John A. Fuerst *Editor*

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Humana Press

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Editor
John A. Fuerst
School of Chemistry and Molecular Biosciences
The University of Queensland
St. Lucia, QLD, Australia

ISBN 978-1-62703-501-9 ISBN 978-1-62703-502-6 (eBook)
DOI 10.1007/978-1-62703-502-6
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013939735

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The planctomycetes have been from the moment of their discovery organisms on the edge of our understanding, on the frontier of our knowledge of what microorganisms might be like, forming new models for microbial life and for biology. They have from this time on posed many fascinating and stimulating problems regarding their true identity, their evolutionary relationships, and their cell structure and biology. Even their name reflects this intriguing ambiguity—encapsulating the idea as actinomycetes once did that a eukaryote affinity might exist. The basis for that early decision—morphological similarity with fungi—is now not supportable since the pioneer work of Jean Schmidt and Mortimer Starr revealed the non-cellular nature of the stalks mistaken for mycelia in those early aquatic rosettes of *Planctomyces bekefii* seen originally only via light microscopy (Chapter 1). Perhaps though the time is not yet ripe to unreservedly apply the name 'planctobacteria' to these organisms as some have done informally. Planctomycetes have since Gimesi's time emerged as organisms with an internal organizational plan which appears to be one of the most complex known in bacteria or archaea—the cell contents are divided by internal membranes into two or even three distinct compartments, the nucleoid DNA is tightly folded, and in some cases membrane vesicles can form, imparting the ability to incorporate macromolecules from the environment analogous to eukaryote endocytosis and probably via similar molecular mechanisms (Chapters 2 and 3). They are indeed new models for cell structure, stretching our imagination of what a bacterial cell can look like and challenging our concept of a 'prokaryote' at the purely organizational level (without even considering the impact of phylogenetics and Archaea on this concept). The pure culture models for planctomycete cell biology and genetics *Gemmata obscuriglobus*, *Planctomyces limnophilus* and *Rhodopirellula baltica* have been central to our progress in these areas. Planctomycetes are of central significance to evolutionary microbiology and cell biology and must be taken into account in any future theories of eukaryote and eukaryote nucleus origins (Chapter 11). Thus, planctomycetes are of wide significance not only to microbiology but also to the biology of most organisms visible to the naked eye and must be taken into account if we are to solve major problems of biology concerning the marked transitions in life's evolution involving cellular

complexity. Changing perspective to our contemporary global problems, planctomycetes are also ready to help. There are now immense bioreactors at industrial scale, from the Netherlands city of Rotterdam to a monosodium glutamate factory in China, where anaerobic ammonium-oxidizing planctomycetes (Chapter 4) help us clean up environmental ammonia-rich waste while saving energy and reducing our CO_2 footprint. In addition, marine versions of these planctomycetes are central to the global nitrogen cycle, responsible for at least 50 % of nitrogen removal from marine ecosystems, and substantial amounts of the nitrogen we breathe may be produced by marine anammox species growing in oxygen-minimum zones of the world's oceans; regions thought to inevitably increase with increasing global warming. This anammox process is dependent on the internal membrane-bounded compartment known simply as the anammoxosome, a body which may be a true energy-generating organelle, one unique within the bacteria, but bearing comparison with eukaryotic mitochondria in some ways (Chapter 4). New unusual habitats such as acid peat bogs have excitingly revealed new taxonomic diversity among the planctomycetes and thrown light on the potential breadth of their ecological roles and importance (e.g. in microbial communities of ecosystems under threat with global climate change) (Chapter 5). If this were not enough microbiological stimulation from one bacterial group, it turns out that they harbour enzymes known previously from C1 transfer pathways involving methane generation and oxidation, and which may be significant for our understanding of how such major geomicrobiological processes for the global carbon cycle may have originated (Chapter 8). Of course, the answers to many of our questions regarding planctomycete cell biology and biochemistry may await the development of genetic systems so powerful for analysing the functions of genes in other bacteria—promising progress is being made to give planctomycetologists these essential molecular tools, and proteomics has already made progress in the understanding of unique features such as the protein cell wall of the model marine planctomycete *Rhodopirellula baltica* (Chapter 6). In the meantime, genomics and bioinformatics are revealing important features for our understanding and will provide a solid necessary basis for any future experimental genetics (Chapter 7). One of the remarkable features of planctomycetes revealed by genomics complemented is their possession of enzymes for pathways manipulating C1 compounds (Chapter 8), but in the apparent absence of methane oxidizing or generating abilities. Whatever their contemporary function, these enzymes are of great evolutionary interest, since they seem to be quite divergent from those known in other bacteria and in archaea, and perhaps go back to the very beginnings of methane biogeochemistry on Earth.

Beyond planctomycetes, we now know that planctomycetes have relatives within the bacteria, in the PVC superphylum, and comparative cell biology and genomics between members of this superphylum may form one of the keys to understanding their evolution. New extremophile PVC verrucomicrobia in the genus *Methyloacidiphilum* (Chapter 9) which, in contrast with planctomycetes, possess both C1 transfer pathways and methane oxidation metabolism, have added to our understanding of the immense physiological diversity within the PVC superphylum, encompassing as it does not only this thermoacidophilic methane oxidizer but also mesophilic aerobic chemoheterotrophs such as *Gemmata obscuriglobus*, the

moderately thermophilic phototactic planctomycete *Isosphaera pallida*, obligately anaerobic chemolithoautotrophs like the anammox planctomycetes, anaerobic human intestinal microbiome organisms like *Akkermansia muciniphila* in the verrucomicrobia and *Victivallis vadensis* in the Lentisphaerae, as well as the obligate intracellular pathogens in the phylum *Chlamydiae* (Chapter 10). Of necessity, this book discusses only some of the many significant PVC species beyond the planctomycetes.

