Systematic and Applied Microbiology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

A phylogenetic analysis of the phylum *Fibrobacteres*

Kelsea A. Jewell, Jarrod J. Scott[1], Sandra M. Adams, Garret Suen*

Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, United States

ARTICLE INFO

Article history:
Received 10 December 2012
Received in revised form 3 April 2013
Accepted 19 April 2013

Keywords:
Fibrobacteres
Fibrobacter
Phylogeny
Host range
Rumen
Phylogeny

ABSTRACT

Members of the phylum *Fibrobacteres* are highly efficient cellulolytic bacteria, best known for their role in rumen function and as potential sources of novel enzymes for bioenergy applications. Despite being key members of ruminants and other digestive microbial communities, our knowledge of this phylum remains incomplete, as much of our understanding is focused on two recognized species, *Fibrobacter succinogenes* and *F. intestinalis*. As a result, we lack insights regarding the environmental niche, host range, and phylogenetic organization of this phylum. Here, we analyzed over 1000 16S rRNA *Fibrobacteres* sequences available from public databases to establish a phylogenetic framework for this phylum. We identify both species- and genus-level clades that are suggestive of previously unknown taxonomic relationships between *Fibrobacteres* in addition to their putative lifestyles as host-associated or free-living. Our results shed light on this poorly understood phylum and will be useful for elucidating the function, distribution, and diversity of these bacteria in their niches.

Introduction

The ability of herbivores to convert plant biomass into usable nutrients is predicated on symbiotic associations with diverse microbial communities [13]. A key example is ruminants, which use a consortium of microbes to degrade and ferment recalcitrant forms of cellulosic biomass into short-chain, host-available volatile fatty acids (for a review, see [49]). Among these important microbes is the bacterium *Fibrobacter succinogenes* [17], a prolific cellulose degrader [4,53] that produces succinic acid as its major fermentation product and lesser amounts of acetic and formic acids. The recently completed genome sequence for the type strain, *F. succinogenes* S85, highlighted the metabolic and cellulolytic specialization of this bacterium in the rumen [45]. *F. succinogenes* belongs to the phylum *Fibrobacteres*, and work within this phylum has focused primarily on this species and *F. intestinalis*, which is typically found associated with non-ruminant mammalian guts [32]. As a result, our knowledge of the *Fibrobacteres*' host range, environmental niche, and species diversity is based almost entirely on *F. succinogenes* and (to a much lesser extent) *F. intestinalis*. A better understanding of this phylum, and its member species, will be useful for guiding research in areas such as agriculture and biofuel production.

The genus *Fibrobacter* was previously classified within the *Bacteroidetes*, but was elevated to its own phylum based on 16S rRNA sequence analysis and physiological differences from other members of the *Bacteroidetes* [32]. The *Fibrobacteres* are currently defined as anaerobic, Gram-negative, non-spore forming, cellulolytic, non-motile rods [32]. However, only two *Fibrobacter* species have been cultured and formally described (*F. succinogenes* and *F. intestinalis*) [32], despite 16S rRNA sequence data that strongly suggests that cryptic species exist [3,18,24,55]. At present, our understanding of *Fibrobacteres* physiology is based entirely upon *F. succinogenes* and *F. intestinalis* isolates associated with animals [18–20,46,55]. These studies do not reflect the wide diversity of 16S rRNA sequences identified as *Fibrobacteres* that have been reported from surveys of environments as disparate as landfills [30], freshwater lakes [29], the ocean [51], limestone cave sulfidic waters [26], termite hindguts [15,52], and a wide variety of animal feces [23,28]. Given the paucity of information available for the *Fibrobacteres*, and the highly desirable cellulolytic properties of its currently described members, it is important to establish a knowledgebase that documents the diversity of species, distribution, and host associations that exist within this phylum.

Here, we capitalize on extensive public databases of existing 16S rRNA sequence data to create and analyze a *Fibrobacteres*-specific phylogeny. This dataset includes many sequences of environmental origin, in addition to host-associated sequences, and we use this data to estimate the diversity present in non-ruminant and non-fecal *Fibrobacteres*. Our analysis reveals a diverse phylogeny that we use to estimate both the species structure and environmental distribution of this poorly understood phylum.

* Corresponding author. Tel.: +1 608 890 3971; fax: +1 608 262 9865.
E-mail address: gsuen@wisc.edu (G. Suen).
[1] Current address: Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, P.O. Box 380, East Boothbay, ME 04544, United States.

0723-2020/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.syapm.2013.04.002

Materials and methods

Sequence data collection, screening, and phylogenetic analysis

All 16S rRNA sequences available through the National Center for Biotechnological Information's (NCBI) nucleotide database marked with the search terms "*Fibrobacteres*," "Fibrobacter," or "Fibrobact*" (where "*" indicates a wild-card search term, accessed: 09/17/2012) were used to construct an initial sequence library. Roche 454-based pyrosequence libraries were not included, as their short average read length complicates the ability to generate usable alignments for downstream analyses [12]. Literature searches were conducted to identify other 16S rRNA sequences likely belonging to the phyla "*Fibrobacteres*" or "*Fibrobacteres*/*Acidobacteria*" but not marked as such in NCBI. The *Fibrobacteres* sequences present in the GreenGenes [11], Ribosomal Database Project [9] and Silva [37] sequence repositories were also included. The total sequence set was annotated to include sequence source and location from both GenBank deposit information and the original publication, if such existed. The complete dataset is presented in Table S1.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2013.04.002.

