Taxonomic heterogeneity within the *Planctomycetales* as derived by DNA–DNA hybridization, description of *Rhodopirellula baltica* gen. nov., sp. nov., transfer of *Pirellula marina* to the genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov. and emended description of the genus *Pirellula*

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Ninety-seven strains of budding bacteria originating from various aquatic habitats and morphologically resembling planctomycetes were investigated taxonomically. Taxonomic differentiation was based on DNA–DNA hybridization, physiological properties and chemotaxonomic tests. Nineteen hybridization groups, containing 79 of the tested strains, were established. Eighteen strains, however, did not fit into any of these groups. *Rhodopirellula baltica* gen. nov., sp. nov. is described, with strain SH 1^T (=IFAM 1310^T=DSM 10527^T=NCIMB 13988^T) as the type strain. *Pirellula marina* is transferred to the genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov., with strain SH 106^T (=IFAM 1313^T=DSM 3645^T=ATCC 49069^T) as the type strain. An emended description of the genus *Pirellula* is also provided. Differentiation between *R. baltica*, *B. marina* and *Pirellula staleyi* was achieved by the integration of morphological, physiological, chemotaxonomic and genetic characteristics.

INTRODUCTION

The budding peptidoglycan-less bacteria of the phylum *Planctomycetes* (Garrity & Holt, 2001) comprise the order *Planctomycetales*, with the single family *Planctomycetaceae* (Schlesner & Stackebrandt, 1986) and the four genera *Planctomyces*, *Pirellula*, *Gemmata* and *Isosphaera* (Staley *et al.*, 1992). The planctomycetes were considered as unculturable bacteria for many decades. Following the first report of a pure culture (Staley, 1973), however, a large number of strains have been isolated from a variety of aquatic habitats differing considerably in salinity, pH or the availability of nutrients (Schlesner, 1994). Other sources of strains were tissue cultures (Fuerst *et al.*, 1991) and postlarvae of the giant tiger prawn *Penaeus monodon* (Fuerst *et al.*, 1997) and the tissue of the Mediterranean sponge *Aplysina aerophoba* (Gade *et al.*, 2004). Recently, the isolation of *Gemmata*-like bacteria from soil was reported (Wang *et al.*, 2002). Detailed information on the history and biology of planctomycetes can be found in the excellent review of Fuerst (1995).

Studies on the evolutionary position of these organisms indicated a great inter- and intrageneric heterogeneity in the 16S rRNA gene sequences (Stackebrandt *et al.*, 1986; Ward *et al.*, 1995; Fuerst *et al.*, 1997; Griepenburg *et al.*, 1999). Sequence-similarity values for the 16S rRNA genes of *Pirellula staleyi*, *Pirellula marina* (here described as *Blastopirellula marina* gen. nov., comb. nov.) and *Pirellula* sp. SH 1^T (here described as *Rhodopirellula baltica* gen. nov., sp. nov.) were below 90 % (see Table 4), supporting the differentiation into three genera. At present, it is generally

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Abbreviation: ASW, artificial sea water.

accepted that individual species of the same genus should have 16S rRNA gene sequence similarity of more than 95 % (Devereux *et al.*, 1990; Fry *et al.*, 1991; Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence heterogeneity was further supported by analysis of the cell-wall components (König *et al.*, 1984; Liesack *et al.*, 1986), phospholipids (Kerger *et al.*, 1988; Sittig & Schlesner, 1993) and polyamines (Griepenburg *et al.*, 1999).

An unusual characteristic of planctomycetes appears to be their large genome sizes. Initial investigations of genome sizes of budding bacteria were performed using DNA-renaturation kinetics, and indicated that *Pirellula* and *Planctomyces* species had considerably larger genomes than most of the other strains of budding bacteria examined (belonging to the genera *Hyphomicrobium*, *Hyphomonas*, *Filomicrobium* and *Pedomicrobium*) (Kölbel-Boelke *et al.*, 1985). These results have been confirmed by the recent publication of the complete genome sequence of *R. baltica* (Glöckner *et al.*, 2003; http://www.regx.de). With a genome size of 7·145 Mb, it is one of the largest circular bacterial genomes known to date. In addition, ongoing genome sequencing projects with *Gemmata obscuriglobus* (http://www.tigr.org) and *Gemmata* sp. Wa 1-1 (http://wit.integratedgenomics.com) support the unusually large genome sizes of planctomycete species. It is generally assumed that early genomes were probably small in size and that larger genomes arose later in evolution as a result of events such as gene duplication or the acquisition of additional genes from other organisms. While it is also known that 'organisms' such as obligate intracellular symbionts or plastids may also reduce their genome size by gene loss (evolutionary reversal), a prerequisite is that the ancestors must have passed through a stage in which the genome size was larger (Anderson & Anderson, 1999; Blanchard & Lynch, 2000; Gil *et al.*, 2002). While planctomycetes have been variously described as rapidly evolving or ancient, it would appear that the large genome size, at least, is probably not a 'primitive' feature.

In the light of these studies on the genomes of various planctomycetes, it is becoming important to re-examine our appreciation of the biology and diversity of this group of organisms. In this study, we investigated the taxonomic positions of 97 pigmented and unpigmented *Pirellula*-, *Planctomyces*- and *Gemmata*-like strains isolated from various aquatic habitats, including the type strains of *Pirellula staleyi*, *B. marina*, *Planctomyces maris* and *G. obscuriglobus*. *R. baltica* was selected as representative of a group of isolates that were genetically related at the species level. This group contained 22 pigmented isolates from brackish water and two strains isolated from the tissue of the sponge *A. aerophoba* (Gade *et al.*, 2004). Here, we describe *Pirellula* sp. SH 1^{T} as the type strain of *R. baltica* gen. nov., sp. nov. Because of the diversity of the respective strains at the genetic and phenotypic levels, we propose that *Pirellula marina* be excluded from the genus *Pirellula* and be transferred to the new genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov. This also means that the description of the genus *Pirellula* must be emended to reflect its changed circumscription.

METHODS

Media for cultivation. Various types of media were used for the cultivation of strains with different nutritional requirements. Media differed essentially with respect to carbon and nitrogen sources and with respect to salinity (Table 1).

Sampling, enrichment and isolation of strains. Experimental procedures were as previously described (Schlesner, 1994).

Microscopy. Morphological studies involving phase-contrast microscopy and electron microscopy were performed as described previously (Schlesner, 1986; Gade *et al.*, 2004). For cryofixation and cryosubstitution, bacteria were fixed using high-pressure freezing (Hohenberg *et al.*, 1994). Samples in 1 % (w/v) osmium tetroxide in acetone were dehydrated in a freeze-substitution unit (AFS Leica) at temperatures of −90, −60 and −30 °C (for 8 h each). Finally, the temperature was increased to 4 °C. The samples were infiltrated with Epon 812 (Epon Kit 45359; Fluka) by incubation with 30 % and 70 % (w/v) resin in acetone for 2 h each and then in pure resin for 12 h at 20 °C. Polymerization occurred at 60 °C for 24 h.