Perhaps one of the reasons that planctomycetes and their relatives are frontier microorganisms is that they indeed include some very ancient bacteria representing some features of the pioneer habitats first available on the early earth (in the case of the anaerobic anammox 'ammonium eaters') and perhaps some features of the very earliest eukaryotes or even a eukaryote-like last common ancestor of the 3 Domains (Chapter 11). Analyses of the likely nitrogen cycle on the early Earth, for example, suggest that anammox planctomycetes were the first producers of nitrate on the planet and that anammox was the only process which could have closed the nitrogen cycle returning fixed nitrogen to the dinitrogen pool in the anaerobic biosphere. If alternatively planctomycetes or their ancestors did later on contribute by gene transfer or more direct vertical inheritance to the molecular basis of eukaryality, those events must have been ancient also. The phylogenetic and bioinformatic analyses are still controversial on how ancient planctomycetes and their closest relatives may be and on how homologous their eukaryote-like features to eukaryotes might be. Whatever the case, due to their widespread presence and activities they are one of the central microbial keys to understanding natural aquatic, terrestrial and perhaps even human microbiome microbial communities, and are a key to understanding the possible mechanisms of origin of the type of cell organization our very own human cells have inherited from the first eukaryote. They may thus form a model for origins of the biology of the modern cell and a key to truly understanding our own biology at the deep evolutionary level. As the late Carl Woese, the great discoverer of the Archaea and the three Domains of life emphasized, without such an evolutionary understanding there is no truly deep understanding of any life, that essentially historical entity.

Planctomycetes and their relatives are an excellent example of how understanding the true extent of microbial diversity can yield insights for science unimaginable if our focus was trained exclusively on *E. coli*. I would like this book also to widen your microbial, biological and scientific horizons to include the planctomycetes, new models for cell structure, origins and biology.

I would like to express my sincere thanks to all our authors—their great contributions have made this first book on planctomycetes focused on their cell biology possible. We would hope that in the future there will be another volume wholly devoted to the significant ecology and environmental significance of the planctomycetes. I also extend my thanks to Springer for publishing this book, one which will be immensely valuable for those in the field of planctomycetology and those entering it for the first time (of whom we hope there will be many more!).

St. Lucia, QLD, Australia John A. Fuerst

Contents

Contributors

Claire Bertelli Institute of Microbiology, University of Lausanne, Lausanne, Switzerland

SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

Ludmila Chistoserdova Department of Chemical Engineering, University of Washington, Seattle, WA, USA

Svetlana N. Dedysh Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia

Damien P. Devos Centre for Organismal Studies (COS) Heidelberg, Ruprecht-Karls-Universitat, Heidelberg, Germany

European Molecular Biology Laboratory, Heidelberg, Germany

Peter F. Dunfield Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

John A. Fuerst School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

Gilbert Greub Institute of Microbiology, University of Lausanne, Lausanne, Switzerland

Daniel H. Haft J. Craig Venter Institute, Rockville, MD, USA

Cheryl Jenkins NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia

Christian Jogler Leibniz-Institut DSMZ, Braunschweig, Germany

Mareike Jogler Leibniz-Institut DSMZ, Braunschweig, Germany

Olga K. Kamneva Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

Stormy J. Knight Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

Irina S. Kulichevskaya Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia

David A. Liberles Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

Sarah Neumann Department of Microbiology, Faculty of Science, Institute for Water & Wetland Research, Radboud University Nijmegen, Nijmegen, The Netherlands

Huub J.M. Op den Camp Department of Microbiology, IWWR, Radboud University Nijmegen, Nijmegen, The Netherlands

Evgeny Sagulenko School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

Christine E. Sharp Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

James T. Staley Department of Microbiology, University of Washington, Seattle, WA, USA

Ivica Tamas Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Laura van Niftrik Department of Microbiology, Faculty of Science, Institute for Water & Wetland Research, Radboud University Nijmegen, Nijmegen, The Netherlands

Muriel C.F. van Teeseling Department of Microbiology, Faculty of Science, Institute for Water & Wetland Research, Radboud University Nijmegen, Nijmegen, The Netherlands

Naomi L. Ward Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

Richard I. Webb School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

Chapter 1
History, Classification and Cultivation of the Planctomycetes

Cheryl Jenkins and James T. Staley

Contents

1.1 History and Classification of the Planctomycetes

1.1.1 The History of the Planctomycetes

The first report of the *Planctomycetes* phylum came from Nándor Gimesi, a Hungarian biologist who observed and photographed an unusual microcolonial form he found in Lake Lagymanyos in Budapest (Gimesi 1924). At this time, this lake although relatively wild was apparently eutrophic with a high organic and also high sulphate content possibly due to pollution from nearby farms (Langó 2005). Because he thought they were planktonic fungi, he named the type species of the

C. Jenkins (✉)
NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568, Australia
e-mail: cheryl.jenkins@dpi.nsw.gov.au

J.T. Staley
Department of Microbiology, University of Washington, 735735, Seattle, WA 98195, USA
e-mail: jtstaley@u.washington.edu

J.A. Fuerst (ed.), *Planctomycetes: Cell Structure, Origins and Biology*,
DOI 10.1007/978-1-62703-502-6_1,

genus, *Planctomyces bekefii* (from Gr. adj. *planktos*, wandering, floating; Gr. n. *mûkes*, fungus; n. *planctomyces*, floating fungus). The species was named to honour a Hungarian abbot, Remigius Békefi (1858–1924), cultural historian, university professor and abbot of the Hungarian Cistercian Order. The organism, as observed in samples from its aquatic habitat, has a very distinctive morphology with several spherical cells each with its own stalk with a holdfast at its tip that holds the cells together to form a microcolonial rosette (Fig. 1.1a, b). Much later, in the 1970s, by which time the lake had been filled in so that its extent had been reduced to a pond several hundred square metres in extent close to a railway bridge across the Danube(Langó 2005), the *Pl. bekefii* morphotype was still able to be documented in this type locality (Schmidt and Starr 1980a—see below).

