REVIEW PAPER

Molecular signatures for the phylum Synergistetes and some of its subclades

Vaibhav Bhandari · Radhey S. Gupta

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Abstract Species belonging to the phylum Synergistetes are poorly characterized. Though the known species display Gram-negative characteristics and the ability to ferment amino acids, no single characteristic is known which can define this group. For eight Synergistetes species, complete genome sequences or draft genomes have become available. We have used these genomes to construct detailed phylogenetic trees for the Synergistetes species and carried out comprehensive analysis to identify molecular markers consisting of conserved signature indels (CSIs) in protein sequences that are specific for either all Synergistetes or some of their sub-groups. We report here identification of 32 CSIs in widely distributed proteins such as RpoB, RpoC, UvrD, GyrA, PolA, PolC, MraW, NadD, PyrE, RpsA, RpsH, FtsA, RadA, etc., including a large >300 aa insert within the RpoC protein, that are present in various Synergistetes species, but except for isolated bacteria, these CSIs are not found in the protein homologues from any other organisms. These

V. Bhandari · R. S. Gupta (⊠) Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON L8N 3Z5, Canada e-mail: gupta@mcmaster.ca CSIs provide novel molecular markers that distinguish the species of the phylum Synergistetes from all other bacteria. The large numbers of other CSIs discovered in this work provide valuable information that supports and consolidates evolutionary relationships amongst the sequenced Synergistetes species. Of these CSIs, seven are specifically present in Jonquetella, Pyramidobacter and Dethiosulfovibrio species indicating a cladal relationship among them, which is also strongly supported by phylogenetic trees. A further 15 CSIs that are only present in Jonquetella and Pyramidobacter indicate a close association between these two species. Additionally, a previously described phylogenetic relationship between the Aminomonas and Thermanaerovibrio species was also supported by 9 CSIs. The strong relationships indicated by the indel analysis provide incentives for the grouping of species from these clades into higher taxonomic groups such as families or orders. The identified molecular markers, due to their specificity for Synergistetes and presence in highly conserved regions of important proteins suggest novel targets for evolutionary, genetic and biochemical studies on these bacteria as well as for the identification of additional species belonging to this phylum in different environments.

Keywords Conserved indels ·

Molecular signatures · Synergistetes phylogeny and taxonomy · Jonquetella-Pyramidobacter-Dethiosulfovibrio clade · Aminomonas-Thermanaerovibrio clade · Lateral gene transfers

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Introduction

The Synergistetes group of bacteria is a recently recognized phylum for which 40 organisms have been isolated and over three hundred 16S rRNA sequences are available (Hugenholtz et al. 2009; NCBI Taxonomy 2012). The phenotypic characteristics shared by the species from this phylum include their gramnegative cell wall, anaerobic existence, and rod/ vibrioid cell shape (Jumas-Bilak et al. 2009). Although the presence of lipopolysaccharides, which is an important characteristic of diderm cell envelopes, in Synergistetes species has not yet been reported, they do contain genes for various proteins that are involved in lipopolysaccharide biosynthesis (Sutcliffe 2010). While a few species have been shown to be asaccharolytic, all Synergistetes have the ability to ferment amino acids (Magot et al. 1997; Baena et al. 1998, 1999a; Surkov et al. 2001; Hongoh et al. 2007; Downes et al. 2009; Jumas-Bilak et al. 2009). The Synergistetes inhabit primarily anaerobic environments including animal gastrointestinal tracts, soil, oil wells and wastewater treatment plants and they are also present in sites of human diseases such as cysts, abscesses and areas of periodontal disease (Godon et al. 2005; Kumar et al. 2005; de Lillo et al. 2006; Horz et al. 2006; Jumas-Bilak et al. 2007; Vartoukian et al. 2007; Zijnge et al. 2010). Due to their presence at illness related sites, the Synergistetes are suggested to be opportunistic pathogens but they can also be found in healthy individuals in the microbiome of the umbilicus and in normal vaginal flora (Vartoukian et al. 2007; Marchandin et al. 2010). Other species from this phylum have been identified as significant contributors in the degradation of sludge for production of biogas in anaerobic digesters and are potential candidates for use in renewable energy production through their production of hydrogen gas (McSweeney et al. 1993; Maune and Tanner 2012; Delbes et al. 2001; Riviere et al. 2009; Ziganshin et al. 2011).

