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REVIEW ARTICLE

The planctomycetes: emerging models for microbial ecology, evolution and cell biology

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Planctomycetes

The planctomycetes are an unusual but widely distributed group of budding bacteria which are proving to be of increasing relevance to at least three major areas of research in microbiology: microbial ecology, molecular evolution and cell biology. They constitute one of the phylogenetically distinct major 'phyla' of the domain Bacteria defined using 16S rRNA sequence analysis (Woese, 1987), and form a group so deeply branching it has been proposed as a new bacterial order, *Planctomycetales*, and family, *Planctomycetaceae* (Schlesner & Stackebrandt, 1986). Cultured planctomycetes which have been analysed with respect to their cell wall chemistry display the unusual feature of not synthesizing the otherwise universal Bacterial cell wall peptidoglycan; this feature is shared only with the chlamydiae and mycoplasmas among the Bacteria (König *et al.*, 1984; Liesack *et al.*, 1986). Planctomycetes also exhibit some unusual molecular features, including short 5S rRNA and, in at least two species, unlinked *rrn* operon organization (Bomar *et al.*, 1988; Liesack & Stackebrandt, 1989). *Gemmata obscuriglobus*, a budding bacterium isolated from a freshwater dam in Queensland, Australia (Franzmann & Skerman, 1984), is a member of the order *Planctomycetales* and is thus within the domain Bacteria, yet possesses a membrane-bounded nuclear body, an organelle hitherto known only in the Eucarya. The unique ultrastructure of this bacterium is of great potential significance to understanding the ways in which eukaryote cell organization may have evolved.

The planctomycetes were initially discovered as aquatic freshwater and planktonic micro-organisms with a distinctive morphology due to their occurrence in rosettes connected by a non-cellular stalk. The type species of the genus *Planctomyces*, *Planctomyces bekefii*, was first observed in a pond in Budapest, Hungary, although interpreted at that time as a planktonic fungus (Gimesi, 1924). '*Planctomyces crassus*', a similar morphotype, was much later also interpreted in this way (Hortobágyi, 1965). *Pl. bekefii* characteristically occurs as rosettes of spherical cells (Fig. 1), joined together by non-cellular stalks, originally interpreted as fungal conidia and fungal conidiophores, respectively (Schmidt *et al*, 1981). The cell pole opposite to the stalk possesses tapering multifibrillar 'spire' appendages while pili occur over the entire cell. *Pl. bekefii* from the original type locality was eventually shown to possess a prokaryotic ultrastructure (Schmidt & Starr, 1980a), as was this species from North American enrich-

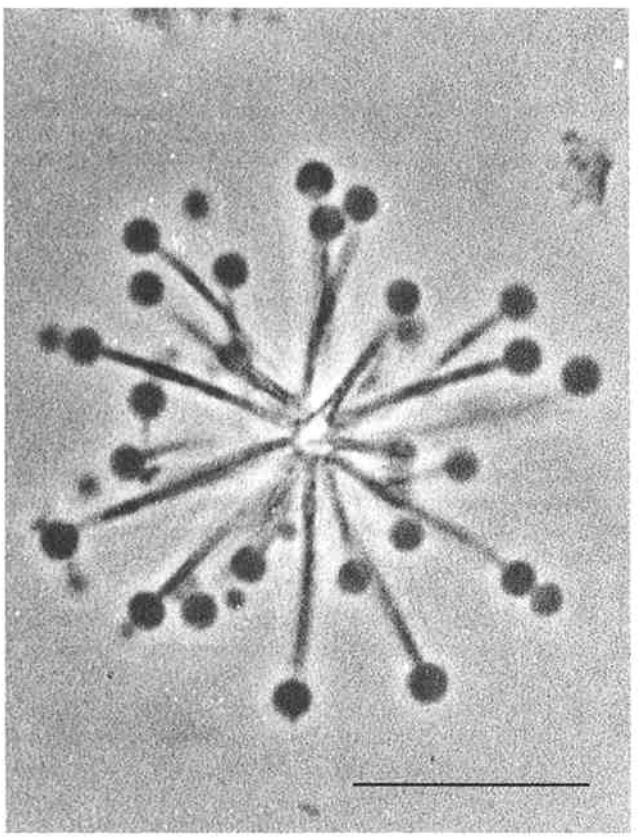

Fig. 1. Phase-contrast micrograph of a rosette of *Pl. bekefii*, showing non-cellular stalks radiating towards a central phase-light granule from spherical cells at the rosette circumference, some of which possess buds. Micrograph taken during collaborative research of the author and J. T. Staley (Department of Microbiology, University of Washington). Bar, 10 μm.

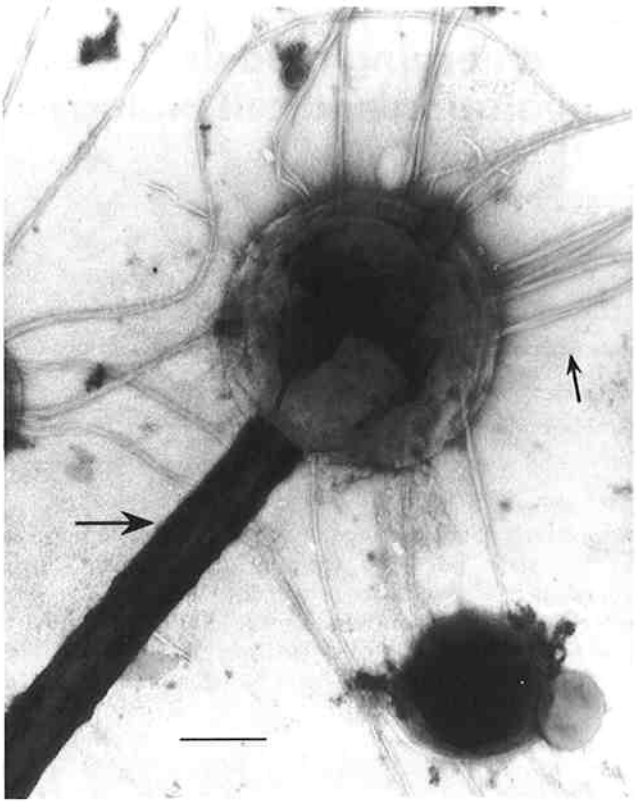

Fig. 2. Electron micrograph of a negatively stained *Pl. bekefii* cell with non-cellular stalk (large arrow), spires (small arrow) and electron-dense stain-accumulating crateriform structures distributed uniformly over the cell surface. Bar, 0·5 μm.

ments (Schmidt & Starr, 1980b). A 'morphotype' system was introduced for distinction of different types of planctomycete from natural enrichments, developed most extensively in Table 1 of Schmidt & Starr (1981) and included in *Bergey's Manual of Systematic Bacteriology* (Starr & Schmidt, 1989). In different morphotypes and in different genera in pure culture, cell shape varies from spherical to ovoid, ellipsoidal, tear-drop-shaped or bulbiform, and stalk or other appendage presence and structure varies. Flagella if present are often sheathed (motile cells have not been observed in *Pl. bekefii* rosettes). In at least some cases these types may be merely manifestations of the same species under different environmental conditions, or reflect varying degrees of iron oxide encrustation in the case of samples from aquatic blooms (Schmidt & Starr, 1981). Although subject to many studies observing their occurrence in natural habitat samples or crude enrichments, freshwater *Planctomyces* rosette-forming stalked 'morphotypes', assigned to species such as *Pl. bekefii*, '*Pl. crassus*', *Pl. stranskae* and *Pl. guttaeformis*, have never been isolated in pure culture. With the application of planctomycete-specific fluorescent oligonucleotide probes, and with PCR applied to 16S rDNA amplification from micromanipulated cells and rosettes, it should now be possible to resolve the true phylogenetic relationships of putative uncultured planctomycetes such as *Pl. bekefii* and similar morphotypes seen in natural enrichments. Their assignment as members of the *Planctomycetales* depends at present solely on morphological and ultrastructural features such as yeast-like budding, 'crateriform structures', absence of peptidoglycan cell wall layer and production of non-cellular multifibrillar stalk-like structures, features known to occur in cultured planctomycetes which have been characterized via molecular phylogenetics. The order *Planctomycetales* and the family *Planctomycetaceae* were defined not only on the basis of 16S rRNA oligonucleotide catalogues and sequence data, but also on the basis of such distinctive phenotypic characters (Schlesner & Stackebrandt, 1986).