Arthur T. Henrici's laboratory in the USA was the first bacteriological group to observe members of the *Planctomycetes* (Henrici and Johnson 1935). In the 1930s they incubated microscope slides in Midwestern lakes and removed and photographed them after several days' incubation. In their investigations, Johnson and Henrici reported budding bacteria that they called *Blastocaulis sphaerica* for the stalked forms (Fig. 1.2a–c) and *Blastobacter* for the non-stalked forms. They were unaware of the previous work by Gimesi. Although they did not isolate any of these organisms, it is clear that they are members of the *Planctomycetes* based upon their distinctive morphology and evidence of budding cell division. Indeed, Peter Hirsch, who later carefully compared *Pl. bekefii* to *Blastocaulis sphaerica*, concluded that they were members of the same genus (Hirsch 1972).

When the Approved List of Bacterial Names was prepared by V. B. D. Skerman in 1980, *Pl. bekefii* was included as one of the few bacteria that had a type species that was not in pure culture. And so it remains to this day (Ward 2010). *Pl. bekefii* has been reported elsewhere in Europe, Asia, Australia and North America where it is found in ponds and lakes. However, it is noteworthy that there are differences in the observed morphology of *Pl. bekefii*-like organisms depending on the locations where it has been reported. For this reason, it is likely that these geographically separated types may comprise different species (Schmidt and Starr 1980a).

A number of limnologists reported observing *Planctomyces* spp.. in freshwater lakes, and many different species names were ascribed to them based on their morphological traits alone (Hirsch and Skuja 1974). Undoubtedly the most morphologically striking species is *Planctomyces guttaeformis* (Hortobágyi 1965) in which the cells are not spherical, but are club shaped, and the mature cells in the rosette bear a long, tapering apical appendage that extends over 20 μm in length (Fig. 1.3). The species name means 'drop shaped'. Club-shaped buds are produced beneath the long apical appendage. The buds lack the apical appendage indicating that it is formed later in the organism's life cycle. Interestingly, the cells are joined together by a holdfast at the narrow pole of the club, so they do not have a stalk like *Pl. bekefii*. The narrow part of the club with its holdfast, which connects the cells together, appears to be cellular and not the acellular filamentous stalk found in *Pl. bekefii*. The long apical appendage of *Pl. guttaeformis* consists of multiple fibrils structurally analogous to the *Pl. bekefii* stalk (Fig. 1.3); however, it does not have a holdfast at its tip (Starr and Schmidt 1984).

Another named but uncultivated species, *Planctomyces stranskae* (named after the discoverer F. Wawrik's biology teacher W.L. Stransky), produces club-shaped cells

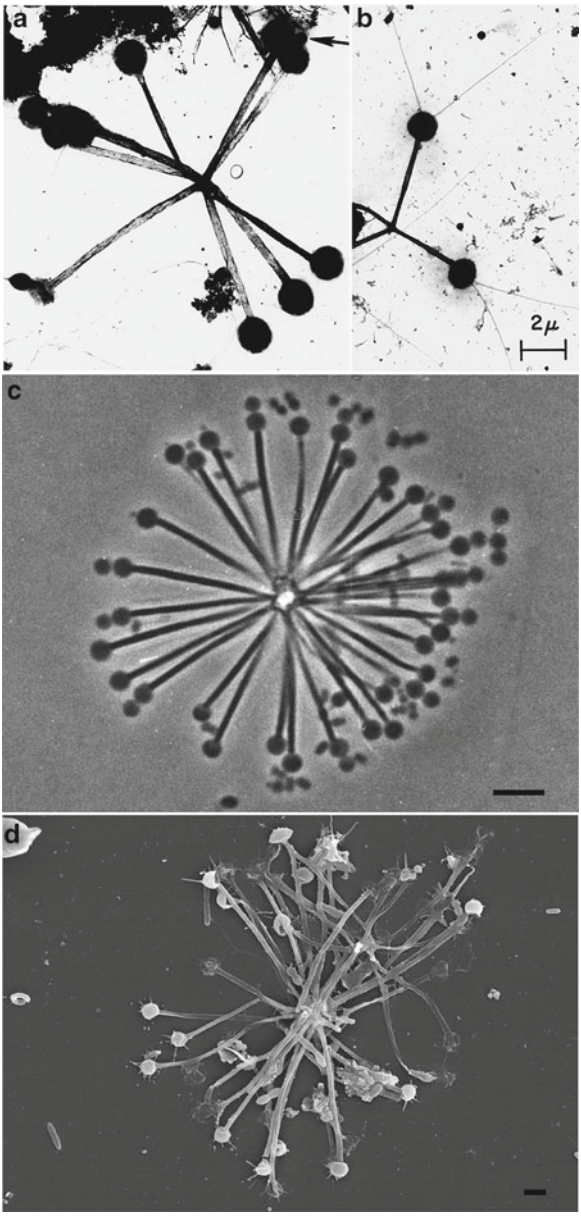

Fig. 1.1 (**a**) Electron micrograph of a microcolony of *Planctomyces bekefii* cells from University Lake near Chapel Hill, North Carolina. Note the single bud forming on one cell (see *arrow*). (**b**) Electron micrograph of another *Pl. bekefii* rosette from University Lake showing the two long apical spines that emanate from each cell. Cell diameters are approximately 1.5–2.0 μm (supplied courtesy of J.T. Staley). (**c**) Phase contrast micrograph of a *Pl. bekefii* rosette from Australian lake water at the University of Queensland (Bar = 5 μm) (from Fuerst (1995). Micrograph by J.A Fuerst and J.T. Staley). (**d**) Scanning electron micrograph of a *Pl. bekefii* rosette from The University of Queensland lake (Bar = 1 μm) (from Margaret K Butler (2006) PhD thesis (The University of Queensland). Supplied courtesy of J.A. Fuerst.)

