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Verrucomicrobia div. nov., a new division of the Bacteria containing three new species of *Prosthecobacter*

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Abstract

Four strains of nonmotile, prosthecate bacteria were isolated in the 1970s and assigned to the genus *Prosthecobacter*. These strains were compared genotypically by DNA/DNA reassociation and 16S rDNA based phylogenetic analyses. Genotypic comparisons were complemented with phenotypic characterizations. Together, these studies clearly indicate each *Prosthecobacter* strain represents a novel species of bacteria. We propose three new species of *Prosthecobacter, P. dejongeii* strain FC1, *P. vanneervenii* strain FC2, and *P. debontii* strain FC3; *P. fusiformis* is reserved for the type strain of the genus, strain FC4. Additionally, we propose the genera *Prosthecobacter* and *Verrucomicrobium*, currently members of the order Verrucomicrobiales, to comprise a novel higher order taxonomic group, the division Verrucomicrobia div. nov. and the class Verrumicrobiae class nov. Many novel members of the Verrucomicrobia, as revealed by molecular ecology studies, await isolation and description.

Introduction

Early taxonomists grouped the prosthecate bacteria in the order Caulobacteriales (Henrici & Johnson 1935). More recently, however, 16S rDNA based phylogenies have suggested that prosthecae are a paraphyletic trait. That is, prosthecate bacteria do not form an evolutionary cluster from which nonprosthecate bacteria are excluded. To the contrary, prosthecate bacteria are now known to reside within several of the major groups of the Bacteria, and prosthecae may offer distinct advantages to each. For example, some such as *Prosthecochloris aestuarii* and *Ancalochloris perfilevii* are prosthecate photosynthetic green sulfur bacteria (Gorlenko 1970; Gorlenko & Lebedeva 1971). The many prosthecae extending from the surface of these bacteria allow the cells to produce high numbers of membrane associated chlorosomes; thus, these bacteria seem to derive photosynthetic potential in turn for materials and energy spent on the production of prosthecae.

Most known prosthecate organisms, however, are planktonic heterotrophs which presumably gain other benefits from possessing prosthecae. First, prosthecae increase the surface to volume ratio of the cell, which should allow increased nutrient uptake in oligotrophic environments which these bacteria commonly inhabit (Semenov & Staley 1993). *Caulobacter crescentus* prosthecae even elongate in response to phosphate starvation (Poindexter 1984). Second, prosthecae decrease cellular sedimentation rates in aquatic environments; prosthecate *Caulobacter* cells resist sedimentation during centrifugation, whereas swarmer cells do not (Poindexter 1978). Finally, some bacteria utilize holdfasts on prosthecal tips to attach to solid materials (Merker & Smit 1988). Attachment may keep bacteria in proximity to a source of nutrients, such as algal cells, provide physical protection from eucaryotic grazers, or for dimorphic organisms, aid in the separation of reproducing cells from their progeny.

Most well-known prosthecate heterotrophic bacteria belong to the α-2 subgroup of the Proteobacteria (Stackebrandt et al. 1988; Schlesner et al. 1989). This group is dominated by both dimorphic and nonmotile prosthecate bacteria as well as by budding bacteria; however, nonprosthecate, nonbudding bacteria are not excluded from this phylogenetic cluster. The taxono-

my and phylogeny among prosthecate Proteobacteria has remained relatively static in recent years.

In contrast, a second phylogenetic cluster of prosthecate heterotrophs has only recently been formally recognized, the order Verrucomicrobiales (Ward-Rainey et al. 1995). At present, the Verrucomicrobiales comprises only two genera of bacteria, both of which possess prosthecae. The first bacterium placed in this group, from which the name of the order is derived, was *Verrucomicrobium spinosum*, a polyprosthecate bacterium with fimbriae extending from its prosthecae termini (Schlesner 1987). Only recently has a second genus, *Prosthecobacter*, been added to the Verrucomicrobiales (Hedlund et al., 1996). Members of the genus *Prosthecobacter* were first observed and photographed in 1935 by Henrici & Johnson who described them as 'fusiform caulobacters' because of their spindle cell shape and, like the genus *Caulobacter*, they had a single polar prostheca. However, no strains were isolated. In 1970 the first report of a pure culture was published by DeBont et al. (1970) who obtained a strain from enrichments from a lake in Michigan. Subsequently three other strains were isolated and all were placed in a single genus and species, *Prosthecobacter fusiformis* (Staley et al. 1976). Besides both having prosthecae, *Verrucomicrobium* and *Prosthecobacter* each are heavily fimbriate, possess menaquinones as predominant respiratory quinones, and specialize in carbohydrate degradation.