The representatives of the planctomycetes isolated in pure culture include four genera and seven species: *Planctomyces maris*, *Pl. limnophilus* and *Pl. brasiliensis*, *Pirellula staleyi* and *Pi. marina*, *Gemmata obscuriglobus* and *Isosphaera pallida* (Bauld & Staley, 1976; Hirsch & Müller, 1985; Schlesner, 1986, 1989; Staley, 1973; Franzmann & Skerman, 1984; Giovannoni *et al.*, 1987b). Of these, *Pi. staleyi*, *Pl. limnophilus* and *G. obscuriglobus* are from freshwater habitats, *Pl. maris* is marine, *Pi. marina* is from the brackish Baltic Sea, *Pl. brasiliensis* is from a hypersaline pond and *I. pallida* is from a hot springs habitat, although only moderately thermophilic (optimum growth temperature 41 °C). Additional strains in pure culture include the freshwater *Pi. staleyi* strain ICPB 4232 (Schmidt CLPM white, Tekniepe BT2 white, ATCC 35122; Tekniepe *et al.*, 1981; Schmidt & Starr, 1982; Starr *et al.*, 1983), bacteria resembling *Pirellula* species from the giant tiger prawn *Penaeus monodon* (Fuerst *et al.*, 1991) and the 257 *Planctomyces*-like, *Pirellula*-like, spherical and some possibly prosthecate planctomycetes from fresh, brackish, marine and hypersaline waters isolated by Schlesner (1994). Isolation methods vary from use of micromanipulation (Franzmann & Skerman, 1984) and relatively non-selective enrichments (Hirsch & Müller, 1986) to the very selective medium based on *N*-acetylglucosamine as carbon source and ampicillin and cycloheximide as selective agents employed with great success by Schlesner (1994). Isolation and identification methods have been usefully reviewed previously (Starr & Schmidt, 1989; Staley *et al.*, 1992).

Electron microscopy has been essential for confirming the yeast-like budding division mode and the presence of planctomycete-characteristic crateriform structures and multifibrillar appendages in putative planctomycete isolates. Future preliminary identification of planctomycetes will apply 16S rRNA sequence-based oligonucleotide probes such as those which can be based on *Escherichia coli* positions 47–53 of the planctomycete 16S rRNA (Liesack & Stackebrandt, 1992; Liesack *et al.*, 1992) as well as electron microscopy. Crateriform structures are cell surface structures characteristic of all known planctomycetes, appearing as electron-dense circular regions after negative staining of whole cells (Figs 2 and 3), with diameters of 12 nm or 5–7 nm; in *I. pallida* such structures are as large as 27 nm in diameter, and in other planctomycetes, enlargement of the structures up to 30 nm can occur with cell maturation (Tekniepe *et al.*, 1981;

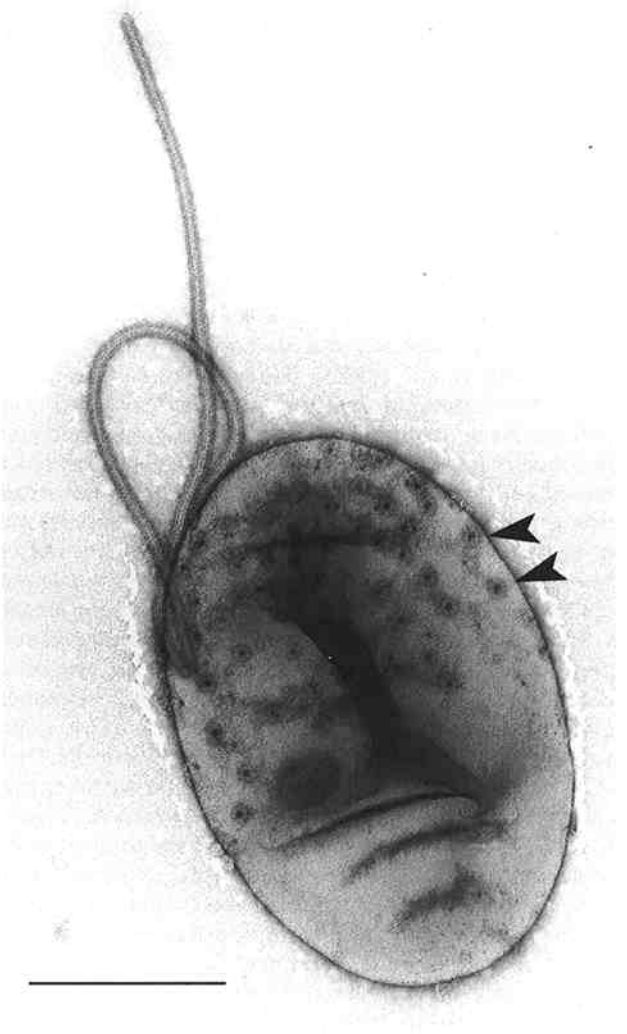

Fig. 3. Electron micrograph of a negatively stained *Pirellula*-like planctomycete from the giant tiger prawn, showing a teardrop-shaped cell possessing crateriform structures (arrows) with polar distribution and a complex sheathed flagellum. Bar, 0·5 μm.

Giovannoni *et al.*, 1987a). Planctomycete crateriform structures appear to be indentations with a raised rim (Tekniepe *et al.*, 1981); circumscription of such structures by a 'grommet' 30–36 nm in outer diameter has been described (Starr & Schmidt, 1989), and conical 'corniculate' surface protrusions seen under some staining conditions with some 'morphotype II' planctomycetes in natural enrichments from freshwater may be related to crateriform structures (Schmidt & Starr, 1979a). Neither those protrusions nor crateriform structures bear any simple relation to the distribution of fimbriae, which are common appendages on planctomycete cells. Crateriform structures may not be holes in the wall but solid structures more similar to circular protein arrays with a geometry allowing stain accumulation (Schmidt & Starr, 1979a). However, isolated planctomycete walls do show perforations consistent with holes, but also with loss of protein, which may normally fill such holes after SDS treatment used in isolation (see later). Presence or absence of crateriform structures has been used along with other structural features to include or exclude planctomycete-like morphotypes in natural enrichments as true members of the planctomycetes (Schmidt & Starr, 1979b; Starr *et al.*, 1984). Their distribution is useful taxonomically to distinguish *Planctomyces* and *Gemmata* from *Pirellula* strains, the latter displaying distribution of the larger form of these structures on the budding, 'reproductive' pole of the cell only (Fig. 3). Although they are claimed to be unique to planctomycetes, the array of 8·5-nm-diameter rings on the surface of *Helicobacter mustelae* (O'Toole *et al.*, 1994), the circular perforations of peptidoglycan layers in cyanobacteria (Guglielmi & Cohen-Bazire, 1982) and the crateriform-like structures of the aerobic bacteriochlorophyll-synthesizer *Porphyrobacter* (Fuerst *et al.*, 1993) should be noted as possible analogous structures. However, they remain a very useful diagnostic feature for members of the planctomycetes.