like those of *Pl. guttaeformis*; however, this species lacks the distinctive long apical appendage produced by *Pl. guttaeformis* (Starr and Schmidt 1984). On the basis of such variable morphology, it seems incongruous that *Pl. guttaeformis* and *Pl. stranskae* are placed in the same genus as *Pl. bekefii*. However, the true taxonomic status of these organisms will remain unclear until pure cultures can be obtained and studied (Schmidt et al. 1981). A number of other morphospecies of *Planctomyces* have been described, but these are regarded as species incertae sedis (Ward 2010).

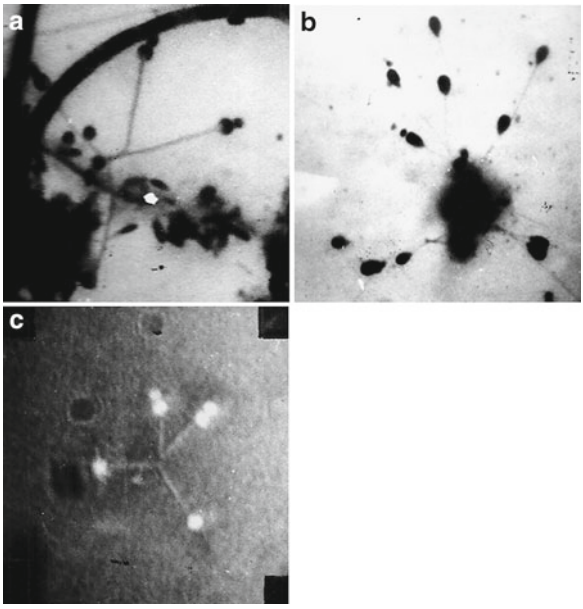

Fig. 1.2 (**a**) Light photomicrograph of the *Blastocaulis sphaerica* organism that Henrici and Johnson published in 1935[1]. (**b**) Light photomicrograph of another *Blastocaulis* rosette from A. T. Henrici's unpublished photos[1]. Note that the cells of this unidentified *Blastocaulis* species are pear shaped. (**c**) Light photomicrograph of what we regard as a *Planctomyces bekefii* rosette (negatively stained) with some budding cells from Henrici's unpublished photographs[1]. While the photo quality is poor, the resemblance to *Pl. bekefii* is clear. Note evidence of the black tape that Henrici used to adhere the photos to folio paper in C.

Jean Schmidt and Mortimer Starr in the USA studied these and other microcolonial forms of planctomycetes from freshwater habitats (e.g. from Arizona but also from the type habitat of *Pl. bekefii* in Hungary) and developed a morphotype system of classification of the genus *Planctomyces* (largely based on cell and stalk morphology) to avoid premature commitment to nomenclature (Schmidt and Starr 1978, 1979a, b, 1980b, 1982). However, the reliance of the morphotype system on stalk dimensions and appearance was problematic in that the acellular stalks can become encrusted with iron and manganese oxides, obscuring their fine structure (Schmidt et al. 1981, 1982). Furthermore, following the isolation of the first few planctomycete representatives in axenic culture, it was evident that culture conditions can affect the appearance of the stalk in *Planctomyces* spp., while in other planctomycete genera, acellular stalks are not formed (Schmidt 1978; Staley 1973; Schlesner 1986).

[1] A. T. Henrici's photomicrographs shown here along with another from his 1935 publication were left with Professor Erling Ordal in the Deparment of Microbiology at the University of Washington after Henrici's death and were given to JTS at the time of Professor Ordal's retirement. They have been returned to Professor Marty Dworkin in the Department of Microbiology at the University of Minnesota

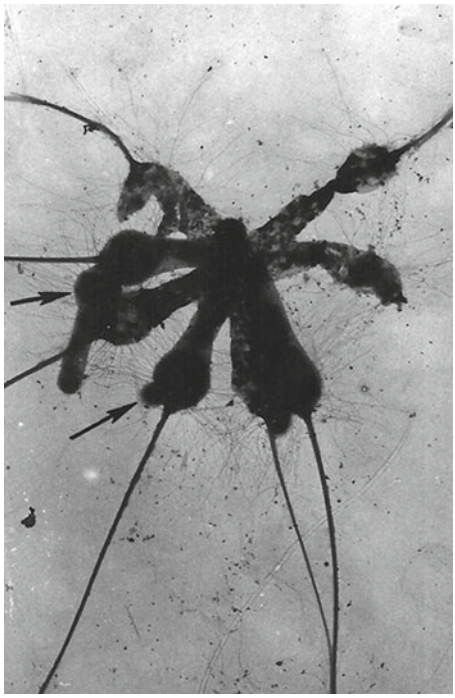

Fig. 1.3 Electron micrograph of *Planctomyces guttaeformis* from University Lake near Chapel Hill, North Carolina. Note that two of the club-shaped cells have buds (*arrows*). The bud from one cell is smaller and spherical and therefore younger than the club-shaped bud. Neither bud has the apical appendage indicating that it is formed later in the life cycle. The upper three cells are lysed and the apical appendage of the one on the upper right appears to be frayed indicating that it consists of multiple fibrils. Cell diameters are approximately 1.5–2.0 μm (supplied courtesy of J.T. Staley)

