Characterization and Evolution of Cell Division and Cell Wall Synthesis Genes in the Bacterial Phyla *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes* and Phylogenetic Comparison with rRNA Genes[▽][†]

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In the past, studies on the relationships of the bacterial phyla *Planctomycetes*, *Chlamydiae*, *Lentisphaerae*, and *Verrucomicrobia* using different phylogenetic markers have been controversial. Investigations based on 16S rRNA sequence analyses suggested a relationship of the four phyla, showing the branching order *Planctomycetes*, *Chlamydiae*, *Verrucomicrobia/Lentisphaerae*. Phylogenetic analyses of 23S rRNA genes in this study also support a monophyletic grouping and their branching order—this grouping is significant for understanding cell division, since the major bacterial cell division protein FtsZ is absent from members of two of the phyla *Chlamydiae* and *Planctomycetes*. In *Verrucomicrobia*, knowledge about cell division is mainly restricted to the recent report of *ftsZ* in the closely related genera *Prosthecobacter* and *Verrucomicrobium*. In this study, genes of the conserved division and cell wall (*dcw*) cluster (*ddl*, *ftsQ*, *ftsA*, and *ftsZ*) were characterized in all verrucomicrobial subdivisions (1 to 4) with cultivable representatives (1 to 4). Sequence analyses and transcriptional analyses in *Verrucomicrobia* and genome data analyses in *Lentisphaerae* suggested that cell division is based on FtsZ in all verrucomicrobial subdivisions and possibly also in the sister phylum *Lentisphaerae*. Comprehensive sequence analyses of available genome data for representatives of *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes* strongly indicate that their last common ancestor possessed a conserved, ancestral type of *dcw* gene cluster and an FtsZ-based cell division mechanism. This implies that *Planctomycetes* and *Chlamydiae* may have shifted independently to a non-FtsZ-based cell division mechanism after their separate branchings from their last common ancestor with *Verrucomicrobia*.

In the past decade, our view of the bacterial cytoskeleton has basically changed. Bacterial homologues of the three major eukaryotic cytoskeletal families, actin, tubulin, and intermediate filaments, have been identified: FtsA/MreB/ParM, FtsZ/BtubAB, and crescentin (reviewed in reference 9). Another bacterial homologue of tubulin (TubZ) was very recently detected in *Bacillus*. It was shown to polymerize in dynamic, linear polymers, which were suggested to be important for plasmid maintenance (21). Bacterial cell division is generally based on the tubulin homologue FtsZ, which is the structural protein forming a Z ring at the division site (5). In *Escherichia coli*, FtsA is the first protein known to be recruited to the Z ring and is thought to be important for stabilization of FtsZ (56, 57). FtsZ is found in almost all bacteria studied so far. However, the analysis of genome-sequencing data suggested that it may be absent in representatives of the three bacterial phyla *Verrucomicrobia*, *Chlamydiae*, and *Planctomycetes* (10, 15, 18, 37, 46).

The report of genuine tubulin genes in the genus *Prosthecobacter* (18), representatives of *Verrucomicrobia*, might have suggested a vicarious takeover of the FtsZ function through the bacterial tubulins in the phylum *Verrucomicrobia*. However, *ftsZ* genes were recently detected and characterized in three different *Prosthecobacter* species and in the incomplete genome sequence data for their closest relative, *Verrucomicrobium spinosum* (36).

Generally, *ftsZ* is located in a cluster of genes involved in cell division and in the synthesis of peptidoglycan precursors (2, 54). A prominent feature of this *d*ivision and *c*ell *w*all (*dcw*) cluster is that it is conserved in many bacterial genomes over a broad taxonomic range (33, 47, 55). This is remarkable, as genome organization, gene content, and gene order within operons or gene clusters are very dynamic in evolutionary terms (reviewed in reference 11). Further, a strong relationship between the organization/conservation of the *dcw* cluster and cell morphology has been detected. In contrast to cocci, the majority of rod-shaped and filamentous bacteria have been found to possess a conserved *dcw* cluster (47). The correlation suggests that cotranslational assembly of some *dcw* cluster gene products links gene order with cell morphology; this mechanism was referred to as genomic channeling (30, 31). As the last common ancestor of extant bacteria has been suggested to have had a rod shape (20), it was inferred that it also had a large *dcw* cluster (similar to that of *E. coli*), which has been maintained in some lineages and has been reduced in others (33). It has been speculated that the ancestral cluster might have arisen from the blending of a primordial *mur* operon with three other gene subsets, differing in their functional roles (30). Alternatively, but very unlikely, the complex gene structure of the *dcw* cluster might have been a recent acquisition

that has arisen independently by convergent evolution in phylogenetically distant bacterial groups. The possibility that the *dcw* cluster spread through the bacterial domain by horizontal gene transfer is improbable because the comparative sequence analyses of the individual genes matches the 16S rRNA phylogeny (30). Indeed, genome analysis of deeply branching bacteria also strongly supports the possibility that the last common ancestor of extant bacteria had a large *dcw* cluster, which was secondarily reduced in different lineages (30).

The *dcw* cluster comprises 16 genes in *E. coli*. The first two genes at the 5-prime terminus (*mraWZ*) code for proteins with unknown functions (6). The *mur* genes, together with *mraY* and *ddl*, are essential and are involved in the synthesis of murein (peptidoglycan) precursors (52). In addition, the *dcw* cluster comprises six other *fts* genes that are essential for cell division; their products have been shown to localize at the division site during septation (27). Gene order and content are conserved to different extents in the different regions of the *dcw* cluster. A variable 5′ region is followed by a conserved block comprising *ftsI* to *murG*, followed by another variable region that may contain the genes *murC*, *murB*, and *ddl*; finally, there is another highly conserved block comprising the division genes *ftsQAZ* (47).

The present knowledge about cell division in the *Verrucomicrobia* is restricted to the characterization of *ftsZ* only in the very closely related genera *Prosthecobacter* and *Verrucomicrobium* (36, 64). However, the phylum *Verrucomicrobia* (13, 16) comprises five subdivisions consisting of very heterogeneous free-living and symbiotic species with only very few cultivable representatives.

On the basis of 16S rRNA gene phylogeny, *Verrucomicrobia*, *Chlamydiae*, and *Planctomycetes* form a monophyletic cluster, also comprising *Lentisphaera* and two additional candidate phyla (*Poribacteria* and OP3). Despite some studies that disagree (12, 16, 59), the establishment of the new PVC (*P*lanctomycetes-*V*errucomicrobia-*C*hlamydiae) superphylum has been proposed on the basis of 16S rRNA phylogeny (58). We tested the hypothesis of the PVC superphylum using comparative sequence analyses of 23S rRNA genes. We characterized the major bacterial cell division gene *ftsZ* and other *dcw* cluster genes in all verrucomicrobial subdivisions with cultivable representatives. Comprehensive sequence analyses and the comparison of the presence of *dcw* genes and their gene order in representatives of all phyla of the PVC superphylum is interpreted here in relation to the cell division mechanism in their last common ancestor.

