Systematic and Applied Microbiology 39 (2016) 297–306

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

A taxonomic framework for cable bacteria and proposal of the candidate genera Electrothrix and Electronema

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ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 6 May 2016
Accepted 9 May 2016

Keywords:
Long distance electron transport (LDET)
Cable bacteria
16S rRNA phylogeny
*dsr*AB phylogeny
Fluorescence *in situ* hybridization (FISH)

ABSTRACT

Cable bacteria are long, multicellular filaments that can conduct electric currents over centimeter-scale distances. All cable bacteria identified to date belong to the deltaproteobacterial family *Desulfobulbaceae* and have not been isolated in pure culture yet. Their taxonomic delineation and exact phylogeny is uncertain, as most studies so far have reported only short partial 16S rRNA sequences or have relied on identification by a combination of filament morphology and 16S rRNA-targeted fluorescence *in situ* hybridization with a *Desulfobulbaceae*-specific probe. In this study, nearly full-length 16S rRNA gene sequences of 16 individual cable bacteria filaments from freshwater, salt marsh, and marine sites of four geographic locations are presented. These sequences formed a distinct, monophyletic sister clade to the genus *Desulfobulbus* and could be divided into six coherent, species-level clusters, arranged as two genus-level groups. The same grouping was retrieved by phylogenetic analysis of full or partial *dsr*AB genes encoding the dissimilatory sulfite reductase. Based on these results, it is proposed to accommodate cable bacteria within two novel candidate genera: the mostly marine "*Candidatus* Electrothrix", with four candidate species, and the mostly freshwater "*Candidatus* Electronema", with two candidate species. This taxonomic framework can be used to assign environmental sequences confidently to the cable bacteria clade, even without morphological information. Database searches revealed 185 16S rRNA gene sequences that affiliated within the clade formed by the proposed cable bacteria genera, of which 120 sequences could be assigned to one of the six candidate species, while the remaining 65 sequences indicated the existence of up to five additional species.

Introduction

The term "cable bacteria" is collectively used for long, multicellular filamentous bacteria affiliated to the deltaproteobacterial family *Desulfobulbaceae* that can mediate electric currents over centimeter-scale distances in marine, freshwater, and salt-marsh sediments [16,20,36,39,41,44]. Cable bacteria have been proposed to perform electrogenic sulfur oxidation *via* long-distance electron transport. Thereby they electrically couple the oxidation of sulfide in anoxic layers with the reduction of oxygen at the sediment surface [29,31]. This unique type of microbial metabolism creates distinct geochemical signals that can be detected by depth profiling of O_2, pH, H_2S, and electric potential [8,29,35], and drastically changes the geochemistry of cable bacteria-populated sediments [24,28,37].

Cable bacteria have so far evaded cultivation in axenic culture, although all specimens reported to date are morphologically conspicuous, cm-long filaments with distinct longitudinal ridges on their surface [31] and belong to an apparently monophyletic sister lineage of the genus *Desulfobulbus* [31,36]. However, their taxonomic delineation and diversity are uncertain, as most studies have only reported short partial sequences that covered different regions of the 16S rRNA gene [16,21,23,46]. Molecular identification of cable bacteria therefore currently relies on matching short environmental sequences with high sequence identity (>97%) to "confirmed" cable bacteria sequences (*i.e.* 16S rRNA gene sequences obtained from single filaments) [31,36,39]. Alternatively, filament morphology and the general *Desulfobulbaceae*-specific,

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http://dx.doi.org/10.1016/j.syapm.2016.05.006

16S rRNA-targeted probe DSB706 [18] are combined to detect and quantify cable bacteria by fluorescence *in situ* hybridization (FISH) without further identification [20,41,44].

The aim of the current study was to establish a robust phylogenetic framework for cable bacteria, which could be used for their taxonomic delineation and the reliable identification of cable bacteria environmental sequences.

Therefore, individual filaments of cable bacteria were picked from freshwater and marine sediments for whole genome amplification, sequencing, and assembly of nearly full-length 16S rRNA and *dsr*AB gene sequences in order to complement the 16S rRNA phylogeny with that of a faster evolving marker gene [25]. The data were used to reconstruct a robust cable bacteria phylogeny, which, together with the available morphological, physiological and ecological information, led to the proposal of six candidate species within two novel candidate sister genera. Using this taxonomic framework, cable bacterial 16S rRNA gene sequences were identified in public databases, and a comprehensive overview of their environmental diversity and distribution is presented together with the design of novel oligonucleotide probes for their specific detection.

Materials and methods

Sites and sample collection

Aarhus Bay, Denmark

Sediment was collected from Aarhus Bay, Denmark (56°8′20.004″N, 10°12′51.084″E) in April 2012 and cable bacteria enrichment cultures were prepared as described previously [31]. In February 2013, sediment from Aarhus Bay was incubated under anoxic, nitrate-amended conditions, according to Marzocchi et al. [23], and incubations were subsampled for cable bacteria collection.

Buzzards Bay, MA, USA

Samples from intertidal salt marsh sediment, Little Sippewissett, MA, USA (41°34′33.9″N, 70°38′04.9″W) were obtained from laboratory incubations during the study of Larsen et al. [16] in August 2012.