Physiological studies. Growth experiments with liquid media were carried out essentially as described by Schlesner (1986, 1994). Cultivation was done with 100 ml Erlenmeyer flasks containing 50 ml medium. Cultures were incubated at 25 °C for 3 weeks on a shaker. To test for carbon sources (0·1 %, w/v) that supported growth, medium M40 was used for *R. baltica* (SH 1^{T}) and *B. marina* (DSM 3645^{T}), whereas medium M40c was used for *Pirellula staleyi* (ATCC 27377^{T}). Anaerobic growth was tested under fermentative conditions and in the presence of nitrate as electron acceptor. Techniques for the preparation of media and cultivation under anoxic conditions were described previously (Widdel & Bak, 1992). Ascorbate was added to the media as an additional reductant (Rabus & Widdel, 1995).

Hydrolysis of gelatin was visualized by flooding the plates with hot (70–80 °C) saturated ammonium sulfate instead of mercury chloride. Lipase activity was tested according to Kouker & Jaeger (1987), using plates that contained trioleic acid and rhodamine B. Activities for the hydrolysis of starch, casein, aesculin and DNA were tested according to standard procedures (Smibert & Krieg, 1994). Formation of hydrogen sulfide from thiosulfate was tested as described previously (Schlesner, 1986).

Salinity tolerance was studied with liquid media containing increasing proportions of artificial sea water (ASW; Lyman & Fleming, 1940) to give final concentrations of 0, 6, 12 and 25 %; 25 % steps were then used to reach a maximum of 300 % ASW (100 % ASW = 34·5 ‰ salinity).

Phospholipids and quinones. These cell components were extracted from lyophilized cells and analysed by TLC as described by Sittig & Hirsch (1992). In addition, polar lipids, respiratory lipoquinones and fatty acids were extracted and analysed as described previously (Tindall, 1990a, b; Strömpl *et al.*, 1999).

DNA base ratio and DNA–DNA hybridization. G+C content determinations and DNA–DNA hybridization experiments were performed as described previously (Rathmann, 1992; Gade *et al.*, 2004).

Table 1. Media used for cultivation

Values are percentages by volume (ASW) or by weight (remaining components).

Medium	Peptone	Yeast extract	Glucose	*N*-Acetylglucosamine	Casamino acids	ASW*	Reference
M1†	–	–	–	0·2	–	–	Staley *et al.* (1992)
M13	0·025	0·025	0·025	–	–	25	Schlesner (1986)
M13a	0·025	0·025	0·025	–	–	50	Schlesner (1994)
M13f	0·075	0·075	0·5	–	–	25	
M20‡	–	–	0·25	–	0·1	25	Schlesner (1986)
M20c‡	–	–	0·25	–	0·1	–	
M22	0·025	0·025	0·025	–	–	–	Schlesner (1994)
M30	–	–	–	0·2	–	25	Staley *et al.* (1992)
M30a	–	–	–	0·2	–	50	Staley *et al.* (1992)
M31	–	–	–	0·2	–	–	Staley *et al.* (1992)
M40§	–	–	–	–	0·1	25	Schlesner (1986)
M40c§	–	–	–	–	0·1	–	

*Composition described by Lyman & Fleming (1940).

†Contained $CaCO_3$ to maintain an alkaline pH. Solidified with gellan gum Gelrite (Kelco) rather than with agar (Staley *et al.*, 1992).

‡Vitamin-free medium for the purpose of testing for vitamin requirements.

§Represents a modification of medium M9 (Schlesner, 1986). Used to test for growth substrates. Casamino acids were added as the nitrogen source; they did not support growth of the tested strains, when added as sole substrate.

RESULTS AND DISCUSSION

Strains investigated and their habitats

We investigated the taxonomic positions of 97 strains that were isolated in this study and that resembled planctomycetes morphologically. Several known species were also included (Table 2). Initially, we categorized all strains mainly according to pigmentation of colonies, the motility of daughter cells and DNA–DNA hybridization groups (Table 2). The novel isolates were obtained from a variety of aquatic habitats, including freshwater, brackish water and marine water. Most habitats had a slightly alkaline pH (around 8), while water bodies of chalk mines reached maximal pH values of 11·6 (Schlesner, 1994). Hypertrophic water samples were retrieved from Apetlon village pond (Austria) and Schrevenpark Pond (Kiel, Germany). The following section deals with the categorization of the entire set of strains investigated.

DNA base ratio and DNA–DNA hybridization groups

The broad range of G+C content from 52 to 69 mol% (Table 2) indicates the genetic diversity of this group. The G+C value for most strains was in the range reported for the genera *Planctomyces* (50–58 mol%), *Pirellula* (54–57 mol%) or *Gemmata* (64 mol%; Staley *et al.*, 1992). Strains SH 404 and SH 449, however, displayed considerably higher values: 69 and 68 mol%, respectively.

DNA–DNA hybridization experiments with labelled DNA of 20 selected strains (underlined in Table 2) resulted in 19 hybridization groups. Definition of hybridization groups was based on 70 % DNA binding (different strains belonging to the same species; Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Six hybridization groups were represented by one strain only. The DNA of 18 strains did not hybridize with labelled DNA of any of the 20 selected strains, with *Planctomyces maris* (ATCC 29201^T) or with *G. obscuriglobus* (DSM 5831^T). These results also point to the genetic heterogeneity among the studied strains. Pigmentation proved to be a useful taxonomic marker, since individual hybridization groups contained exclusively either pigmented or unpigmented strains.

Hybridization group I contained 25 pigmented strains of *R. baltica*, which displayed the typical *Pirellula* morphology. All of them possessed motile daughter cells. The majority of strains (19) were isolated from Kiel Fjord (Baltic Sea) over a period of 25 years, suggesting that these bacteria belong to the autochthonous microbial community of this habitat. Interestingly, two novel strains were recently isolated from the Mediterranean sponge *A. aerophoba* (Gade *et al.*, 2004), indicating the widespread occurrence of this species. In addition, DeLong *et al.* (1993) obtained molecular clones of 16S rRNA gene sequences with high similarity to *R. baltica* from marine snow (Pacific Ocean).

Hybridization group II consisted of eight strains of *B. marina*, including the type strain (DSM 3645^T). The main difference with respect to hybridization group I was the lack of pigmentation. While four strains were isolated from Kiel Fjord, others originated from other brackish habitats.

Hybridization group III consisted of six strains of *Pirellula staleyi*, including the type strain (ATCC 27377^T). Like those in hybridization group II, the colonies were unpigmented.