MATERIALS AND METHODS

Cultures. All strains were cultured with shaking in 50 ml liquid media. Cultures of *Prosthecobacter dejongeii* DSM12251, *Prosthecobacter debontii* DSM14044, *Prosthecobacter vanneervenii* DSM12252, and *Opitutus terrae* DSM11246 were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany). *Akkermansia muciniphila* CIP107961 was obtained from the Pasteur Institute Collection (France). *Chthoniobacter flavus* (43) and isolate Ellin514 (44) were obtained from Peter H. Janssen (University of Melbourne, Melbourne, Australia). *Prosthecobacter* species were grown aerobically at 28°C in DSM medium 628. *O. terrae* was grown anaerobically at 30°C in DSM medium 298g. *A. muciniphila* was grown anaerobically at 30°C in brain heart infusion medium (Becton Dickinson and Company). Media for anaerobic cultures were degassed using a vacuum, and the gas atmosphere was exchanged for dinitrogen. *C. flavus* and strain Ellin514 were grown aerobically at 30°C in medium VL55 (41).

DNA extraction. DNA from *Prosthecobacter* was isolated according to the method of Wisotzkey et al. (63). DNA from *A. muciniphila*, *C. flavus*, *O. terrae*, and isolate Ellin514 was extracted using a modified protocol to inhibit high nuclease activity. Liquid cultures were centrifuged, and the pellet was resuspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA disodium salt, pH 8). The lysis of the cells was directly performed in a high sodium dodecyl sulfate concentration without previous lysozyme treatment. Sodium dodecyl sulfate (10%) was added in a 1:1 ratio to the cell resuspension, followed by the addition of proteinase K (0.625 mg/ml) and RNase (0.25 mg/ml). The mixture was incubated for 1 h at 55°C. The subsequent steps were as described by Wisotzkey et al. (63).

Sequencing of 23S rRNA genes. 23S rRNA genes were characterized for *P. debontii*, *P. dejongeii*, *P. vanneervenii*, *A. muciniphila*, *C. flavus*, isolate Ellin514, and *O. terrae*.

Partial rRNA operons were amplified using the primers listed in the supplemental material. The PCR program was as follows: 35 cycles with 30 s of 50°C annealing and 4 min of 72°C extension. Direct sequencing of the 23S rRNA genes was performed by MWG Biotech AG (Germany) using the sequencing primers listed in the supplemental material.

Sequencing of *ddl* genes. In our previous study, consensus degenerate hybrid oligonucleotide primers (CODEHOP) (39, 40) were used to detect *ftsZ* in *Prosthecobacter* (36). Attempts to detect *ftsZ* in other *Verrucomicrobia* using a high number of different combinations of CODEHOP in PCR failed.

Therefore, CODEHOP were designed to target conserved domains of the D-Ala:D-Ala ligase (*ddl*) gene, which is usually located upstream of the *ftsQAZ* cluster. A fragment of the *ddl* gene was initially amplified using the same forward primer for all investigated species, targeting the *ddl* nucleotide sequences coding for the deduced protein domain GEDG. The reverse primers targeted the *ddl* nucleotide sequences coding for the deduced protein domains FYDI (*P. debontii* and *P. dejongeii*), FYDY (*P. vanneervenii*), EINT (*A. muciniphila*), GKEL (*C. flavus*), and NTIP (strain Ellin514 and *O. terrae*). Primer sequences are listed in the supplemental material. The PCR program was as follows: 5 cycles with 30 s of 45°C annealing and 1 min of 72°C extension, followed by 30 cycles with 15 s of 55°C annealing and 1 min of 72°C extension. PCR products were analyzed on 2% agarose gels. Selected fragments were cut from the gel, and DNA was extracted using the Perfectprep kit (Eppendorf, Germany). Fragments were cloned using a commercially available TOPO TA cloning kit (Invitrogen), and inserts were sequenced by MWG Biotech AG (Germany) using vector-specific primers.

Sequencing of the genomic environment of *ddl* and *ftsZ*. In *P. dejongeii* and *P. debontii*, specific primers targeting *ddl* and *ftsZ* were used in PCR to specifically amplify and subsequently sequence the intervening region comprising *ftsQA*. For the remaining investigated *Verrucomicrobia*, two-step gene walking, as described by Pilhofer et al. (35), was applied to characterize the genomic environment of the *ddl* gene. Attempts to walk downstream of the *O. terrae ddl* gene were not performed, as there are two ongoing genome projects on *Opitutaceae* isolates. The sequencing results were verified, and chimera formation during gene walking was excluded using both specific primers and genomic DNA as templates in PCR and Southern hybridization experiments (data not shown). The procedure was described previously (35).

Detection of *dcw* cluster genes in genome project data. The Integrated Microbial Genomes System (28) and BLASTP (1) were used to screen for the presence of *dcw* cluster genes in available genome project data.

At present, genome sequence data (unpublished genomes) for five isolates and strains belonging to *Verrucomicrobia* or *Lentisphaerae* (the closest relatives of *Verrucomicrobia*) are publicly available. The strains are *V. spinosum* (*Verrucomicrobia*, subdivision 1, *Verrucomicrobiae*), isolate DG1235 (*Verrucomicrobia*, subdivision 4, *Opitutaceae*), isolate TAV2 (*Verrucomicrobia*, subdivision 4, *Opitutaceae*), *Lentisphaera araneosa* (*Lentisphaerae*), and *Victivallis vadensis* (*Lentisphaerae*). The sequence data for isolate TAV2 and *Victivallis vadensis* were produced by the U.S. Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/). Preliminary sequence data for isolate DG1235 and *L. araneosa* were obtained from the J. Craig Venter Institute or from the Gordon and Betty Moore Foundation Marine Microbial Genome Sequencing Project website (https://research.venterinstitute.org/moore/).

For *Chlamydiae*, the published genomes of *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydophila caviae*, *Chlamydophila felis*, *Chlamydophila abortus*, and "*Candidatus* Protochlamydia amoebophila" were analyzed (3, 15, 19, 37, 38, 51). For *Planctomycetes*, the genomes of *Rhodopirellula baltica* (10), "*Candidatus* Kuenenia stuttgartiensis" (46), *Planctomyces maris* (unpublished), and *Blastopirellula marina* (unpublished) were analyzed. Preliminary sequence data for *Planctomyces maris* and *Blastopirellula marina* were obtained from the J. Craig Venter Institute website (http://www.jcvi.org/).

RNA isolation and transcription analyses. Total RNA was isolated from *Prosthecobacter* cultures (20 ml; mid-log phase) by using Trizol reagent (Invitrogen). The protocol of the manufacturer was modified. After cells were pelleted by centrifugation, the cells were lysed by adding 1 ml Trizol reagent. The cells

were resuspended by vortexing them for 30 s and then incubated for 5 min at 20°C and for 1 h at −80°C. The subsequent steps were as described in the instructions of the manufacturer.