1.1.2 Classification of the Planctomycetes

1.1.2.1 Morphological Features of the Phylum Planctomycetes

While the morphotype system has been superseded by molecular taxonomy, morphological features can be useful for presumptive identification of planctomycetes. As mentioned previously, most species divide by budding and may or may not possess acellular stalks (Fig. 1.4). Stalked species often have a holdfast at the exposed tip of the stalk which allows cells to connect to one another to form a rosette (but not by the mechanism of *Caulobacter* spp. where cells attach directly to each other) or to attach to other organisms or detritus. In addition, cells are usually larger in diameter than other bacteria (up to 3.5 μm and occasionally larger), can be spherical, pear shaped, ellipsoid or club shaped and may exhibit both sessile and swarmer phases (Staley 1973; Schlesner 1994; Tekniepe et al. 1981).

Electron microscopy often reveals the presence of the hair-like surface appendages known as fimbriae. The planctomycetes are amongst the most hirsute bacteria known. Most recognised species have fimbriae, and the fimbriae may have particular locations on the cell surface depending on the genus and species. Some are perifimbrial (Fig. 1.5), where the fimbriae are located completely around the cell (Bauld and Staley

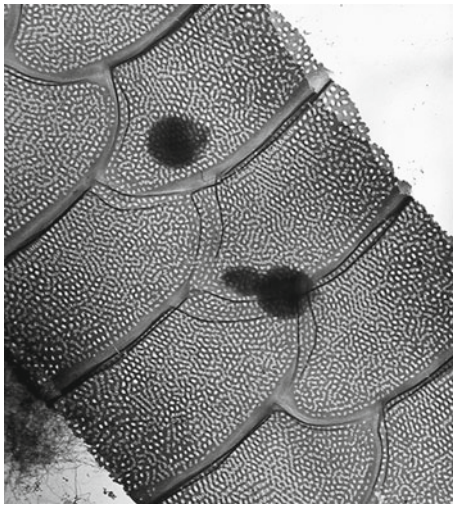

Fig. 1.4 An electron micrograph showing two non-stalked planctomycete cells attached to a diatom frustule (*Asterionella*) from Lake Washington. Note that one cell has a distinctive bud emerging from the broader reproductive pole of its cell. Note also how their cells are almost transparent to the electron beam (supplied courtesy of J.T. Staley)

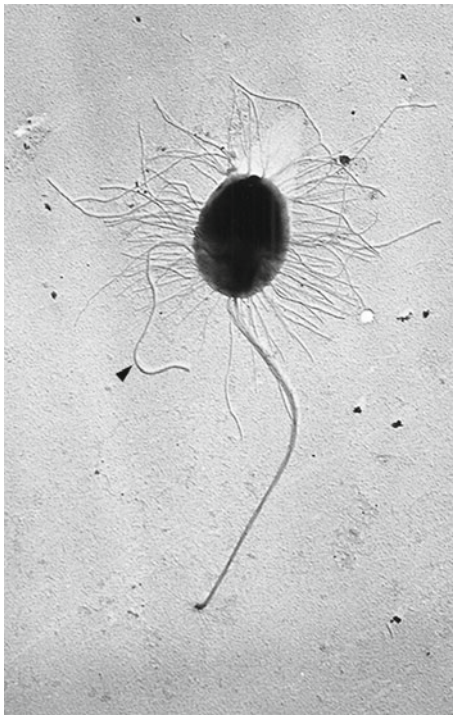

Fig. 1.5 *Planctomyces maris* as shown in a whole cell electron micrograph. Note that the cell is perifimbrial and that the stalk consists of several fimbriae-like fibrils bundled together. Also, note that the stalk is bent indicating its flexibility and that the cell has a flagellum (*arrow*) (supplied courtesy of J.T. Staley)

1976; Schmidt and Starr 1978). Others have polar fimbriae that are found at one pole and not the other (Staley 1973; Schlesner 1986). The fimbriae can be bundled together to form stalks (Hirsch and Müller 1985) and other related appendages such as the apical appendages of *Pl. guttaeformis* (Starr and Schmidt 1984). Fimbriae may be associated with crateriform structures, which are distinctive recessed areas in the cell wall;

however, a specific relationship between these structures is yet to be identified (Schmidt and Starr 1979a; Fuerst 1995). Nearly all members of the planctomycetes studied to date possess these structures.

In thin section, a unique proteinaceous cell wall that lacks a layer of peptidoglycan (a component of nearly all bacterial cell walls) is evident in all members of the planctomycetes (König et al. 1984). In addition, all planctomycetes have a compartmentalised cell plan consisting of a diverse range of membrane-bound internal cell structures (Lindsay et al. 2001; Fukunaga et al. 2009; see Chap. 2 in this volume). All species that have been examined to date possess distinctive internal membranes; the precise nature of which depends on the genus, but with some shared organisational features (Chap. 2 in this volume). As would be predicted, the separation of the cellular components by membranes may provide special organisational advantages for these organisms, including metabolic compartmentalisation (Damsté et al. 2002; van Niftrik et al. 2004), but they also pose barriers to transport between different sections of the cell presumably making intracellular communication more complex. This may in part explain the relatively large genomes of many of the planctomycetes (4–9 Mb).