The phylogenetic position of members of the Verrucomicrobiales within the framework of bacterial phyla defined by Woese (1987) has been investigated by different groups using several 16S rDNA based phylogenetic methods (Albrecht et al. 1987; Rainey-Ward et al. 1995; Hedlund et al. 1996). The results of each of these analyses suggest that the Planctomycetales and Chlamydiales are the closest known relatives of the Verrucomicrobiales; however, bootstrap support for such associations are weak (< 50% support). Furthermore, strict analyses using only 16S rDNA nucleotides of certain alignment suggest the Verrucomicrobiales are more deeply rooted than the Planctomycetales or Chlamydiales (Hedlund et al. 1996). The evolutionary scenario in which the Verrucomicrobiales are deeply rooted suggests that a common ancestor of the Planctomycetales and Chlamydiales lost the ability to synthesize peptidoglycan following its divergence from ancestors of the Verrucomicrobiales.

Despite the scarcity of bacterial isolates within the Verrucomicrobiales, phylogenetic relatives of this group appear to be widespread in the environment. Molecular ecology studies in which 16S rDNA was PCR-amplified, cloned, and sequenced from environmental DNA preparations have detected bacteria related to *Verrucomicrobium* and *Prosthecobacter* in several distinct environments. These environments include forest soil (Liesack & Stackebrandt, 1992), agricultural fields (Ueda et al. 1995; Ueda, T., unpubl.; Lee, S.Y., J. Bollinger, D. Bedicek & A. Ogram, unpubl.), freshwater environments (Hiorns et al., 1996; Wise et al. 1996), and the pelagic environment (Fuhrman et al. 1993). As these molecular ecology studies indicate relatives of the Verrucomicrobiales are widely distributed and possibly abundant in the environment, it has become clear that these bacteria deserve further investigation. Such studies should include attempts to culture and describe new microbes from the environment as well as more complete studies of Verrucomicrobia present in pure culture. In this paper we investigate the members of the genus *Prosthecobacter* and, in addition, address the higher order taxonomy of the Verrucomicrobiales.

Materials and methods

Microscopy. Stationary phase cells of *P. vanneervenii* FC2 were shadowed with platinum-palladium and viewed with a JEM-100B electron microscope at 60 KV.

Carbon source tests. Late exponential-phase *Prosthecobacter* strains were added to defined media (Staley et al. 1976) containing 0.2% of the carbon source of interest in microtiter wells. Growth was monitored by measuring an increase in turbidity at 600 nm using Automated Microplate Reader EL311sx (Bio-Tek, Winooski, VT) and Delta Soft II (BioMetallics, Princeton, NJ) after three, five, and ten day incubations at room temperature. Growth was defined as two or more cell doublings. Each test was carried out in quadruplicate.

Determination of generation time. Prosthecobacter cells were grown in MMB (Staley & Mandel 1973) broth at room temperature with rapid shaking. Growth was quantified by measuring absorbance at 600 nm.

Phylogenetic analysis. Prosthecobacter 16S rDNA was purified, sequenced, and aligned with similar sequences as described previously (Hedlund et al. 1996). Additional sequences used in the phylogenetic analysis were accessed from the RDP (Larsen

Table 1. Genetic evidence supporting proposal of three new species of *Prosthecobacter*[1]

Species	DNA/DNA reassociation	16S rDNA homology			
		P. fusiformis FC4	*P. debontii* FC3	*P. vanneer-venii* FC2	*P. dejongeii* FC1
P. fusiformis FC4	100	100			
P. debontii FC3	3	97.1	100		
P. vanneer-venii FC2	1	95.3	94.6	100	
P. dejongeii FC1	5	97.9	97.3	94.8	100

[1] Table modified from Staley et al. (1996).

