Coexistence of Tubulins and *ftsZ* in Different *Prosthecobacter* Species

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Abstract

Prosthecobacter, one of the few cultivable representatives of the bacterial phylum *Verrucomicrobia*, is of increasing interest to the scientific community due to the presence of tubulin genes in its genome and the apparent absence of the bacterial homologue FtsZ that is normally involved in prokaryotic cell division. These findings suggested the possibility of a vicarious takeover of the FtsZ function through these novel tubulins and opened new scenarios on the possible evolution of bacterial cytoskeleton and cell division. In the present manuscript, we report the characterization of *ftsZ* and *ftsA* homologues in different *Prosthecobacter* species that also possess tubulin genes. Based on these findings, we propose an FtsZ-based cell division mechanism in *Verrucomicrobia*. The analysis of available genome data of *Verrucomicrobia* suggests that tubulins are not a feature common to all members of this phylum. Therefore, it can be assumed that *Prosthecobacte*r acquired tubulins through horizontal gene transfer. The functional role of tubulins in *Prosthecobacter* remains enigmatic.

Key words: bacterial tubulin (*btub*), FtsZ, *Prosthecobacter, Verrucomicrobia.*

The hypothesis that bacteria contain a cytoskeleton that is related to the eukaryote cytoskeleton was first established when the bacterial Z-ring, which plays a key role during bacterial cell division, was visualized using green fluorescent protein–labeled FtsZ. FtsZ is a protein with a secondary structure that mirrors tubulin (Lowe and Amos 1998; Nogales et al. 1998) and displays in vitro similar dynamic properties (reviewed in Addinall and Holland 2002; Stricker et al. 2002). Although FtsZ is incapable to form microtubule-like structures, the combined structural and functional properties make it unlikely that FtsZ and tubulin proteins evolved twice (Erickson 1998); therefore, eukaryotic tubulin and bacterial FtsZ are considered to be homologous proteins.

Despite microtubule-like structures have been reported several times in bacteria (Bermudes et al. 1994; Petroni et al. 2000), the first molecular indications of the presence of tubulin genes in the bacteria are rather recent. In 2002, during the analysis of the genome sequence (95% completion) of *Prosthecobacter dejongeii*, Jenkins et al. reported the presence of 2 genes showing a higher similarity to eukaryotic tubulin than to bacterial *ftsZ*. These genes were referred to as bacterial A tubulin (*btubA*) and bacterial B tubulin (*btubB*) because of their apparent similarity to eukaryotic alpha and beta tubulins. However, no FtsZ genes were found in the genome sequence of *P. dejongeii* (Jenkins et al. 2002). Later biochemical studies showed that BtubA and BtubB are able to associate in vitro into heterodimers that form long filaments. (Schlieper et al. 2005; Sontag et al. 2005).

Prosthecobacter dejongeii is one of the few cultivable representatives of the still poorly investigated bacterial phylum *Verrucomicrobia*, which is phylogenetically related to *Chlamydiae* and *Planctomycetes* (Wagner and Horn 2006). Intriguingly, the latter 2 are the only bacterial phyla that do not possess FtsZ and rely on a yet unknown cell division mechanism (Read et al. 2000; Gloeckner et al. 2003; Horn et al. 2004; Strous et al. 2006).

These findings suggested the possibility of a vicarious takeover of the FtsZ function through the *btub*s in *Verrucomicrobia*, thus opening novel scenarios on the evolution of the eukaryotic cell.

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To evaluate this hypothesis, we accurately screened several *Prosthecobacter* species and their closest cultivated relative, *Verrucomicrobium spinosum*, for the presence of *ftsZ* and tubulin genes.