The following phylogenetic analysis was performed on the compiled sequence dataset. All sequences were imported into ARB [25] and a full alignment was created against the current Silva 16S/18S rRNA non-redundant sequence database (SSU Ref NR; release 102; 262,092 total sequences); sequences with closer affinity to known *Fibrobacteres* than to any other phyla were considered as belonging to the phylum *Fibrobacteres*. The *Fibrobacteres*-associated sequences were processed in MOTHUR (v.1.26.0, commands used in the following description denoted in italics) [42]. Sequences >900 bp with 5 or fewer ambiguous nucleotides and nine or fewer homopolymers were retained (*screen.seqs*), duplicate sequences were removed (*unique.seqs*), and a preliminary alignment was created (*align.seqs*, Needleman–Wunsch pairwise alignment method, gap extension penalty = −1, gap opening penalty = −1, and match = +1, mismatch penalty = −1, *k* size = 7). Chimera detection (*chimera.uchime*) used the Silva 16S/18S rRNA non-redundant sequence database (SSU Ref NR, accessed 09/2012). Aligned sequences and were filtered (*filter.seqs*, trump=., vertical = T, soft = 50) and used to create a distance matrix (*dist.seqs*, calc = onegap, cutoff = 0.2). The distance matrix used to calculate the estimated number of operational taxonomic units (OTUs) present at 90%, 95%, and 97% similarity cut-offs (*cluster*, nearest neighbor algorithm). Representative sequences were chosen for each OTU (*get.oturep*) for use in constructing a tree in MrBayes (v3.1) [16,39] (ngen = 10,000,000, chain = 4), with the resulting tree visualized using FigTree (v1.3.1) [38]. In addition, a Neighbor-Joining tree was generated from all sequences [40] using the Maximum Composite Likelihood method [47] in MEGA5 [48] and visualized using the Interactive Tree of Life project [22]. The complete 16S rRNA sequence for *Bacteroides fragilis* NCTC9343 was included in our phylogenetic analyses as an outgroup (GenBank genome accession number: NC_003228.3).

A second Neighbor-Joining tree was constructed for sequences of 450 bp or greater in length using these same methods. Some modifications to the sequence manipulations were required due to this shortened minimal sequence length, and were as follows: no ambiguous nucleotides were allowed, and the alignment filter did not include *trump*=. After alignment and filtering the dataset was used to create a p-distance pair-wise distance matrix in MEGA5, and those sequences for which it was not possible to estimate evolutionary distances were removed.

Results

Distribution and definition of the Fibrobacteres

From an initial database of 1166 putative *Fibrobacteres* sequences we generated a database of 863 confirmed *Fibrobacteres* sequences of >900 bp (henceforth, "long sequence database") and 1095 sequences >450 bp (henceforth, "short sequence database") after all filtering steps were performed (Table S1 and Fig. S1). The 900 bp length cut-off was chosen to retain a maximum number (at least 70%) of the original sequences while minimizing the effects of reduced alignment quality (number of columns removed during alignment filtering). The shorter *Fibrobacteres* database was constructed for comparative purposes. The results presented here, except when stated otherwise, refer to analyses performed using the long sequence database.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2013.04.002.

An analysis of our sequences revealed that they were generated from both animal [2–4,15,20,23,24,31,43,52,55] and environmental [5,26,30,36,51] sources, including a wide range of locations spanning the globe (Fig. 1). This range of sampling locations likely reflects the distribution of research groups and interests rather than the dominant natural reservoirs for *Fibrobacteres* bacteria. The two largest groups of sequences belonged to either those associated with mammals (17%, of which 9% were specifically from ruminants) or termites (81%), with the remaining sequences isolated from non host-associated environments (2%) and a single isolate from the water flea *Ventiella sulfuris*. The rumen samples were nearly evenly split between sheep (40 sequences) and cows (32 sequences), with the remaining two sequences from goats. Among the non-ruminants there was a high degree of diversity (Fig. 1 and Table S1), with the most represented non-ruminant animal hosts being Somali wild asses (23 sequences), Yunnan snub-nosed monkeys (12 sequences), and the Eastern black and white colobus (7 sequences). Of the non-host associated environments, 3 were from marine sources, 7 from freshwater sources, 2 from soil, and the remaining 8 from landfills. Among the freshwater sources, surface water, acid-impacted lakes, and sulfidic cave waters were all represented.

Further analysis of the 245 *F. succinogenes* sequences only present in our short sequence database revealed that they were generated primarily from free-living environments, the bulk of which were from landfills (24%), soil (16%), and aquatic (38%) sources. The 43 host-associated short sequences were dominated by rumen (10%) and ostrich cecum (7%) sources. Specific locations unique to the short sequence set included oil sands, yak and buffalo rumens, hot springs, ostrich cecae, and a swine anaerobic lagoon.

An initial Fibrobacteres phylogeny

To gain an initial understanding of the phylogenetic relationship between our identified *Fibrobacteres* sequences, we generated Neighbor-Joining (NJ) trees using the combined long and short sequence databases (Fig. S2). In general, we found that *Fibrobacteres* sequences grouped according to their host or environmental association. For example, the higher and lower termites formed clades distinct from other host-associated sequences, while there was very little mixing of environmental (aquatic or soil) and host-associated sequences within terminal clades. Furthermore, we found a single branch point that includes almost all of the host-associated sequences, with the *F. succinogenes* and *F. intestinalis* sequences forming distinct clades from that common branch point.