et al. 1993) or from GenBank. The following sequences, with GenBank accession numbers in parentheses, were included: *Prosthecobacter fusiformis* FC4 ATCC 25309 (U60015), *Prosthecobacter vanneervenii* FC2 (U60013), *Pirellula staleyi* ATCC 27277 (M3412), *Chlamydia psittaci* str. 6BC ATCC VR125 (M13769), *Verrucomicrobium spinosum* IFAM 1439 (X90515), clone env. MC17 (X64381), clone env. MC18 (X64374), clone env. MC31 (X64380), *Fibrobacter succinogenes* str. A3C ATCC 51219 (M62683), *Spirochaeta halophila* str. RS1 ATCC 29478 (M88722, M34262), *Caulobacter crescentus* str. CB2A (M83799), *Caulobacter subvibrioides* str. CB81 (M83797), *Neisseria gonorrhoeae* str. B5025 ATCC 19424 (X07714), *Escherichia coli* (J01695), *Desulfovibrio desulfuricans* ATCC 27774 (M34113), and *Streptomyces lividans* str. TK21 (Y00484). Since the data set included distantly related sequences, certain regions of the 16S rRNA gene could not be confidently aligned. Comparisons of nucleotides which are not evolutionary counterparts misguides phylogenetic analyses (Olsen & Woese 1993); therefore, we deleted these nucleotides from all sequences (*E. coli* positions 69–101, 163–169, 182–226, 433–504, 838–853, and 997–1044 (Brosius et al. 1978)). The data set was analyzed using maximum-likelihood (Olsen et al. 1994; Felsenstein 1981), parsimony (Swafford 1991), and distance (Felsenstein 1989) methods and projected using the TreeDraw program. The position of the *Verrucomicrobium Prosthecobacter*, and MC clone cluster within resulting consensus tree was evaluated using the method of Kishino & Hasegawa (1989).

Results and discussion

Genotypic comparisons. The nearly complete (1470 base pairs) *Prosthecobacter* 16S rDNA sequences were obtained as described previously (Hedlund et al. 1996). With the exception of FC1 and FC4, all pairwise *Prosthecobacter* 16S rDNA sequence comparisons yielded values of less than 97.5% identity, the value proposed by Stackebrandt & Goebel (1994) to validate the separation of bacterial strains into distinct species (Table 1). In support that each *Prosthecobacter* strain represents a unique species, DNA/DNA reassociation data obtained previously (Moore et al. 1978) indicate strains FC1, FC2, and FC3 are only distantly related to the type strain of *P. fusiformis*, strain FC4. In fact DNA/DNA, reassociation values are well below 70%, the value currently used to circumscribe species of bacteria (Wayne et al. 1987). A 16S rDNA phylogenetic analysis showing the relationships between *Prosthecobacter* strains was published previously (Hedlund et al. 1996).

Microscopy. All strains of *Prosthecobacter* share similar morphological traits. For example, cells possess prosthecae at the older pole of the cell and often remain attached at to one another following cell division; thus, after division cells form a mirror image of one another (Figure 1). Also, all *Prosthecobacter* strains are heavily fimbriate (Figure 2).

Examination of the four *Prosthecobacter* strains by light microscopy, however, indicated that each strain is morphologically unique (Figure 1). Cells of *P. fusiformis* str. FC4 are usually straight with cells long (3–10 μm) and thin (0.5 μm). *P. vanneervenii* str. FC2 closely resembled *P. fusiformis*, however cells were typically shorter in length (2–8 μm). *P. debontii*