Table 2. Strains investigated in this study and their growth media, pigmentation, motility and G+C content

Strains are arranged according to their DNA–DNA hybridization groups (HG). DNA of underlined strains was labelled. ND, Not determined. Sources are identified as: 1, Kiel Fjord, part of the Baltic Sea; 2, public aquarium of the Institute for Marine Research at the University of Kiel, Germany (the basins in the aquarium contained water from Kiel Fjord); 3, *Aplysina aerophoba*; 4, wastewater aeration lagoons of a sugar-processing plant near Schleswig, Germany (highly eutrophic); 5, Fjord Schlei, part of the Baltic Sea; 6, chalk mine at Lägerdorf, Holstein, Germany; 7, pebble wash water from a gravel pit at Rastorfer Kreuz near Kiel, Germany; 8, Lake Felder See, Holstein, Germany (highly eutrophic); 9, Winogradski column made up with sediment and water from Kiel Fjord; 10, Strande Beach; 11, Karlsminde Beach, Baltic Sea near Eckernförde, Schleswig-Holstein, Germany; 12, Schrevenpark Pond, a pond in a public park in Kiel, Germany; 13, Lake Mondsee, a pond in a public park in Kiel, Germany (highly eutrophic because of flocks of water fowl being fed by visitors to the park); 14, gypsum mine at Klein-Nordende, Holstein, Germany; 15, Campus pond, University of Kiel; 16, Lake Fuhlensee, a shallow, hypertrophic, slightly brackish lake north of Kiel, Germany; 17, rubbish dump at a rubbish-depositing plant at Rastorfer Kreuz near Kiel, Germany; 18, Kiel Bight; 19, surface and groundwater lagoon within Schrevenpark Pond; 20, Apetlon pond, a hypertrophic village pond east of Lake Neusiedler See, Burgenland, Austria; 21, 'Nebelhaus' greenhouse in the botanical garden of Kiel University (the greenhouse has high humidity but a moderate temperature); 22, Karlsminde Pond, a pond near Karlsminde camping area, about 50 m from the shore of the Baltic Sea on the northern coast of Eckernförde Bight, Germany; 23, Strohbrück, an experiment in a pilot project to convert cattle manure to fertilizer; 24, Lake Heidensee, west of Plön, Germany; 25, Groß Barkau, fire pond west of Preetz, Germany.

Strain	Source	Culture medium	Pigmented colonies	Motile daughter cells	G+C content (mol%)	HG
Rhodopirellula baltica						
SH 1^T (=IFAM 1310^T)	1	M13a	+	+	55	I
SH 12 (=IFAM1428)	1	M13a	+	+	ND	I
SH 26 (=IFAM 1429)	1	M13a	+	+	ND	I
SH 28 (=IFAM 1430)	1	M13a	+	+	55	I
SH 29 (=IFAM 1431)	1	M13a	+	+	57	I
SH 32 (=IFAM 1695)	1	M13a	+	+	ND	I
SH 39 (=IFAM 1449)	1	M13a	+	+	55	I
SH 121 (=IFAM 1433)	1	M13a	+	+	ND	I
SH 123 (=IFAM 1434)	1	M13a	+	+	ND	I
SH 126 (=IFAM 1435)	1	M13	+	+	ND	I
SH 155 (=IFAM 1436)	1	M13a	+	+	55	I
SH 156 (=IFAM 1437)	1	M13a	+	+	56	I
SH 157 (=IFAM 1438)	1	M13a	+	+	56	I
SH 159 (=IFAM 1455)	1	M13a	+	+	56	I
SH 165 (=IFAM 1451)	1	M13a	+	+	53	I
SH 188 (=IFAM 1580)	1	M13a	+	+	ND	I
SH 190 (=IFAM 1582)	1	M13a	+	+	ND	I
SH 198 (=IFAM 1735)	1	M13a	+	+	ND	I
99/2	1	M13a	+	+	55	I
SH 385 (=IFAM 3255)	2	M13a	+	+	ND	I
SH 386 (=IFAM 3184)	2	M13a	+	+	ND	I
SH 398 (=IFAM 3246)	2	M13a	+	+	55	I
SH 400 (=IFAM 3187)	2	M13a	+	+	ND	I
SH 796	3	M13a	+	+	54	I
SH 797	3	M13a	+	+	54	I
Blastopirellula marina						
DSM 3645^T	–	M13a	−	+	57	II
SH 150 (=IFAM 1557)	1	M13a	−	+	ND	II
SH 152 (=IFAM 1547)	1	M13a	−	+	ND	II
SH 166 (=IFAM 1570)	1	M13a	−	+	55	II
SH 168 (=IFAM 1453)	1	M13a	−	+	54	II
SH 405 (=IFAM 3258)	4	M13	−	+	56	II
SH 406 (=IFAM 3277)	4	M13	−	+	56	II
SH 452 (=IFAM 3298)	5	M13	−	+	ND	II
Pirellula staleyi						
ATCC 27377^T	–	M31	−	+	57	III
SH 353 (=IFAM 3411)	6	M1	−	+	ND	III

Table 2. cont.

Strain	Source	Culture medium	Pigmented colonies	Motile daughter cells	G+C content (mol%)	HG
SH 355 (=IFAM 3191)	6	M1	−	+	56	III
SH 356 (=IFAM 3281)	6	M1	−	+	ND	III
SH 479 (=IFAM 3323)	7	M31	−	+	60	III
SH 488 (=IFAM 3411)	8	M1	−	+	ND	III
Other strains						
SH 140 (=IFAM 1319)	1	M13a	+	−	56	IV
SH 410 (=IFAM 3275)	2	M13a	+	−	58	IV
SH 411 (=IFAM 3186)	2	M13a	+	−	ND	IV
SH 520 (=IFAM 3483)	9	M30	+	−	56	IV
SH 143 (=IFAM 1358)	10	M13a	+	+	56	V
SH 453 (=IFAM 3390)	11	M30	+	+	56	V
SH 455 (=IFAM 3300)	11	M30	+	+	54	V
SH 158 (=IFAM 1452)	1	M13a	+	+	55	VI
SH 282 (=IFAM 3016)	12	M22	+	+	54	VII
SH 287 (=IFAM 3011)	13	M22	+	+	54	VII
SH 241 (=IFAM 3207)	12	M22	+	+	64	VIII
SH 269 (=IFAM 3050)	12	M22	+	+	66	VIII
SH 116 (=IFAM 1432)	1	M13a	−	+	55	IX
SH 118 (=IFAM 1447)	1	M13a	−	+	55	IX
SH 203 (=IFAM 1999)	14	M13	−	+	52	X
SH 218 (=IFAM 2078)	14	M13	−	+	55	X
SH 238 (=IFAM 2076)	12	M13	−	+	53	X
SH 239 (=IFAM 2077)	11	M13	−	+	ND	X
SH 245 (=IFAM 2296)	15	M22	−	+	55	X
SH 248 (=IFAM 2297)	15	M22	−	+	ND	X
SH 255 (=IFAM 2299)	12	M22	−	+	ND	X
SH 267 (=IFAM 3013)	12	M22	−	+	ND	X
SH 277 (=IFAM 2247)	15	M13	−	+	ND	X
SH 280 (=IFAM 3014)	12	M22	−	+	ND	X
SH 286 (=IFAM 3010)	13	M22	−	+	56	X
SH 217 (=IFAM 1945)	16	M13	−	+	62	XI
SH 221 (=IFAM 2001)	16	M13	−	+	ND	XI
SH 460 (=IFAM 3202)	17	M31	−	+	65	XI
SH 292 (=IFAM 3017)	16	M13	−	+	61	XII
SH 293 (=IFAM 3015)	16	M13	−	+	61	XII
SH 295 (=IFAM 3018)	16	M13	−	+	61	XIII
SH 139 (=IFAM 1318)	18	M13a	+	+	57	XIV
SH 302 (=IFAM 3198)	5	M13	+	+	52	XV
SH 440 (=IFAM 3302)	19	M31	+	+	56	XV
SH 441 (=IFAM 3245)	5	M31	+	+	ND	XV
SH 458 (=IFAM 3391)	3	M31	+	+	56	XVI
SH 404 (=IFAM 3288)	19	M31	+	+	70	XVII
SH 449 (=IFAM 3304)	5	M1	+	+	68	XVIII
SH 380 (=IFAM 3182)	2	M30	+	+	ND	XIX
SH 382 (=IFAM 3183)	2	M30	+	+	57	XIX
SH 240 (=IFAM 3001)	15	M31	+	+	59	−
SH 279 (=IFAM 3051)	12	M22	+	+	ND	−
SH 331 (=IFAM 3248)	20	M31	−	+	ND	−
SH 367 (=IFAM 3262)	20	M30	+	+	68	−
SH 381 (=IFAM 3269)	2	M30	+	+	ND	−
SH 389 (=IFAM 3270)	21	M13	−	+	ND	−
SH 391 (=IFAM 3241)	2	M13	−	+	54	−
SH 392 (=IFAM 3393)	2	M13a	−	+	ND	−

