Research

Gene discovery within the planctomycete division of the domain Bacteria using sequence tags from genomic DNA libraries
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Abstract

**Background:** The planctomycetes comprise a distinct group of the domain Bacteria, forming a separate division by phylogenetic analysis. The organization of their cells into membrane-defined compartments including membrane-bounded nucleoids, their budding reproduction and complete absence of peptidoglycan distinguish them from most other Bacteria. A random sequencing approach was applied to the genomes of two planctomycete species, *Gemmata obscuriglobus* and *Pirellula marina*, to discover genes relevant to their cell biology and physiology.

**Results:** Genes with a wide variety of functions were identified in *G. obscuriglobus* and *Pi. marina*, including those of metabolism and biosynthesis, transport, regulation, translation and DNA replication, consistent with established phenotypic characters for these species. The genes sequenced were predominantly homologous to those in members of other divisions of the Bacteria, but there were also matches with nuclear genomic genes of the domain Eukarya, genes that may have appeared in the planctomycetes via horizontal gene transfer events. Significant among these matches are those with two genes atypical for Bacteria and with significant cell-biology implications - integrin alpha-V and inter-alpha-trypsin inhibitor protein - with homologs in *G. obscuriglobus* and *Pi. marina* respectively.

**Conclusions:** The random-sequence-tag approach applied here to *G. obscuriglobus* and *Pi. marina* is the first report of gene recovery and analysis from members of the planctomycetes using genome-based methods. Gene homologs identified were predominantly similar to genes of Bacteria, but some significant best matches to genes from Eukarya suggest that lateral gene transfer events between domains may have involved this division at some time during its evolution.
terrestrial habitats [13-15]. In many of these environments planctomycetes make up a significant proportion of the microbial population [7,16], indicating that they may have a significant role in the cycling of organic or inorganic compounds. The recent discovery that the “missing lithotroph” responsible for the anaerobic oxidation of ammonium (anammox process) is an autotrophic planctomycete [8], and the existence of other planctomycetes with similar activities [9], highlights the potential importance of these bacteria for the flux of nutrients in the environment and indicates the potentially wide physiological diversity of the division.

Consistent with their phylogenetic distinctiveness, the planctomycetes possess a series of unusual phenotypic characteristics common to members of the division [5]. These include budding reproduction, peptidoglycan-less (proteinaceous) cell walls and a complex internal ultrastructure [17]. Most notably, cells of at least three species exhibit eukaryote-like membrane-bounded nuclear regions: the genomic DNA of *Gemmata obscuriglobus* is enclosed by two membranes [17,18], whereas that of *Pirellula marina* and *Pi. staleyi* is enveloped by a single membrane [19]. Membranes surrounding the nucleoid are unique to the planctomycetes among members of the domain Bacteria. This feature is only one aspect of a unique type of cell organization shared by all planctomycetes examined so far, involving compartmentalization via intracytoplasmic membranes [17]. Despite the interesting ecological and cell biological aspects of the planctomycetes, molecular studies on this group have been relatively few. To date, DNA sequencing studies on the planctomycetes have involved genes for small subunit (SSU) and large subunit (LSU) rRNA [1,20,21], and a small number of protein-coding genes, including those for the β-subunit of ATPase from *Pi. marina* [22], the DnaK heat-shock protein (HSP70) from *Pirellula* and *Planctomyces* species [23] and the gene *rpoN* for sigma factor 54 from *Planctomyces limnophilus* [24]. Phylogenetic analyses using these gene sequences have failed to elucidate the evolutionary relationship of the planctomycetes relative to the other divisions of the Bacteria. Some sequence analyses show that the planctomycetes form a sister-group of the chlamydiae [1,25], while others place them as a deeply branching division [1,22]. In more recent analyses using the gene for the conserved protein, elongation factor-Tu, inconsistency in the branch position of the planctomycetes was attributed to long-branch attraction effects [26].