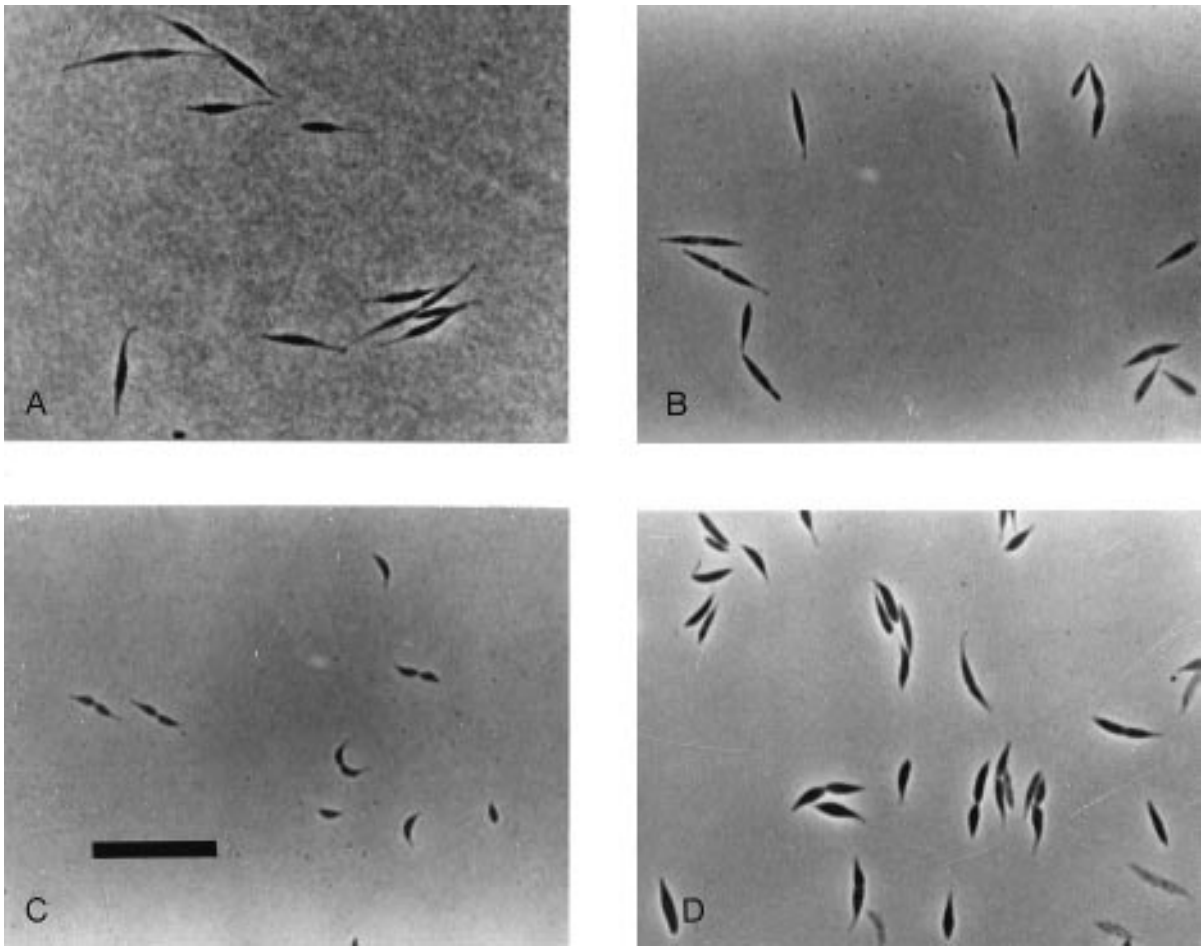

Figure 1. Phase micrograph of four *Prosthecobacter* species. A) *P. fusiformis* (FC4); B) *P. vanneervenii* (FC2); C) *P. debontii* (FC3); and *P. dejongei* (FC1). Bar represents 10 μM. Figure reproduced from Staley et al. (1976).

str. FC3 was short (2–8 μm) and vibrioid in shape. *P. dejongeii* str. FC1 was straight, like FC2 and FC4, however, wider in diameter (1 μm). These morphologies appeared to be consistent whether cells were grown in defined or complex media and were independent of growth phase.

Carbon source tests. *Prosthecobacter* strains fail to use amino acids, sugar alcohols, or C1 or C2 compounds as growth substrates (Staley et al. 1976). *Prosthecobacter*, like its relative *Verrucomicrobium* uses mainly carbohydrates as carbon sources. We examined the ability of all four *Prosthecobacter* strains to use individual carbohydrates as sole cources of carbon and energy. As shown in Table 2, each *Prosthecobacter* strain used a unique group of carbon sources. These unique carbon source utilization patterns, as well as other differences indicated in Table 2, are congruent with the proposition that the *Prosthecobacter* strains represent separate species.

Higher taxonomy of the Verrucomicrobiales. It has been suggested that members of the Verrucomicrobiales and related 16S rDNA clones from uncultured organisms represent a novel division of the Bacteria (Albrecht et al. 1987; Liesack & Stackebrandt, 1992; Ward-Rainey et al. 1995; Hedlund et al. 1996). This proposal is based on the results of phylogenetic analyses using 16S rDNA sequences which show that Verrucomicrobia are only distantly related to other bacteria; furthermore, although they seem to be related to the Planctomycetales and Chlamydiales, bootstrap support placing the Verrucomicrobia with these or any other bacterial group is weak. In addition to their phyloge-