DNase (RQ1 RNase-Free DNase; Promega) treatment of total RNA was performed according to the instructions of the manufacturer. Subsequently, reverse transcription was carried out with the Revert Aid First Strand cDNA kit (Fermentas, Germany) according to the instructions of the manufacturer and by using random hexamer primers. One microliter of the obtained cDNA was used for PCR (25-μl final volume), together with specific primers (see the supplemental material) for the genes *ddl*, *ftsQ*, *ftsA*, and *ftsZ*.

Sequence analyses. Open reading frames (ORFs) were detected using the ORF-Finder at http://www.ncbi.nlm.nih.gov (62). Similarities to known protein sequences and to conserved protein domains were searched using protein-protein BLAST (1). The program MOTIF Search (http://motif.genome.jp/) was used in order to detect conserved domains in Ddl and FtsQAZ protein sequences.

Phylogenetic sequence analyses. Comparative sequence analyses were performed using the ARB program package (24). The 23S rRNA ARB database comprised the new 23S rRNA sequences obtained in this study and sequences of the major bacterial groups. For tree calculation, 96 (860 for RAxML) sequences were selected representing all available sequences of *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, *Planctomycetes*, and selected species of the major bacterial groups, some *Archaea*, and some 28S rRNA sequences of *Eukarya*. Minimum-similarity filters were calculated, which retained only positions conserved in at least 0%, 20%, or 50% of the selected sequences. The phylogenetic analyses were performed using each filter in combination with each treeing program. The treeing methods used were distance matrix methods (Phylip NEIGHBOR and FITCH), maximum parsimony (Phylip DNAPARS), and maximum likelihood (RAxML, AxML, and PHYML DNA). All programs are implemented in the ARB program package (24). Maximum-parsimony and maximum-likelihood trees were bootstrap resampled (1,000 bootstraps). As a nucleotide substitution model, the Kimura-2-Parameter was used for distance matrix methods and PHYML; the HKY85 model was used for RAxML.

The FtsZ_ClustalW ARB database published by Pilhofer et al. (36) was extended with the new sequences obtained in this study. A Ddl ARB database was established importing the Ddl protein sequences obtained in this study and Ddl proteins of other major bacterial groups. The initial alignment was produced by using ClustalW (50) of the ARB program package (24) and was refined manually. Minimum-similarity filters were calculated in each database, which retained only positions conserved in at least 0% or 30% of the selected sequences, respectively. Phylogenetic analyses were performed using distance matrix methods (the programs ARB neighbor joining and Phylip UPGMA, FITCH, and KITSCH), maximum parsimony (Phylip PROTPARS), and maximum likelihood (Phylip PROML and TREE-PUZZLE [45]). All programs are implemented in the ARB program package (24). For distance matrix methods and the maximum-likelihood method, the model of substitution used was the Dayhoff PAM matrix (8); for the TREE-PUZZLE method, the Muller-Vingron model of substitution (32) was used. Maximum-parsimony trees were bootstrap resampled (10,000 bootstraps); the number of puzzling steps for TREE-PUZZLE trees was 100,000.

Nucleotide sequence accession numbers. Nucleotide sequences were submitted to the EMBL nucleotide database with accession numbers AJ96882 to AJ96884 and AM905423 to AM905426. All characterized nucleotide sequences of the *dcw* clusters were submitted to the EMBL nucleotide database with accession numbers AM905291 to AM905296. Entries AJ888907 and AJ888908 were updated.

RESULTS

Phylogenetic sequence analyses of 23S rRNA genes. 23S rRNA genes were characterized for *P. dejongeii*, *P. debontii*, *P. vanneervenii*, *A. muciniphila*, *C. flavus*, Ellin514, and *O. terrae*. For the remaining investigated *Verrucomicrobia* and *Lentisphaerae*, 23S rRNA gene sequences were retrieved from the genome project data. The data set used for calculation of phylogenetic trees included all available 23S rRNA sequences of members of the *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes* and selected representatives of the major bacterial groups, some *Archaea*, and some *Eukarya*. Trees were calculated using 0%, 20%, and 50% minimum-similarity filters in combination with the different treeing methods (see Materials and Methods). (For one representative maximum-likeli-

hood tree, see Fig. 4A; a further selection of trees is available as supplemental material.) All 18 calculated trees showed a monophyly of all included members of the PVC superphylum. The tree topology within the PVC superphylum was also stable, showing the monophyletic groups always branching in the order *Planctomycetes*, *Chlamydiae*, *Lentisphaerae*, and *Verrucomicrobia*. The verrucomicrobial subdivisions (1 to 4) also showed a monophyletic and stable treeing pattern.

Overall, the 23S rRNA trees were in absolute agreement with many published 16S rRNA phylogenies (7, 16, 43, 58) in regard to the monophyly of the PVC superphylum, the branching order of the different phyla within the PVC superphylum, and the tree topology within *Verrucomicrobia*.

Detection and genomic organization of *ddl* and *ftsQAZ* in *Verrucomicrobia* and *Lentisphaerae*. Sequences with similarities to *ddl* and *ftsZ* could be detected in all verrucomicrobial subdivisions with cultivable representatives.

With the exception of *P. vanneervenii* and *A. muciniphila*, the intervening region of *ddl-ftsZ* (generally *ftsQA*) was shown to be conserved (Fig. 1). Downstream of the *P. vanneervenii ddl* gene, an ORF with similarities to *rarD* genes (coding for predicted permeases) (26) could be detected. Upstream of the complete *ftsQ* sequence, an ORF with similarities to the conserved protein domain duf1501, a protein family with unknown function, was identified (Fig. 1). In *A. muciniphila*, *ftsQ* was detected downstream of the *ddl* gene, followed by a gene with similarities to *recA* (a bacterial recombination protein). Downstream of the *recA* homolog, a single ORF with similarities to both *amiA* (*N*-acetylmuramoyl-L-alanine amidase; N terminal) and *lipB* (lipoyltransferase; C terminal) was detected (Fig. 1).

ddl, *ftsQ*, *ftsA*, and a sequence with some similarities to *ftsZ* were also detected in *L. araneosa* (*Lentisphaerae*, closest relatives of *Verrucomicrobia*), but the single genes were organized isolated at different locations on the genome (Fig. 1). In the incomplete *V. vadensis* (*Lentisphaerae*) genome sequence data, only the *ddl* gene could be identified (Fig. 1).

Genomic organization of *dcw* cluster genes in the phyla *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes*. The upstream region of *ddl* in *Verrucomicrobia*/*Lentisphaerae* showed either *murB* (*A. muciniphila* and Ellin514), a *murCB* fusion (DG1235), or the *ddl* gene fused with *murB* or *murBC* (*V. vadensis* and *L. araneosa*, respectively) (Fig. 1). In some other cases the upstream region was not conserved and apparently not related to the *dcw* cluster (*P. debontii*, *P. dejongeii*, and *V. spinosum*) (Fig. 1).