The other unusual major surface structure of *Pl. bekefii*, the non-cellular non-prosthecate multifibrillar stalk, which in that species radiates from the centre of rosettes, is not of universal occurrence among the planctomycete genera. It is however a feature characteristic of the genus *Planctomyces*, occurring in *Pl. limnophilus*, *Pl. maris* and *Pl. brasiliensis* of the strains in pure culture. Although the stalks of *Pl. bekefii* and similar morphotypes are thick enough (at least 0·3 μm wide) to be visible by light microscopy, those of *Pl. maris* are too thin at 0·1 μm to be above the usual resolution limits (Bauld & Staley, 1976). Stalks may be tubular, ribbon-like or rope-like, comprised of twisted fibrils. Another more irregular form of multifibrillar appendage is found in at least *Pi. staleyi* among other planctomycetes; the significance of such appendages was one of the bases for the distinction of the genus *Pirellula* (Schlesner & Hirsch, 1984) and molecular evidence has confirmed this distinction on much firmer grounds (Liesack *et al.*, 1992). Multifibrillar stalk-like appendages have also been found outside the planctomycetes, in the proteobacterium *Porphyrobacter* (Fuerst *et al.*, 1993). Although the non-cellular stalks of *Gallionella ferruginea* contain fibres, these are claimed to be completely inorganic, consisting of ferric hydroxide only (Hanert, 1989).

At times in the history of planctomycete studies, nomenclature has been confusing, and those entering the field should be aware of the fact that the organism now known as *Pi. staleyi* was once thought to be a rediscovery of *Pasteuria ramosa* described in the 19th century by Elie Metchnikoff (Staley, 1973), and that this species has also been classified as a member of the genus *Planctomyces* (Starr *et al.*, 1983). The latter view is now obsolete as a result of a reassignment (Schlesner & Hirsch, 1984) and in the light of later 16S rRNA sequence data. However, it is still confusingly preserved in many phylogenetic trees and small subunit rRNA databases because of a GenBank 16S rRNA sequence entry (M34126) under *Planctomyces staleyi* for the important type strain of this species, ATCC 27377. An additional source of confusion was the need to reject the genus name *Pirella* and replace it with *Pirellula*, due to

prior use of *Pirella* for a fungal genus (Schlesner & Hirsch, 1987). The planctomycetes were once commonly referred to as the *Blastocaulis–Planctomyces* group, reflecting a genus name used for uncultured freshwater lake planctomycetes (Henrici & Johnson, 1935; Hirsch, 1972; Schmidt & Starr, 1981).

Ecology

Classical ecology

Originally studied as freshwater organisms, the planctomycetes are proving to be quite widely distributed in marine, hypersaline and even terrestrial soil habitats. Even in freshwater and brackish water habitats, planctomycetes can be isolated from habitats of diverse trophic status, including oligotrophic, eutrophic, very eutrophic and frankly polluted habitats (Schlesner, 1994). By use of selective media based on ampicillin resistance of the peptidoglycan-less planctomycetes and utilization of *N*-acetylglucosamine as sole carbon and nitrogen source, isolates have been derived from such diverse sources as sewage treatment sludge, cattle manure, garbage dump leakage water, the meromictic saline Ekho lake in Antarctica, alkaline chalk mines, acid bog water and the pitcher of the insectivorous plant *Nepenthes* sp., as well as brackish water of the Baltic Sea (Schlesner, 1994). *In situ* morphological evidence suggests that groundwater may also be a planctomycete habitat (Hirsch & Rades-Rohkohl, 1983; Ekendahl *et al.*, 1994).

Little is known concerning physiology and metabolism of planctomycetes, but strains in pure culture are all chemoheterotrophs capable of growth in air, with some such as *Pl. maris*, *G. obscuriglobus* and *I. pallida* being obligate aerobes (Bauld & Staley, 1976; Franzmann & Skerman, 1984; Giovannoni *et al.*, 1987b). Others, such as *Pi. marina* and *Pl. limnophilus*, are facultative in the sense of being capable of carbohydrate fermentation (Schlesner, 1986; Hirsch & Müller, 1985). Presence of cytochrome *c* oxidase and nitrate reduction ability in at least some strains, such as *Pi. marina* and *Pl. brasiliensis*, suggest possession of electron transport chains similar to other facultative aerobic Bacteria (Schlesner, 1986, 1989), but quinone composition is unusual for aerobes (see later). It may also be relevant to planctomycete electron transport chain composition that oxygen uptake in *I. pallida* is cyanide sensitive (Giovannoni *et al.*, 1987b). Considering the physiological diversity of members of an equivalent distinct Bacterial phylum such as the Proteobacteria, anaerobic, chemoautotrophic and photosynthetic representatives of planctomycetes may well be discovered. Carbohydrates have been identified as the major carbon sources, yet gelatin hydrolysis can be performed by *Pl. limnophilus*, *Pl. brasiliensis* and *Pi. marina* (Hirsch & Müller, 1985; Schlesner, 1986, 1989). Starch is hydrolysed by *Pl. brasiliensis*, *Pi. marina* and *G. obscuriglobus*. Planctomycetes typically exhibit slow growth, with the shortest generation time recorded for *Pl. maris* at 13 h (Bauld & Staley, 1976), identical to the generation time recorded for *G. obscuriglobus* (Franzmann & Skerman, 1984), but a generation time in excess of 100 h has been observed for *Pl. maris* in a glucose-seawater salts medium. Many planctomycete strains may require relatively dilute media, as nutrient-rich media (above 0·75 g peptone, yeast extract and glucose l^{-1}) have been found to be inhibitory to *Pi. marina* (Schlesner, 1994), and the obligate oligotrophy of *I. pallida* has already been noted.

I. pallida possesses several especially interesting ecophysiological features; moderate thermophily (growth up to 55 °C), obligate oligophily (growth inhibited with glucose above 0·025 %), gliding motility (unique among budding Bacteria), phototactic ability (unique among heterotrophic Bacteria), and ability to form gas vesicles (Giovannoni *et al.*, 1987b; Giovannoni & Castenholz, 1989). Since these organisms occur in hot springs as plankton or as components of cyanobacteria-dominated microbial mats, the latter two abilities may be related to vertical axis positioning. Phototaxis in a non-phototroph without bacteriochlorophylls (but with carotenoids) may be useful for study of phototaxis independently of photosynthesis (Giovannoni *et al.*, 1987b). *I. pallida* may be widely distributed, since in addition to its type locality in Warm Springs, Oregon, isolations have been achieved from other North American hot springs (Giovannoni & Castenholz, 1989), and cloned 16S rDNA sequences identified as from *I. pallida* reported from the Octopus Spring cyanobacterial mat, Yellowstone National Park, USA, confirm evidence from microscopy that this species is abundant in the top mat layers at this location (Ward *et al.*, 1987, 1990). *I. pallida* has been reported to grow in co-culture with a hot-springs phototroph, *Heliothrix oregonensis*, a co-culture maintained successfully only under aerobic conditions in the light, and apparently obligate for growth of *H. oregonensis* (Pierson *et al.*, 1985). In the mats of the alkaline hot springs at Warm Springs, Oregon, *H. oregonensis* was consistently found associated with *I. pallida* and a few specific cyanobacteria in a bright orange layer in O_2-saturated water at 45–56 °C and with high solar radiation exposure.