Table 2. cont.

Strain	Source	Culture medium	Pigmented colonies	Motile daughter cells	G+C content (mol%)	HG
SH 420 (=IFAM 3285)	3	M31	−	+	ND	−
SH 423 (=IFAM 3293)	3	M31	−	−	ND	−
SH 427 (=IFAM 3291)	19	M31	+	+	ND	−
SH 431 (=IFAM 3252)	3	M13	−	+	ND	−
SH 439 (=IFAM 3395)	19	M31	+	+	ND	−
SH 461 (=IFAM 3267)	12	M1	+	+	ND	−
SH 462 (=IFAM 3396)	22	M30	+	+	ND	−
SH 468 (=IFAM 3203)	3	M30	+	+	ND	−
SH 474 (=IFAM 3394)	16	M1	+	+	ND	−
SH 531 (=IFAM 3322)	23	M1	+	−	62	−
SH 567 (=IFAM 3405)	24	M1	+	+	54	−
SH 592 (=IFAM 3421)	25	M1	+	+	ND	−
Planctomyces maris ATCC 29201^T	–	M13a	−	+	51	
Gemmata obscuriglobus DSM 5831^T	–	629*	+	+	64	

*See the DSMZ Catalogue of Strains (http://www.dsmz.de).

While members of hybridization groups I and II originated from brackish to marine habitats, group III strains were isolated from freshwater habitats in northern Germany. *Pirellula staleyi* originated from Lake Lansing (Michigan, USA; Staley, 1973).

A detailed differentiation of the 19 hybridization groups according to salinity tolerance, hydrolysis of polymers and the presence of phosphatidylcholine is summarized in Table 3. Strains within an individual hybridization group displayed only minor phenotypic differences. Although the

Table 3. Characteristics useful for differentiating between DNA–DNA hybridization groups (HG)

+, Positive or main component; (+), weak or present in small amounts; −, negative or not present; ND, not determined; PC, phosphatidylcholine.

HG	Species/strain	ASW (%)	Hydrolysis of:					H_2S from thiosulfate	Lipase (pH 7)	PC
			Casein	DNA	Aesculin	Gelatin	Starch			
I	*R. baltica*	12·5–200	−	+	+	+	+	+	−	+
II	*B. marina*	12·5–175	−	+	+	+	+	+	+	−
III	*P. staleyi*	0–50	+	−	+	+	+	+	−	−
IV	SH 140	12·5–150	−	+	+	+	+	+	−	+
V	SH 453	12·5–325	+	+	+	+	−	+	−	+
VI	SH 158	25–175	−	−	−	+	+	+	+	+
VII	SH 282	0–25	−	−	+	−	−	−	−	−
VIII	SH 269	0–25	+	−	+	+	+	−	−	+
IX	SH 116	12·5–175	+	+	+	+	+	+	+	−
X	SH 245	0–125	−	−	+	+	+	+	−	−
XI	SH 217	6–125	+	+	+	+	+	+	−	−
XII	SH 292	0–100	−	−	−	+	+	+	−	−
XIII	SH 295	0–50	+	+	−	+	+	−	−	+
XIV	SH 139	25–175	+	−	+	+	+	+	−	+
XV	SH 302	0–50	+	+	+	+	+	−	−	+
XVI	SH 458	0–25	+	+	+	+	−	+	−	−
XVII	SH 404	0–25	+	(+)	+	+	−	−	−	−
XVIII	SH 449	12·5–50	−	−	−	+	−	−	−	+
XIX	SH 382	12·5–175	−	+	+	+	−	+	+	+

presence of MK-6 as the major respiratory quinone did not allow differentiation within the group, it allows this group to be distinguished from all other prokaryotes that produce menaquinones of longer chain length. Similar results were found by Sittig & Schlesner (1993). Detailed studies on the fatty acids and polar lipids of a range of strains indicated that it was possible to differentiate distinct groups, highlighting the evolutionary heterogeneity of this group. Of the 97 strains investigated here, only six had immotile daughter cells. In the following sections, we focus on the taxonomic differentiation of *R. baltica*, *B. marina* and *Pirellula staleyi*.

Morphological characteristics

The pink-coloured *R. baltica* (SH 1^T) was isolated from Kiel Fjord, from the same sample as the unpigmented *B. marina* [=*Pirellula marina*; strain SH 106^T (=IFAM 1313^T=DSM 3645^T); Schlesner, 1986]. The morphology of *R. baltica* resembled that of other members of the *Planctomycetales*, in particular of the group of *Pirellula*-like bacteria, as indicated by the mode of budding, the presence of fimbriae and crateriform structures and the holdfast substance excreted directly from the smaller cell pole (Schlesner & Hirsch, 1984; Fig. 1a). In contrast to that of *B. marina* (Schlesner, 1986), the bud was not bean-shaped but was a smaller mirror image of the mother cell. Thin cryosubstituted sections of cells (Fig. 1b) showed membranous structures surrounding the nuclear material and the majority of the ribosomes. Analysis of cross-sections of strain SH 1^T as well as SH 796 (Gade *et al.*, 2004) revealed several small structures in addition to a large central one. This microscopic appearance differs from that of the pirellulosome structure described for *Pirellula staleyi* and *B. marina* (Lindsay *et al.*, 2001).