Please cite this article in press as: K.A. Jewell, et al., A phylogenetic analysis of the phylum *Fibrobacteres*, Syst. Appl. Microbiol. (2013), http://dx.doi.org/10.1016/j.syapm.2013.04.002

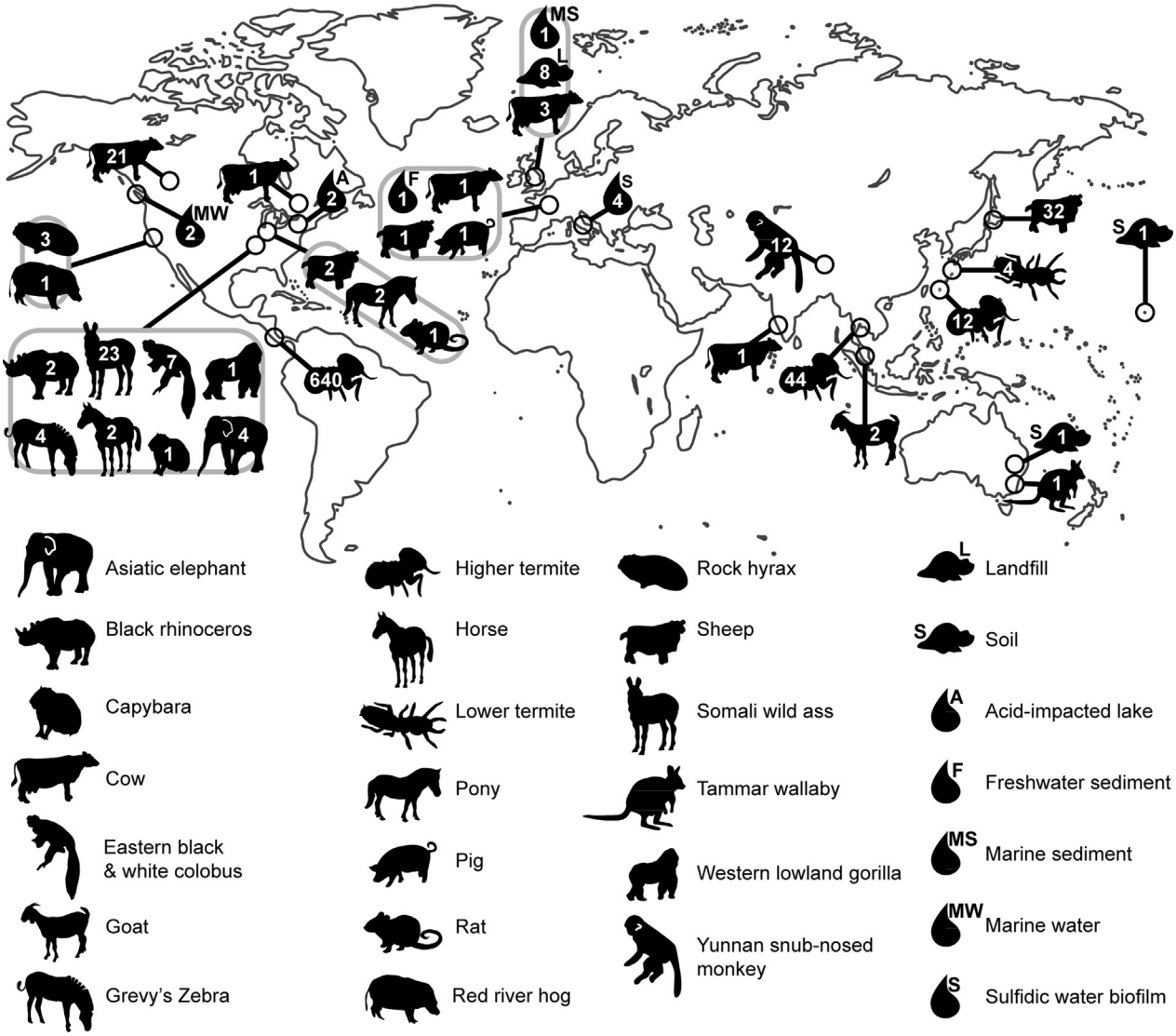

Fig. 1. A world map showing the distribution and number of *Fibrobacteres* sequences used in this study. Sequence source is indicated by the silhouette, with the number of sequences per source given within the silhouette. Sample locations are indicated by the circles, and are based upon the reported locations in the relevant publication or GenBank accession information. Not all sequences are shown due to incomplete sampling location information in GenBank.

An OTU analysis of the phylum Fibrobacteres

A typical challenge in analyzing sequence libraries is the bias toward those samples that have the largest numbers of sequences. This is true for our sequence database, as there is apparent numerical superiority of sequences from two sources: (a) the large number of historical studies focused on *Fibrobacteres* from ruminants, and (b) the inclusion of a particularly exhaustive study on the hindgut bacterial populations in higher termites [52]. One approach to reduce this complexity is to evaluate sequence libraries using operational taxonomic units (OTUs), which use percentages of sequence similarity to define phylogenetic relationships at different taxonomic levels, independent of sequence count. An OTU analysis of the long sequence database using MOTHUR [42] revealed that there are at least 55 genera present within the phylum *Fibrobacteres* and at least 88 species (Table 1, OTUs at 97% similarity for species and 95% for genus [41]). As an example of how OTU usage decreases the impact of sampling bias, of the 129 OTUs present at 97% sequence similarity in the short sequence database, those from higher termite guts were the source of slightly less than half of the OTUs (47.7%) despite encapsulating 80.6% of all sequences. A single OTU for all

Table 1
Number and approximate taxonomic level of *Fibrobacteres* OTUs by percentage of 16S rRNA gene sequence similarity.