The only verrucomicrobial organism for which the available sequence data could prove the conservation of the gene order of the complete *dcw* cluster was isolate DG1235 (Fig. 2). Notably, the gene content and order were highly similar to the proposed ancient *dcw* cluster type (Fig. 2) (48). The only differences from the ancient type were (i) the replacement of *ftsL* through a hypothetical protein, (ii) the insertion of *lysM* between *murD* and *ftsW*, and (iii) the *murC-murB* fusion (Fig. 2). The first feature is shared with *L. araneosa* and all *Chlamydiae*; the second feature is shared only with *Chlamydiae*.

Ongoing and finished genome projects of the phyla *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes* were also investigated for the presence of *dcw* cluster genes. With the exception of *ftsL*, *L. araneosa* was found to also possess all genes of the proposed ancient *dcw* cluster; however, the genes generally

Organism	Phylogenetic group	Cell morphology	Evidence	Genomic organization of some *dcw* cluster genes
Prosthecobacter debontii	*Verrucomicrobia* subdivision 1 *Verrucomicrobiae*	fusiform vibroid rod	This study Pilhofer et al., 2007	abg ddl ftsQ ftsA ftsZ PS-DA
Prosthecobacter vanneervenii	*Verrucomicrobia* subdivision 1 *Verrucomicrobiae*	fusiform rod	This study Pilhofer et al., 2007	ddl rarD * hyp. protein duf1501 ftsQ ftsA ftsZ
Prosthecobacter dejongeii	*Verrucomicrobia* subdivision 1 *Verrucomicrobiae*	fusiform rod	This study Pilhofer et al., 2007	abg ddl ftsQ ftsA ftsZ
Verrucomicrobium spinosum	*Verrucomicrobia* subdivision 1 *Verrucomicrobiae*	prosthecate rod	Genome project	narK ddl ftsQ ftsA ftsZ
Akkermansia muciniphila	*Verrucomicrobia* subdivision 1 *Verrucomicrobiae*	oval	This study	murB ddl ftsQ recA amiA-lipB
Chthoniobacter flavus	*Verrucomicrobia* subdivision 2 *Spartobacteria*	oval rod	This study	ddl ftsQ ftsA ftsZ
Isolate Ellin514	*Verrucomicrobia* subdivision 3	cocci	This study	murB ddl ftsQ ftsA ftsZ corB
Opitutus terrae	*Verrucomicrobia* subdivision 4 *Opitutaceae*	cocci	This study	ddl
Isolate TAV2	*Verrucomicrobia* subdivision 4 *Opitutaceae*	cocci	Genome project	ddl ftsQ ftsA ftsZ
Isolate DG1235	*Verrucomicrobia* subdivision 4 *Opitutaceae*	cocci	Genome project	murCB ddl ftsQ ftsA ftsZ
Uncultivated species	*Verrucomicrobia* subdivision 5			
Victivallis vadensis	*Lentisphaerae*	cocci	Genome project	murB-ddl
Lentisphaera araneosa	*Lentisphaerae*	sphere	Genome project	murBC-ddl * ftsQ * ftsA * ftsZ

FIG. 1. Genomic organization of the 3′-terminal part of the *dcw* cluster in *Verrucomicrobia* and *Lentisphaerae*. The genes shaded in gray or black are generally organized within the *dcw* cluster. The genes in white are not functionally related to and normally not organized within the *dcw* cluster. The asterisk indicates that contigs are isolated on the chromosome. The dashed lines indicate that the sequence of the ORF is partial. With two exceptions, *Verrucomicrobia* show the conserved gene order *ddl-ftsQAZ*, whereas in *Lentisphaerae*, the individual genes are isolated on the chromosome.

located in the 3′-terminal region of the cluster (*ftsQ*, *ftsA*, and *ftsZ*-like) were dispersed in the genome (Fig. 2).

In *Chlamydiae* representatives, *ftsZ*, *ftsA*, *ftsQ* (with the exception of "*Ca*. Protochlamydia"), *ftsL*, and *mraZ* are absent. The remaining *dcw* genes are arranged in two isolated clusters, which are remarkably more similar to DG1235 than to the ancestral cluster (*ftsL* replaced by a gene coding for a hypothetical protein; *lysM* insertion). *Chlamydia* and *Chlamydophila* show a fused *murC-ddl* gene, whereas in "*Ca*. Protochlamydia," *murC* is followed by *ftsQ* and the *ddl* gene is isolated (Fig. 2).

In representatives of *Planctomycetes*, the *dcw* gene content and order conservation vary widely between the different representatives. Most of the genes of the ancient *dcw* cluster are present in "*Candidatus* Kuenenia stuttgartiensis." They are organized in a cluster that is very similar to that predicted for the ancestral type; *ftsA*, *ftsZ*, *murD*, and *murB* are absent, and three ORFs coding for hypothetical proteins are inserted. In

contrast, *R. baltica* and *B. marina* genome data present only some isolated *mur* genes and none of the *fts* genes (*ftsQ* in *B. marina*). *P. maris* possesses most of the *dcw* genes, but they are clustered at different locations in the genome (Fig. 2).

Transcription analyses. The transcription of *ddl*, *ftsQ*, *ftsA*, and *ftsZ* was verified in *P. dejongeii* and *P. vanneervenii* using reverse transcription of mRNA (Fig. 3). Total RNA was isolated from *Prosthecobacter* cultures and was subsequently treated using DNase. Total RNA was reverse transcribed using random hexamers. The cDNA was subsequently used as a template in PCR with gene-specific primers. Control reactions for each PCR were one reaction without template and one control sample processed without reverse transcriptase (Fig. 3). Specific amplification from cDNA was detected for all investigated genes in both species.

Search for conserved protein domains and residues. In order to verify the sequence similarities detected using BLASTP, Ddl and FtsQAZ protein sequences of *Verrucomicrobia/Len-*

Group	Organism	mraZ	mraW	ftsL	ftsI	murE	murF	mraY	murD	ftsW	murG	murC	murB	ddl	ftsQ	ftsA	ftsZ
	ancestral cluster	mraZ	mraW	ftsL	ftsI	murE	murF	mraY	murD	ftsW	murG	murC	murB	ddl	ftsQ	ftsA	ftsZ
V	*V. spinosum*		mraW		ftsI	murE		mraY	murD	ftsW	murG	murC-murB		ddl	ftsQ	ftsA	ftsZ
	Isolate DG1235	mraZ	mraW	H	ftsI	murE	murF	mraY	murD lysM	ftsW	murG	murC-murB		ddl	ftsQ	ftsA	ftsZ
L	*Lentisphaera araneosa*	mraZ	mraW	H	ftsI	murE	murF	mraY	murD	ftsW	murG	murB-murC--ddl			ftsQ	ftsA	ftsZ
C	*Chlamydia* spp.		mraW	H	ftsI	murE	murF	mraY	murD lysM	ftsW	murG	murC----------ddl					
	Chlamydophila spp.		mraW	H	ftsI	murE	murF	mraY	murD lysM	ftsW	murG	murC----------ddl					
	Cand. P. amoebophila		mraW	H	ftsI	murE	murF	mraY	murD lysM	ftsW	murG	murC	ftsQ	ddl-ddl			
P	*Cand.* K. stuttgartiensis	mraZ	mraW	ftsL	ftsI	H murE	murF	mraY		ftsW	H murG	H murC		ddl	ftsQ		
	Rhodopirellula baltica		mraW			murE					murG		murB	ddl			
	Blastopirellula marina	mraZ				murE							murB		ftsQ		
	Planctomyces maris	mraZ	mraW	lysM	ftsI	murE	murF	mraY		ftsW	murG	murC	murB	ddl			