The complete nucleotide sequence coding for the 2 *btub* genes, *btubA* and *btubB*, of *P. dejongeii* was already published as well as the partial sequences of these genes in *Prosthecobacter vanneervenii* and *Prosthecobacter debontii* (Jenkins et al. 2002). We confirmed the presence of 1 A tubulin and 1 B tubulin gene in *P. vanneervenii*, and the sequence of both open reading frames together with a connecting spacer was completed. In addition, we could detect and completely sequence 2 further A and B tubulin genes in *P. debontii*. This finding was also confirmed by Southern blot and hybridization experiments. *Prosthecobacter debontii btubA* and *btubB* partial sequences characterized by Jenkins et al. (2002) do not exist as adjacent loci, but each of them is adjacent to the newly identified *btub* genes. Therefore, we renamed the *btub* genes in *P. debontii*. Henceforth, *P. debontii btubA* (Jenkins et al. 2002) is renamed *btubA2* and is followed by the newly characterized *btubB2*; the newly characterized *btubA1* precedes *P. debontii btubB* (Jenkins et al. 2002) that is renamed *btubB1* (table 1). Several combinations of primers were used in polymerase chain reaction (PCR) attempts to detect tubulin genes in *V. spinosum* but without any success (table 1). This negative result was later confirmed by Blast analysis of *V. spinosum* genome data (sequence complete and all gaps closed, update 7 May 2005).

Table 1
Presence of *btubA*, *btubB* and *ftsZ* in Representatives of *Verrucomicrobia*, *Chlamydiae*, and *Planctomycetes*

	btubA, *btubB*	Evidence	*ftsZ*	Evidence
Prosthecobacter vanneervenii	*btubA*, *btubB*	This study, Jenkins et al. 2002	+	This study
	btubA1	This study	+	This study
	btubB1	This study, Jenkins et al. 2002		
	btubA2	This study, Jenkins et al. 2002		
Prosthecobacter debontii	*btubB2*	This study		
Prosthecobacter dejongeii	*btubA*, *btubB*	Jenkins et al. 2002	+	This study
Prosthecobacter fusiformis	*btubA*, *btubB*	Jenkins et al. 2002	nd	
Verrucomircobium spinosum	—	Genome project	+	Genome project
Chlamydiae	—	Genome projects	−	Genome projects
Planctomycetes	—	Genome projects	−	Genome projects

Note.—+, gene present; −, gene(s) absent; nd, not determined; *Chlamydiae* stands for *Chlamydia*, *Chlamydophila*, *Protochlamydia*; *Planctomycetes* stands for *Candidatus* Kuenenia stuttgartiensis and *Rhodopirellula baltica.*

Despite the apparent absence of *ftsZ* in *P. dejongeii* (Jenkins et al. 2002; Staley et al. 2005), we could detect a sequence coding for FtsZ in that organism using consensus degenerate hybrid oligonucleotide primers (Rose et al. 1998) in PCR. Moreover, *ftsZ* was also identified in *P. debontii* and *P. vanneervenii* (accession numbers AJ888907, AJ888908, AM498604). The retrieved sequences were used to detect an open reading frame with protein sequence similarities to FtsZ also in the sequence data of the ongoing *V. spinosum* DSM 4136 genome project (TIGR_240016, contig 534) (table 1).

Prosthecobacter and *Verrucomicrobium* FtsZs exhibit most of the typical FtsZ features and some peculiar characteristics. Like typical bacterial FtsZ, they can be divided into the 4 domains (N-terminus, core, spacer, and C-terminus) as defined by Vaughan et al. (2004).

The sequences present the typical features of functional FtsZ. First, 6 out of 6 characteristic motifs of FtsZ were identified by PRINTS fingerprint scan (Attwood et al. 2003) (probability values between 3.4×10^{-49} and 3.9×10^{-44}; see table 2 and its extended version in supplementary fig. S1, Supplementary Material online). Second, the tubulin signature motif [S/A/G]GGTG[S/A/T]G (PROSITE motif PS00227) is always present and perfectly conserved (supplementary fig. S1, Supplementary Material online). Third, amino acids which contact guanosine diphosphate (Lowe and Amos 1998; Nogales et al. 1998) are conserved or conservatively exchanged with the exception of position N70H according to

Methanocaldococcus jannaschii sequence (supplementary fig. S2, Supplementary Material online). Other nonconservative substitutions in the core domain are 1) position D235G (supplementary fig. S2, Supplementary Material online), a highly conserved position located within the T7-loop which is considered to be important for GTPase activity (Scheffers and Driessen 2001) and FtsZ polymerization (Cordell et al. 2003); and 2) the C-terminal end of the core domain, generally represented by the conserved tripeptide ATG and replaced in *Verrucomicrobia* by the tripeptide SSL. In all characterized *Verrucomicrobia*, the substituted amino acids are conserved, thus suggesting that functional constraints are still present at these positions although the substitutions are different from those occurring in other bacteria.