% Similarity	Long sequence database Number of OTUs	Short sequence database Number of OTUs	Taxonomic level
97	88	129	
96	69	105	
95	55	89	Species[a]
94	40	76	
93	32	64	
92	29	53	
91	23	44	Genus[b]
90	22	36	
81	1	2	Phylum[c]
80	1	1	

[a] At 95% similarity the *F. succinogenes*-identified long sequences formed a single OTU.
[b] At 91% similarity the *Fibrobacter*-identified long sequences formed a single OTU.
[c] At 81% similarity all Fibrobacteres-identified long sequences formed a single OTU.

Please cite this article in press as: K.A. Jewell, et al., A phylogenetic analysis of the phylum *Fibrobacteres*, Syst. Appl. Microbiol. (2013), http://dx.doi.org/10.1016/j.syapm.2013.04.002

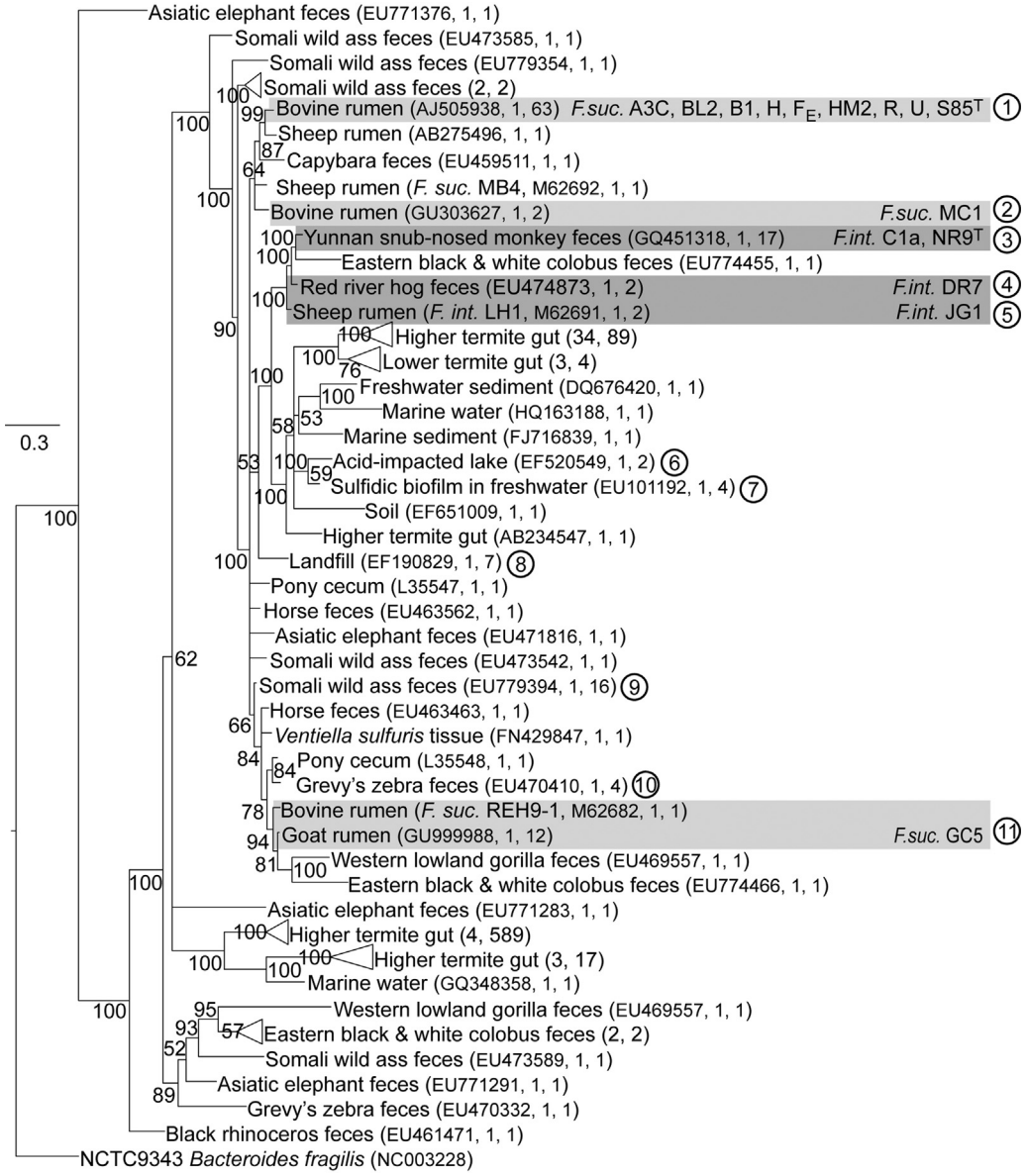

Fig. 2. A Bayesian phylogenetic reconstruction of the representative 16S rRNA OTUs at 97% similarity with *Bacteroides fragilis* NCTC9343 as an outgroup. All posterior probability values are shown (ngen = 10,000,000). Values in parentheses indicate the GenBank accession number, number of total OTUs in that clade, and sequences represented by those OTUs (#Genbank, #OTUs, #sequences). GenBank accession numbers are not given for collapsed clades. Clades have been collapsed only when all sequences in the sub-clades are from the same location type. Representative sequences including *F. succinogenes* are highlighted in light gray, while those including *F. intestinalis* are highlighted in dark gray. *F. succinogenes* = *F. suc.*; *F. intestinalis* = *F. int*. Arbitrary OTU numbers (in circles) have been assigned to correspond with Fig. 3. All sequences used were at least 900 bp in length and were aligned against the full-length Silva 16S/18S rRNA non-redundant sequence database.