1.1.2.2 Molecular Taxonomy

Despite the fact that Gimesi first recorded the presence of planctomycetes in water samples in 1924, for many years the planctomycetes were considered 'unculturable', and the first isolates of the phylum were not obtained until the 1970s. *Pirellula staleyi* was the first species isolated in pure culture in 1973, initially as the neotype strain of 'Pasteuria ramosa' (Staley 1973) but which was later renamed *Pirellula staleyi* (Schlesner and Hirsch 1984, 1987). The first species isolated from the genus *Planctomyces* was the stalked marine species, *Planctomyces maris* (Bauld and Staley 1976). Soon after, Schmidt (1978) reported the isolation of a number of members of the genus *Planctomyces* from freshwater habitats. Since then, the numbers of strains, species and genera have increased dramatically (Table 1.1). A description of isolation methods and of the various taxa is provided in greater detail in Sects. 1.2–1.4.

In 1986, the genera *Planctomyces* and *Pirellula* were assigned to a new family (*Planctomycetaceae*) and order (*Planctomycetales*) (Schlesner and Stackebrandt 1986) based on 16S rRNA cataloguing and phenotypic features. This order later expanded to include the genera *Gemmata* and *Isosphaera* (Ward et al. 1995). The diversity within these genera as well as the discovery of new strains has since resulted in the division of some of these clades into multiple genera as well as the addition of several new genera. Based on 16S rRNA analyses, the *Planctomycetes* are now considered their own phylum (Ward 2010) and this currently comprises 11 genera and 6 Candidatus genera, many of which are monospecific (Table 1.1). Some of these taxa still contain organisms with considerable genetic diversity and are likely to be split into further genera in the future.

The phylum *Planctomycetes* is considered to contain three distinct classes, the *Planctomycetia* (Ward 2010), the *Phycisphaerae* (Fukunaga et al. 2009) and the deep-branching anammox planctomycetes of the order *Candidatus* 'Brocadiales'

Table 1.1 Currently recognised genera and species within the phylum Planctomycetes

Class	Order	Family	Genus	Species	Taxonomic comments	Notable features
Planctomycetia	Planctomycetales	Planctomycetaceae	*Planctomyces*	*Pl. bekefii*	Type species. Named based on morphological features only. Not isolated in pure culture	Forms rosettes via thick tube-like stalks
				Pl. guttaeformis	Named based on morphological features only. Not isolated in pure culture	Large club-like cells. Long apical appendage. Buds form under the appendage
				Pl. stranskae	Named based on morphological features only. Not isolated in pure culture	Large club-shaped cells. No apical appendage observed
				Pl. maris	First species of the genus isolated in pure culture isolate	Marine species. Forms thin, flexible stalks. Colonies are non-pigmented. Genome sequence available
				Pl. limnophilus		Freshwater species. Forms a stalk. Colonies are red pigmented. Genome sequence and genetic tools available

		Pl. brasiliensis		Isolated from a hypersaline lagoon. Forms a stalk and possesses a single unicorn-like prostheca. Colonies are orange pigmented. Genome sequence available
	Schlesneria	*Sc. paludicola*	Distinct genus within the Planctomyces clade	Isolated from a peat bog. Moderately acidophilic. Forms short stalk-like structures. Polar distribution of crateriform structures
	Pirellula	*Pi. staleyi*	Type species of the genus. First planctomycete isolated in pure culture. Formerly *Pasteuria ramosa* (Staley 1973) and *Pirella staleyi* (Schlesner and Hirsch 1984)	Freshwater species. Lacks stalks but occasionally a 'fascicle' is observed. Crateriforms are distributed over the upper half of the cell. Genome sequence available
	Blastopirellula	*B. marina*	Type species. Formerly *Pirellula marina* (Schlesner 1986; Schlesner and Hirsch 1987)	Marine species. Crateriform structures distributed over the upper half of the cell. Genome sequence available

(continued)

Table 1.1 (continued)

Class	Order	Family	Genus	Species	Taxonomic comments	Notable features
			Rhodopirellula	*R. baltica*	Type species. Formerly '*Pirellula* sp. Strain 1'	Marine species. Forms pink colonies. Crateriform structures distributed over upper fifth of cell. Genome sequence available. Contains a large number of sulphatases
			Gemmata	*G. obscuriglobus*		Freshwater species. Possesses a double-membrane-bounded nuclear body and an endocytosis-like system. Draft genome sequence available
			Zavarzinella	*Z. formosa*	Distinct genus within the Gemmata clade	Isolated from an acidic peat bog. Forms rosettes. Possesses thick stalks that appear to be involved in the budding process

	Isosphaera	*I. pallida*		Thermophilic species isolated from hot springs. Forms filaments via intercalary budding. Exhibits gliding motility and phototaxis. Contains gas vesicles. Oligotrophic. Genome sequence available
	Singulisphaera	*Si. acidiphila*	Distinct genus within the Isosphaera clade. Displays 95 % similarity to 'Nostocoida limicola III'	Isolated from acidic wetlands. Colonies are non-pigmented. Spherical cells that do not form shapeless aggregates rather than filaments. Moderately acidophilic
		Si. rosea	Distinct genus within the Isosphaera clade. Displays 95 % similarity to *Si. rosea*	Isolated from acidic sphagnum peat. Forms pink colonies. Spherical cells found in pairs or short chains. Do not form long filaments. Moderately acidophilic

(continued)

Table 1.1 (continued)