In this study we applied a random-sequencing approach to the genomes of two planctomycete species, *G. obscuriglobus* and *Pi. marina*, in order assist discovery of genes that may be relevant to their ecology, phylogeny and cell biology. These species are members of phylogenetically distinct genera within the planctomycete division as judged by 16S rRNA sequence analysis [20]. While it is noteworthy that members of both the genus *Pirellula* (*Pirellula* sp. strain 1) and the genus *Gemmata* (*Gemmata* strain Wa1-1) are currently the subject of whole-genome studies by the REGX project and Integrated Genomics, respectively [27,28], to date, no reports have been published concerning analyses of the resulting genome sequence data. Furthermore, sequence data from these whole-genome projects is not currently accessible to the public. Thus the random-sequencing approach used in this study provides the first insight into the genomes of planctomycetes. Such sequence tag approaches have proved an effective method of gene discovery in other prokaryotes [29-32].

**Results and discussion**

Sequence tags from *G. obscuriglobus* and *Pi. marina* that represent putative protein-coding genes were identified by comparison of individual clone nucleotide sequence translated in all reading frames against protein-sequence databases using the BLASTX algorithm (Tables 1,2). Only sequence matches with expected (e) values below E-4 (as determined by BLASTX) were considered to be significant [33] and are presented here. Of the clones sequenced from *G. obscuriglobus*, 27% (43/160) showed significant matches with known proteins or hypothetical proteins, whereas 32% (29/91) of *Pi. marina* sequences had significant database matches. In addition to these matches, nucleotide sequences for several clones were shown by BLASTN analyses against nucleotide-sequence databases to represent SSU and LSU rRNA gene sequences (data not shown). Best matches for protein-coding genes were predominantly with gene homologs from Bacteria, but there were also some best matches with genes from Archaea and most notably from Eukarya (see below). Within the Bacteria, best matches did not cluster within any one division, although matches with members of Gram-positive and cyanobacterial divisions were common in the case of *G. obscuriglobus*.

Random sequence tags from the two planctomycete species displayed homology with proteins of diverse function, including metabolic enzymes (30 and 38% of total for *G. obscuriglobus* and *Pi. marina* respectively), and proteins involved with transport (7 and 10%), regulation (9 and 10%) and the central processes of translation (9% and 7%) and DNA replication (16 and 10%). Selected proteins from these functional groups are described in detail below.

**Metabolism and biosynthesis**

A large proportion of the metabolism and biosynthesis genes identified in *G. obscuriglobus* and *Pi. marina* were homologous to those coding for enzymes involved in amino-acid and vitamin biosynthesis. For *G. obscuriglobus*, close database matches were found to the enzymes isopropyl malate dehydratase, diaminopimelate epimerase, asparagine synthetase and 2-amino-3-ketobutyrate coenzyme A ligase, which are involved in the biosynthesis of leucine, lysine, asparagine and glycine, respectively. In addition, a homolog
Table 1