FIG. 2. Genomic organization of *dcw* genes in *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Planctomycetes*. Comparison of *dcw* gene content and gene order conservation in the PVC superphylum. The hypothetical ancestral cluster (47) is shown in the first line. The first column designates phylogenetic groups: V, *Verrucomicrobia*; L, *Lentisphaerae*; C, *Chlamydiae*; P, *Planctomycetes*. The vertical lines indicate isolated organization on the chromosome. Hyphens denote gene fusions. H stands for hypothetical protein. DG1235 shows a good conservation of the *dcw* cluster with some peculiarities compared to the ancestral type. *L. araneosa* presents most of the *dcw* genes, whereas the gene order is lost. *Chlamydiae dcw* clusters are disrupted in two parts but are remarkably similar to DG1235, except that they contain no *fts(Q)AZ* genes. *Planctomycetes* show high variability in the conservation of gene content and gene order.

tisphaerae were searched for conserved domains of the Pfam library (4). The matching scores of the query sequences to the respective Pfam domains are listed as E values in Table 1. All detected Ddl sequences showed matches to both D-Ala:D-Ala ligase Pfam domains with comparable high scores, except for two partial sequences (*O. terrae* and TAV2). For all available investigated FtsQA sequences, there were matches to the POTRA domain FtsQ type (42) or to the cell division protein FtsA domain, respectively. Due to the high sequence divergence of these proteins, the scores for the matches were generally lower than Ddl matches (Table 1). All complete verrucomicrobial FtsZ sequences showed a match to the tubulin/FtsZ family GTPase domain, with comparable high scores. The
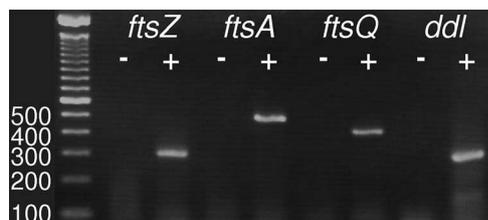

FIG. 3. Transcriptional analyses of *dcw* cluster genes in *Prosthecobacter*. Five-microliter aliquots of PCR products were loaded on a 2% agarose gel. The template for PCR was the cDNA sample (+) or a control sample processed without reverse transcriptase (−). A 100-bp ladder (Invitrogen) was loaded on the left side; the numbers refer to fragment lengths in base pairs. The expected sizes of the fragments were 296 bp (*ftsZ*), 435 bp (*ftsA*), 354 bp (*ftsQ*), and 283 bp (*ddl*). Transcription was detected for all investigated genes in *P. dejongeii* (shown) and *P. vanneervenii*.

sequence of *L. araneosa* (*Lentisphaerae*) with similarities to FtsZ did not match the tubulin/FtsZ family GTPase Pfam domain, nor did it match the FtsZ PROSITE pattern, the BLOCKS database, or the PRINTS motif (the search was performed using MOTIF search [http://motif.genome.jp/]). However, the sequence was initially detected using BLASTP and showed similarities to the conserved domain FtsZ (cd02191) (26) and to FtsZ from *P. debontii* with E values of $5e^{-06}$ and $6e^{-04}$, respectively.

FtsZ sequences were additionally searched for the tubulin signature motif (S/A/G)GGTG(S/A/T)G (PROSITE motif PS00227), which is present and perfectly conserved in all investigated verrucomicrobial FtsZ proteins. In contrast, the putative *L. araneosa* FtsZ homolog did not show the tubulin signature motif. A similar observation was made during the analysis of amino acid positions that contact GDP (23, 34). The analysis performed by Pilhofer et al. (36) was extended using the newly obtained FtsZ sequences (data not shown). All important positions were conserved among *Verrucomicrobia*, even if the new FtsZ sequences of subdivisions 2 to 4 were added. The *L. araneosa* FtsZ-like protein showed no conservation in the majority of these amino acid residues. BLASTP analysis of *ddl* ORFs revealed that *Verrucomicrobia* show the common structure comprising one Ddl domain. Whereas, similar to *ddl* genes of *Verrucomicrobia*, *ddl* genes of *Planctomycetes* comprise only one Ddl domain, Ddls of *Lentisphaerae* and *Chlamydiae* present different types of fused genes (Fig. 2). *ddl* genes were found to be fused with *murBC* (*L. araneosa*), *murB* (*V. vadensis*), and *murC* (*Chlamydia* and *Chlamydophila*) or with another *ddl* ("*Ca.* Protochlamydia").

TABLE 1. E values[a] of *dcw* genes for Pfam domains

Isolate	E value				
	Ddl		FtsQ (POTRA domain; FtsQ type)	FtsA (cell division protein FtsA)	FtsZ (tubulin/FtsZ family; GTPase)
	D-Ala:D-Ala ligase C terminus	D-Ala:D-Ala ligase N terminus			
P. dejongeii	9.8e−53	2.1e−15	0.01	1.3e−38	5.9e−49[b]
P. debontii	2.9e−59	1.5e−16	0.0042	7.8e−40	3.3e−50
P. vanneervenii	2.3e−64	2.0e−14	4.7e−11	8.9e−42	5.8e−54
V. spinosum	2.0e−69	2.1e−11	1.5e−07	1.3e−42	5.4e−48
A. muciniphila	3.5e−56	2.8e−18	7.9e−06		
C. flavus	9.7e−54	3.4e−13	5.4e−10	2.4e−50	5.5e−60[b]
Ellin514	3.5e−57	3.7e−19	1.8e−09	2.0e−46	7.1e−67
O. terrae	1.2e−48[b]	−[b,c]			
TAV2	−[b,c]	−[b,c]	5.4e−12	1.2e−19	0.27[b]
DG1235	2.6e−45	1.3e−13	4.5e−12	1.7e−24	1.2e−53
V. vadensis	5.8e−53	9.6e−21			
L. araneosa	3.4e−47	1.3e−26	3.4e−15	7.0e−05	−[c]
E. coli	3.4e−119	2.9e−47	8.7e−37	6.7e−79	7.8e−91

[a] Based on scoring matches to the motifs.
[b] Partial sequence.
[c] No significant hit.

In BLASTP analyses, the first hits detected for *Verrucomicrobia*, *Planctomycetes*, *Chlamydia*, and *Chlamydophila* Ddl sequences were other bacterial Ddls (data not shown). Notably, the "*Candidatus* Protochlamydia amoebophila" sequence comprising two Ddl domains showed the best matches (E value, 3e−161) to a nuclear-encoded gene in *Physcomitrella patens* (Viridiplantae), a moss for which *ddl* and related genes seem to be important for plastid division (25).