Synergistetes species were first identified with the isolation of *Synergistes jonesii* from which the phylum name "Synergistetes" is derived. *S. jonesii* was isolated from the rumen of a goat in 1992 and described as a gram-negative staining, anaerobic, rod-shaped, commensal bacteria with the ability to degrade the toxic compound pyridinediol 3-hydroxy-4-1(*H*)-pyridone (Allison et al. 1992; McSweeney et al. 1993). *S. jonesii* 16S rRNA was not closely related to that of any bacteria

characterized at the time but the species was later misclassified as a member of the Deferribacteres group (Garrity et al. 2004). Around the same time, the species Thermoanaerovibrio acidaminovorans was isolated from methanogenic sludge and indicated to be a member of the Selenomonas genus within the Firmicutes phylum (Guangsheng et al. 1992; Baena et al. 1999b). These misclassifications of Synergistetes continued, as species from the genera Aminobacterium and Dethiosulfovibrio were described as forming a deep-branching clade beside cluster V within Clostridia, consisting of a group composed of Thermoanaerobacter species (Magot et al. 1997; Baena et al. 1998). Several other organisms now considered Synergistetes were also placed among the Syntrophomonadaceae family of the Firmicutes (Garrity et al. 2004; Dahle and Birkeland 2006; Diaz et al. 2007). Eventually, efforts based on 16S rRNA sequences by Jumas-Bilak et al. (2009), identified the monophyletic nature of the Synergistetes within the bacterial domain and proposed that these "Synergistia jonesii-like" species form a distinct phylum, now named the Synergistetes (Jumas-Bilak et al. 2009). All characterized Synergistetes species are currently placed under the class Synergistia, the order Synergistiales and the family Synergistaceae (Jumas-Bilak et al. 2009). This family until recently was comprised of 11 genera, namely: Aminiphilus, Aminobacterium, Aminomonas, Anaerobaculum, Cloacibacillus, Dethiosulfovibrio, Jonquetella, Pyramidobacter, Synergistes, Thermoanaerovibrio and Thermovirga (Hugenholtz et al. 2009; Jumas-Bilak et al. 2009; NCBI Taxonomy 2012). Recently, a new genus Fretibacterium has also been described, which contains a single species Fretibacterium fastidiosum that was previously known as Synergistes bacterium SGP1. A candidate genus Tammella, composed of a group of related and uncultured species found within termite guts, has also been suggested to belong to the phylum Synergistetes (Hongoh et al. 2007; Hugenholtz et al. 2009).

While the Synergistetes are currently classified as belonging to a separate phylum based on their 16S rRNA sequences, no characteristic of these bacteria is known that can easily differentiate a Synergistetes species from other bacteria. Though all cultured Synergistetes can ferment amino acids, various species from other taxa also share this ability (Hou et al. 2004; Fonknechten et al. 2010). The availability of genome sequences has allowed for the employment of comparative genome approaches for the identification of molecular markers that are specific for different bacterial groups at various taxonomic levels (Gupta 1998; Griffiths et al. 2005; Gupta and Bhandari 2011; Gupta and Shami 2011). Using genomic sequences, our lab has pioneered the discovery of conserved signature insertions/deletions (i.e. indels, CSIs) present in protein sequences that are specific for particular groups of organisms (Gupta 1998, 2009; Gao and Gupta 2005; Griffiths and Gupta 2006; Gupta and Bhandari 2011). The group specific presence of CSIs can be parsimoniously explained through rare genetic changes occurring in a common ancestor to the particular groups of species and then being passed down through vertical descent (Gupta 1998, 2000, 2009). Such CSIs, which are present in a related group of species and absent in other organisms, are useful as molecular markers for the identification of species belonging to a taxonomic group and the demarcation of the group's boundaries. Additionally, through comparison of sequences and based on the presence or absence of the indicated CSIs in outgroup species, a rooted phylogenetic relationship can be inferred among the species (Rivera and Lake 1992; Baldauf and Palmer 1993; Gupta 1998, 2001).

From the species identified as Synergistetes, complete or annotated draft genomes are now available for nine species (described below). In the present work, we have carried out detailed comparative analyses on protein sequences from these genomes to identify molecular markers (CSIs) that are specific for the phylum Synergistetes and some of its subgroups, as well as those that provide information regarding its relationship to other bacterial phyla. Our work has identified numerous CSIs that provide highly specific markers for all sequenced members of the Synergistetes phylum as well as a number of its sub-groups. Additionally, several CSIs that are commonly shared by Synergistetes and some species from other bacterial phyla suggest potential cases of lateral gene transfers. These CSIs provide novel and powerful means for the identification/ circumscription of species from the phylum Synergistetes and for different types of studies on them.