Putative protein-coding genes identified in *Gemmata obscuriglobus*

| Clone number | GenBank accession | Best BLASTX match | E value of best match | Species of best match (GenBank accession number) | Domain of best match*
|--------------|-------------------|-------------------|-----------------------|-----------------------------------------------|-----------------------------|
| **Metabolism and biosynthesis** | | | | | |
| G63 | BH001096 | Putative isopropylmalate dehydratase | 9.00E-09 | Methanococcus jannaschii (Q85673) | B |
| G60 | BH001097 | Heptaprenyl diposphosphate synthase component 5- | 6.00E-18 | Bacillus stearothermophilus (P55785) | B |
| G63 | BH001098 | Peptide methionine sulfoxide reductase | 4.00E-11 | Deinococcus radiodurans (Q9RTB6) | B |
| G64 | BH001099 | N4-(beta-N-acetylglucosaminy1)-L-asparaginase precursor | 6.00E-27 | Flavobacterium meningosepticum (Q47898) | B |
| G87 | BH001100 | 3-Oxoadipate enol-lactonase | 2.00E-08 | Acinetobacter calcoaceticus (P00632) | B |
| G136 | BH001104 | 1,4-Alpha-glucan branching enzyme | 4.00E-11 | Bacillus caldolyticus | B |
| G179 | BH001105 | Putative asparagine synthetase | 2.00E-07 | Rattus norvegicus (P07308) | B |
| G184 | BH001106 | 2-Amino-3-ketobutyrate coenzyme A ligase | 5.00E-25 | Burkholderia cepacia | B |
| G108 | BH001102 | FDHD protein homolog | 2.00E-45 | Streptomyces coelicolor | B |
| G136 | BH001104 | I,4-alpha-glucan branching enzyme | 4.00E-11 | Bacillus caldolyticus (P30537) | B |
| G179 | BH001105 | Putative asparagine synthetase | 2.00E-07 | Mycobacterium tuberculosis (Q10374) | B |
| G184 | BH001106 | 2-Amino-3-ketobutyrate coenzyme A ligase | 5.00E-25 | Bacillus subtilis (O31777) | B |
| G222 | BH001107 | Threonine dehydratase biosynthetic | 9.00E-53 | Listeria monocytogenes (P53607) | B |
| G204 | BH001108 | Ribonucleotide reductase large chain | 1.00E-05 | Schizosaccharomyces pombe (P36602) | E |
| **Transport** | | | | | |
| G74 | BH001109 | Large conductance mechanosensitive channel | 8.00E-10 | Haemophilus influenzae (P44789) | B |
| G139 | BH001110 | Cadmium efflux system accessory protein | 4.00E-05 | Listeria monocytogenes (Q56405) | B |
| G185 | BH001111 | Sulfate transport ATP-binding protein CysA | 6.00E-35 | Synechocystis PCC6803 (P74548) | B |
| **Regulation and signal transduction** | | | | | |
| G99 | BH001112 | Ompr homolog | 6.00E-11 | Guillardia theta chloroplast (P704168) | E(c) |
| G114 | BH001113 | Nitrogen regulation protein NbrB | 2.00E-16 | Bradyrhizobium sp. (P10578) | B |
| G217 | BH001114 | Transcriptional regulatory protein Af5Q1 | 3.00E-09 | Streptomyces coelicolor (P50492) | B |
| G223 | BH001115 | Transcriptional regulator SmtB | 2.00E-05 | Synechococcus PCC7942 (P30340) | B |
| **DNA replication/insertion** | | | | | |
| VG23 | BH001116 | Replicative DNA helicase | 2.00E-11 | Synechocystis PCC6803 (Q55418) | B |
| G86 | BH001117 | H repeat-associated protein in RHSC-PHRB intergenic region | 1.00E-17 | Escherichia coli (P57541) | B |
| G142 | BH001118 | Tral protein (DNA helicase I) | 2.00E-12 | Escherichia coli (P14565) | B |
| G165 | BH001119 | Insertion element IS402 hypothetical 24 kd | 6.00E-05 | Burkholderia cepacia (P24536) | B |
| G182 | BH001120 | Putative helicase HelY | 5.00E-06 | Mycobacterium tuberculosis (Q10701) | B |
| G196 | BH001121 | Tral protein (DNA helicase I) | 3.00E-12 | Escherichia coli (P14565) | B |
| G235 | BH001122 | Probable transposase for insertion sequence element IS702 | 3.00E-18 | Freynella diplosiphon (Q00462) | B |
| **Translation** | | | | | |
| G51 | BH001123 | SOS ribosomal protein L23 | 1.00E-13 | Aquifex aeolicus (O66433) | B |
| G154 | BH001124 | SOS ribosomal protein L4 | 3.00E-10 | Bacillus stearothermophilus (P28601) | B |
| G156 | BH001125 | Arginyl-tRNA synthetase | 4.00E-21 | Escherichia coli (P11875) | B |
| G230 | BH001126 | Probable tRNA methyl transferase | 5.00E-05 | Synechocystis PCC6803 (P73755) | B |
| **Miscellaneous** | | | | | |
| VG17 | BH001127 | Serine-threonine protein kinase | 1.00E-22 | Myxococcus xanthus (P54737) | B |
| G92 | BH001128 | Cell division protein FtsH | 4.00E-04 | Lactococcus lactis subsp. lactis (P46469) | B |
| G149 | BH001129 | Integrin alpha-V (vitronectin receptor alpha subunit precursor) | 9.00E-05 | Homo sapiens (P06756) | E |
| G159 | BH001130 | Serine protease DO-like precursor | 7.00E-10 | Chlamydia trachomatis (P18584) | B |
| G189 | BH001131 | H repeat-associated protein in RHSE-NARV intergenic region | 2.00E-13 | Escherichia coli (P28917) | B |
| G191 | BH001132 | Ribonuclease inhibitor | 6.00E-05 | Sus scrofa (P10775) | E |
| **Hypothetical proteins** | | | | | |
| VG21 | BH001133 | Hypothetical 47.5 kDa protein Cy9c4.09 | 1.00E-04 | Mycobacterium tuberculosis (Q50739) | B |
| G76 | BH001134 | Hypothetical protein in DHLA 3' region | 3.00E-17 | Xanthobacter autotrophicus (P22644) | B |
| G96 | BH001135 | Hypothetical 42.1 kDa protein | 5.00E-04 | Bacillus subtilis (O31712) | B |
| G137 | BH001136 | Hypothetical protein in DHLA 3' region | 4.00E-31 | Xanthobacter autotrophicus (P22644) | B |
| G180 | BH001137 | Hypothetical 30.2 kDa protein SLR1717 | 1.00E-29 | Synechocystis strain PCC6803 (P73846) | B |
| G231 | BH001138 | Hypothetical protein MJ0682 | 2.00E-35 | Methanococcus jannaschii (Q58095) | A |