863 sequences was derived at 81% similarity, approximately equal to the commonly used phylum-level cutoff of ≥80% similarity [41].

A comparison of OTUs identified in the long and short sequence databases showed that, as expected, our short sequence database had a greater apparent diversity at each sequence similarity level examined down to 80%, at which point all sequences converged into a single OTU (Table 1). The inclusion of additional environmental sequences in the short sequence database resulted in the formation of both unique OTUs and mixing with OTUs already defined from the long sequence database. We found the largest difference in OTU counts at 97%, with an additional 41 OTUs identified using the short sequence database. A total of 12 of these OTUs were composed entirely of sequences unique to the short sequence database and each of these OTUs included at least two sequences. Two of these 12 OTUs were of animal origin (ostrich cecae and bovine rumen) while the remainder were of environmental origin (freshwater, marine water, soil, and oil sands); none of the OTUs had mixed origin (*e.g.*, no OTU containing both fresh and marine water sequences). By comparing the sequences within the OTUs we found that the inclusion of short sequences did not strengthen our phylogeny, except in suggesting that additional work should be done to generate longer *Fibrobacteres* sequences from environmental sources.

Fibrobacter is the only described genus in the phylum *Fibrobacteres* and our OTU analysis did not collapse the *F. intestinalis* and *F. succinogenes* sequences into a single genus at 95% sequence similarity (Table 1), highlighting the difficulty in using arbitrary values in determining taxonomic identity. The *Fibrobacter* genus did not achieve resolution at a single OTU until 93% similarity, which mirrors the results found previously of relatedness at 91–93% [3]. Without additional gene sequence information it is difficult to

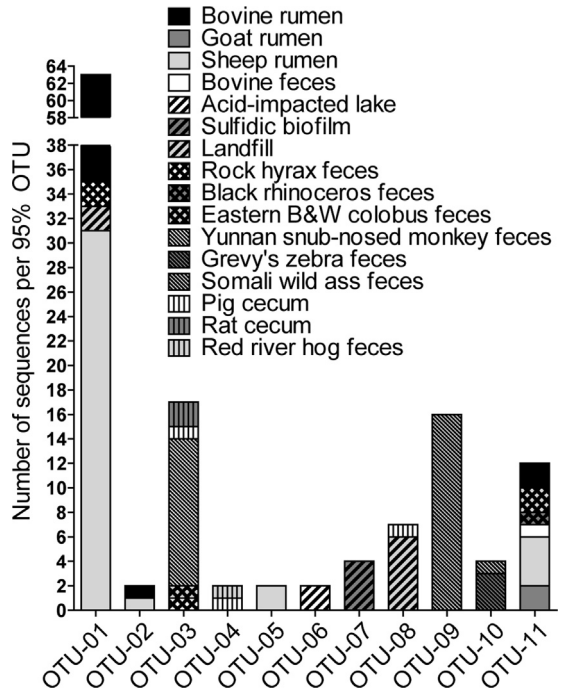

Fig. 3. Distribution of sequence sources in non-termite 95% OTUs with two or more sequences. Arbitrary OTU numbers have been used, and correspond to those given in Fig. 2 (in circles).

determine whether this relatively low percent identity is due to high diversity within the previously defined *Fibrobacter* genus or the 16S rRNA gene sequences used in this and previous studies.

An OTU-based phylogeny of the Fibrobacteres and Fibrobacter spp.

In order to create a comparative phylogeny we used representative sequences from each OTU at 97% sequence similarity in our long sequence database to create a Bayesian phylogenetic tree, as shown in Fig. 2. Despite being considered the same species, the various strains of *F. succinogenes* and *F. intestinalis* failed to collapse into singular representative OTUs at this degree of sequence similarity. Of the 88 OTUs represented in Fig. 2, 27 included 2 or more sequences and 16 were composed entirely of termite-derived sequences. The remaining 11 OTUs were each dominated by specific sequence sources, such as ruminants; a full distribution is given in Fig. 3. As with the short sequence trees, the organization and topology of the sequences (Fig. S2) shows strong differences between environmental or insect- and mammal-derived *Fibrobacteres*.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2013.04.002.

To date, only 16 *F. succinogenes* cultured isolates have been reported and at least partially characterized: strains 128 [14], 095 [14], A3c [10], B1 [44], BL2 [44], F_E [4], GC5 [3], H [4], HM2 [3], MB4 [3], MC1 [3], MM4 [3], R [4], REH9-1 [33], the type strain S85 [7], and U [4]. These *F. succinogenes* 16S rRNA sequences were classified into five species-level OTUs using a 97% identity for species (Table 1). The largest of these OTUs contained ruminal strains A3C, B1, BL2, S85, and U all from cattle rumens; HM2 from a sheep rumen; and 56 other sequences of which 52 were ruminal and 2 each were from rock hyrax feces or landfills. Both *F. succinogenes* REH9-1 (cattle rumen) and MB4 (sheep rumen) were single-sequence OTUs, while GC5 (cattle feces) with 11 other sequences formed a diverse 97% OTU with origins in cattle, sheep, and goat rumens (7 sequences total), Eastern black and white colobus feces (2 sequences), black rhinoceros feces (1 sequence), and the Tammar wallaby foregut (1 sequence). Finally, *F. succinogenes* MC1 (sheep rumen) formed an OTU with a single sequence from a cattle rumen.