Many valuable but isolated limnological observations concerning planctomycetes exist, and we know that *Pl. bekefii* morphotypes, at least, occur in freshwater at widely dispersed geographical locations, including Hungary, Denmark and many other European sites, Vietnam, Venezuela, Israel, Australia and the USA (Schmidt & Starr, 1980a,b; Kristiansen, 1971; Hortobágyi, 1968; Lewis *et al.*, 1986; Schmaljohann *et al.*, 1987; Staley *et al.*, 1992; Pedrós-Alió & Brock, 1982). Association with eutrophic or polluted water has been reported, along with high pH values (6·8–9·4), high conductivity and temperate summer temperatures, and it has been suggested that the decomposition of phytoplankton maxima resulting in H_2S production and rising iron and manganese concentrations may favour *Pl. bekefii* blooms (Kristiansen, 1971). *Pl. bekefii* and '*Pl. condensatus*' have been correlated with a seasonal increase in heterotrophic phytoplankton in a municipal water reservoir (Granberg, 1969). Culturable freshwater planctomycetes have also been reported as being most numerous in eutrophic habitats (Staley *et al.*, 1980). Continued ecological research on *Pl. bekefii* will be hampered by the absence of culture techniques for this

organism, and by the present lack of any molecular technique for its identification as a planctomycete and determination of phylogenetic homogeneity. This should be resolved easily with methods such as cloning of 16S rDNA amplified from micromanipulated cells and *in situ* hybridization using planctomycete-specific fluorescent oligonucleotide probes. The question of whether *Pl. bekefii* may serve as a useful environmental indicator organism for eutrophic conditions can then be addressed. *Planctomyces* rosettes can occur in densities of as high as $3{\cdot}5 \times 10^3$ ml^{-1} in British lakes, but in a thorough multiple regression and seasonal study of Esthwaite Water, English Lake District, no clear relationship with any physico-chemical variable was found, though numbers increased at the onset of overturn in September (Jones, 1978). *Pl. bekefii*, *Pl. guttaeformis* and *Pl. stranskae* have been noted to often occur in freshwater bodies following or accompanying algal or cyanobacterial blooms, laboratory enrichments of planctomycetes may be favoured by ambient light and consequent phototroph development (Schmidt & Starr, 1980b; Starr & Schmidt, 1989) and freshwater *Pirellula* species can occur as epiphytes of ensheathed cyanobacteria, which may relate their occurrence to cyanobacterial bloom formation (Staley, 1981).

An interesting biogeochemical aspect of rosette- and stalk-forming *Planctomyces* morphotypes observed in native freshwater habitats is their accumulation of iron and/or manganese on the non-cellular stalk (Schmidt *et al.*, 1981, 1982). Energy-dispersive X-ray spectroscopy has shown encrustation with such elements, with a possibility that at least in some specific locations, manganese may be exclusively accumulated by one morphotype (morphotype 1a; *Pl. bekefii*) and both iron and manganese by another (morphotype 1b; '*Pl. crassus*') (Schmidt *et al.*, 1981, 1982). Passive accumulation of oxidized iron is a likely explanation for iron encrustation, but biologically catalysed oxidation of reduced manganese is a property to be considered in investigating the eco-physiology of such bacteria, if and when they are isolated in pure culture.

Possible associations of planctomycetes with crustacea have been an issue ever since Metchnikoff's report of assocation of pear-shaped parasitic micro-organisms with cladoceran 'water fleas' of *Daphnia* spp. (Metchnikoff, 1888). It now seems more likely that this parasite was the non-planctomycete endospore-forming *Pa. ramosa*, phenotypically similar to *Pa. penetrans* and *Pa. thornei*, pathogens of plant-parasitic nematodes (Sayre *et al.*, 1983; Starr & Sayre, 1988). True *Pirellula*-like bacteria were later found occurring attached to the carapace of *Daphnia pulex* (Staley, 1973). More recently, the isolation of bacteria resembling *Pirellula* species has been recorded from the hepatopancreas of the giant tiger prawn *Penaeus monodon* (Fuerst *et al.*, 1991); these bacteria and further isolates from post-larvae of that crustacean have been shown to be phylogenetically closely related to *Pirellula* or *Planctomyces* species on the basis of 16S rRNA sequences (J. A. Fuerst and others, unpublished). Such bacteria may inhabit the prawn haemolymph as well as the gut, and may possibly be selected for especially within baculovirus-infected prawns, though they occur in healthy prawns in at least low numbers. The exact nature of their association with this crustacean remains to be determined.

Molecular ecology

The application of the methods of molecular ecology were first applied to the problem of planctomycete distribution and diversity in a study of acid soil (pH 4·2) from a subtropical Australian environment (Liesack & Stackebrandt, 1992; Stackebrandt *et al.*, 1993). The clone library resulting from amplification of 16S rRNA genes derived from direct lysis of the bacterial community within the soil matrix revealed seven clones hybridizing with planctomycete-specific oligonucleotides under stringent conditions. Sequencing revealed that planctomycete-specific signature sequences (a valuable list of which is presented in Table 1 of Liesack & Stackebrandt, 1992) were shared also by these clones, and that the phylogenetic relationships of the sequences spanned the diversity of at least three of the described genera of the family *Planctomycetaceae*, a surprising finding since members of these genera were all originally described and isolated from aquatic habitats and *Isosphaera* only from hot springs. While one clone was related to *I. pallida*, and another to *Pl. limnophilus*, the remaining clones branched off adjacent to *G. obscuriglobus*, and shared with that species a unique bulge loop of 13 nucleotides around position 1005 (*E. coli* 16S rRNA numbering), a slightly enlarged bulge loop between positions 1024–1036, and a higher G+C base ratio for 16S rDNA than for *Planctomyces* and *Pirellula*, a feature also shared with *I. pallida*. However, except for the case of the close relationship of clone MC100 to *Pl. limnophilus*, these authors stressed the speculative nature of the allocation of the clones clustered with *Isosphaera* and *Gemmata* to those genera. In addition to these clear-cut planctomycete clones, a further group of clones (soil cluster III) appeared to be related to planctomycetes more distantly, forming a novel main line of descent within the Bacteria, sharing a common ancestry with planctomycetes and with chlamydia, and also sharing synapomorphic 16S rDNA signature nucleotides with both the latter groups.

That planctomycetes may prove to be of global significance for nutrient cycling processes in marine habitats is suggested by the recognition of their possible significance as components of macroscopic marine phytodetrital aggregates (> 0·5 mm) also known as 'marine snow'. Macroscopic detrital aggregates have been suggested to be important agents in the flux of biogenic carbon from the sea surface to the deep ocean, with biological and chemical transformations on sinking aggregates having a profound influence on the quantity and quality of organic matter reaching the deep sea. Such aggregates can serve as localized microhabitats and may harbour transient microaerophilic or anaerobic microhabitats in otherwise aerobic environments. Therefore, processes not occurring in planktonic non-particulate seawater habitats but important to marine chemistry, such as methanogenesis and denitrification, as well as aerobic processes utilizing the products of anaerobic decomposition, may be catalysed by macroaggregate or particulate-associated microflora

(Alldredge & Cohen, 1987; Bianchi *et al.*, 1992). Understanding the diversity of such particulates, which have now been found to include planctomycetes, thus becomes important to understanding global marine biogeochemistry. On phytodetrital macroaggregates composed of dense populations of *Rhizosolenia* sp. and *Phaeocystis* sp. collected off the coast of Southern California (5 km offshore in the Santa Barbara Channel, at a depth of approximately 10 m), a molecular ecology approach analysing cloned 16S rDNA sequences showed that planctomycetes were the second most abundant aggregate-associated clonal class, including clone AGG8 sharing highest similarity with *Pirellula* sp. IFAM1310, and clone AGG27 at least peripherally related to the planctomycetes (De Long *et al.*, 1993). Planctomycetes may have a role to play in such globally significant processes important for both marine processes and interchanges between sea and atmosphere affecting climate change. Recently, De Long and co-workers have also examined aggregates from the Adriatic Sea as well as zooplankton-derived aggregates from the Californian coast, and in all cases planctomycetes appear to be a common and apparently abundant constituent of such aggregate communities (E. F. De Long, personal communication). Within the consistent and informative biodiversity patterns coming to be detected on aggregate-associated bacterial assemblages, planctomycetes are a significant component signal of those patterns (E. F. De Long, personal communication).