* B, Bacteria; A, Archaea; E, Eukarya; E(c), eukaryal chloroplast.
of threonine deaminase was identified. This enzyme catalyzes the formation of \( \alpha \)-ketobutyrate from threonine, an intermediary step in isoleucine biosynthesis [34]. In *Pl. marina*, a gene putatively coding for the NADH/NADPH-dependent enzyme glutamate synthase was found. This enzyme, a glutamine oxoglutarate aminotransferase (GOGAT), is important in the incorporation of inorganic nitrogen into cell material by conversion of 2-oxoglutarate and L-glutamine to L-glutamate [35].

Homologs of genes involved in the biosynthesis of vitamins and cofactors were also identified in *Pl. marina*. These include glutamate-1-semialdehyde-2,1-aminomutase, which catalyzes the final step in the conversion of glutamate to 4-aminolevulinate (the precursor of tetrapyrrole synthesis), uroporphyrin-III C-methyltransferase and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXP). Uroporphyrin-III C-methyltransferase is involved in the synthesis of both cobalamin (vitamin B12) and siroheme (a cofactor for sulfite reduction).
and nitrite reductases) [36], whereas DXP catalyzes the initial reactions in biosynthesis of isoprenoids, which are precursors of some vitamins such as vitamin A [37].

The large proportion of amino-acid and vitamin biosynthesis genes identified in the two planctomycete species suggests that they may be able to synthesize many of these growth factors de novo. This finding is consistent with the ability of planctomycetes to grow in oligotrophic conditions and habitats [38,39]. Many planctomycetes, including Pi. marina can be cultured on minimal media with only a modest or no requirement for the addition of vitamins [38-41].

Other sequences homologous to metabolic genes of interest that were identified include heptaprenyl diphosphate synthase from G. obscuriglobus and glucosamine fructose-6-phosphate aminotransferase (GlmS) from Pi. marina. Heptaprenyl diphosphate synthase has been shown in Bacillus species to catalyze the synthesis of the prenyl side chain of menaquinone-7 [42]. The presence of a menaquinone biosynthesis gene in G. obscuriglobus is consistent with a previous report demonstrating that the planctomycetes possess menaquinones [43], despite the fact that ubiquinones rather than menaquinones are typically associated with aerobic bacteria.

GlmS reversibly catalyses the formation of D-glucosamine-6-phosphate and L-glutamate from D-fructose-6-phosphate, using L-glutamine as the ammonia source [44]. This is the initial step in a pathway that produces N-acetyl-D-glucosamine as the final product, a compound that is a major component of the bacterial cell-wall constituent peptidoglycan. Interestingly, peptidoglycan is not a component of the predominantly protein cell walls of planctomycetes, including those of Pirellula and Gemmata species [20,45,46]. Therefore, it is possible that the net reaction catalyzed by GlmS in planctomycetes is the reverse reaction - the conversion of glucosamine-6-phosphate to fructose-6-phosphate (an intermediate compound in the glycolysis pathway). Reactions flowing in this direction would be consistent with the ability of planctomycetes to utilize N-acetylglucosamine as a sole source of carbon and nitrogen [39]. Alternatively, it is possible that N-acetylglucosamine is synthesized but utilized in the formation of glycoprotein or polysaccharide rather than in that of peptidoglycan.