Physiological properties

The pigment of *R. baltica* could be extracted with methanol and ethanol but not with chloroform, ether or petroleum ether. The absorption spectrum of a methanol extract had a maximum at 495 nm and two shoulders at 460 and 520 nm and thus showed similarity to carotene. The sulfuric acid test for polyene carotenoids, however, was negative, and carotene is also soluble in chloroform and petroleum ether.

R. baltica and *B. marina* can be considered as marine bacteria since they do not grow in freshwater media. Growth of *R. baltica* occurred in media containing rising concentrations (12–175 %) of ASW (100 % ASW corresponds to a salinity of 34·5 ‰). Similar values were observed for *B. marina*. Essential components of ASW were Ca^{2+}, Na^+ and Cl^-. In contrast, the freshwater bacterium *Pirellula staleyi* tolerated only up to 50 % ASW. *R. baltica* required the addition of vitamin B_{12} to the medium, while *B. marina* and *Pirellula staleyi* were able to grow in vitamin-free media (Table 4).

Despite the 16S rRNA gene sequence difference between *R. baltica*, *B. marina* and *Pirellula staleyi*, their physiological properties were very similar. Substrates serving as carbon and energy sources were mainly carbohydrates. *N*-Acetylglucosamine also served as a nitrogen source. Chondroitin sulfate was an excellent carbon source for *R. baltica* and *B. marina*. All three organisms displayed catalase and cytochrome oxidase activities, but no urease activity; they produced H_2S from thiosulfate, but did not produce acetoin or indole. Initial tests on the mesophilic cells of *R. baltica* indicated that they appeared to be strictly aerobic, since they were unable to use nitrate as an electron

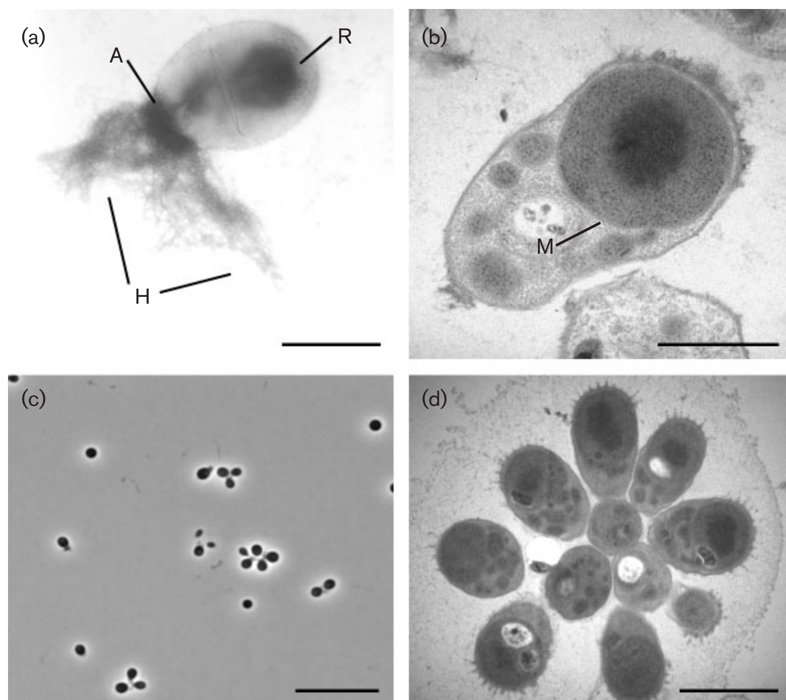

Fig. 1. Microscopic images of *R. baltica* strains SH 1^T (a–c) and SH 796 (d). (a) Electron-microscopic image of a single cell displaying the polar organization of *R. baltica* cells. R, Reproduction pole; A, attachment pole; H, holdfast substance. Bar, 0·5 μm. (b) Electron-microscopic image displaying the intracellular compartmentalization. M, Membrane engulfing the pirellulosome-like structures. Bar, 0·5 μm. (c) Light-microscopic image of rosette-like aggregates. Bar, 10 μm. (d) Electron-microscopic image of a rosette showing the attachment of cells via the attachment poles. Bar, 1 μm.

Table 4. Characteristics useful for differentiating between *R. baltica*, *B. marina* and *Pirellula staleyi*

+, Positive, main component; −, negative, not present; (+), present in small amounts.

Characteristic	*R. baltica* SH 1^T	*B. marina* DSM 3645^T	*Pirellula staleyi* ATCC 27377^T
Cell size (μm)	1·0–2·5 × 1·2–2·3	0·7–1·5 × 1·0–2·0	0·9–1·0 × 1·0–1·5
Pigmentation	Pink to red	Unpigmented	Unpigmented
Salinity tolerance (% ASW)	12–200	12–175	0–50
Vitamin requirement	B_{12}	−	−
Carbon source utilization:			
Fucose	−	+	+
Glycerol	+	+	−
Glutamic acid	−	+	−
Chondroitin sulfate	+	+	−
Hydrolysis of casein	−	−	+
Lipase	−	+	−
Polyamines [μmol (g dry weight)$^{-1}$]:[a*]			
Putrescine	19·5	−	−
Cadaverine	15·3	−	−
Spermidine	−	−	0·8
sym-Homospermidine	19·6	11·3	50·2
Phosphatidylcholine	+	−	−
Cell-wall amino acids (molar ratios):			
Threonine	9·0[b]	3·8[c]	3·0[b]
Glutamate	36·3[b]	11·3[c]	9·0[b]
Cysteine	9·2[b]	1·2[c]	3·6[b]
Valine	8·2[b]	2·4[c]	1·7[b]
Fatty acids (relative %):			
14 : 0	0·5	−	4·9
15 : 0	0·5	5·9	−
i-16 : 0	−	4·9	−
16 : 1Δ9	8·0	4·1	3·5
16 : 0	39·2	27·5	33·8
17 : 1Δ9	4·0	−	14·4
17 : 0	1·2	−	5·3
18 : 1Δ9	40·8	26·6	26·6
18 : 1Δ11	1·6	2·3	2·0
18 : 0	4·3	2·5	3·3
19 : 1Δ11	−	2·6	−
19 : 0	−	2·7	−
20 : 1Δ11	−	1·2	15·7
16S rRNA gene sequence similarity (%) to ATCC 27377^T/SH 1^T[d]	85·0/100	87·5/87·1	100/85·0

*Results from previous studies were taken from: *a*, Griepenburg *et al.* (1999); *b*, Liesack *et al.* (1986); *c*, König *et al.* (1984); *d*, Ward *et al.* (1995).

acceptor or to grow fermentatively with glucose. In agreement with a strictly aerobic metabolism, the genome of *R. baltica* revealed no evidence for an anaerobic ribonucleotide reductase. Thus, the predicted capacity for lactic acid fermentation (Glöckner *et al.*, 2003) may serve maintenance only.