Phylogenetic analysis of the genome sequenced Synergistetes

The complete genomes for *Aminobacterium (Amb.)* colombiense (Chertkov et al. 2010), *T. acidaminovorans*

(Chovatia et al. 2009) and *Thermovirga (Tv.) lienii* (Dahle and Birkeland 2006) have been published while annotated draft genomes were accessible for *Dethiosulfovibrio peptidovorans* (Labutti et al. 2010), *Aminomonas (Amm.) paucivorans* (Pitluck et al. 2010), *Anaerobaculum (An.) hydrogeniformans, Jonquetella anthropi* and *Pyramidobacter piscolens* (NCBI genomic database 2012). Limited sequence data for *F. fastidiosum*, which is currently referred to as *Synergistetes bacterium SGP1* in the NCBI database, was also available (Vartoukian et al. 2012). These species represent nine of twelve characterized genera from the phylum. Some characteristics of these organisms and their genomes are provided in Table 1.

The relationships of the species in the Synergistetes phylum have thus far been primarily analyzed through 16S rRNA sequence data. However, it is now recognized that trees based on a larger dataset of genes or proteins representing diverse functional categories are more reliable in resolving phylogenetic relationships than a single gene such as the 16S rRNA or a single protein (Rokas et al. 2003; Ciccarelli et al. 2006; Wu and Eisen 2008). Therefore, in order to visualize the relationship among the sequenced Synergistetes species, phylogenetic trees based upon concatenated sequences of ten housekeeping proteins were constructed. The 10 proteins that were used for phylogenetic analysis (viz. ArgRS, GyrB, Hsp70, ribosomal proteins L1 and L5, RpoB, RpoC, TrxB, UvrD and ValRS) are found in most bacteria and they have been extensively used for other phylogenetic studies (Bocchetta et al. 2000; Ciccarelli et al. 2006; Soria-Carrasco et al. 2007; Zhaxybayeva et al. 2009; Naushad and Gupta 2012). In addition to the Synergistetes species, the dataset that was employed for phylogenetic analyses also contained information for species from several other bacterial phyla including those in whose proximity the Synergistetes species were observed to branch in earlier studies (Guangsheng et al. 1992; Magot et al. 1997; Baena et al. 1998; Garrity et al. 2004; Diaz et al. 2007; Herlemann et al. 2009). The results for the multi-protein concatenated phylogenetic analysis are presented in Fig. 1a. In parallel, a 16S rRNA tree was also created to investigate the congruence with the protein tree (Fig. 1b).

In both the protein tree and the 16S rRNA tree, the Synergistetes species formed a monophyletic clade that was distinct from all other bacterial groups,

Synergistetes species	GC (%)	Isolation source	Asaccharolytic	Optimum temperature (°C)	Size (Mb)	Reference
Aminobacterium colombiense	45.3	Anaerobic lagoon of dairy wastewater treatment plant	Yes	37	1.98	DOE-JGI ^a
Aminomonas paucivorans	43	Anaerobic lagoon of dairy wastewater treatment plant	Yes	35	2.6	DOE-JGI ^a
Anaerobaculum hydrogeniformans	46.6	Oil-well production water	No	55	2.3	GSC-WashU ^b
Dethiosulfovibrio peptidovorans	54.4	Oil well	Yes	42	2.6	DOE-JGI ^a
Jonquetella anthropi	59.4	Peritoneal fluid, Breast and pelvic abscess, sebaceous cyst and wounds	Yes	37	1.7	DOE-JGI ^a
Pyramidobacter piscolens	59	Human oral cavity	Yes	37	2.6	JCV ^c
Thermanaerovibrio acidaminovorans	63.8	Granular methanogenic sludge	No	55	1.85	DOE-JGI ^a
Thermovirga lienii	46.6	Oil-well production water	Yes	58	2.06	DOE-JGI ^a
Fretibacterium fastidiosum	63	Subgingival plaque	Yes	37	Unknown	Vartoukian et al. (2010)

Table 1 Characteristics of the Synergistetes species with sequenced genomes

^a DOE-JGI-these genomes have been sequenced by the United States Department of Energy Joint Genomic Institute

^b GSC-WashU—Genome Sequencing Center (GSC) at Washington University (WashU) School of Medicine

^c JCV-J. Craig Venter Institute

supporting their assignment into a separate phylum. The species such as Syntrophomonas wolfei or Selenomonas sputigena in whose proximity some of the Synergistetes species were indicated to branch in earlier studies, branched distinctly from them. Although the relationships among other bacterial species/phyla differed within the two trees and they were mostly unresolved, within the Synergistetes clade both the concatenated protein tree and the rRNA tree displayed a similar branching order. In both trees, the Synergistetes species showed a split into two clades at the highest level. One clade is comprised of Thermanaerovibrio and Aminomonas sharing a distant relationship with the Thermovirga and Anaerobaculum species while the other clade is comprised of the five species from the genera Aminobacterium, Jonquetella, Pyramidobacter, Dethiosulfovibrio and Fretibacterium. The concatenated protein tree shows, with high statistical support, that the J. anthropi and P. piscolens species branch together and that D. peptidovorans is the closest relative of these two species. The trees also show a well-supported, close relationship between T. acidaminovorans and Amm. paucivorans. The species Amb. colombiense and F.