Table 2
Sequence Analysis of Different FtsZ, Btub, and Eukaryotic Tubulin Protein Sequences

			FtsZ		Tubulin	
Organism	Phylogenetic group	Protein	No. of motifs	*P* value	No. of motifs	*P* value
Escherichia coli	*Proteobacteria*	FtsZ	**6 of 6**	**3.4×10^{-79}**	2 of 9	6.3×10^{-07}
Prosthecobacter dejongeii	*Verrucomicrobia*	FtsZ	**6 of 6**	**3.4×10^{-49}**	2 of 9	3.4×10^{-07}
Prosthecobacter vanneervenii	*Verrucomicrobia*	FtsZ	**6 of 6**	**2.1×10^{-48}**	2 of 9	1.6×10^{-06}
Prosthecobacter debontii	*Verrucomicrobia*	FtsZ	**6 of 6**	**1.0×10^{-46}**	2 of 9	2.6×10^{-07}
Verrucomicrobium. spinosum	*Verrucomicrobia*	FtsZ	**6 of 6**	**3.9×10^{-44}**	—	—
P. dejongeii	*Verrucomicrobia*	BtubA	2 of 6	8.3×10^{-06}	**9 of 9**	**1.4×10^{-62}**
P. dejongeii	*Verrucomicrobia*	BtubB	3 of 6	4.3×10^{-05}	**9 of 9**	**6.4×10^{-73}**
P. vanneervenii	*Verrucomicrobia*	BtubA	2 of 6	1.4×10^{-06}	**9 of 9**	**3.4×10^{-61}**
P. vanneervenii	*Verrucomicrobia*	BtubB	2 of 6	1.5×10^{-08}	**9 of 9**	**1.2×10^{-72}**
P. debontii	*Verrucomicrobia*	BtubA1	2 of 6	8.2×10^{-06}	**9 of 9**	**3.5×10^{-61}**
P. debontii	*Verrucomicrobia*	BtubB1	2 of 6	4.1×10^{-09}	**9 of 9**	**1.6×10^{-72}**
P. debontii	*Verrucomicrobia*	BtubA2	2 of 6	8.3×10^{-06}	**9 of 9**	**4.5×10^{-58}**
P. debontii	*Verrucomicrobia*	BtubB2	3 of 6	1.4×10^{-10}	**9 of 9**	**2.2×10^{-73}**
Arabidopsis thaliana	*Eukarya*	TUA3	2 of 6	1.1×10^{-08}	9 of 9	$2.7 \times 10^{-}$

Note.—Protein sequences analyzed with PRINTS (Attwood et al. 2003); *P* value, probability value (based on scoring matches to the motifs). In bold are reported the values for the FtsZ–FtsZ and bacterial tubulin-tubulin matches.

Residues that have been demonstrated to be involved in protein–protein interaction, for example, with FtsA (Yan et al. 2000; Haney et al. 2001) are located in the C-terminal domain of FtsZ. These amino acids are arranged in a nonapeptide and are followed by a stretch of variable length, which is rich in basic amino acids (Vaughan et al. 2004). This feature is considered typical of a functionally active FtsZ and is also present in *Verrucomicrobia*. Moreover, the nonapeptide of the investigated *Verrucomicrobia* shows a good conservation in comparison to the bacterial consensus sequence (Vaughan et al. 2004) especially in positions which, in *Escherichia coli*, have been shown to be important for the protein conformation (Mosyak et al. 2000) or are thought to be involved in interactions with FtsA (Haney et al. 2001) (supplementary fig. S3, Supplementary Material online).

Phylogenetic analyses were performed on the core domain protein sequences using the ARB program package (Ludwig et al. 2004). They indicate a steady monophyly of verrucomicrobial FtsZ independently from the applied algorithm. One representative tree is shown in figure 1; the other calculated trees are available in FtsZ_ClustalW ARB database at http://www.arb-home.de. Calculated trees clearly indicate that the phylogenetic information retained by FtsZ is relatively limited and, in most cases, is not sufficient to resolve relationships above the phylum level, as it was also shown in earlier studies (Faguy and Doolittle 1998; Gilson and Beech 2001). Verrucomicrobial FtsZ always cluster together as independent lineage, thus supporting the existence of specific evolutionary constraints for these genes.