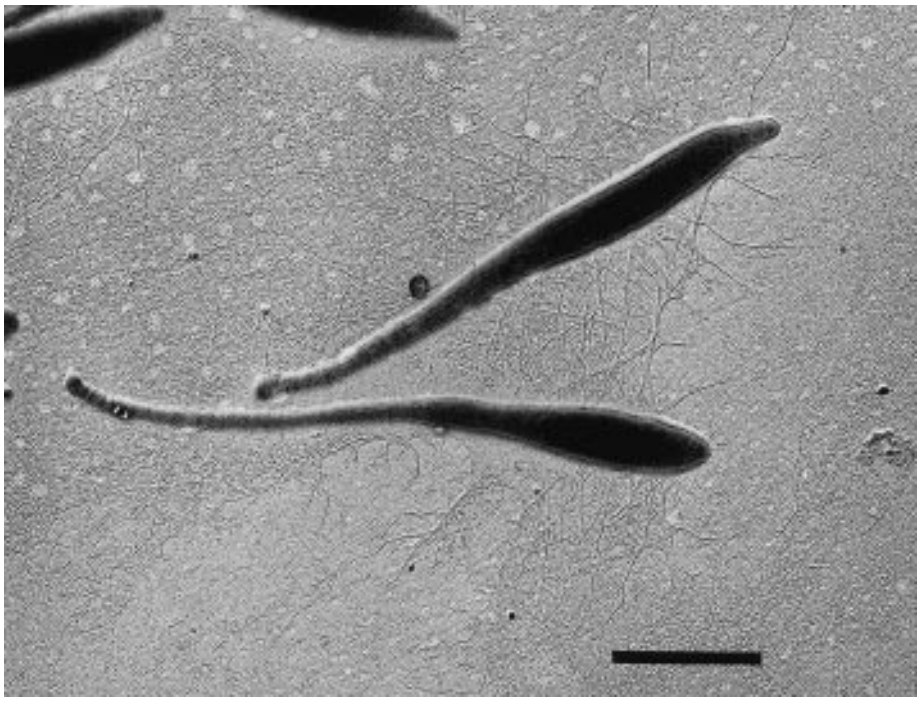

Figure 2. Shadowed electron micrograph of *Prosthecobacter vanneervenii* strain FC2. Note the numerous fimbriae. Bar equals 2 μm.

netic independence, the members of the Verrucomicrobiales each have rigid peptidoglycan cells walls, a trait that is absent from the known members of the *Planctomyces/Chlamydia* phylogenetic cluster (Koenig et al. 1984).

We performed phylogenetic analyses using Verrucomicrobia 16S rDNA sequences and taxa representing different divisions of the Bacteria (Figure 3). The resulting consensus dendrogram was evaluated using Kishino Hasegawa analysis (Kishino & Hasegawa 1989). When the branch joining *Prosthecobacter, Verrucomicrobium,* and the Cluster III MC clones was rejoined from the Gram positives, the spirochetes, the fibrobacteria, or the Proteobacteria, the resulting trees were significantly worse than the consensus tree (see Figure 3), indicating *Verrucomicrobium* and relatives can be excluded from these groups with confidence. Trees formed when the Verrucomicrobia were joined with the Chlamydiales or the Planctomycetales, although worse than the consensus tree, were not significantly different. Thus, the precise branching order between the Verrucomicrobiales, the Planctomycetales, and the Chlamydiales is not certain as has been previously reported (Hedlund et al. 1996) and as has been found for many deep branches in the Bacteria (Van de Peer et al. 1994).

Using available information we formally propose that the Verrucomicrobiales and uncultured microbes with related 16S rDNA sequences comprise a unique division and class of the Bacteria (Table 3). This proposal should be considered preliminary given recent studies on the reliability of 16S rDNA based phylogenies. Notably, it is well-documented that the inclusion of certain taxa, for example *Planctomyces* spp. and relatives (Liesack et al. 1992), in 16S rDNA phylogenetic analyses can produce dendrograms which contradict those which are commonly accepted (Woese 1987). Also, high numbers of replications of 16S rDNA phylogenetic analyses indicate deep nodes of the 16S rDNA tree, particularly those at the division level, are not resolvable with a high degree of confidence (Van de Peer et al. 1994). Most importantly, recent studies using other well conserved molecular sequences have produced dendrograms with variable degrees of agreement with 16S rDNA trees (Gupta & Golding 1996). Thus, even though we advocate moving toward a phylogenetic description of taxonomic groups, we are aware that 16S rDNA phylogenies will continue to be challenged with analyses of other molecular sequences. It is also presently unclear whether individual organisms can accurately be viewed as products of linear evolution. That is, large scale genetic exchange