Homologs of the genes for the enzymes phosphoribosylamine-glycine ligase (GARS) and orotate phosphoribosyl transferase (OPRT) were also found in Pi. marina. These enzymes are involved in the pathways of pyrimidine and purine biosynthesis, respectively. Furthermore, a sequence homolog of ribonucleotide reductase, an enzyme central to deoxyribonucleotide synthesis, was discovered in G. obscuriglobus. Significantly, two out of three of these gene sequences had their closest BLASTX matches with organisms from domains other than the Bacteria. The closest match to the putative Pi. marina OPRT gene was with Methanococcus jannaschii, a member of the Archaea, whereas the putative gene for ribonucleotide reductase had its closest match with a eukaryotic organism. The GARS sequence homolog had a top match with a cyanobacterium; however, some of the next best matches were with eukaryotes and other bacteria (data not shown). Certain enzymes involved in the eukaryotic purine and pyrimidine biosynthesis pathways are believed to have been derived from prokaryotes by horizontal gene transfer [47,48]. If the genes involved in these pathways are subject to frequent horizontal transfers, this may also explain the presence of the eukaryote-and archaea-like genes in the planctomycetes.

### Transporters

A number of genes homologous to those involved in transport across membranes were sequenced from both G. obscuriglobus and Pi. marina. A homolog of a gene encoding a large-conductance mechanosensitive channel (MscL) was identified in G. obscuriglobus. In Escherichia coli, MscL forms a channel in the inner membrane that has been shown to gate in response to tension in the lipid bilayer. The MscL is believed to protect cells from lysis upon osmotic shock [49]. MscL channels may have a particularly important role in planctomycetes, which frequently inhabit osmotically stressful oligotrophic environments but lack the rigidifying peptidoglycan component in their cell walls.

Several transport protein gene homologs were also identified in Pi. marina. Of particular interest were two genes putatively involved in the synthesis and transport of capsular and O polysaccharide, RfbB [50] and ABCA protein [51], suggesting that this organism may be able to produce and secrete extracellular polysaccharides or lipopolysaccharides, in a similar manner to Gram-negative organisms. This is consistent with previous studies on planctomycete lipids, which suggested the presence of lipopolysaccharide lipid A in Planctomyces, Pirellula [52] and Isosphaera [53] species on the basis of 3-hydroxy fatty acid detection.

Also in Pi. marina, a gene homologous to that encoding the FlhA protein was identified. In various bacteria this protein is believed to be essential for the export of the flagellar hook protein and hook-capping protein to the periplasm [54]. This protein may play a similar role in Pi. marina, as it possesses a single sheathed flagellum [40].

### Regulation and signal transduction

Several genes putatively involved in signal transduction and regulation of transcription were identified. Homologs of the osmotic shock response regulator OprR and the nitrogen regulation sensor kinase NtrB were identified in G. obscuriglobus. Thus, in this organism, there is evidence for signal transduction proteins that form ‘two-component
systems’, simple regulatory systems operating through protein phosphorylation cascades that allow adaptation to environmental changes.

In *Pi. marina*, there is evidence for the presence of a chemotaxis signal transduction system. Homologs of two proteins involved in bacterial chemotaxis in *Bacillus subtilis*, CheB and CheC, were identified. CheB has been shown to display methylesterase activity and is involved in the modification of the cytoplasmic domains of the chemoreceptor methyl-accepting chemotaxis proteins [55], while CheC is involved in determining the direction of flagellar rotation [56].

**DNA replication**

A number of sequence tags with matches to enzymes involved in DNA replication were identified, including several genes putatively encoding helicases. A gene for a replicative DNA helicase (DnaB), which acts at the replication fork by disrupting the hydrogen bonds between the complementary base pairs [57], was identified in *G. obscuriglobus*. *Pi. marina* also contains a putative DNA helicase that had its closest match with the Hus2 helicase of the eukaryote *Schizosaccharomyces*. The Hus2 helicase is highly homologous to the RecQ family of helicases of bacteria and other eukaryotes [58]. Bacterial RecQ helicases were also among the top BLASTX hits for this sequence tag (data not shown); thus, the eukaryote match for this sequence may not be highly significant.

Two additional helicases were putatively identified from *G. obscuriglobus*. These included an RNA helicase (HelY) and the TraI protein. TraI has been shown to possess both site-specific nicking and DNA unwinding activity, and is specifically involved in the conjugal DNA transfer of plasmid DNA [59]. The presence of plasmids in *G. obscuriglobus* has not yet been investigated; however, another member of the planctomycetes, *P. limnophilus*, has been shown by pulse-field gel electrophoresis to contain an extrachromosomal element [60]. The transfer of plasmid DNA may thus be a useful function in planctomycetes as in other bacteria, consistent with the demonstrated ability of a planctomycete, *P. maris*, to acquire conjugative plasmids [61].