In the context of increasing interest in the true extent of microbial diversity in nature, and the application of molecular methods to this problem, the planctomycetes are proving an important and widely distributed component of that diversity. The potential extent of their contribution is great, yet was unsuspected before application of molecular ecology methods. Understanding their roles within the communities of which they form a part will be an emerging challenge for marine and terrestrial microbial ecologists. The distribution of *N*-acetylglucosamine utilization among recent isolates (Schlesner, 1994) suggests that participation in chitin degradation or mineralization may be one possible role which could be shared by both terrestrial and marine planctomycetes.

Because they are phylogenetically distinct and widely distributed and because retrieval of planctomycetes in culture is possible though often difficult, they may prove useful indicators of the sensitivity of new methods for diversity estimation and for retrieval of the cryptic hitherto unculturable portion of natural microbial communities.

Evolution

A distinct group of the domain Bacteria

The initial phylogenetic study of planctomycetes using analysis of 16S rRNA oligonucleotides established their membership of the Bacteria (Stackebrandt *et al.*, 1984). Grounds for considering the planctomycetes as members of the domain Bacteria, apart from 16S rRNA catalogues and sequence, include a negative diphtheria toxin reaction (protein synthesis resistant to this ADP-ribosylating toxin), lipids with glycerol esters of fatty acids and DNA-dependent RNA polymerase with subunit pattern characteristic of eubacteria (Schlesner & Stackebrandt, 1986). 16S rRNA oligonucleotide cataloguing using *Planctomyces* or *Planctomyces*-like organisms and *Pirellula* (then *Pirella*), including *Pl. limnophilus*, *Pi. staleyi* and *Pi. marina*, also indicated their membership of a single line of descent equivalent in depth to other major lines of the eubacterial kingdom (Stackebrandt *et al.*, 1986a). The similarity in phenotype (e.g. reproduction, morphology and aerobic physiology) of extant genera is, however, unusual relative to other aerobic prokaryotes of comparable phylogenetic depth. A family *Planctomycetaceae* and order *Planctomycetales* for *Planctomyces* and *Pirella* was proposed on the basis of genotypic and phenotypic coherency and a phylogenetic depth comparable to that of other eubacterial divisions (Schlesner & Stackebrandt, 1986). 'Planctomyces and relatives' was recognized as one of the major (eu)bacterial divisions based on 16S rRNA oligonucleotide catalogues, of status equivalent to such extensive divisions as what is now the class *Proteobacteria* and the Gram-positive Bacteria (Woese, 1987).

There is direct molecular evidence for the distinctiveness of the planctomycetes relative to other bacteria. Certain oligonucleotides present in 16S rRNA of isochronically evolving eubacteria, that is, CACAAG, CUAACG, AAUACG, UUCCCG, AUACCCUG and AUUCCUACG, were found to be missing from the planctomycetes originally examined by oligonucleotide cataloguing, including the brackish water strains from Kiel Fjord, Baltic Sea, IFAM 1310 and IFAM 1317, later assigned to the genus *Pirellula* and to *Pl. maris*, respectively (Schlesner & Hirsch, 1984), and strain IFAM 1319, a strain of uncertain position later assigned to *Pirellula* (Liesack *et al.*, 1986). Of these, CUAACG, AUACCCUG and AUUCCUACG can be confirmed as absent from all known planctomycete 16S rRNA sequences on the basis of more recent sequence data. The absence of these otherwise universal oligonucleotides can be interpreted as implying that these universal oligonucleotides evolved later in the evolution of the eubacteria, after the ancestor of *Planctomyces* and relatives had branched off, and the lack of peptidoglycan in planctomycetes from this perspective may likewise be explained by evolution of peptidoglycan synthesis at a stage later than that at which planctomycetes diverged (Stackebrandt *et al.*, 1984). These interpretations apply only if a relatively ancient lineage for the planctomycetes is assumed. Examination of planctomycete 16S rRNA sequences for signature positions characteristic of the domains shows that all planctomycetes possess a G:C pair at position 52:359, like Archaea and Eucarya but unlike most Bacteria (except the chlamydias and the deep-branching radioresistant micrococci and relatives phylum), which have a pyrimidine:purine pair, an unusual G:U pair at position 53:358 shared only with chlamydias and the deep-branching green non-sulphur phylum, and at position 933:1384 an A:U in common with Archaea and Eucarya

but unknown in any other Bacteria except the green sulphur phylum. The latter signature is also absent from the acknowledged deepest phyla of the Bacteria such as the thermotogas and *Aquifex*. The 5S rRNA of planctomycetes exhibits an unusual and very short primary structure of only 109–111 nucleotides relative to the 118 base minimal length for other Bacteria and Archaea, the absence of an insertion at position 66 unique among prokaryote 5S rRNA molecules, and the presence of numerous base-pair transversions relative to the minimal 5S rRNA secondary structure for Bacteria (Bomar *et al.*, 1988).

Are they related to chlamydiae?

An important question for future resolution is the nature of the relationship of planctomycetes to the chlamydiae. On the basis of 16S rRNA sequences, a remote relationship of *Pi. staleyi* to *Chlamydia psittaci* supported by shared higher order structures has been postulated (Weisburg *et al.*, 1986). Cloned 16S rRNA gene sequences of *C. psittaci* and *Pi. staleyi* were used in comparisons with representatives of other Bacteria and a remote but specific relationship was found between the chlamydiae and that single planctomycete representative when signature positions with a composition unique to and characteristic of particular groupings were compared; evolutionary distances showed no specific relationship of the chlamydiae to any other of 400 partial 16S rRNA sequences of eubacteria (Weisburg *et al.*, 1986). Twenty-five such related signature positions were found, higher than the chlamydiae shared with any other eubacterial sequence. Secondary structure of the 16S rRNA also indicated some shared features with chlamydiae distinct from other Bacteria in the region covering positions 40 to 400. There is however only one known shared phenotypic character, the absence of peptidoglycan in their cell walls (otherwise a universal feature of walled Bacteria, i.e. excluding mycoplasms from consideration). With respect to the possible connection with *Chlamydia* and the possible ancient status of planctomycetes, it is of interest that phylogenetic trees based on nucleotide and amino acid sequences for elongation factor Tu have indicated possible early divergence of *Chlamydia* (as well as *Thermotoga*) from the domain Bacteria root (Cousineau *et al.*, 1992). The question of possible relationships of planctomycetes to chlamydiae has been closely connected with the problems of shifting branch position of the planctomycetes in phylogenetic analysis and the problem of whether planctomycetes represent an ancient lineage or whether a rapid evolutionary rate has distorted their position within phylogenetic tree topologies.

Ancient or rapidly evolving?

There have been two interpretations of phylogenetic analyses of the planctomycetes based on 16S rRNA sequences, one in which the planctomycetes appear to be an ancient lineage, and the other in which the planctomycetes are seen as a rapidly evolving lineage. These interpretations are complicated by the variable results of phylogenetic analyses including the group. From the first perspective, they might represent descendants of an ancient group of Bacteria branching immediately after divergence of the 'ur-eubacteria' from the progenote (universal common ancestor) and before the main radiation of the Bacterial lines of descent (Stackebrandt *et al.*, 1984). From the second perspective, planctomycetes may appear to be an older group than actually is the case due to a higher evolutionary rate causing artificially deep branching within phylogenetic trees (Schlesner & Stackebrandt, 1986). A third position could be imagined consistent with both of these views, where planctomycetes branched early from other phyla and also diverged via a rapid evolutionary rate.

The well-known tree based on 16S rRNA oligonucleotide catalogues (Fig. 11 in Woese, 1987) shows 'planctomyces and relatives' as a relatively shallow branching division very distant from the deepest branching *Thermotoga*. In a recent maximum likelihood analysis based on 16S rRNA sequences using 253 species of Bacteria and Archaea, clustering of planctomycetes with chlamydia is also obtained (Olsen *et al.*, 1994). Such conclusions differ from an earlier 16S rRNA oligonucleotide catalogue analysis yielding a deep-branched position of planctomycetes and the fimbriate prosthecate non-budding bacterium *Verrucomicrobium spinosum*, with both separated from chlamydia (Albrecht *et al.*, 1987).