Table 2. Phenotypic comparison of *Prosthecobacter* species

Carbon source/ phenotype	Species			
	P. dejongeii FC1	*P. vanneervenii* FC2	*P. debontii* FC3	*P. fusiformis* FC4
D-glucose	+	+	+	+
D-galactose	+	+	+	+
D-mannose	+	+	+	+
D-sucrose	+	+	+	+
D-lactose	+	+	+	+
D-melibiose	+	+	+	+
D-maltose	+	+	+	+
D-xylose	+	+	+	+
L-rhamnose	+	+	+	+
D-trehalose	+	+	+	+
Cellobiose	+	+	+	+
D-ribose	+	+	+	-
D-arabinose	-	-	+	+
D-fructose	-	+	+	-
D-raffinose	-	+	-	-
Glycogen	+	+	-	-
D-glucosamine	-	+	-	-
N-acetyl glucosamine	+	+	+	-
DNA% G + C[1]	54.6	60.1	57.1	56.1
Colony color	opaque pale yellow	opaque pale yellow	opaque yellow	opaque yellow
Temp. range[1] (°C)	1–35	10–38	8–38	4–40
Gen. time in MMB (hours)	6	24	ND[2]	ND[2]

[1] Data from Staley et al. (1976).
[2] ND indicates not determined.

between organisms may occur, giving an individual organism two or more distinct lines of descent (Irwin 1994; Gupta & Singh 1994; Lake & Rivera, 1994; Gupta & Golding 1996). Perhaps such issues of uncertainty will only be resolved following robust comparisons of larger genetic elements which will become available upon completion of genome sequencing projects.

Despite these uncertainties, we believe 16S rDNA trees currently best represent bacterial evolution. First, analyses using a large number of molecular sequences, for example, ATPase subunits (Iwabe et al. 1989; Gogarten et al. 1989), RNA polymerase (Puhler et al. 1989) and elongation factors (Iwabe et al. 1989), produce trees with overall topologies that are similar to 16S rDNA phylogenies. Second, 16S rDNA trees form groups which are supported by certain important phenotypic traits. For example, 16S rDNA trees separate the Archaea from the Gram positives, which seems appropriate based on differences in cell wall content, lipid structure, and components involved in transcription and translation. Trees produced using the most universally conserved protein sequence, heat shock protein (HSP) 70, suggest the Archaea are paraphyletic within the Gram positives (Gupta & Singh 1994). Notwithstanding some of the concerns of phylogenetic analyses using 16S rDNA for assessing deep roots within the Bacteria, and with the intent of furthering bacterial classification, we propose the creation of the division Verrucomicrobia. Nevertheless, we encourage critical analysis of this and other major clades by further analyses of additional molecular sequences.