Chemotaxonomic characteristics

Chemotaxonomic markers proved to be more useful than physiological properties for differentiating between *R. baltica*, *B. marina* and *Pirellula staleyi*. The chemotaxonomic markers that have been analysed are the fatty acid and phospholipid profiles (Kerger *et al.*, 1988; Sittig & Schlesner, 1993), the amino acid composition of cell walls (König *et al.*, 1984; Liesack *et al.*, 1986) and the polyamine patterns (Griepenburg *et al.*, 1999). In contrast, analysis of the quinone profile was not useful within the group, since all planctomycetes investigated so far have possessed MK-6 as the only quinone (Sittig & Schlesner, 1993; B. J. Tindall & H. Schlesner, unpublished). However, the presence of this

short-chain lipoquinone is useful for delineating this group and also in distinguishing it from other menaquinone-producing prokaryotes with longer isoprenoid side chains. It should be noted that the chemical composition of the cells (polar lipids, fatty acids and respiratory lipoquinone composition) provides a way of differentiating organisms within this group, but also indicates that the planctomycetes are chemically distinct from any other taxa examined to date. Detailed analyses of the fatty acids examined in this study are given in Table 4. All species produce 16 : 1Δ9, 16 : 0, 18 : 1Δ9 and 18 : 0 fatty acids. While these fatty acids are fairly common in members of the α-, β- and γ-subclasses of the *Proteobacteria*, this combination, together with the presence of MK-6, clearly distinguishes these species from these major evolutionary groups. Among the strains examined, the presence/absence of 14 : 0, 15 : 0, i-16 : 0, 17 : 1Δ9, 17 : 0 and 20 : 1Δ11 can be used to distinguish between different taxa. While Kerger *et al.* (1988) reported the presence of hydroxy fatty acids in planctomycetes, they only deduced that they originated from lipopolysaccharides, providing no direct proof. In this study, the methods used would detect the presence of lipopolysaccharide-derived hydroxy fatty acids present in *Escherichia coli*. Thus we conclude that the absence of measurable amounts of hydroxy fatty acids is indicative of the absence of significant amounts of lipopolysaccharides in the cell wall, despite the fact that these organisms are Gram-negative. Sittig & Schlesner (1993) provided the first indications of the chemical heterogeneity of this group, but the full significance of this is now evident when different (phenotypic and genetic) datasets are integrated.

The polar lipid patterns determined by two-dimensional TLC are shown in Fig. 2. The polar lipid compositions of the three type strains are clearly different. Investigations performed on a wider range of strains (Sittig & Schlesner, 1993; B. J. Tindall and H. Schlesner, unpublished) indicate that this chemical diversity also correlates well with the 16S rRNA gene diversity. Thus the presence of phosphatidylcholine in *R. baltica* is not just a feature of this species, but is also found in other strains that group with this species in 16S rRNA gene studies. Similarly, the polar lipid patterns of *Pirellula staleyi* and *B. marina* also indicate features that allow them to be differentiated not only from *R. baltica*, but also from one another. The use of spray reagents that render all lipid-like material visible shows that a significant percentage of the cellular lipids are novel. To date, there are no indications that these unidentified lipids are present in any other taxa beyond planctomycetes.

Resistance to antibiotics

All three strains were resistant to ampicillin and penicillin (1000 μg ml^{-1}), cephalotin (100 μg ml^{-1}), streptomycin (500 μg ml^{-1}) and cycloserine (100 μg ml^{-1}), but not to tetracycline (10 μg ml^{-1} was lethal).

Genome of *R. baltica*

The complete sequence of the 7·145 Mb genome of *R. baltica* (SH 1^T) was only recently reported (Glöckner *et al.*, 2003). Sequence analysis revealed the complete genetic blueprint for glycolysis, the pentose phosphate cycle and the tricarboxylic acid cycle, which agrees with the specialization in carbohydrate utilization found for this strain in the present study. A surprising finding was the presence of more than 100 genes possibly encoding sulfatases. It could be speculated that growth with chondroitin sulfate, as found here, may require the activity of a specific sulfatase that liberates the carbohydrate moiety. The first insights into the regulation of carbohydrate metabolism were recently obtained by means of a proteomic approach (Rabus *et al.*, 2002).

The presence of known phospholipids (phosphatidylcholine and phosphatidylglycerol), together with the presence of novel compounds (of as yet unknown structures),

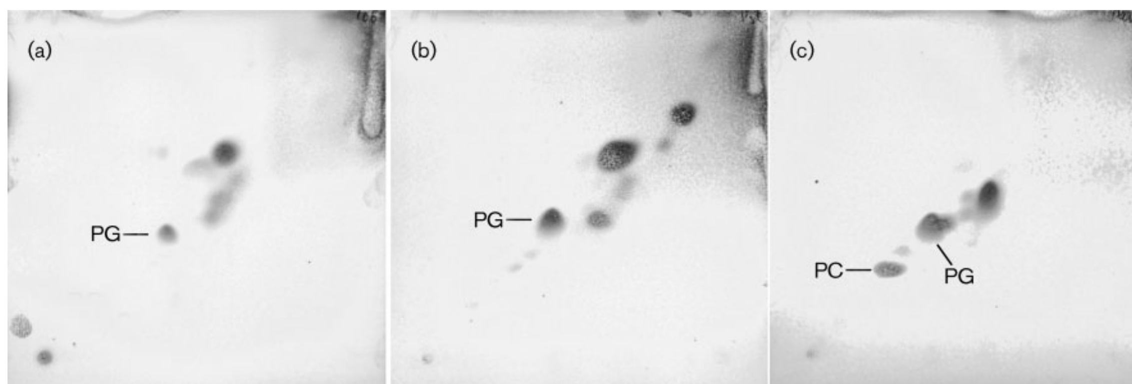

Fig. 2. Two-dimensional thin-layer chromatograms of the polar lipids of *B. marina* (a), *Pirellula staleyi* (b) and *R. baltica* (c). All polar lipids were stained with 5 % (v/v) ethanolic molybdophosphoric acid. Solvents: chloroform/methanol/water (65 : 25 : 4, by vol.), first dimension; chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.), second dimension. Unlabelled lipids could not be fully characterized or assigned to known structures. PG, Phosphatidylglycerol; PC, phosphatidylcholine.

will provide a stimulus for examining the biosynthesis and gene regulation of these cellular components, and for locating the genes responsible. The presence of phosphatidylcholine in *R. baltica* as the sole nitrogen-containing phospholipid is interesting, since it is generally accepted that phosphatidylcholine is synthesized via progressive methylation of phosphatidylethanolamine (Pieringer, 1989). Polar lipid patterns containing phosphatidylethanol and phosphatidylcholine are typical of certain actinomycetes and some major evolutionary groups within the α-subclass of the *Proteobacteria* (Ratledge & Wilkinson, 1989). However, there are currently no reliable reports of prokaryotes containing phosphatidylcholine as the sole nitrogen-containing phospholipid, and this may be indicative of an alternative pathway leading to the synthesis of phosphatidylcholine in these organisms.