A second class of enzyme involved in DNA replication and repair was identified in *Pi. marina*. DNA polymerase I is responsible for processing Okazaki fragments on the lagging strand during replication [62]. In bacteria, this enzyme also possesses 5'-3' exonuclease activity and in some organisms, including *E. coli*, 3'-5' exonuclease activity [63]. The 3'-5' exonuclease activity is encoded by the central portion of the *polA* gene and organisms deficient in this activity lack three essential protein motifs in this region [64]. The DNA polymerase I sequence tag from *Pi. marina* encompasses two out of three of these motifs (NLKYD and YAAE), both of which are conserved relative to the *E. coli* *polA* gene. This suggests that the *Pi. marina* DNA polymerase I may also possess the 3'-5' exonuclease activity, an hypothesis testable by enzyme assay.

**Translation**

Several sequence tags putatively coding for proteins central to translation were identified. These included two classes of proteins: ribosomal proteins and aminoacyl-tRNA synthetases. In *G. obscuriglobus*, two homologs of ribosomal protein genes were found, one for ribosomal protein L23 and one for L4. In the assembled ribosome, L23 is located within the A site of the 50S subunit [65] and is one of the few ribosomal proteins that directly binds LSU rRNA. In bacteria, the L4 protein is implicated in both ribosomal peptidyltransferase activity and in some cases, autoregulation of the S10 ribosomal protein operon [66].

Three sequence tags putatively coding for aminoacyl-tRNA synthetases, enzymes catalyzing the esterification of amino acids to their respective tRNA molecules, were identified in *G. obscuriglobus* and *Pi. marina*. Two of these belong to the class I tRNA synthetases (arginyl- and leucyl- tRNA synthetase), which are typically monomeric, and one belongs to class II tRNA synthetases (alanyl-tRNA synthetase), which are di- or tetrameric.

Both the ribosomal proteins and the aminoacyl-tRNA synthetases are central to the translational process; thus, they have been employed as phylogenetic markers in a number of studies. In particular, ribosomal proteins (when used in concatenated analyses in conjunction with rRNAs) and leucyl-tRNA synthetases appear to uphold the ribosomal RNA-based phylogeny of the three domains as well as a large proportion of the inter-bacterial relationships [67,68]. From this perspective, the ribosomal protein sequences from *G. obscuriglobus* and the leucyl-tRNA synthetase sequence from *Pi. marina* may prove useful in re-examining the phylogenetic position of the planctomycetes within the Bacteria. In contrast, both the alanyl- and arginyl-tRNA synthetases do not uphold the rRNA tree, displaying multiple horizontal gene transfers between lineages [68]. Nonetheless, the alanyl-tRNA synthetase of certain bacterial taxa, including the *Chlamydia* group, is believed to contain an important amino-terminal signature sequence [69]. The alanyl-tRNA synthetase sequence tag generated from *Pi. marina* does not encompass this region, and the retrieval of the remaining portion of this gene made possible by using the sequence tag as basis for a probe may aid in determining whether the planctomycetes share this signature with the chlamydiaceae and thus whether the purported relationship between these two groups [1,25] is supported.

**Eukaryote-like genes**

Several sequence tags from *G. obscuriglobus* and *Pi. marina* displayed closest BLASTX matches with proteins from members the domain Eukarya, including an acyl-CoA fatty-acid desaturase, a ribonucleotide reductase large
chain, and a ribonuclease inhibitor in the case of *G. obscurglobus*, and a phosphoribosylamine-glycine ligase and the ATP-dependent DNA helicase HUS2 in the case of *Pi. marina*. However, only two of these matches were with proteins that are considered atypical in Bacteria. These include a homolog of integrin alpha-V in *G. obscurglobus* and a homolog of inter-alpha-trypsin inhibitor protein in *Pi. marina*.

In higher eukaryotes, integrins are important in transmembrane signal transduction from the extracellular matrix and in organization of the cytoskeleton [70], and members of the inter-alpha-trypsin inhibitor protein family play a major role in extracellular matrix stability [71].