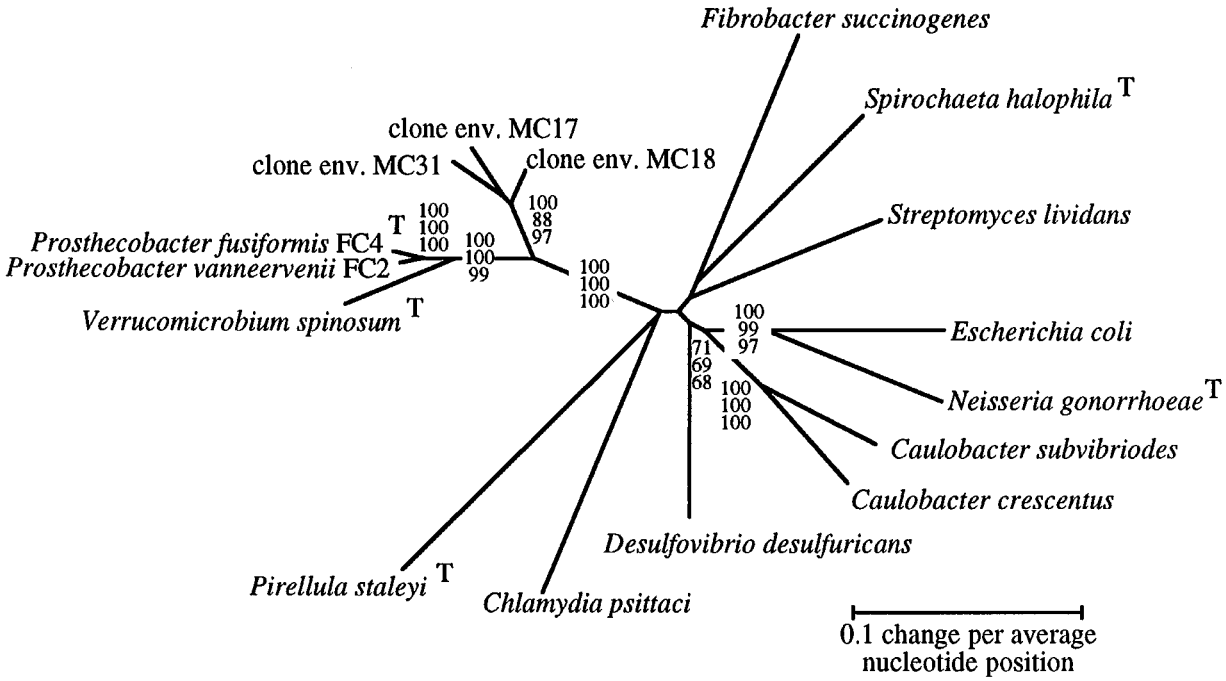

Figure 3. 16S rDNA phylogenetic analysis of *Prosthecobacter, Verrucomicrobium* and Cluster III MC clones as well as bacteria representing established divisions. Numbers at branch nodes represent bootstrap values out of 100 resamplings for neighbor-joining (maximum likelihood distances) (top), maximum likelihood (middle), and parsimony (bottom). Only bootstrap values greater than 50% are shown. The scale bar represents 0.1 average change per nucleotide position.

Conclusions

Description/redescription of taxa

Description of Verrucomicrobia *div. nov.*
ver.ru'co.mi.cro'bi.a, *Verrucomicrobiae* type class of the division, *-a* ending to denote division. *Verrucomicrobia* the *Verrucomicrobiae* division.

Gram negative bacteria that contain diaminopimelic acid and are sensitive to ampicillin. Some members of the division produce prosthecae and cells of species may have many fimbriae. Division is defined on phylogenetic basis by 16S rRNA sequence analyses of isolated strains and uncultured representatives from numerous natural soil and aquatic habitats.

Description of Verrucomicrobiae *class nov.*
ver.ru'co.mi.cro'bi.ae. *Verrucomicrobiales* type order of the class, *-ae* ending to denote a class, *Verrucomicrobiae* the *Verrucomicrobiales* class.

Gram negative bacteria that contain peptidoglycan. Cells of some species possess prosthecae and fimbriae may be produced. Found in freshwater and marine aquatic habitats as well as soils.

Emended description of Verrucomicrobiales to include genus Prosthecobacter
Gram negative bacteria that contain peptidoglycan. Cells of some species may produce from one to many prosthecae for each cell. Fimbriae may be produced. Found in freshwater and marine aquatic habitats and soils.

Emended description of Verrucomicrobiaceae to include genus Prosthecobacter
Gram negative unicellular bacteria that produce one or more prosthecae per cell. Fimbriae may also be produced. Known species are aerobic or facultatively aerobic. Found in freshwater and marine aquatic habitats and soils. DNA base composition of known species ranges from 54 to 61 mol% G + C.

Description of Prosthecobacter debontii *sp. nov. Prosthecobacter debontii,* (de.bont'i.i. N.L. gen. *debontii* named in honor of J.A.M. de Bont, a Dutch

Table 3. Bacterial isolates and 16S rDNA clone sequences within the division Verrucomicrobia