In re-examining the taxonomy of the *Pirellula* group within the planctomycetes, we have attempted to integrate as much current knowledge about this group as possible. Although DNA–DNA hybridization studies are generally considered to be problematic from a methodological standpoint, as well as with respect to determining which fragments of DNA are involved in binding, this method still serves as one of the best ways of gaining an indirect insight into overall similarities between genomes. The results presented in this work clearly indicate that the strains currently available in pure culture not only constitute a diverse range of strains (according to 16S rRNA gene studies), but also that they are represented by numerous, different species. Differences in 16S rRNA gene sequences of more than 3 % (Stackebrandt & Goebel, 1994) are generally indicative of different species (when the species groups are tested by DNA–DNA hybridization). Thus, differences in the 16S rRNA gene sequence are indicative of differences at the 'genetic level', which will certainly be evident in the genomes of the organisms concerned. Unfortunately, this genetic (and of course evolutionary) diversity does not appear to be reflected in the physiology of the organisms concerned, as was experimentally determined using classical phenotypic tests. While there are clearly marine and freshwater strains, the ability of the organisms to utilize the range of substrates tested would suggest that members of this group are rather uniform. However, this uniformity at the level of substrate utilization does not tell us anything about the potential diversity in the underlying biochemical pathways, or about the structural diversity of the enzymes concerned. Further studies on genomes of different species and different strains within the planctomycetes will help to elucidate this point.

In contrast to the apparently 'limited' physiological diversity, the diversity in chemical composition indicates that the use of this polyphasic taxonomic approach, as in other prokaryotes, reflects the evolutionary diversity that we also detect via constrained elements, such as the ribosome. Thus, strains that share a high degree of genetic similarity (as reflected by DNA–DNA hybridization and high 16S rRNA gene similarity values) are difficult to distinguish chemically, but, with increasing genetic diversity (as reflected by decreasing DNA–DNA hybridization values and decreasing 16S rRNA gene sequence similarity), the chemical differences become increasingly evident. In such instances, changes in the chemical composition may reflect changes either in regulatory mechanisms or in the biochemical pathways leading to the synthesis of the end-products. Such changes are as significant in the evolution of the cell as the changes in the sequences of genes, such as the 16S rRNA gene. Taking these aspects into consideration, we suggest that it is possible to examine their significance in the evolution of prokaryotes and the taxonomy upon which this is based.

While planctomycetes have been described as rapidly evolving or ancient, it is evident from the genome size that this is probably representative of a later stage in evolution and it is debatable whether this is a feature of a 'primitive' group of organisms. Arguments centring on the fact that planctomycetes are rapidly evolving (Liesack *et al.*, 1992) are based on Simpson's work on 'tempo and mode' in evolution (Simpson, 1944). Key arguments in favour of rapid evolution are the large 16S rRNA gene differences between species within the genera *Planctomyces* and *Pirellula* as well as the low 16S rRNA gene similarity values between genera (Liesack *et al.*, 1992). In addition, idiosyncrasies in the 16S rRNA gene sequence, together with other peculiarities of planctomycetes, are used in support of the hypothesis that these organisms are evolving rapidly. However, when evaluating rates of evolution, both Simpson (1944) and Mayr (1969) have considered the geological time-scale, a feature usually missing from the majority of gene sequence comparisons. The problem is compounded by the fact that calibration rates are ultimately based on a known fossil record (Doolittle, 1997; Doolittle *et al.*, 1996; Feng *et al.*, 1997; Lee, 1999), and even then rates may vary between lineages (Ochman *et al.*, 1999; Soltis *et al.*, 2002). In addition, there are no reliable reports of 'molecular fossils' that are more than 50–100 million years old (Poinar *et al.*, 1996; Willerslev *et al.*, 2003). Such problems have been highlighted by Sneath (1974) and are beginning to be discussed again.

In recent years, detailed three-dimensional structures of ribosomes have been published: these have indicated the significance not only of secondary structure (in both proteins and RNA molecules), but also of the diverse close RNA–RNA and RNA–protein interactions (Ban *et al.*, 2000; Brimacombe, 2000; Schluenzen *et al.*, 2000; Wimberly *et al.*, 2000). Thus, the idiosyncrasies in the 16S rRNA gene sequence should be considered in the light of these interactions, rather than the gene sequence alone. Similarly, the fact that planctomycetes are characterized by a set of other peculiarities (to which we can also add the polar lipid composition) certainly serves to underline the uniqueness of this group of organisms, but are not necessarily indicative of rapid rates of evolution. Morse

et al. (1996) have also called into question the suggestion that *Oenococcus oeni* is evolving rapidly.

On the basis of the data presented here, we consider that planctomycetes are diverse across a range of properties, and that this diversity is a reflection of extensive evolution. While we cannot easily say whether the evolution of this group has taken place over short or long periods of geological time, it is evident that the current taxonomy does not reflect the diversity that we find in this group. We propose, therefore, a number of taxonomic changes, based on the closer study of a limited number of strains, which may also further serve as a basis for evaluating the systematics of this group of organisms.

Properties that differentiate the genera *Rhodopirellula*, *Blastopirellula* and *Pirellula*

Properties of the type strains of *R. baltica*, *B. marina* and *Pirellula staleyi* that allow differentiation of the three genera are summarized in Table 4. Pigmented cells are observed only for *R. baltica*. Growth of *R. baltica* and *B. marina* requires high concentrations of sodium chloride and calcium, whereas *Pirellula staleyi* has only limited tolerance to ASW. Even though all type strains grow with carbohydrates, some difference can be noted. *R. baltica* and *Pirellula staleyi* are not able to utilize fucose and chondroitin sulfate, respectively. Only *Pirellula staleyi* can utilize glutamic acid and hydrolyse casein, while lipase activity can be observed only in *B. marina*. Polyamine patterns differ between the type strains. While all contain *sym*-homospermidine, putrescine and cadaverine are found only in *R. baltica*, and spermidine is found only in *Pirellula staleyi*. Only *R. baltica* possesses phosphatidylcholine. Differences are also observed with respect to the molar ratios of the cell-wall amino acids. The fatty acid patterns of the three organisms differ as follows: *R. baltica* and *Pirellula staleyi* lack i16 : 0, 19 : 1Δ11 and 19 : 0, which are found in *B. marina*. In contrast, 17 : 1Δ9 and 17 : 0 are present in the former two but absent in the latter. The intracellular compartmentalization is formed by different structures. The pirellulosomes described for *B. marina* and *Pirellula staleyi* (Lindsay *et al.*, 2001) show a single large structure in the electron microscopic image, whereas multiple smaller structures are visible in addition to a large one in the case of *R. baltica*. These considerable differences between the type strains are also reflected at the genetic level, as indicated by DNA–DNA hybridization (Table 2) and the low similarity of the 16S rRNA gene sequences (<90 %; Table 4).