For *F. intestinalis*, 16S rRNA sequences are available for all five published isolates including strains C1a [50] and DR7 [3] from porcine ceca; JG1 [3] and LH1 [3] from sheep ceca; and the type strain NR9 [33] from a rat cecum. At 97% sequence similarity we found three separate *F. intestinalis*-containing OTUs. Specifically, *F. intestinalis* JGI and LH1 (sheep rumen) formed one OTU, while DR7 and a single sequence from red river hog feces formed a second. The third and largest OTU contained NR9 (rat cecum) and C1a (pig cecum) along with 15 other sequences: 12 from black and white colobus feces and singlets from a rat cecum, rock hyrax feces, and an eastern black and white colobus. All *F. intestinalis* sequences formed a single OTU at 95% similarity, separate from the *F. succinogenes* OTU.

Higher and lower termite OTUs

In addition to the mammal-associated *Fibrobacter* species, numerous 16S rRNA sequences correlated to the *Fibrobacteres* have been reported from termites. These include the lower (wood-consuming) and higher (fungus-farming or detritivoric) termites [6]. Our OTU analysis agreed with previous work [15] in that the four lower termite-originating sequences do not combine with any higher termite sequences, at least not until the 90% OTU cutoff. The higher termite *Fibrobacteres* sequences formed multiple lineages (Fig. 2), and both lower and higher termite representative clades were intermixed with aquatic and soil-derived sequences. Even at the 90% OTU there was no mixing of termite with non-termite sequences within an OTU.

Discussion

Members of the phylum *Fibrobacteres* are typified by two species, *F. succinogenes* and *F. intestinalis*, which formulate the current assumption that all members of this phylum are host-associated, non-motile, obligate anaerobes [32]. Scientific interest in the *Fibrobacteres* is centered primarily on *F. succinogenes* S85, which is a prolific and efficient microbe capable of degrading plant cell wall polysaccharides [4,27,45]. Given the importance of this species, it is probable that there are other members of the *Fibrobacteres* that would also be of interest in terms of biomass processing and cellulose degradation. Our broad phylogenetic analysis of this phylum is a first step toward understanding their diversity, environmental associations, and geographic distribution. Specifically, we have presented a global analysis of the geographic and phylogenetic distribution of the *Fibrobacteres* by generating an extensive phylogeny using all publicly available 16S rRNA gene sequences of 900 bp or longer. Shorter sequences were not included in our primary phylogeny due to the difficulties associated with separating spurious from actual diversity when mixing shorter-read data sets generated from non-overlapping variable regions of 16s rRNA sequence [12,21], but were used to construct a second, less stringent phylogeny for comparative purposes.

Sequences classified as belonging to the *Fibrobacteres* are present in widely disparate host-associated and free-living environments, indicating that the distribution and diversity within the *Fibrobacteres* is greater than previously thought. Our analysis revealed several distinct clades corresponding to both environmental or host associations, including a clear separation of mammal, insect, and environment-associated sequences. In many cases sequences were found from niches not known to include *Fibrobacteres*. For example, a single sequence was found from the water

flea *Ventiella sulfuris* taken from a hydrothermal vent. This may represent truly novel *Fibrobacteres* populations, transient sequence detection, or even sample contamination, and thus caution must be used to consider the niche distribution of these bacteria. Based on our phylogeny and recent phylogenetic and microscopic work [15,29,30], it is clear that our definition of the phylum *Fibrobacteres* must be re-evaluated. The current classification of *F. succinogenes* as a mammalian gut-specific microbe, for example, does not encompass those sequences that have been recovered from non-host associated environments (such as [5,26,29,30,34,51]). These data underscore the need for future work, specifically in isolating and characterizing non-host-associated cultures of *Fibrobacteres*, to refine our definition and understanding of this phylum.

In addition, we found that, based on commonly accepted levels of sequence similarity [41], the sequences currently defined as *F. succinogenes* and *F. intestinalis* do not represent single species or combine as a genus until highly relaxed similarity values are applied. In particular, the published 16S rRNA sequences from isolates of different *F. succinogenes* strains did not become a single OTU until 95% sequence similarity, below the 97% typically used to define a species [41]. Previous work by Amann et al. and Shinkai et al. using 16S rRNA gene sequencing showed that strains of *F. succinogenes* can be divided into four groups [3,43], with *F. intestinalis* strains forming two groups [3]. In our work, at 97% sequence similarity, the *F. succinogenes* sequences fell into five groups whereas *F. intestinalis*, formed three major groups (Fig. 2). In particular, *F. succinogenes* MM4, MB4, and HM2, which were shown previously as a separate group [3,43], were found intermixed with other named sequences in our results. The separation of named strains for *F. intestinalis* (Fig. 2) was identical to previously reported clusters [3]. It is probable that the sequence diversity within both "species" represents at least sub-species difference, as has been previously suggested [3] and codified for *F. succinogenes* subsp. *succinogenes* (type strain S85) and *F. succinogenes* subsp. *elongatus* (type strain HM2) [32].