Additional evidence for homology between the planctomycete sequence tags and integrin alpha-V and inter-alpha-trypsin inhibitor was found by conducting iterative database searches with the position-specific iterative program PSI-BLAST. In both cases, PSI-BLAST converged on the integrin and inter-alpha-trypsin inhibitor proteins respectively. Furthermore, multiple sequence alignment of the translated sequence tags with these proteins revealed a number of regions of conservation (Figures 1 and 2). A motif characteristic of integrin alpha-chain proteins, [G][ILV]...[D][DN]...[FILMV][FILMV][ILMV] (single-letter amino-acid code), was found in the *G. obscurglobus* homolog as GLSVAIGDVNGDAGDVIVV (Figure 1). This integrin alpha-chain motif is part of one of the putative Ca2+-binding regions of the alpha subunit of the vitronectin receptor and other integrins [72].

Genes homologous with integrins (beta4 and alpha6) have also been detected in the genome of the cyanobacterium *Synechocystis* sp. PCC6803 [73] and are assumed to be the result of horizontal transfer from eukaryotes. Homologs of the inter-alpha-trypsin inhibitor heavy chain were also detected by us via the ERGO genome sequence database [74] in the genomes of the cyanobacteria *Nostoc punctiforme* (sequence ID RNP0g803) and *Anabaena* sp. (sequence ID RAN0g682). Integrin and inter-alpha-trypsin inhibitor homologs thus appear to be present in two separate divisions of the Bacteria, the planctomycetes and the cyanobacteria. This may suggest a lateral gene transfer from eukaryotes to bacteria that occurred before the separation of these two divisions, or retention of such genes only in Eukarya and these two bacterial divisions accompanied by gene loss in others.

Studies of bacterial genome sequences routinely reveal genes homologous to those from other domains, for example, the relatively large proportion of archaeal and eukaryal homologs in the genomes of *Thermotoga maritima* [75] and

|                | H. sapiens | M. musculus | G. obscurglobus |
|----------------|------------|-------------|-----------------|
| 20             | CTYVYAPCRD | CTYVYAPCRD  | TSGLGGG-NTNTNVTAQ----| |
| 40             | *          | *           | LLDRLRVLDEGD    | |
| 60             | SFYWQGQLISD | SFYWQGQLISD | STSLGDAWPLS    | |
| 80             | *          | *           | VYKQTAQIAEDDS   | |
| 100            | YG--G--SVVGDEEDGID | YG--G--SVVGDEEDGID | FGGRGTVRLGD   | |
| 120            | *          | *           | FGGRGTVRLGD    | |
| 140            | SLYRFTEQCA | SLYRFTEQCA  | LGVFTGMAAYEGESVAATDINGDYAVETICAPFL | |
| 160            | *          | 160         | LGVFTGMAAYEGESVAATDINGDYAVETICAPFL | |
|                | [integrin α motif] | | |

Figure 1
Alignments for clone M3G149 from *Gemmata obscurglobus* with integrin alpha-V sequences from the eukaryotes *Homo sapiens* and *Mus musculus*. Reference sequences are from SWISS-PROT database (P06756 for *H. sapiens*, P43406 for *M. musculus*). Shading indicates level of conservation of amino acids. Black, 100% similarity; dark gray, ≥ 80% similarity; light gray, ≥ 60% similarity; white, < 60% similarity. Asterisks indicate every 10th amino-acid position where this is not already indicated by a number.
Genome /G74 M. auratus (ACM 2246) was cultured on M1 agar plates [39] at 28°C. G. obscuriglobus (Australian Collection of Microorganisms (ACM) 3344) was cultured on half-strength marine agar [19] at 28°C.

Conclusions
In summary, the random-sequence-tag approach is a valuable and economic means of generating genomic sequence information for members of the distinct phylum Planctomycetes. This hypothesis could be tested by consideration of ge gene organization, base composition and codon usage, which may be deduced only with a more extensive dataset derived from a planctomycete genome sequence.

Materials and methods
Bacterial strains and culture
*G. obscuriglobus* (Australian Collection of Microorganisms (ACM) 2246) was cultured on M1 agar plates [39] at 28°C. *Pi. marina* (ACM 3344) was cultured on half-strength marine agar [19] at 28°C.