The phylogenetic difficulties were illustrated clearly in one study in which the planctomycete position varied according to which sequences were included in the analysis (Liesack *et al.*, 1992). When either *Planctomyces* or *Pirellula* sequences only were included as planctomycete representatives, planctomycetes clustered with *C. psittaci*, and dendrogram topology resembled that emerging without inclusion of planctomycetes. If, however, *G. obscuriglobus* was the only representative planctomycete sequence, the planctomycete line branched off deeply, between lines defined by *Bacteroides/Clathrochloris* and *Thermus/Chloroflexus*. Inclusion of *I. pallida* resulted in its position as the deepest branch in the Bacteria, and influenced the relationships between *Thermus thermophilus* and *Thermotoga maritima* by formation of a common root, a situation also found when both *G. obscuriglobus* and *I. pallida* were included. If all six planctomycete sequences available were included, a new position of planctomycetes between the *Thermotoga* and the *Chloroflexus/Thermus* lines of descent resulted. Such inconsistencies were considered unique for analyses of Bacteria except perhaps for mycoplasmas. A 'fast clock' interpretation invoking elevated mutation rate in the planctomycetes, and within that group, an even more rapid rate for *G. obscuriglobus* and *I. pallida*, was used to explain these results.

Recent publications from three separate research groups contain trees derived from 16S rRNA gene sequences which exhibit relatively deep-branch positions for the planctomycetes (Neefs *et al.*, 1993; Van de Peer *et al.*, 1994; Embley *et al.*, 1994). Van de Peer *et al.* (1994) employed a novel statistical method for determining the confidence of the tree topology involving repeated

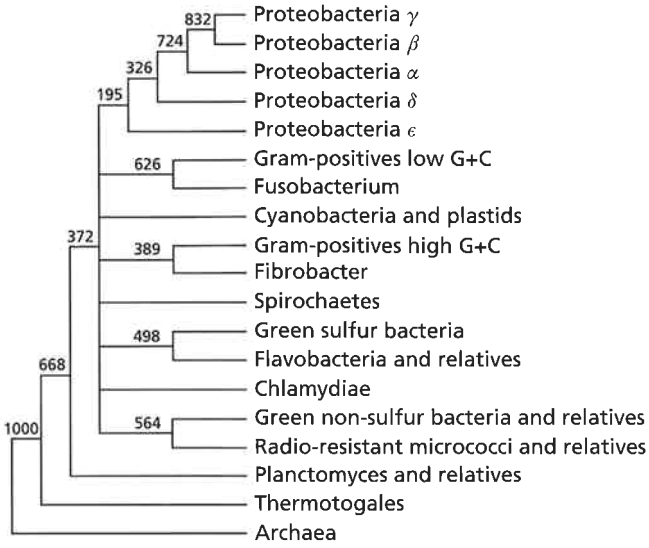

Fig. 4. Consensus tree of Van de Peer *et al.* (1994) constructed from 1232 bacterial small ribosomal subunit RNA sequences. Clusters apparent were selected because their composition does not conflict with previously selected clusters in a sorting of 16000 clusters in 1000 trees. The frequency of each cluster is listed at the corresponding node. In 372 trees out of 1000, both Planctomyces and relatives and Thermotogales are found to have diverged before the other bacterial groups. Branches where the divergence order cannot be decided are drawn as if diverging simultaneously. Horizontal distances between branching points are arbitrary and unrelated to evolutionary distance.

random sampling of selected sequence sets from a dataset of different representatives of 18 taxa corresponding to the divisions or phyla of the Bacteria but including also all the subdivisions of one of these, the *Proteobacteria*. By examining the frequency of different clusters among 1000 trees resulting from this protocol, a consensus tree resulted in which planctomycetes branch as the sister group of all remaining taxa except the thermotogales, and separately from the chlamydiae (Fig. 4). A higher probability was attributed to the early divergence of planctomycetes than to their forming a binary cluster with the chlamydiae, a topology which did occur but only at a lower frequency.

Analysis of the translated amino acid sequence of partial sequence (79%) for a protein-coding gene in planctomycetes, *atpD*, coding for the β-subunit of ATPase in *Pi. marina*, indicated a deeper branch position for this species than other Bacteria for which homologous sequence was available (Rönner *et al.*, 1991). Sequencing of further examples of ancient conserved proteins, such as elongation factors, topoisomerases, DNA-dependent RNA polymerase, and heat-shock proteins, for planctomycetes will contribute valuable data for evolutionary analysis of the group. Although a 23S rRNA sequence exists for *Pi. marina* (Liesack *et al.*, 1988), one phylogenetic analysis using this sequence disagrees with 16S rRNA and *atpD* analyses in that *Pi. marina* groups with the *Proteobacteria* (Schleifer & Ludwig, 1989), while another distinguishes *Pi. marina* as a well-defined separate phylum of the Bacteria and notes also the absence of helices 8 and 9 within domain I of the 23S rRNA molecule in this species (Höpfl *et al.*, 1989).

One 5S rRNA-based phylogenetic analysis recognizes the planctomycetes as a cluster including *Planctomyces*, *Pirellula*, *Gemmata* and *Isosphaera*, but they occur in a loose grouping with the low mol% G+C Gram-positive Bacteria and quite distinct from the *Proteobacteria* (Van Den Eynde *et al.*, 1990), yet some common structural features shared between the 5S rRNA molecules of planctomycetes and proteobacteria have suggested phylogenetic affinities (Bomar *et al.*, 1988).

What then are the arguments for and against the case of planctomycetes being an ancient lineage or a rapidly evolving (tachytelic) but relatively recent lineage? In favour of an ancient lineage is the internal phylogenetic depth of the group, comparable with that of other Bacterial phyla or divisions, and the absence of certain universal Bacterial oligonucleotides in planctomycete 16S rRNA, as well as the absence of peptidoglycan, both the latter suggesting evolution of the group before acquisition of either the particular signature oligonucleotides or ability to synthesize peptidoglycan. Deep branching in itself may not indicate ancient lineage if the group has exhibited a rapid evolutionary rate giving rise to extreme sequence divergence. On the side of rapid evolutionary rate may be the lower similarity values of planctomycetes with members of other Bacterial phyla as compared with values found between those phyla themselves (Liesack *et al.*, 1992). In a similar manner to the tachytelic mycoplasmas, but also to the deep branching green non-sulphur (*Chloroflexus*) division, planctomycetes display uniqueness in variants of highly conserved oligonucleotide families (Woese *et al.*, 1985). Also, the occurrence of many unusual phenotypic and genotypic idiosyncrasies, including short 5S rRNA and split *rrn* operons, may be an indication of highly derived unique features which result from a fast clock (Liesack *et al.*, 1992). At present the question must be considered unresolved, at least until complete relative rate tests are performed. 'Relative rate tests' using comparison of sequence changes between planctomycetes and other Bacteria against a third outgroup reference sequence might be able to resolve the question of how much planctomycetes differ in their evolutionary rate from other Bacteria, but such analysis has not appeared, and might be difficult to interpret due to dependence on assumed phylogeny (Sarich & Wilson, 1967; Fitch, 1976). More complex approaches involving determination of decay of internal symmetry of a gene may be needed to read the planctomycete molecular clock (Gibbs & Dugaiczyk, 1994). Establishment beyond reasonable doubt whether planctomycetes and chlamydiae are sister groups may also be needed to resolve this phylogenetic problem (Woese, 1991).

The disparities between phylogenetic conclusions concerning the planctomycetes from different molecules and analyses reinforce the significance of planctomycetes as a model group for testing hypotheses concerning the

evolution of Bacteria via molecular phylogenetics. Changes in branch positions and phylogenetic depth with the molecule sequenced and organisms included challenge presently available analysis techniques. The interesting and difficult features of this model, which strain the limits of contemporary phylogenetic methods, may generate approaches to analysis which may even be useful with less divergent and problematic groups of micro-organisms.