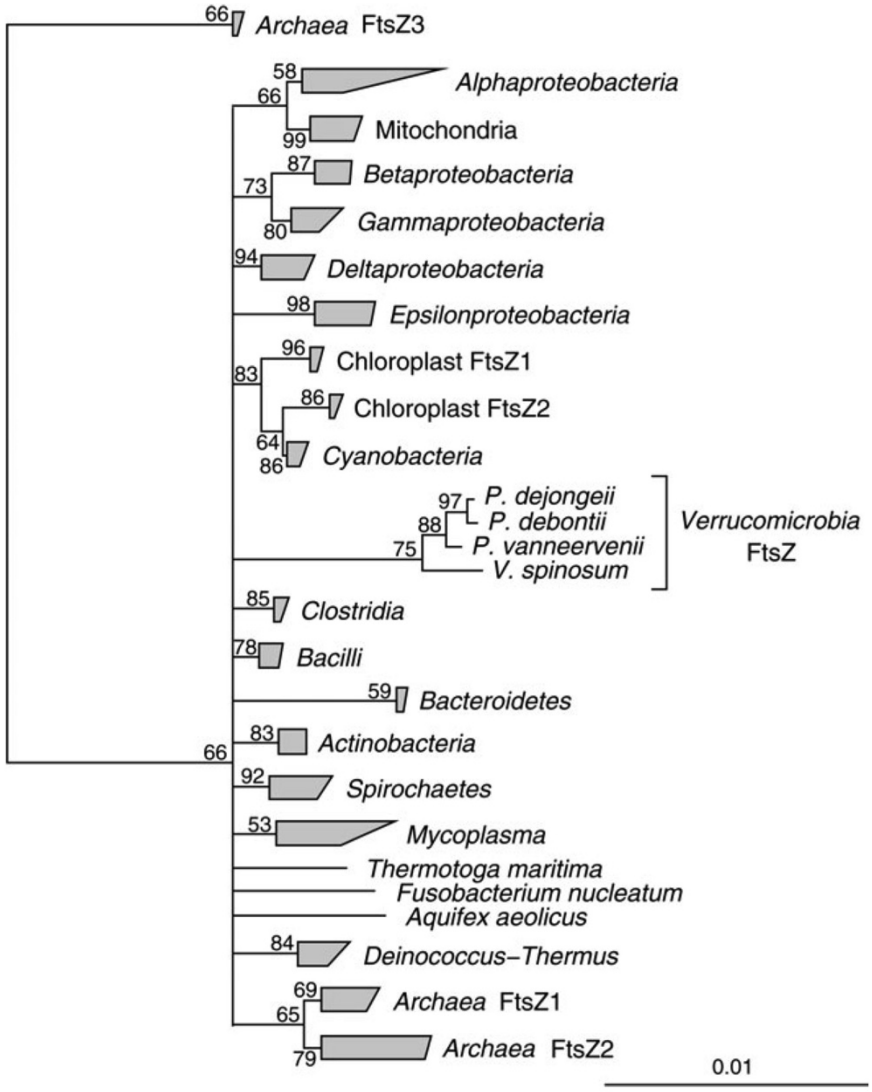

Fig. 1. Comparative sequence analysis of FtsZ protein sequences of bacteria, archaea, and eukaryotic organelles representatives. Phylogenetic tree produced using Tree-Puzzle (Schmidt et al. 2002) (prot_30 filter, 1,000 puzzling steps, mixed rate of heterogeneity). Only the core domain was used for calculation. The *Prosthecobacter dejongeii* sequence was not complete. Archaeal FtsZ3 was used as outgroup. Numbers represent confidence values in percent. Verrucomicrobial FtsZs cluster together forming a monophyletic group, also the other major bacterial groups are recovered. Compared with the majority of other groups, verrucomicrobial FtsZs present a longer branch indicative of their sequence peculiarities.