Class	Order	Family	Genus	Species	Taxonomic comments	Notable features
			Candidatus 'Nostocoida limicola III'	'Nostocoida limicola III'	Originally named based on morphology but since recognised as a member of the Planctomycetes within the Isosphaera clade	Present in activated sludge. Cells are spherical to discoid. Forms filaments
			Aquisphaera	*A. giovannonii*	Distinct genus within the Isosphaera clade. Displays 92 % similarity to *Si. acidiphila*	Freshwater species. Forms pink-pigmented colonies. Non-motile
Phycisphaerae	Phycisphaerales	Phycisphaeraceae	*Phycisphaera*	*Ph. mikurensis*	Only species isolated within the Class Phycisphaerae	Isolated from a marine alga. Divides by binary fission rather than budding. Very high %GC content in genome (>73 %). Facultative anaerobe that can ferment D-xylose
Candidatus 'Brocadiae'	'Brocadiales'	'Brocadiaceae'	*Candidatus* 'Brocadia'	'Brocadia anammoxidans'	*Candidatus* species not yet isolated in pure culture	First anammox organism discovered. Contains an anammoxosome
				'Brocadia fulgida'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater. Oxidises acetate. Autofluorescent

Candidatus 'Kuenenia'	'Kuenenia stuttgartiensis'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater. Genome sequence available
Candidatus 'Anammoxoglobus'	'Anammoxoglobus propionicus'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater. Mixotrophic metabolism. Can oxidise propionate
Candidatus 'Scalindua'	'Scalindua brodae'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater
	'Scalindua wagneri'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater
	'Scalindua sorokinii'	*Candidatus* species not yet isolated in pure culture	Marine species found in low-oxygen regions of the Black Sea
	'Scalindua arabica'	*Candidatus* species not yet isolated in pure culture	Marine and freshwater species. Found in low-oxygen regions
	'Scalindua richardsii'	*Candidatus* species not yet isolated in pure culture	Marine species found in low-oxygen regions of the Black Sea
Candidatus 'Jettenia'	'Jettenia asiatica'	*Candidatus* species not yet isolated in pure culture	Species found in wastewater

(Jetten et al. 2010). The latter group we will term here class *Candidatus* 'Brocadiae'. Although this classification differs from the recent classification of the *Planctomycetes* in the second edition of Bergey's Manual of Systematic Bacteriology (Ward 2010), in which the phylum is separated into two groups at the ordinal level, recent evidence from new strains that have been isolated and phylogenetic analyses of as yet uncultivated strains support the three classes (Nogales et al. 2001; Fukunaga et al. 2009; Jetten et al. 2010). Thus, we agree with several other researchers that the divergence between the groups is sufficiently great phylogenetically for them to be considered three separate classes (Janssen 2006; Elshahed et al. 2007; Fukunaga et al. 2009; Fuchsman et al. 2012). Some phenotypic features of members of the three classes currently support this view also. For example, the members of the *Planctomycetia* and *Candidatus* 'Brocadiae' all reproduce by budding and possess crateriform structures, while the known strains of *Phycisphaerae* reproduce by binary transverse fission and appear to lack crateriform structures. Furthermore, all known members of the *Planctomycetia* and *Phycisphaerae* are chemoheterotrophs, while members of *Candidatus* 'Brocadiae' are autotrophic (or mixotrophic) in their metabolism. In addition, there is evidence that C1 transfer genes are found only in the *Planctomycetia* and not in the anammox planctomycetes (Woebken et al. 2007).

The position of phylum Planctomycetes within the domain Bacteria has been the subject of some debate. They are generally believed to form a 'superphylum' with the *Verrucomicrobia*, *Lentisphaerae* and *Chlamydiae* phyla (PVC superphylum) based on analysis of ribosomal proteins and RNA polymerase subunits (Hou et al. 2008) and rDNA analyses (Wagner and Horn 2006), although some early sequence analyses did not support this relationship (Jenkins and Fuerst 2001; Ward et al. 2000). Some shared phenotypic traits are also used as evidence to link these phyla. For example, all known planctomycetes lack the cell wall component peptidoglycan and the cell division protein FtsZ features shared with members of the *Chlamydiae* phylum. Both *Planctomycetes* and *Verrucomicrobia* possess a compartmentalised cell plan and share unusual membrane coat proteins (Santarella-Mellwig et al. 2010). These features, which are shared with the Eukarya and some Archaea, have also been used to argue for a deep phylogenetic origin for the PVC superphylum within the Bacteria, or that the last universal common ancestor was a relative of the PVC group (Brochier and Philippe 2002; Reynaud and Devos 2011; Fuerst and Sagulenko 2012). Indeed planctomycetes possess additional eukaryote-like features including endocytosis-like processes, condensed DNA, sterols, integrin genes and membrane-bound DNA, as well as archaeal features such as ether- and ester-linked lipids, and genes for C1 transfer reactions (Chistoserdova et al. 2004; Damsté et al. 2002; Devos and Reynaud 2010; Fuerst and Webb 1991; Fuerst and Sagulenko 2010; Jenkins et al. 2002; Lindsay et al. 2001; Lonhienne et al. 2010; Pearson et al. 2003). Many of these features are described in detail in later chapters. Some molecular analyses also support the view that the planctomycetes, rather than hyperthermophilic organisms as has been traditionally posited, form an ancestral bacterial lineage (Brochier and Philippe 2002).

In contrast, other researchers have argued that many of the features of the planctomycetes and/or PVC superphylum are merely analogous to eukaryotic

or archaeal features, rather than homologous (membrane-bound DNA), have been derived by horizontal gene transfer (C1 transfer genes), or represent degenerative evolution (loss of FtsZ and peptidoglycan) rather than ancestral traits (McInerney et al. 2011). Regarding the lack of peptidoglycan in planctomycete cell walls, possible evidence for degenerative or reductive evolution is implied by the presence of some or even most of the genes required for peptidoglycan biosynthesis in the genomes of some species (Glöckner et al. 2003; Strous et al. 2006).