Molecular cell biology

Cell wall and membranes

The first unusual feature of planctomycete cell biology to be found was the absence of peptidoglycan in their cell walls. This had been indicated first from ultrastructural evidence, and was confirmed by determination of wall chemical composition with respect to absence of muramic acid and diaminopimelic acid in *Pl. maris*, *Pi. staleyi*, *Pi. marina* and related strains, and in *G. obscuriglobus* and *I. pallida* (König *et al.*, 1984; Liesack *et al.*, 1986; Stackebrandt *et al.*, 1986b; Giovannoni *et al.*, 1987a). A strain of *Pi. marina* (IFAM 1313) was found to have an SDS-resistant wall composed mainly of a protein rich in glutamate (König *et al.*, 1984). An additional five strains of *Pirellula*, including *Pi. staleyi*, and three *Planctomyces* strains, including *Pl. maris*, displayed a major protein component rich in proline and stabilized to a significant degree by disulphide bonds as indicated by a high cystine content, explaining resistance of envelopes to 10% SDS (Liesack *et al.*, 1986). *G. obscuriglobus* has a wall relatively low in cystine (Stackebrandt *et al.*, 1986b). Perforations consistent with planctomycete-specific crateriform structures appear also in isolated planctomycete walls (Liesack *et al.*, 1986; Stackebrandt *et al.*, 1986b; Giovannoni *et al.*, 1987a). Lack of peptidoglycan was consistent with the known resistance of these bacteria to peptidoglycan-synthesis-targeting antibiotics such as penicillin-G, ampicillin, vancomycin, D-cycloserine and cephalotin (König *et al.*, 1984). The resistance to β-lactam antibiotics observed for the uncultured species *Pl. bekefii* in enrichments (Schmidt & Starr, 1989) is thus consistent with its probable true relationship to the planctomycetes, as is its cell wall structure. It has been suggested that the absence of peptidoglycan from planctomycetes may be consistent with an ancient origin for this division of the Bacteria, perhaps representing retention of the characteristics of a bacterium evolving prior to invention of peptidoglycan (Stackebrandt *et al.*, 1984). This view may be a useful source of hypotheses with respect to cell wall synthesis pathways.

Cellular fatty acid and chemotaxonomic analysis has revealed some unusual features in the planctomycetes. Lipids of planctomycetes examined possess fatty acids in the ester linkage typical of the Bacteria and Eucarya rather than of Archaea (Kerger *et al.*, 1988; Giovannoni *et al.*, 1987a). In *Pirellula* sp. and *Planctomyces* sp., including *Pi. staleyi*, *Pi. marina*, *Pl. limnophilus* and *Pl. maris*, the major ester-linked fatty acids of phospholipids are palmitic, palmitoleic and oleic acids, a pattern more typical of microeukaryotes than of eubacteria (Kerger *et al.*, 1988). Presence of the 18-carbon monounsaturated 18:1ω9c, not normally a major component of bacteria, is characteristic of the order *Planctomycetales* (Kerger *et al.*, 1988). Evidence for presence of lipopolysaccharide lipid A, presumably from the planctomycete wall, was also obtained (though not direct evidence of LPS as such), as indicated by the presence of hydroxy fatty acids of unique 3-hydroxy pattern sufficiently unusual to perhaps form the basis of signature biomarkers for planctomycete presence in a habitat (Kerger *et al.*, 1988). Evidence for 3-hydroxy acids has also been obtained for *I. pallida* (Giovannoni *et al.*, 1987a). Presence of even-numbered 3-hydroxy fatty acids was confirmed in a more recent study where distribution of different types of those compounds was found to correlate well with the distinction between *Planctomyces* and *Pirellula*, but such compounds also occurred in non-planctomycete prosthecate bacteria such as *Prosthecomicrobium* (Sittig & Schlesner, 1993). A unique cyclopropane fatty acid 13,14cy21:0 is found in some strains resembling *Planctomyces*. In contrast to prosthecate proteobacteria, menaquinones rather than ubiquinones are found in planctomycetes even though grown under aerobic conditions (Sittig & Schlesner, 1993).

The above features emphasize the phenotypically distinct nature of the planctomycetes, consistent with the molecular sequence evidence for their novelty.

Genetic organization

Unusual features of planctomycete molecular cell biology include the unusually short 5S rRNA with special structural characters and the unusual *rrn* operon organization involving separation of the 16S rRNA gene from the 23S rRNA gene. Evidence for such a separation has been found in at least two genera so far, in *Pi. marina* (Liesack & Stackebrandt, 1989) and *Pl. limnophilus* (Menke *et al.*, 1991). In *Pi. marina*, each of the two sets of 16S rRNA genes are separated by at least 8·5 and 4·4 kb, respectively, from the closely linked 23S–5S rRNA genes. *Pl. limnophilus* possesses two unlinked 16S and 23S rRNA genes separated from each other by at least 4·3 kb, while the organization of the five *rrn* operons found in *G. obscuriglobus* could not be unambiguously determined (Menke *et al.*, 1991). Although linkage of 16S and 23S rRNA genes into an *rrn* operon with order 5′-16S-23S-5S-3′ is typical for Bacteria, there are enough exceptions, such as some proteobacteria in addition to the planctomycetes and the deep-branching *Thermus*, to indicate an absence of phylogenetic significance for this trait. A by-product of this research was the discovery of a −10 Pribnow box-like promoter region (TATCTAT) in the 5′-flanking regions of the 23S–5S operons from *Pi. marina* similar to P2 of the *rrn* G operon in *E. coli*, although homologies to a typical −35 region were not found (Liesack & Stackebrandt, 1989). The putative Pribnow box was followed by a GC-rich sequence similar to the discriminator region under stringent control in stable RNA and ribosomal protein genes of *E. coli*. Conserved elements similar to sequences involved in antitermination control in rRNA operons of *E. coli* and *Thermus thermophilus* were also identified in these flanking regions. A