The genomic environment of *P. debontii* and *P. vanneervenii* FtsZ was additionally investigated. It shows the presence of an open reading frame similar to *ftsA*. FtsA is an actin homologue that is also involved in bacterial cell division. Moreover, the *V. spinosum* genome reveals a cluster of genes involved in cell division, comprising open reading frames with similarities to D-alanine-D-alanine-ligase, *ftsQ*, *ftsA*, and *ftsZ* (fig. 2). This gene order is highly conserved and also found in other distantly related organisms (e.g., *E. coli* CFT073) (Faguy and Doolittle 1998).

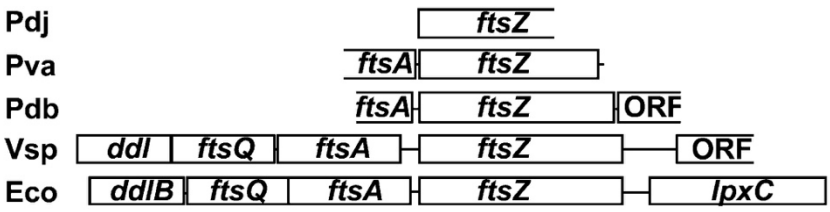

Fig. 2. Detected *ftsZ* genes and their genomic environment in *Verrucomicrobia* and *Escherichia coli*. *Prosthecobacter vanneervenii* (Pva) and *Prosthecobacter debontii* (Pdb) show an open reading frame with similarities to *ftsA* upstream of *ftsZ*; *Verrucomicrobium spinosum* (Vsp) presents 3 open reading frames functionally related to cell division: *ftsA*, *ftsQ*, *ddl* (D-ala D-ala ligase). This gene order is conserved also in distantly related species, for example, in *E. coli* CFT073 (Eco). Partial *ftsZ* was characterized in *P. dejongeii* (Pdj).

The following properties indicate that the identified *ftsZ* genes are functionally active in *Verrucomicrobia*: 1) all characteristics typical of functional FtsZ are present; 2) verrucomicrobial FtsZ is evolutionary constrained; and 3) other typical bacterial cell division genes are present in these organisms.

The simultaneous presence of functional FtsZ in *Prosthecobacter* spp. and *Verrucomicrobium* together with tubulin genes in the genus *Prosthecobacter* is a strong indication that FtsZ and not tubulin is the major protein involved in cell division in the *Verrucomicrobia*.

The comparison of *Prosthecobacter* tubulins and verrucomicrobial FtsZs shows only a low sequence similarity (see table 2 and its extended version in supplementary fig. S1, Supplementary Material online) and indicates that *Prosthecobacter* tubulins did not directly derive from *Prosthecobacter* FtsZ. The apparent absence of tubulin genes in *V. spinosum* and the great divergence between *Prosthecobacter* FtsZ and tubulins would favor the hypothesis that tubulin sequences were acquired by *Prosthecobacter* through horizontal gene transfer as it was already suggested by other authors (Schlieper et al. 2005). In any case, the origin and especially the function of *Prosthecobacter* tubulins and of those tubulins supposed to be present in other representatives of the phylum, that is, epixenosomes (Rosati et al. 1993; Petroni et al. 2000), remain to be elucidated.

Supplementary Material

Materials and Methods and figures SI-S3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjoumals.org/). The DNA sequences reported in this work have been deposited in the EMBL nucleotide database (accession numbers AJ888907, AJ888908, AM041148-AM041150, AM498604). ARB database is available at http://www.arb-home.de.

Acknowledgments

This work was supported by a German Research Organization (DFG) grant to K.H.S., W.L., and G.P. Italian Research Ministry (MUR) is acknowledged for additional support to G.P. (PRIN protocol 2006053200_002). The Bayerische Forschungsstiftung is acknowledged for a research and mobility grant to M.P. Christina Eckl is acknowledged for her help with some of the experiments. The authors wish to thank Simone Gabrielli for computer image assistance. Preliminary sequence data were obtained from The Institute for Genomic Research Web site at http://www.tigr.org. Sequencing of *V. spinosum* DSM4136 was accomplished with support from National Science Foundation.

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