Whether the presence of these remarkable features represent a case of analogy or homology, the planctomycetes are increasingly playing a major role in understanding the evolution of cellular complexity and organisation.

1.2 Methods for the Enrichment, Isolation and Cultivation of the Planctomycetes

Low nutrient or oligotrophic enrichments have been used as a primary step in the isolation of particular organisms from aquatic environments. The use of low nutrient enrichments came from the method of Houwink (1951) who added low concentrations of peptone, 0.01 % to aquatic samples, for the enrichment and isolation of *Caulobacter* spp. This same approach, using 0.01 % peptone or 0.005 % peptone and 0.005 % yeast extract combined, was subsequently used to enrich and isolate prosthecate Proteobacteria as well as strains of planctomycetes (Staley 1968, 1973; Bauld and Staley 1976). These liquid enrichments are incubated at room temperature and examined microscopically after 1–2 weeks or more for evidence of budding bacteria that appear to be members of phylum Planctomycetes. Inoculum from these enrichments can then be streaked onto similar solid oligotrophic media for their isolation. Extended incubation of water samples without the addition of nutrients, in the dark to prevent cyanobacterial and algal growth, has also been used successfully in the enrichment of planctomycete strains (Hirsch and Müller 1986).

Most planctomycetes are not strictly oligotrophic, and while many do reside in relatively low-nutrient aquatic habitats, they are also found in eutrophic water, soils, wastewater and other nutrient-rich environments. In particular, in a survey of one mesotrophic and several oligotrophic Australian lakes as well as eutrophic ponds, the viable concentrations of the *Planctomycetes* group were found to be highest in the eutrophic ponds where their numbers were as high as 240 per ml. However, their numbers relative to total viable heterotrophs remained similar regardless of trophic state (Staley et al. 1980). Nonetheless, all planctomycetes have relatively long generation times and as such, enrichment in dilute solutions favours planctomycete growth, where the use of nutrient-rich media can result in the overgrowth of more rapidly dividing bacteria. Low nutrient enrichment has been successfully applied to the isolation of a number of freshwater planctomycete strains (Staley 1973; Schmidt 1978) and has proven to be a very effective technique for isolation of planctomycetes from soil (Yee et al. 2008) where overgrowth of fungi can otherwise prove problematic. Marine

strains have been successfully isolated using dilute peptone enrichment medium made with artificial seawater and on commercially available marine agar at half or lower strength (Bauld and Staley 1976; Fuerst et al. 1997; Fukunaga et al. 2009). Remarkably, the type strain of the species *Isosphaera pallida*, a true oligotroph, was initially isolated on a solid medium suitable for autotrophs, without the addition of any specific organic carbon sources. This organism is chemoheterotrophic rather than autotrophic in its metabolism and was apparently able to survive and multiply using only the organic contaminants within the agar itself (Giovannoni et al. 1987). Specific testing of *I. pallida* strains on a variety of carbon sources indicated that growth of this organism is inhibited by glucose concentrations of just 0.05 %. Some strains are also inhibited by low concentrations of ribose, fructose, maltose or glycolate (Giovannoni et al. 1987).

The ability of some planctomycete genera to attach to surfaces via a holdfast structure has also been exploited in some isolation techniques such as the 'petri dish method' of Hirsch and Müller (1986). In this method, sterile glass coverslips are placed upright in a petri dish containing the water sample of interest and incubated for several days to allow planctomycete organisms to attach to the glass. The coverslip is then placed face down on a nutrient-containing agar to allow colonies to develop. More recently, this technique has been combined with molecular detection methods for the isolation of planctomycete strains from acidic peat. In that study, planctomycetes were enriched on coverslips immersed in peat water and fluorescent in situ hybridisation, employing planctomycete-specific probes, was used to monitor enrichment of planctomycetes within the coverslip biofilms (Kulichevskaya et al. 2006). The petri dish method has been used successfully for the isolation of planctomycete strains possessing holdfasts such as *Planctomyces, Pirellula* spp. (Hirsch and Müller 1986) and *Zavarzinella formosa* (Kulichevskaya et al. 2009) as well as strains exhibiting glycocalyx formation such as *Singulisphaera* (Kulichevskaya et al. 2006, 2008).

The distinctive morphology of many planctomycetes, their relatively large cell size and/or tendency to form rosettes, enables their presumptive identification within a complex sample. The distinctive appearance of these organisms can then be exploited in their isolation through the use of microtools. Micromanipulation, a technique employing a fine glass tool attached to a low-powered microscope lens, and the forces of surface tension (Skerman 1968) enabled the capture and subsequent cultivation of cells of *Gemmata obscuriglobus* (Franzmann and Skerman 1984) and *Candidatus* 'Nostocoida limicola III' (Liu et al. 2001). More modern but equivalent methods, such as the use optical (laser) tweezers (Fröhlich and König 2000) or gel microdroplet encapsulation (Zengler et al. 2002), are potentially promising techniques for the isolation of uncultivated planctomycete strains.

Increased knowledge regarding the ecology and physiology of planctomycetes facilitated the development of selective media for their isolation, and a comprehensive study of selective chemoheterotrophic enrichment and isolation techniques by Schlesner (1994) resulted in the isolation of a large collection of planctomycetes from diverse aquatic habitats varying in salinity, pH and nutrient levels. A notable finding from this study was the ability of many of these strains to utilise *N*-acetyl-*D*-glucosamine as a sole source of carbon and nitrogen. *N*-acetyl-