It is intriguing to note that the phylogenetic division between the higher and lower termites is recapitulated in the division of their gut *Fibrobacteres* sequences, as was suggested by previous work done on the phylogeny of insect-associated *Fibrobacter* sequences [15]. It is possible that this separation is due to differences in the diets or physiology of the termites (lower termites consume wood, while higher termites cultivate fungi [6]), or that there are specific host-associated factors involved in a *Fibrobacteres*-termite symbiosis. Further work in the termites is needed to determine if there exist distinct phylogenetic lineages of *Fibrobacteres* that are linked to host evolution, as has been found for other insect symbioses [1,35].

In conclusion, we have presented an extensive phylogenetic view of the phylum *Fibrobacteres* created from a synthesis of sequences reported from a wide range of scientific studies representing disparate sample sources and locations. With this framework it is now possible to redefine our understanding of this phylum, and we expect that our sequence-based diversity analysis will help guide and renew efforts to obtain isolates of *Fibrobacteres* from novel locations. For example, the presence of verified *Fibrobacteres* sequences in oxygenated waters [26] strongly suggests that the current definition of this phylum containing only strict anaerobes may be inaccurate. It is also conceivable, given the wide host and environmental distribution reported on here, that additional *Fibrobacteres* isolates may be found that are more amenable to potential industrial exploitation than *F. succinogenes* [8,54,56] (*e.g.*, by aerotolerance or less-stringent nutritional requirements). The phylogenetic work presented here will help us better understand the diversity and potential environmental niches for these bacteria.

Acknowledgements

This work was funded by the Wisconsin Bioenergy Initiative supporting S.M.A and G.S. J.J.S. was supported by a University of Wisconsin Distinguished Graduate Fellowship. We would like to thank P. Weimer, F. Aylward, K. Grubbs, and A. Hanshew for useful comments and discussion.

References

[1] Aanen, D.K., et al. (2002) The evolution of fungus-growing termites and their mutualistic fungal symbionts. PNAS 99, 14887–14892.
[2] Amann, R., Krumholz, L., Stahl, D.A. (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. 172, 762–770.
[3] Amann, R., et al. (1992) Diversity among *Fibrobacter* isolates: towards a phylogenetic classification. Syst. Appl. Microbiol. 15, 23–31.
[4] Bera-Maillet, C., Ribot, Y., Forano, E. (2004) Fiber-degrading systems of different strains of the genus *Fibrobacter*. Appl. Environ. Microbiol. 70, 2172–2179.
[5] Briée, C., Moreira, D., López-García, P. (2007) Archaeal and bacterial community composition of sediment and plankton from a suboxic freshwater pond. Res. Microbiol. 158, 213–227.
[6] Brune, A., Friedrich, M. (2000) Microecology of the termite gut: structure and function on a microscale. Curr. Opin. Microbiol. 3, 263–269.
[7] Bryant, M., Robinson, I., Chu, H. (1959) Observations on the nutrition of *Bacteroides succinogenes* - a ruminal cellulolytic bacterium. J. Dairy Sci. 42, 1831–1847.
[8] Chen, B.-Y., Wang, H.-T. (2008) Utility of enzymes from *Fibrobacter succinogenes* and *Prevotella ruminicola* as detergent additives. J. Ind. Microbiol. Biotechnol. 35, 923–930.
[9] Cole, J.R., et al. (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37, D141–D145.
[10] Dehority, B.A. (1963) Isolations and characterization of several cellulolytic bacteria from *in vitro* rumen fermentations. J. Dairy Sci. 46, 217–222.
[11] DeSantis, T., et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench campatible with ARB. Appl. Environ. Microbiol. 72.
[12] Engelbrektson, A., et al. (2010) Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J. 4, 642–647.
[13] Flint, H.J., et al. (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev. Microbiol. 6, 121–131.
[14] Gaudet, G., Cheng, K.J. (1990) Use of glucose and cellobiose by 3 strains of *Fibrobacter succinogenes*. Repr. Nutr. Dev. 2, 201s–202s.
[15] Hongoh, Y., et al. (2006) Phylogenetic diversity, localization, and cell morphologies of members of the candidate phylum TG3 and a subphylum in the phylum *Fibrobacteres*, recently discovered bacterial groups dominant in termite guts. Appl. Environ. Microbiol. 72, 6780–6788.
[16] Huelsenbeck, J., Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
[17] Hungate, R. (1950) The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. 14, 1–49.
[18] Kim, M., Morrison, M., Yu, Z. (2011) Status of the phylogenetic diversity census of ruminal microbiomes. FEMS Microbiol. Ecol. 76, 49–63.
[19] Kobayashi, Y., Shinkai, T., Koike, S. (2008) Ecological and physiological characterization shows that *Fibrobacter succinogenes* is important in rumen fiber digestion: review. Folia Microbiol. 53, 195–2000.
[20] Kong, Y., Teather, R., Forster, R. (2010) Composition, spatial distribution, and diversity of the bacterial communities in the rumen of cows fed different forages. FEMS Microbiol. Ecol. 74, 612–622.
[21] Kunin, V., et al. (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ. Microbiol. 12, 118–123.
[22] Letunic, I., Bork, P. (2007) Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23, 127–128.
[23] Ley, R.E., et al. (2008) Evolution of mammals and their gut microbes. Science 320, 1647–1651.
[24] Lin, C., Stahl, D.A. (1995) Taxon-specific probes for the cellulolytic genus *Fibrobacter* reveal abundant and novel equine-associated populations. Appl. Environ. Microbiol. 61, 1348–1351.
[25] Ludwig, W., et al. (2004) ARB: a software environment for sequence data. Nucleic Acids Res. 32, 1363–1371.
[26] Macalady, J.L., et al. (2008) Niche differentiation among sulfur-oxidizing bacterial populations in cave waters. ISME J. 2, 590–601.
[27] Maglione, G., Russell, J.B., Wilson, D. (1997) Kinetics of cellulose digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 63, 665–669.
[28] Matsui, H., Ban-Tokuda, T., Wakita, M. (2010) Detection of fiber-digesting bacteria in the ceca of ostrich using specific primer sets. Curr. Microbiol. 60, 112–116.
[29] McDonald, J.E., et al. (2009) Molecular biological detection and quantification of novel *Fibrobacter* populations in freshwater lakes. Appl. Environ. Microbiol. 75, 5148–5152.
[30] McDonald, J.E., et al. (2008) Detection of novel *Fibrobacter* populations in landfill sites and determination of their relative abundance via quantitative PCR. Environ. Microbiol. 10, 1310–1319.