Emended description of the genus *Pirellula* Schlesner and Hirsch 1987

The description of the genus *Pirellula* is largely based on physiological, biochemical and morphological properties, and it would be appropriate to emend the description to take into account both additional data and our changing appreciation of the taxonomy of this group. The biochemical, physiological and morphological characteristics are described by Schlesner & Hirsch (1984). The major polyamine is *sym*-homospermidine. The major respiratory lipoquinone present is MK-6. The major phospholipid present is phosphatidylglycerol. A number of other lipids are present that have characteristic R_f values, but whose structures are not currently known. However, the lipid pattern is characteristic of this genus. The major fatty acids present are 14 : 0, 16 : 1Δ9, 16 : 0, 18 : 1Δ9, 18 : 1Δ11, 18 : 0 and 20 : 1Δ9. It is also evident that 16S rRNA gene sequence similarity values are of significance in delineating this genus. Nevertheless, the extent cannot be defined at present, since strains with less than 95 % sequence similarity to members of this genus should probably be placed in separate genera. The type species of the genus is *Pirellula staleyi.*

Description of *Rhodopirellula* gen. nov.

Rhodopirellula (Rho.do.pi.rel′lu.la. Gr. neut. n. *rhodon* a rose; N.L. fem. n. *Pirellula* name of a bacterial genus; N.L. fem. n. *Rhodopirellula* a red *Pirellula*).

Cells are ovoid, ellipsoidal or pear-shaped, occurring singly or in rosettes by attachment at the smaller cell pole. Buds are formed at the broader cell pole. Buds may have a single flagellum inserted subpolarly at the proximal pole. Adult cells are immobile. Crateriform structures and fimbriae are found in the upper cell region. Colonies are pink to red in colour. Non-sporulating. Strictly aerobic. Catalase- and cytochrome oxidase-positive. The proteinaceous cell wall lacks peptidoglycan. The major polyamines are putrescine, cadaverine and *sym*-homospermidine. The major menaquinone is MK-6. The major fatty acids are 16 : 1Δ9, 16 : 0, 17 : 1Δ9, 17 : 0, 18 : 1Δ9, 18 : 1Δ11 and 18 : 0. The major phospholipids are phosphatidylcholine and phosphatidylglycerol. Additional, unidentified polar lipids are also present. This genus is a member of the phylum *Planctomycetes*, order *Planctomycetales*, family *Planctomycetaceae*, as currently defined primarily on the basis of 16S rRNA gene sequence analysis. The type species is *Rhodopirellula baltica.*

Description of *Rhodopirellula baltica* sp. nov.

Rhodopirellula baltica (bal′ti.ca. L. fem. adj. *baltica* pertaining to the Baltic Sea, the place of isolation).

Cells are 1·0–2·5 × 1·2–2·3 μm in size. A single flagellum is subpolarly inserted at the proximal pole. Colonies are round, smooth and pink to red in colour. Growth is optimal between 28 and 30 °C. Growth is not observed above 32 °C. Vitamin B_{12} and sea water are required for growth. The bacterium is strictly aerobic. Glucose is not fermented. Nitrate cannot serve as an electron acceptor. Carbon sources utilized are as follows: cellobiose, fructose, galactose, glucose, lactose, lyxose, maltose, mannose, melibiose, melezitose, raffinose, rhamnose, ribose, sucrose, trehalose, xylose, dextrin, *N*-acetylglucosamine, glycerol, aesculin, amygdalin, gluconate, glucuronate, salicin and chondroitin sulfate. Carbon sources not utilized are as follows: fucose,

sorbose, methylamine, methylsulfonate, methanol, ethanol, erythritol, adonitol, arabitol, dulcitol, inositol, mannitol, sorbitol, acetate, adipate, benzoate, caproate, citrate, formate, fumarate, glutarate, lactate, malate, 2-oxoglutarate, phthalate, propionate, pyruvate, succinate, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, urea, indole, inulin and pectin. Peptone, Casamino acids, yeast extract, gelatin, ammonium, nitrate and *N*-acetylglucosamine are each utilized as a nitrogen source, but neither nicotinate nor urea is utilized. Aesculin, gelatin and starch are hydrolysed, but alginate, casein, cellulose, chitin and Tween 80 are not hydrolysed. No haemolytic activity is found with horse, calf or sheep blood. Catalase and cytochrome oxidase activities are observed, but urease activity is not observed. H_2S, but not acetoin or indole, is produced. Cells are sensitive to tetracycline, but resistant to streptomycin, ampicillin and penicillin. The G+C content of the DNA is 53–57 mol% (55 mol% for the type strain). The chemical composition is identical to that in the genus description. The main habitat is brackish water of Kiel Fjord (Baltic Sea).

The type strain is SH 1^T (=IFAM 1310^T=DSM 10527^T =NCIMB 13988^T).

Description of *Blastopirellula* gen. nov.

Blastopirellula (Blas.to.pi.rel′lu.la. Gr. masc. n. *blastos* bud, shoot; N.L. fem. n. *Pirellula* name of a bacterial genus; N.L. fem. n. *Blastopirellula* a budding *Pirellula*).

Cells are ovoid, ellipsoidal or pear-shaped, occurring singly or in rosettes by attachment at the smaller cell pole. Buds are formed at the broader, proximal cell pole. Adult cells are immobile. Crateriform structures and fimbriae are found in the upper cell region. Colonies are greyish to brownish white. Non-sporulating. Strictly aerobic. Catalase- and cytochrome oxidase-positive. The proteinaceous cell wall lacks peptidoglycan. The major polyamine is *sym*-homospermidine. The major menaquinone is MK-6. The major phospholipid present is phosphatidylglycerol. Additional unidentified polar lipids are also present, at least one of which appears to be identical (in R_f value) to one of the major components present in members of the genus *Pirellula*. The major fatty acids present are 15:0, i-16:0, 16:1Δ9, 16:0, 17:1Δ9, 17:0, 18:1Δ9, 18:1Δ11, 18:0, 19:1Δ11 and 20:1Δ9. This genus is a member of the phylum *Planctomycetes*, order *Planctomycetales*, family *Planctomycetaceae*, as currently defined primarily on the basis of 16S rRNA gene sequence analysis. The type species is *Blastopirellula marina*.

Description of *Blastopirellula marina* comb. nov.

Blastopirellula marina (ma.ri′na. L. fem. adj. *marina* of, or belonging to, the sea, marine).

Basonym: *Pirellula marina* (Schlesner 1987) Schlesner and Hirsch 1987

The description is the same as that published for *Pirellula marina* (Schlesner, 1986), with the following additions. The species is apparently strictly aerobic, as glucose is not metabolized anaerobically either by fermentation or with nitrate as the electron acceptor. The chemical composition is identical to that in the genus description.

The type strain is SH 106^T (=IFAM 1313^T=DSM 3645^T =ATCC 49069^T).

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