Phylogenetic analyses using Ddl and FtsZ sequences. The newly obtained FtsZ and Ddl sequences were used for phylogenetic-tree reconstructions. The FtsZ_ClustalW ARB database published by Pilhofer et al. (36) was extended with the new sequences obtained in this study. FtsZ sequences of *Verrucomicrobia* and *Lentisphaerae* (excluding partial TAV2 FtsZ) were used to produce two different minimum-similarity filters. Phylogenetic trees were calculated using both filters in combination with distance matrix methods, maximum parsimony, and maximum likelihood. Attempts to calculate global trees could recover a relationship of the *L. araneosa* FtsZ-like protein and verrucomicrobial FtsZs in most cases, whereas the relationships between the other major bacterial groups were unstable (data not shown; compare reference 36). Therefore, subsequent phylogenetic analyses were restricted to *Lentisphaerae* and *Verrucomicrobia*. All calculated trees showed stable tree topologies, with stable monophyletic groups representing the different subdivisions. In all trees, the branching patterns of the FtsZ sequences of the different verrucomicrobial subdivisions (1, 2, 3, and 4) were consistent with the pattern obtained in rRNA phylogeny (Fig. 4) (7, 16, 43, 58). One representative maximum-likelihood tree is shown in Fig. 4B.

A Ddl ARB database was established by importing the Ddl protein sequences obtained in this study and Ddl sequences of other bacterial groups from the EMBL nucleotide database. Initial attempts to calculate global trees using different methods and filters showed that in most cases the major bacterial groups could be recovered, whereas the relationships between the major bacterial groups were unstable (data not shown), as is also the case for FtsZ phylogeny (36). Therefore, subsequent

phylogenetic analyses were restricted to *Lentisphaerae* and *Verrucomicrobia*. Notably, all global Ddl trees showed a stable relationship of "*Candidatus* Protochlamydia amoebophila" Ddl with *P. patens* (Viridiplantae) Ddl (nuclear encoded). The "*Ca*. Protochlamydia" Ddl was duplicated in the database, so each of the Ddl domains could be aligned once.

Ddl sequences of *Verrucomicrobia* and *Lentisphaerae* (excluding partial TAV2 Ddl) were used to calculate two minimum-similarity filters. Phylogenetic trees were reconstructed using both filters in combination with distance matrix methods, maximum parsimony, and maximum likelihood. As the isolate TAV2 Ddl sequence is partial and therefore very short, it was added separately to each tree using ARB Interactive Parsimony and a special filter considering the length of TAV2 Ddl. One representative maximum-likelihood tree is shown in Fig. 4C. All calculated trees showed stable tree topology, with stable monophyletic groups representing the two phyla *Verrucomicrobia* and *Lentisphaerae*. Within the *Verrucomicrobia*, each subdivision (1, 2, 3, and 4) was recovered monophyletically; however, the bootstrap support for the subdivision 4 cluster was not high. The branching pattern is consistent with the pattern obtained using comparative sequence analyses of 16S rRNA genes (7, 16, 43, 58), 23S rRNA genes (Fig. 4A), and FtsZ proteins (Fig. 4B).

DISCUSSION

Phylogenetic relationships between *Planctomycetes*, *Chlamydiae*, *Lentisphaerae* and *Verrucomicrobia*. A stable monophyly of *Planctomycetes*, *Chlamydiae*, *Lentisphaerae* and *Verrucomicrobia* could be detected using 23S rRNA phylogeny (Fig. 4A). Also, the bootstrap support was high for this clade (14). In the past, there were also other studies, addressing the phylogenetic relationships between *Planctomycetes*, *Chlamydiae*, and *Verrucomicrobia* based on 16S rRNA (7, 13, 22, 58, 61), 23S rRNA (59), or protein sequences (12, 17, 46, 49, 60), in which the formation of a monophyletic cluster of all three lineages was controversial. The study by Ward et al. (59), also using 23S
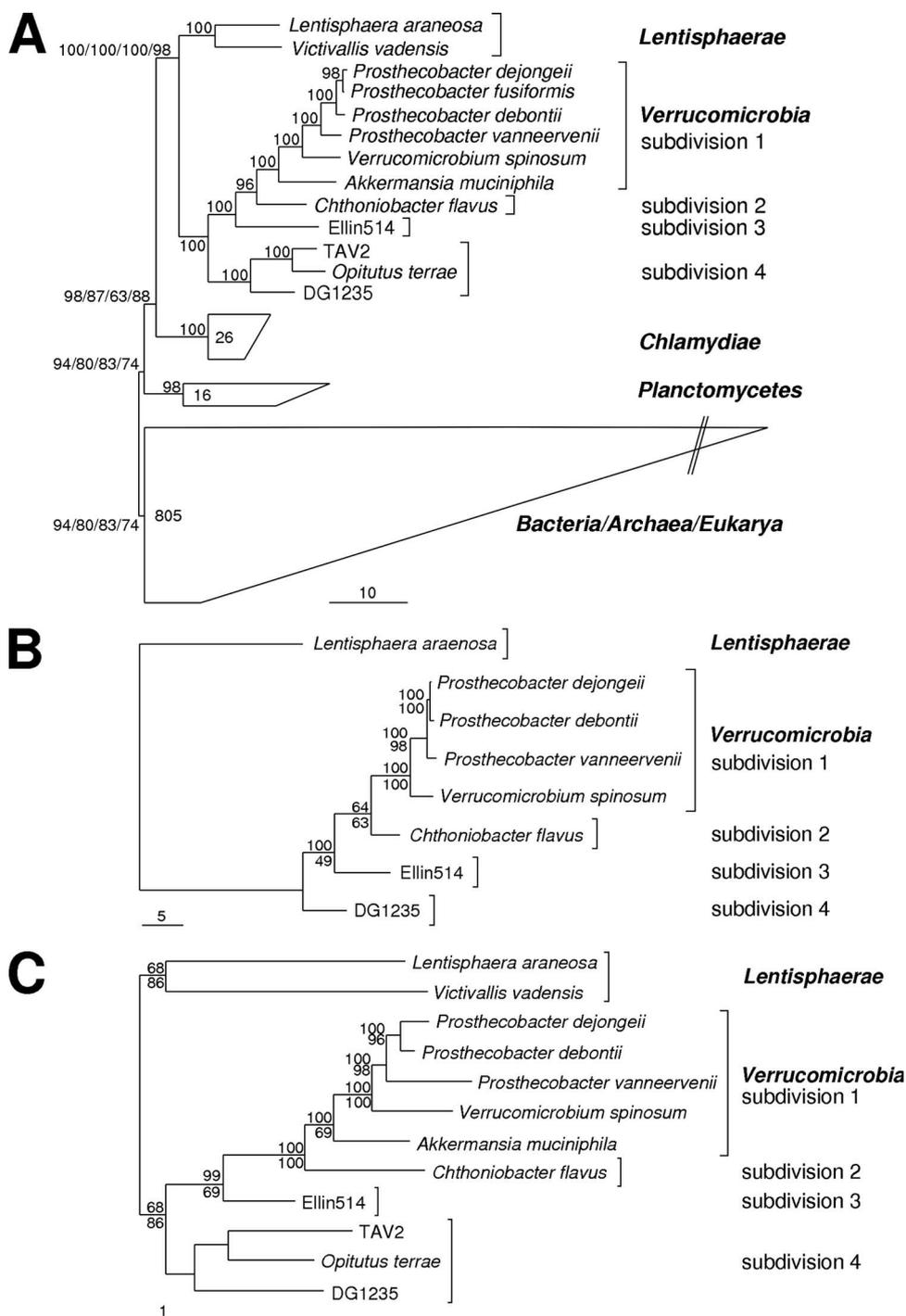
FIG. 4. Comparative sequence analyses using 23S rRNA (A), FtsZ (B), and Ddl (C). All calculated 23S rRNA trees showed a stable monophyletic group of *Planctomycetes*, *Chlamydiae*, *Lentisphaerae*, and *Verrucomicrobia* with a stable branching order within this PVC superphylum. Verrucomicrobial subdivisions were recovered monophyletically and showed a branching order matching the 16S rRNA trees. Other 23S rRNA trees are available in the supplemental material. Verrucomicrobial FtsZ and Ddl trees show the same tree topologies as 16S and 23S rRNA trees. (A) Phylogenetic 23S rRNA tree produced using the maximum-likelihood method (RAxML) and a minimum-similarity filter (50%). Selected representatives of major bacterial groups, *Archaea*, and *Eukarya* were used as outgroups. Note that the tree is unrooted. The branching pattern within the group "Bacteria/Archaea/ Eukarya" is shown in the supplemental material. The numbers in closed groups denote the numbers of sequences included. The numbers at the nodes represent bootstrap values (1,000 bootstraps) (percent). Four bootstrap values are shown for the branchings of the phyla (maximum likelihood, 0% minimum-similarity filter; maximum likelihood, 50% minimum-similarity filter; maximum parsimony, 0% minimum-similarity filter; maximum parsimony, 50% minimum-similarity filter); one bootstrap value is shown for the remaining nodes (maximum parsimony, 50% minimum-similarity filter). The bar represents the estimated evolutionary distance (percent). (B) Phylogenetic FtsZ (core domain) tree produced using the maximum-likelihood method and a minimum-similarity filter (30%). The numbers represent bootstraps/confidence values of a maximum-parsimony tree (lower numbers, 10,000 boot-straps) and a TREEPUZZLE tree (upper numbers, 100,000 puzzling steps) (percent). The bar represents the estimated evolutionary distance (percent). (C) Phylogenetic Ddl tree produced using the maximum-likelihood method and a minimum-similarity filter (30%). The numbers represent bootstraps/ confidence values of a maximum-parsimony tree (lower numbers, 10,000 bootstraps) and a TREEPUZZLE tree (upper numbers, 100,000 puzzling steps) (percent). The bar represents the estimated evolutionary distance (percent).