Bacterium/clone	Environmental source	GenBank Accession number	References
Verrucomicrobium spinosum IFAM 1439	Lake Plußsee (Germany)	X90515	Albrecht et al. 1987; Schlesner 1987; Ward-Rainey et al. 1995
Prosthecobacter dejongeii (FC1) ATCC 27091	Sewage (NC, USA)	U60012	DeBont et al. 1970; Staley et al., 1976; Hedlund et al. 1996
vanneervenii (FC2)	Freshwater (NC, USA)	U60013	
debontii (FC3)	Freshwater (WA, USA)	U60014	
fusiformis (FC4) ATCC 25309	Freshwater (MI, USA)	U60015	
Clone MC15	Forest soil	X64382	Liesack &
MC17	(Australia)	X64381	Stackebrandt 1992
MC18		X64374	
MC31		X64380	
Clone PAD7	Agricultural soil	D26194	Ueda et al. 1995;
PAD18	(Japan)	D26205	Ueda, T. unpubl.
PAD50		D26237	
FIE19		D26269	
Clone ACK.M6	Freshwater lakes	NA[1]	Hiorns et al. 1996
ACK.M10	Adirondack range		
ACK.DE36	(NY, USA)		
ACK.41			
ACK.DH1			
ACK.DH7			
Clone RB01	Freshwater	U62825	Wise et al., 1996
RB02	Carolina Bay	U62826	
RB14	(SC, USA)	U62837	
RB22		U62843	
RB24		U62845	
RB31		U62850	
RB35		U62854	
Clone EA25	Agricultural soil	U51864	Lee, S.Y., J. Bollinger,
EA29	(WA, USA)	NA[1]	D. Bedicek, and A.
EA49		NA[1]	Ogram, unpubl.
Clone env.25.19	Pacific Ocean 100 m	L11970	Fuhrman et al. 1993

[1] NA indicates not available.

microbiologist who isolated and described the first member of the genus, *P. fusiformis* FC4).

Fusiform cells are usually vibrioid, short (2–8 μm), and thin (0.5 μm). Growth occurs in defined media using ammonium salts as nitrogen source. Carbon sources include D-glucose, D-galactose, D-mannose, D-sucrose, D-lactose, D-melibiose, D-maltose, D-xylose, L-rhamnose, D-trehalose, cellobiose, D-ribose, D-fructose, D-arabinose, and N-acetyl glucosamine. Vitamins not required for growth. Temperature range for growth 8–38 °C. Colonies of type strain are pale yellow, opaque, circular, convex, and with entire margins. DNA% G+C 57.1.

The type strain is str. FC3. A culture has been deposited in the American Type Culture Collection.

Description of Prosthecobacter dejongeii *sp. nov. Prosthecobacter dejongeii*, (de.jonge‘i.i N.L. gen.

dejongeii, named in honor of Klaaske de Jonge, a Dutch microbiologist who studied *Prosthecobacter*).

Fusiform cells are usually straight, long (3–10 μm), and wide (1 μm). Growth occurs in defined media using ammonium salts as nitrogen source. Carbon sources include D-glucose, D-galactose, D-mannose, D-sucrose, D-lactose, D-melibiose, D-maltose, D-xylose, L-rhamnose, D-trehalose, cellobiose, D-ribose, glycogen, and N-acetyl glucosamine. Vitamins not required for growth. Temperature range for growth 1–35 °C. Colonies of type strain are pale yellow, opaque, circular, convex, and with entire margins. DNA% G + C 54.6.

The type strain is ATCC 27091 (FC1).

Description of Prosthecobacter vanneervenii *sp. nov. Prosthecobacter vanneervenii*, (van.neer.ven'i.i. N.L. gen. *vanneervenii* named in honor of Alex van Neerven, a Dutch microbiologist who studied *Prosthecobacter* and other prosthecate bacteria).

Fusiform cells are usually straight, long (3–10 μm), and thin (0.5 μm). Growth occurs in defined media using ammonium salts as nitrogen source. Carbon sources include D-glucose, D-galactose, D-mannose, D-sucrose, D-lactose, D-melibiose, D-maltose, D-xylose, L-rhamnose, D-trehalose, cellobiose, D-ribose, D-fructose, D-raffinose, glycogen, D-glucosamine, and N-acetyl glucosamine. Vitamins not required for growth. Temperature range for growth 10–38 °C. Colonies of type strain are yellow, opaque, circular, convex, and with entire margins. DNA% G + C 60.1.

The type strain is str. FC2. A culture has been deposited in the American Type Culture Collection.

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Note added in proof: Novel, anaerobic members of the Verrucomicrobia have recently been isolated from rice paddy soil. These nonprosthecate bacteria were described as ultramicrobacteria on the basis of their small size (Janssen PH, Schuhmann A, Mörshel E & Rainy FA (1977). Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Appl. env. Microbiol. 63: 1382–1388.)