DNA extraction
Genomic DNA was extracted from *G. obscuriglobus* using a modification of the DNAzol technique (Gibco BRL). *G. obscuriglobus* cells were harvested from plate cultures and suspended in 10 ml TE buffer. The cells were pelleted by centrifugation, resuspended in 10 ml DNAzol and incubated at 65°C for 30 min. Cell debris was pelleted by centrifugation at 14,000g for 15 min. An equal volume of absolute ethanol was added to the supernatant and DNA precipitated overnight at -20°C. Precipitated DNA was recovered by centrifugation at 14,000g for 10 min at 4°C. The DNA pellet was washed with 70% ethanol and air-dried. DNA was resuspended in TE buffer containing 20 μg/ml RNase A.

Genomic DNA was extracted from *Pi. marina* as follows. Growth from half-strength marine agar plates was harvested and suspended in 10 ml STE buffer (0.75 M sucrose, 50 mM Tris-HCl pH 8.3, 40 mM EDTA). The cells were treated with 2% SDS for 1 h at 42°C followed by 50 μg/ml proteinase K at 55°C for 30 min. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the lysate and the sample centrifuged at 7,000g for 45 min. DNA contained in the aqueous phase was precipitated with 0.1 volumes of 3 M sodium acetate and 2 vols absolute ethanol. DNA was pooled onto a glass rod, washed with 70% ethanol, dried and resuspended in TE buffer for storage at -20°C.

Clone library preparation
Plasmid (*G. obscuriglobus*) and lambda phage (*Pi. marina*) clone libraries were prepared from restricted...
Genomic DNA. *G. obscuriglobus* DNA was digested with Sau3AI to give fragments of 400 base-pairs (bp) to 3 kilobases (kb), and ligated into the *BamHI* site of the pBlue-script II SK(-) phagemid. The ligated DNA was electroporated into *E. coli* XL1-Blue. Recombinant *E. coli* were selected on LB agar supplemented with ampicillin. Plasmid DNA was isolated using a High Pure plasmid isolation kit (Boehringer Mannheim).

Genomic DNA from *Pi. marina* was restricted with the enzyme BclI to give DNA fragments in the range of 2-12 kb. The restricted DNA fragments were ligated into the *BamHI* arms of the lambda Zap Express vector (Stratagene). The vector DNA, containing inserts, was packaged into lambda phage heads using Gigapack III Gold packaging extract (Stratagene). *E. coli* cells (strain XL1-Blue MRF) were infected with the packaged lambda phage according to the manufacturer's instructions. Recombinant phage were blue-white screened and titered on LB agar plates overlayed with LB top agarose containing isopropylthiogalactose and X-gal. The phage library was amplified according to the manufacturer's instructions. Plaques of recombinant phage were picked randomly and stored in 96-well microtiter plates. The vector inserts were amplified by PCR according to the protocol of the Filarial Genome Network [78].

**Cycle sequencing**

Plasmids and PCR products were sequenced from the respective *G. obscuriglobus* and *Pi. marina* clone libraries using the BigDye dideoxy terminator sequencing mix (Applied Biosystems) and T3 or T7 primers. A total of 160 sequencing reactions were performed using the BigDye dideoxy terminator sequencing mix (Applied Biosystems) and T3 or T7 primers. A total of 160 bases available within the National Center for Biotechnology Information (NCBI) website [79]. To detect and localize homologous sequences, a BLASTX or BLASTN search was initiated using PSI-BLAST (NCBI) and aligned with homologous sequences using PILEUP within BioNavigator (BioNavigator.com provided by Entgen Corporation) within the Australian National Genomic Information Service (ANGIS). GeneDoc [80] was used to edit final alignments and produce residue shading. Nucleotide accession numbers for nucleotide sequences from the clones are given in Tables 1 and 2.

**Sequence analysis**

The resulting single-pass sequences were edited and analyzed to identify the represented genes using BLASTX or BLASTN algorithms for sequence comparison with databases available within the National Center for Biotechnology Information (NCBI) website [79]. Selected sequence tags were analyzed with the position-specific-iterative search algorithm PSI-BLAST (NCBI) and aligned with homologous sequences using PILEUP within BioNavigator (BioNavigator.com provided by Entgen Corporation) within the Australian National Genomic Information Service (ANGIS). GeneDoc [80] was used to edit final alignments and produce residue shading. Nucleotide accession numbers for nucleotide sequences from the clones are given in Tables 1 and 2.

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