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rRNA as a phylogenetic marker, could not detect a significant relationship of the three phyla. However, the data set was rather limited: *Planctomycetes* were represented by 13 sequences (16 representatives in this study), *Chlamydiae* by one sequence (26 representatives in this study), and *Verrucomicrobia* by three sequences (11 representatives in this study), and *Lentisphaerae* were completely lacking (2 representatives in this study). The much more comprehensive phylogenetic analyses in this study showed very good agreement between trees derived from 23S and 16S rRNAs (Fig. 4). Therefore, these analyses support the suggestion of a PVC superphylum based on 16S rRNA phylogeny in which the sister phyla *Lentisphaerae* and *Verrucomicrobia* are more closely related to *Chlamydiae* than to *Planctomycetes* (58).

Origin of the "*Candidatus* Protochlamydia amoebophila" *ddl-ddl* gene. An intriguing feature derives from the analysis of "*Candidatus* Protochlamydia" Ddl-Ddl. This gene shows unexpectedly high similarity to plant Ddl-Ddl, involved in chloroplast division (25), and apparently low similarity to bacterial Ddl. The plant *ddl* genes have been characterized up to now in *Arabidopsis*, in *Oryza*, and in the moss *Physcomitrella*. For the first two organisms, genomic data are available, and they show the presence of many introns interrupting the coding region of the *ddl* genes. The observed high similarity between plants and "*Ca*. Protochlamydia" *ddl* suggests that either plants acquired *ddl* from a "*Ca*. Protochlamydia" ancestor and later inserted introns in the coding region or "*Ca*. Protochlamydia" acquired *ddl* from plants, most likely via reverse-transcription-mediated mechanisms (due to the "necessity" to remove introns). The latter case would imply that some bacterial genes of the *dcw* cluster might be lost and reacquired through horizontal gene transfer.

FtsZ-based cell division mechanism in *Verrucomicrobia* and *Lentisphaerae*. In contrast to previous assumptions, *ftsQAZ* and *ddl* genes could be detected in representatives of all verrucomicrobial subdivisions. The failure to detect *ftsZ* in *A. muciniphila* and *O. terrae* by PCR is not significant, due to the sequence divergence of *ftsZ* (see Materials and Methods); we predict that these organisms also possess *ftsZ*. Phylogenetic sequence analyses using the obtained FtsZ and Ddl sequences are in full agreement with the rRNA trees (Fig. 4) (7, 16, 43, 58). This consistency suggests the absence of horizontal gene transfer influence on FtsZ and Ddl evolution in *Verrucomicrobia* and strongly indicates that the genes are conserved due to functional constraints. This also applies to the isolated genes of the nonconserved *dcw* clusters (in terms of gene order) of *P. vanneervenii* and *A. muciniphila* (Fig. 1). Comprehensive analyses of the FtsZ sequences revealed that all verrucomicrobial FtsZ proteins matched the criteria of functional FtsZ as defined by Vaughan et al. (53). The sequences showed a match to the conserved Pfam domain tubulin/FtsZ family GTPase, the tubulin signature motif could always be detected without mismatches, and the analysis of amino acid positions that contact GDP (23, 34) also showed good conservation. The constant presence of *ftsQAZ* in *Verrucomicrobia*, the phylogenetic conservation of FtsZ, the fulfilment of all criteria of functional FtsZ within the gene sequence, and the proof of transcription of *ftsQAZ* in actively growing cultures provide a strong indication of functionality and suggest an FtsZ-based cell division mechanism in all members of the phylum *Verrucomicrobia*.