Tokyo Bay, Japan

Sediment cores from Tokyo Bay, Japan (35°32′12.11″N, 139°55′0.12″E; water depth, 20 m) were collected by Scuba diving in November 2013, and the uppermost 20 cm were preserved in 50% (v/v) ethanol and later used for cable bacteria extraction without prior enrichment. The site was seasonally hypoxic and bioturbated with brittle stars and polychaetes.

Giber Å, Denmark

For freshwater cable bacteria, four recently published full-length 16S rRNA gene sequences (GenBank acc. no. KP728462-65) were used, which had been retrieved from freshwater sediment enrichments of the lowland stream Giber Å (56°3′53.9″N, 10°10′20.3″E), at a site located approximately 5 km inland from the coast of Aarhus Bay, Denmark [36].

Filament preparation, genome amplification, sequencing, and genome reconstruction

Cable bacteria were extracted from sediments as described previously [31]. Briefly, single filaments of cable bacteria were picked from sediments with custom-made capillary glass hooks under dissection microscope guidance (Leica M125; Leica, Wetzlar, Germany). All filaments were washed in sterile artificial seawater (salinity: 25‰, pH 8; Reef Crystal) and lysed by ultrasonic bead-beating (Sonoplus HD2070; Bandelin, Berlin, Germany; sonication parameters: 3 min, continuous mode, amplitude setting: 30% ≈ 21 W). Genomes were amplified using the GenomePlex® Single Cell Whole Genome Amplification Kit (Sigma–Aldrich). Sequencing of the amplified genomic material was performed on an Ion Torrent PGM™ sequencer (Life Technologies, USA) using 316v1 chips and 200 or 400 bp chemistry according to the manufacturer's protocols. Read trimming and adapter clipping were carried out as described earlier [36]. The trimmed and clipped reads were assembled using (i) gsAssembler version 2.6 (Roche 454 Life Sciences, Branford, CT) with 10 different configurations: minimum overlap settings of 50 or 100 bp, respectively, and minimum similarity values of 96–100% with 1% steps; and (ii) SPAdes version 2.2.1 (Aarhus Bay MCF) or 3.5 (all other filaments) [2]. Additionally, a set of 10 reduced-complexity assemblies was generated, where 500,000 randomly-selected reads were assembled using gsAssembler 2.6 with a minimum overlap of 100 bp and a minimum sequence identity of 98%. The resulting 21 assemblies were combined, and contigs shorter than 1000 bp were excluded from further analysis. Cable bacterial 16S rRNA genes were extracted from the assemblies as described previously [36]. Open reading frames (ORFs) were predicted on the extracted contigs using FragGeneScan version 1.19 [34]. ORFs coding for DsrA and DsrB were identified and extracted using MEGAN version 4.70.4 [13] based on BLASTp hits to NCBI's nr database (January 25th, 2015 version). Due to the multiple assemblies, this resulted in multiple *dsr*A and *dsr*B ORFs extracted from most single filaments, which were aligned and merged using ARB [49], yielding the final *dsr*A and *dsr*B sequences used for further analysis. Several of the reconstructed *dsr*AB sequences were incomplete, or even absent in filaments F1 and F5 (Table S2), most likely due to incomplete and biased genome recovery after the genome amplification step.

Phylogenetic analyses

All 16 cable bacteria 16S rRNA gene sequences were aligned using the SINA online tool [32], added to the SILVA Release 119 SSU Ref database [33] using ARB [49], and the alignment was manually inspected. Phylogenetic trees were calculated by maximum likelihood (ML; RAxML [43]) and maximum parsimony (MP; Phylip [9]) methods implemented in ARB with 1000 bootstraps and a 50% base frequency filter. Full-length 16S rRNA gene sequences from 36 *Desulfobulbaceae* isolates, extracted from RDP [http://rdp.cme.msu.edu/] were included as reference sequences (Table S1). A consensus tree was constructed from ML and MP trees with multifurcations at nodes that differed between the two treeing methods, indicating that the tree topology at these nodes could not be unambiguously resolved. Pairwise 16S rRNA gene sequence similarities were calculated using the ARB neighbor-joining tool.

The *dsr*A and *dsr*B sequences (Table S2) were concatenated and added into a published reference alignment [25] using ARB. Nucleotide identities between aligned *dsr*AB sequences were calculated using MEGA version 6.06 [45]. Nucleotide *dsr*AB sequences were translated to amino acid sequences and aligned based on the corresponding nucleotide alignment using ARB. Phylogenetic trees were calculated based on almost full-length DsrAB sequences (≥788 amino acids long) by ML analysis with RAxML version 8.2.4, and MP analysis with Phylip version 3.696 (http://evolution.genetics.washington.edu/phylip.html). ML analysis was performed using the Γ model of rate heterogeneity and the WAG amino acid substitution matrix. Node stability of ML and MP phylogenies was evaluated by 1000 bootstrap replicates. Short DsrAB sequences (<788 amino acids long) were added to the ML phylogeny using an evolutionary placement algorithm (EPA) [4] implemented in RAxML version 8.2.4.