Please cite this article in press as: K.A. Jewell, et al., A phylogenetic analysis of the phylum *Fibrobacteres*, Syst. Appl. Microbiol. (2013), http://dx.doi.org/10.1016/j.syapm.2013.04.002

[31] Monteils, V., et al. (2008) Potential core species and satellite species in the bacterial community within the rabbit caecum. FEMS Microbiol. Ecol. 66, 620–629.
[32] Montgomery, L., Flesher, B., Stahl, D. (1988) Transfer of Bacteroides succinogenes (Hungate) to Fibrobacter gen. nov. as *Fibrobacter succinogenes* comb. nov. and description of *Fibrobacter intestinalis* sp. nov. Int. J. Syst. Bacteriol. 38, 430–435.
[33] Montgomery, L., Macy, J. (1982) Characterization of rat cecum cellulolytic bacteria. Appl. Environ. Microbiol. 44, 1435–1443.
[34] Nüsslein, K., Tiedje, J. (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl. Environ. Microbiol. 65, 3622–3626.
[35] Ohkuma, M., et al. (2001) Coevolution of symbiotic systems of termites and their gut microorganisms. RIKEN Rev. 41, 73–74.
[36] Percent, S.F., et al. (2008) Bacterial community structure of acid-impacted lakes: what controls diversity? Appl. Environ. Microbiol. 74, 1856–1868.
[37] Pruesse, E., et al. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB 35., 21.
[38] Rambaut, A. FigTree v1.3.1 http://tree.bio.ed.ac.uk/software/figtree/
[39] Ronquist, F., Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
[40] Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
[41] Schloss, P.D., Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl. Environ. Microbiol. 71, 1501–1506.
[42] Schloss, P.D., et al. (2009) Introducing MOTHUR: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
[43] Shinkai, T., et al. (2009) Fibrolytic capabilities of ruminal bacterium *Fibrobacter succinogenes* in relation to its phylogenetic grouping. FEMS Microbiol. Lett. 294, 183–190.
[44] Stewart, C., et al. (1981) Selective isolation and characteristics of *Bacteroides succinogenes* from the rumen of a cow. Appl. Environ. Microbiol. 41, 504–510.
[45] Suen, G., et al. (2011) The complete genome sequence of *Fibrobacter succinogenes* S85 reveals a cellulolytic and metabolic specialist. PLoS ONE 6, e18814.
[46] Tajima, K., et al. (1999) Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. FEMS Microbiol. Lett. 29, 159–169.
[47] Tamura, K., Nei, M., Kumar, S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. PNAS. 101, 11030–11035.
[48] Tamura, K., et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
[49] van Houtert, M.F.J. (1993) The production and metabolism of volatile fatty acids by ruminants fed roughages: a review. Anim. Feed Sci. Technol. 43, 189–225.
[50] Varel, V., Fryda, S., Robinson, I. (1984) Cellulolytic bacteria from pig large intestine. Appl. Environ. Microbiol. 47, 219–221.
[51] Walsh, D.A., et al. (2009) Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. Science 326, 578–582.
[52] Warnecke, F., et al. (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450, 560–565.
[53] Weimer, P.J., French, A., Calamari, T., Jr. (1991) Differential fermentation of cellulose allomorphs by ruminal cellulolytic bacteria. Appl. Environ. Microbiol. 57, 3101–3106.
[54] Wen, T.-N., et al. (2005) A truncated *Fibrobacter succinogenes* 1,3-1,4-β-D-glucanase with improved enzymatic activity and thermotolerance. Biochemistry 44, 9197–9205.
[55] Whitford, M.F., et al. (1998) Phylogenetic analysis of rumen bacteria by comparative sequence analysis of cloned 16S rRNA Genes[ß]. Anaerobe 4, 153–163.
[56] Zeikus, J.G., Jain, M.K., Elankovan, P. (1999) Biotechnology of succinic acid production and markets for derived industrial products. Appl. Microbiol. Biotechnol. 51, 545–552.

Please cite this article in press as: K.A. Jewell, et al., A phylogenetic analysis of the phylum *Fibrobacteres*, Syst. Appl. Microbiol. (2013), http://dx.doi.org/10.1016/j.syapm.2013.04.002