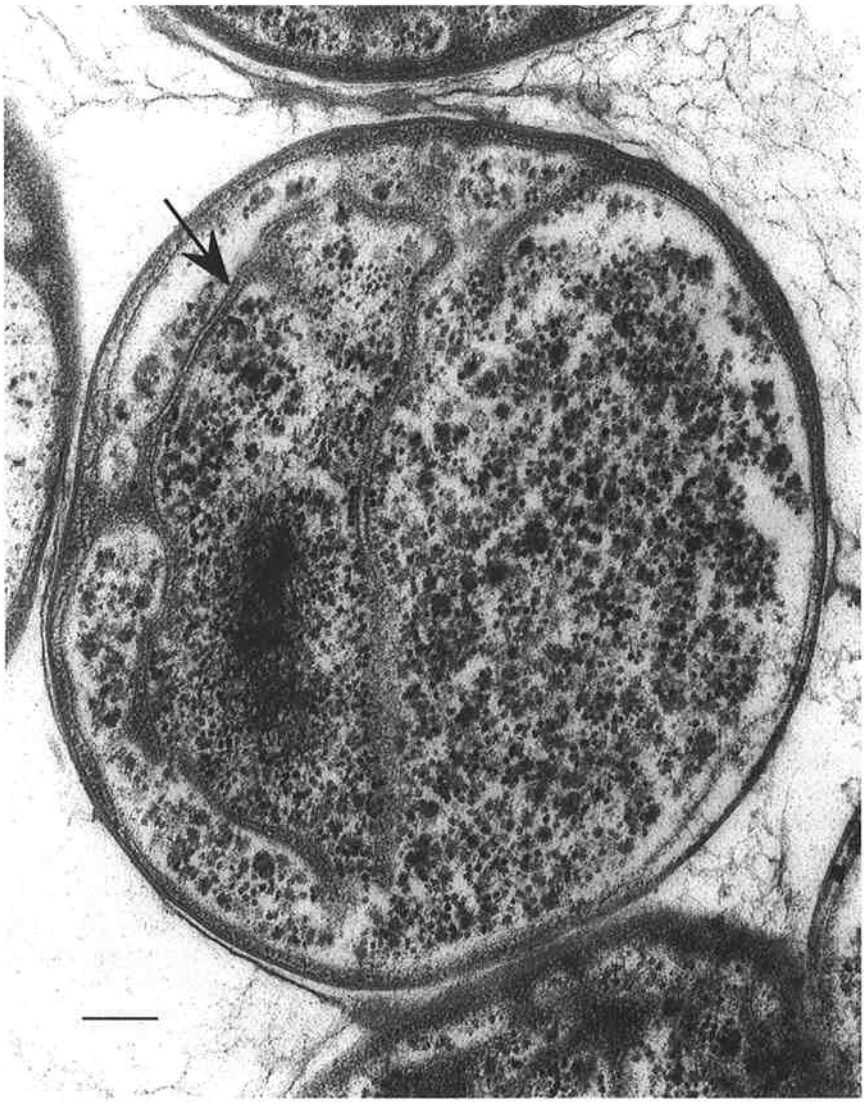

Fig. 5. Electron micrograph of *G. obscuriglobus*, cryofixed and cryosubstituted before embedding and thin-sectioning, showing the nuclear body (arrow) surrounded by two nuclear membranes and enclosing a fibrillar nucleoid. Micrograph taken during collaborative research with R. I. Webb (Centre for Microscopy and Microanalysis, and Department of Microbiology, University of Queensland) and M. L. Lindsay (Department of Microbiology, University of Queensland). Bar, 0·2 μm.

putative processing signal and rho-independent terminator with GC-rich hairpin were found in the 3′-terminal region of the 23S–5S rDNA operons. Homologies may exist between organization of the antitermination boxes in the 23S–5S operons of *Pi. marina* and those of *Thermus thermophilus*. This remains our only knowledge of the possible organization of genes with respect to transcriptional control in the planctomycetes.

The membrane-bounded nuclear body: new plans for cell architecture and new models for cell biology?

The budding mode of reproduction in the planctomycetes is one which could well form a model for cell differentiation in a similar way to the successful use of *Caulobacter* for this purpose. The life-cycle of at least one *Pirellula*-like strain suggests division of this cycle into at least three distinct phases: swarmer cell maturation, budding and mother cell 'resting' between buddings, and pilus synthesis and flagellum loss are correlated with the maturation phase (Tekniepe *et al.*, 1981). Multiple successive daughter cells can appear from one locus of the mother cell.

The character which most clearly positions the planctomycetes as a significant group for cell biology, however, is the membrane-bounded nuclear body of *G. obscuriglobus* (Fuerst & Webb, 1991). This body is visible after cryofixation techniques such as cryo-substitution (Fig. 5) known to prevent artifactual membrane organelles such

as mesosomes, as well as after conventional chemical fixation without freezing. Recent results have demonstrated that the shape and structure of *G. obscuriglobus* can be markedly influenced by osmotic effects of buffer concentration during conventional chemical fixation for electron microscopy (Lindsay *et al.*, 1995). The structure of chemically fixed cells had originally suggested that the DNA of this species might be 'packaged' in a phase-dark inclusion (Franzmann & Skerman, 1984). *I. pallida* has been reported to possess nuclear material located in a distinct region of the cell when 4′,6-diamidino-2-phenylindole (DAPI) staining of DNA is applied, and may also prove to have a compartmentalized cell organization (Staley *et al.*, 1992). Possible occurrence of membrane-bounded nuclear regions in this and other planctomycete species is presently under investigation. In *G. obscuriglobus*, as in eukaryote nuclei, there are two nuclear membranes, separated by a relatively electron transparent space. The nuclear body is visible in DAPI-stained cells via fluorescence microscopy, DNA can be demonstrated inside the body by anti-dsDNA monoclonal antibody, and all the cell DNA appears to be contained within the body.

Several evolutionary scenarios may account for the occurrence of such an organelle in what is phylogenetically a member of the *Bacteria*. These structures might be evolutionarily homologous with eucaryal nuclei, and represent retention of a character shared with a common nucleated proto-eukaryote ancestor. Alternatively, convergent evolution may have resulted in a structure analogous to the eukaryotic nucleus, perhaps even similar in function as well as structure. A third alternative is that the planctomycete nucleus merely reflects a fortuitous internal membrane hypertrophy with a different function to membranes of eucaryal nuclei. The first two hypotheses imply that molecular cell biological features unique within the Bacteria may be discovered. For example, nuclear-targeted proteins bearing nuclear localization signals, perhaps with homology to those used by eucarya, may be discovered if the first hypothesis is true. Alternatively, proteins with unique planctomycete-specific signals would be expected if the second applies.

The occurrence of nuclei in planctomycetes implies that it may not be necessary to invoke either a central role for phagocytosis (Cavalier-Smith, 1975) or an endosymbiotic mechanism involving phagocytic engulfment (Lake & Rivera, 1994; Sogin, 1991) to explain the origin of the eukaryotic nucleus. The planctomycete case demonstrates that an analogous structure can occur within a rigid-walled non-phagocytic cell, presumably one with a non-phagocytic ancestor in which the original genome engulfment by a purely internal membrane had occurred.

If evolutionary homology of planctomycete nuclei with those of eucarya is assumed, hypothetical planctomycete phylogenies consistent with possible modes for eucaryal origins include considering planctomycetes (1) as 'ancient living fossils' retaining an ancestral feature shared with the last common ancestor of the three domains (this implies a proto-eucaryal common ancestor) or (2) as advanced 'missing links' between Bacteria and Eucarya or more precisely, descendants of such links retaining their significant structural innovations (which implies a late eucaryal origin and may be inconsistent with trees clustering Archaea closer to Eucarya than Bacteria). If we assume these nuclei are the result of convergent evolution, phylogenetic problems are avoided.

Regardless of the true phylogeny of the planctomycete nucleus, the occurrence of a membrane-bounded genome in such organisms has many intriguing implications for the study of cell biology. Some predictions which can be made are that there should be some type of nuclear localization signal in nuclear-directed proteins such as DNA-binding topoisomerases and polymerases (e.g. gyrase and DNA-dependent RNA polymerase), and some form of nuclear pore analogue. Weaker predictions are that introns may be found in some protein-coding genes of planctomycetes (which would have major implications for the 'introns-early' vs 'introns-late' controversy) together with correlated RNA-splicing mechanisms involving spliceosomes and small nuclear ribonucleoprotein particles, and that cytoskeletal proteins necessary for distribution of membrane-bounded nuclei during division might occur. Sequences with homology to nuclear localization signals have already been claimed to occur in the proteobacteria *Agrobacterium tumefaciens* and *Neisseria gonorrhoeae*, bacteria not known to possess membrane-bounded nuclei, but with intimate eukaryotic host relationships (Citovsky *et al.*, 1992; Pohlner *et al.*, 1990).

In summary, the planctomycetes are a novel group of organisms with great potential for increasing our knowledge of microbial ecology, bacterial evolution and cell biology. Their wide distribution in nature implies important and hitherto unsuspected roles for them in ecosystem processes, especially concerning the marine ecosystem and contributions of particulate fractions of seawater to global biogeochemistry. The problems posed by their evolutionary relationships challenge contemporary phylogenetic analysis. Finally, and perhaps most significantly, they have challenged our understanding of possible fundamental plans for structural organization of the cell. The molecular implications of their ultrastructure may have wide implications for cell biology and an emerging perspective on the expanding common ground shared by prokaryote and eukaryote biology.

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