria* group and on a *Prosthecomicrobium* sp. *Arch Microbiol* **138**, 200–205.
- Liesack, W. & Stackebrandt, E. (1989). Evidence for unlinked rrn operons in the planctomycete *Pirellula marina*. *J Bacteriol* **171**, 5025–5030.
- Liesack, W., König, H., Schlesner, H. & Hirsch, P. (1986). Chemical composition of the peptidoglycan-free envelopes of budding bacteria of the *Pirella/Planctomyces* group. *Arch Microbiol* **145**, 361–366.
- Liesack, W., Söller, R., Stewart, T., Haas, H., Giovannoni, S. & Stackebrandt, E. (1992). The influence of tachytelically evolving sequences on the topology of phylogenetic trees – intrafamily relationships and the phylogenetic position of *Planctomycetaceae* as revealed by comparative analysis of 16S ribosomal RNA sequences. *Syst Appl Microbiol* **15**, 357–362.
- Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Munro, G. F., Hercules, K., Morgan, J. & Sauerbier, W. (1972). Dependence of the putrescine content of *Escherichia coli* on the osmotic strength of the medium. *J Biol Chem* **247**, 1272–1280.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996a). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Rainey, F. A., Ward-Rainey, N., Janssen, P. H., Hippe, H. & Stackebrandt, E. (1996b). *Clostridium paradoxum* DSM 7308^T contains multiple 16S rRNA genes with heterogeneous intervening sequences. *Microbiology* **142**, 2087–2095.
- Scherer, P. & Kneifel, H. (1983). Distribution of polyamines in methanogenic bacteria. *J Bacteriol* **154**, 1315–1322.
- Schlesner, H. (1986). *Pirella marina* sp. nov., a budding, peptidoglycan-less bacterium from brackish water. *Syst Appl Microbiol* **8**, 177–180.
- Schlesner, H. (1994). The development of media suitable for the microorganisms morphologically resembling *Planctomyces* ssp., *Pirellula* ssp., and other *Planctomycetales* from various aquatic habitats using dilute media. *Syst Appl Microbiol* **17**, 135–145.

- Schlesner, H. & Hirsch, P. (1984). Assignment of ATCC 27377 to *Pirella* gen. nov. as *Pirella staleyi* comb. nov. *Int J Syst Bacteriol* **34**, 492–495.
- Schlesner, H. & Stackebrandt, E. (1986). Assignment of the genera *Planctomyces* and *Pirella* to a new family *Planctomycetaceae* fam. nov. and description of the order *Planctomycetales* ord. nov. *Syst Appl Microbiol* **8**, 174–176.
- Sittig, M. & Schlesner, H. (1993). Chemotaxonomic investigations of various prosthecate and/or budding bacteria. *Syst Appl Microbiol* **16**, 92–103.
- Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.
- Staley, J. T., Fuerst, J. A., Giovannoni, S. & Schlesner, H. (1992). The order *Planctomycetales* and the genera *Planctomyces*, *Pirellula*, *Gemmata*, and *Isosphaera*. In *The Prokaryotes. A Handbook on the Biology of Bacteria Ecophysiology, Isolation, Identification, Applications*, pp. 3710–3731. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Ward, N., Rainey, F. A., Stackebrandt, E. & Schlesner, H. (1995). Unravelling the extent of diversity within the order *Planctomycetales*. *Appl Environ Microbiol* **61**, 2270–2275.
- Ward-Rainey, N., Rainey, F. A., Wellington, E. M. H. & Stackebrandt, E. (1996). Physical map of the genome of *Planctomyces limnophilus*, a representative of the phylogenetically distinct planctomycete lineage. *J Bacteriol* **178**, 1908–1913.
- Woese, C. R. (1987). Bacterial evolution. *Microbiol Rev* **51**, 221–271.
- Yamamoto, S., Shinoda, S., Kawaguchi, M., Wakamutsu, K. & Makita, M. (1983). Polyamine distribution in *Vibrionaceae*: norspermidine as a general constituent of *Vibrio* species. *Can J Microbiol* **29**, 724–728.
- Yamamoto, S., Yoshida, M., Nakao, N., Koyama, M., Hashimoto, Y. & Shinoda, S. (1986). Variations of cellular polyamine compositions and contents of *Vibrio* species during growth with various NaCl concentrations. *Chem Pharm Bull (Tokyo)* **34**, 3038–3042.