Concerning the phylum *Lentisphaerae*, represented by the cultivated species *V. vadensis* and *L. araneosa*, the situation is less clear. Considering that the genome sequence of *V. vadensis* is still unfinished (324 contigs), the present inability to detect *ftsQAZ*-like sequences in the genome data is not significant. Indeed, its closest relative, *L. araneosa*, possesses *ftsQ*, *ftsA*, and an *ftsZ*-like gene, although all of them are spread out among different positions on the chromosome. Concerning *L. araneosa* FtsZ, similarities to *Prosthecobacter* FtsZ and to the conserved domain FtsZ could be clearly detected, whereas the criteria of functional FtsZ (53) are not fulfilled at all. *L. araneosa* FtsQA have matches to the respective Pfam motifs with high E values (Table 1), which could be an indication of functionality. Up to now, no bacteria that had a non-FtsZ-based cell division mechanism and at the same time possessed *ftsA* (encoding the first protein recruited to the Z ring and directly interacting with FtsZ) could be identified. In summary, the present data may suggest that *Lentisphaerae* also perform FtsZ-based cell division like *Verrucomicrobia* and the majority of other bacteria. However, the failure to meet the criteria of functionality could indicate a modified mechanism or even that the gene is (at least in cell division) nonfunctional. Future studies of the *L. araneosa* FtsZ-like protein could verify whether the fundamental criteria of functional FtsZ (53) have to be revised.

Conservation of the gene content and gene order of the *dcw* cluster in *Verrucomicrobia* and *Lentisphaerae*. The analyses of the available sequence data demonstrated that in at least one verrucomicrobial organism (DG1235) the *dcw* cluster is nearly perfectly conserved in comparison to the ancient type of cluster, which comprises 16 genes (Fig. 2) (48). In at least five other cases (all investigated subdivision 1 organisms), gene order in the cluster showed variations (Fig. 1). With one exception (*ftsL*), *Lentisphaera* maintained the gene content, whereas the gene order was conserved only in the 5′-terminal part of the *dcw* cluster (Fig. 2).

A comparison of the *dcw* clusters from several bacterial genomes showed that there is a relationship between the organization of the gene cluster and the shape of the cells, so that the cluster tends to be conserved in rod-shaped and filamentous bacteria and tends to be disrupted in bacteria with other shapes (48). This observation is supported in this study, as none of the *Verrucomicrobia*/*Lentisphaerae* with dispersed *dcw* cluster genes formed classical cylindrical rods with hemispherically capped ends. The types of morphology in *Verrucomicrobia*/*Lentisphaerae* range from spheres (*L. araneosa*), cocci (*V. vadensis*, DG1235, TAV2, *O. terrae*, and Ellin514), oval rods (*C. flavus*), oval shapes (*A. muciniphila*), and prosthecate rods (*V. spinosum*) to fusiform rods (*Prosthecobacter*) (Fig. 1). It is notable that there are two different types of *dcw* cluster organizations (concerning the generally highly conserved 3′ region) within the genus *Prosthecobacter* that have very similar morphologies (*P. vanneervenii*-*P. dejongeii* and *P. debontii*) (Fig. 1). Mingorance et al. (30, 31) proposed a model, called genomic channeling, in which the selective pressure to maintain the *dcw* cluster arises from the need to efficiently coordinate the process of elongation and septation in rod-shaped bacteria. Although *Prosthecobacter* and *Verrucomicrobium* have rod-like morphologies, they also possess one or many prosthecae. It can be suspected that the presence of these prosthecae is likely to

force a growth mechanism rather divergent from the one found in classical rods. Thus, it is hard to predict if and how peptidoglycan elongation and septation machineries compete. Therefore, it seems likely that genomic channeling of the *dcw* cluster does not play a major role in these organisms, and it is very probable that other mechanisms for the determination of cell shape exist in *Prosthecobacter*.

Conservation of gene content and gene order of the *dcw* cluster in *Planctomycetes* and *Chlamydiae* compared to *Verrucomicrobia*/*Lentisphaerae*. The high similarity of *dcw* clusters within *Chlamydiae* could be derived from adaptation to their similar intracellular lifestyles and to the consequent functional constraints. Although up to now the existence of peptidoglycan in *Chlamydiae* has not been proven, the genes coding for a nearly complete pathway for peptidoglycan synthesis were suggested to be functional (reviewed in reference 29). In contrast to *Chlamydiae*, *Planctomycetes* show a high degree of variability in the *dcw* cluster, which could be related to the very different lifestyles of its representatives.

The hypothesis that the last common ancestor of extant bacteria harbored a conserved *dcw* cluster and that some lineages lost this gene order during evolution (33) is strongly supported by the observations obtained in this study. In comparison to the ancestral *dcw* cluster type, DG1235 shows only one gene replacement, one gene insertion, and one gene fusion; *Chlamydiae* show the gene order conservation of many *dcw* genes; and 12 out of 16 genes are conserved in the *Planctomycetes* representative "*Candidatus* Kuenenia stuttgartiensis" (Fig. 2). These results are also a strong indication that the last common ancestor of the PVC superphylum possessed a conserved *dcw* cluster. Combined with the phylogenetic analyses, these data clearly support the view that many representatives of *Planctomycetes*, *Chlamydiae*, and *Lentisphaerae* independently lost some of the *dcw* genes or the gene order within the *dcw* cluster. An exception to this might be the replacement of *ftsL* by a hypothetical protein in DG1235 (*Verrucomicrobia*), *L. araneosa* (*Lentisphaerae*), and all *Chlamydiae*; this replacement might represent a characteristic of the last common ancestor of *Verrucomicrobia-Lentisphaerae-Chlamydiae*. In the same way, the insertion of *lysM* between *murD* and *ftsW* might represent a synapomorphic feature of *Verrucomicrobia* and *Chlamydiae* (the absence of *lysM* in *V. spinosum* in the expected position could be interpreted as a secondary loss).

In the scenario postulated here, after the separate branchings of *Chlamydiae* and *Planctomycetes* from their last common ancestor with *Verrucomicrobia*, their cell division mechanisms most probably shifted independently from an FtsZ-based mechanism to a non-FtsZ-based mechanism. The major cell division gene *ftsZ* was completely lost or diverged so far from the original *ftsZ* that a relationship is no longer recognizable. FtsZ could have been replaced by two different division mechanisms (concerning the structural protein of the division ring) in the different phyla and therefore does not necessarily have to be homologous. Another possibility is that there was an independent shift to the same division mechanism in *Chlamydiae* and *Planctomycetes*. If this was the case, it seems probable that the mechanism could have already been present in the last common ancestor of *Planctomycetes*/*Chlamydiae*/*Lentisphaerae*/*Verrucomicrobia*. To the best of our knowledge, two *Ureaplasma* species are the only examples in bacteria besides *Chla-*

mydiae and *Planctomycetes* that were identified to lack *ftsZ*. Thus, a special predisposition of the last common ancestor of the PVC superphylum to evolve an FtsZ-independent type of cell division can be hypothesized.

The single presently identifiable *L. araneosa ftsZ*-like gene (in the unfinished genome sequence) shows the highest similarities to verrucomicrobial FtsZs. At the same time, the sequence divergence in comparison to other bacterial FtsZ proteins is higher than that of verrucomicrobial FtsZ. Thus, from an evolutionary perspective, *L. araneosa* could be seen as an organism on the way to developing an FtsZ-independent cell division mechanism or one that has already developed such a mechanism.

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