fastidiosum are observed to robustly branch together, though their relationship to the *Dethiosulfovibrio– Pyramidobacter–Jonquetella* clade was strongly supported only by the NJ concatenated protein tree. The ML analysis and the rRNA tree weakly supported this relationship. The position of *An. hydrogeniformans* and *Tv. lienii* species within the phylum was poorly resolved in both the concatenated protein and the rRNA trees. In both the trees, short branches connect these species to the *Thermoanaerovibrio–Aminomonas* clade and their grouping in this clade was weakly supported by the ML tree and the rRNA tree.

Identification of CSIs that are specific for the Synergistetes species

For the identification of CSIs, BlastP searches against the non-redundant protein sequence (nr) database were carried out on all proteins from the genome of the species *Amb. colombiense DSM 12261* and *T. acidaminovorans DSM 6589* from the Synergistetes phylum (Altschul et al. 1997, 2005). Using the ClustalX program, multiple sequence alignments were



Fig. 1 Phylogenetic tree for sequenced Synergistetes species and representative species from some closely related bacterial phyla. **a** A neighbour joining (NJ) distance tree based upon concatenated sequences for 10 highly conserved and widely distributed proteins (ArgRS, GyrB, Hsp70, ribosomal proteins L1 and L5, RpoB, RpoC, TrxB, UvrD and ValRS). The numbers on the node indicate the % bootstrap score (or statistical support)

created for all proteins for which high scoring homologues were available from most Synergistetes species as well as several other groups of organisms. The aligned proteins were visually inspected to identify insertions or deletions that were flanked by conserved amino acids on both sides. Insertions and deletions that were not flanked by at least 4-5 conserved residues within the neighbouring 30-40 residues were not further considered as they do not provide useful molecular markers (Gupta 1998, 2001; Gupta and Bhandari 2011). More detailed BlastP searches (searching for 250 of the closest sequence matches) were then carried out on 50-80 aa long segments (longer in some cases) containing the indels and its flanking conserved regions to determine the species distribution for the identified indels. Indels predominantly found in Synergistetes species or those that were found in Synergistetes along with some other taxonomic group of organisms were retained and compiled into signature files. The signature files shown here contain sequence alignments of various

for each node in the NJ and maximum-likelihood analyses, respectively. The *dashes* (–) at nodes indicate that the statistical support for this particular branching relationship was <50 % in the NJ or ML analysis. **b** A NJ tree for the same species as shown in (**a**) based upon 16S rRNA sequences. The trees were constructed as described in our earlier work (Gupta and Bhandari 2011)

detected indels along with the flanking conserved regions for all Synergistetes and representative species from other taxonomic groups for which information was detected in the Blast searches. However, due to spatial considerations sequence information for only limited numbers of species from other groups are shown here. In a few cases, where more than one homologue of a protein was detected for the same species, sequence information for different homologues was included only if they showed differing characteristics (viz. one homologue contained the indel while the other(s) did not). All of the indels reported in this work are independent of each other and they are not part of indels for other larger clades.

CSIs that are specific for the Synergistetes phylum

CSIs in proteins brought about by rare genomic changes that are restricted to phylogenetically well-

defined groups are useful as molecular markers that provide means for evaluating evolutionary relationships (Gupta 1998; Rokas and Holland 2000). Our analyses of genome sequences from Synergistetes species have identified 32 CSIs that help define and demarcate the species of this phylum. Some characteristics for these Synergistetes specific CSIs are listed in Table 2 and two examples are provided in Fig. 2. Figure 2a depicts two inserts that are present in close proximity within the β' subunit of the RNA polymerase enzyme (RpoC), an essential enzyme responsible for transcription of genes in all organisms. The region of the protein shown is highly conserved among all organisms and it contains a 2 aa insert that is specifically present in all homologues of the RpoC enzyme from species of the phylum Synergistetes. Another example of a conserved indel that is specific for all Synergistetes species is shown in Fig. 2b. In this case, the α -subunit of DNA polymerase III contains a 1 aa insert that is specifically present in all Synergistetes species (Fig. 2b) but not in any other bacteria. The absence of amino acid residue both the CSIs shown in Fig. 2 in all other organisms except Synergistetes indicates that these CSIs constitute inserts rather than deletions. Within the RpoC protein, in the neighborhood of the conserved insert that is shown in Fig. 2a, another very large insert consisting of between 311 and 316 aa is also uniquely present in all sequenced Synergistetes species. The sequence region corresponding to this large insert is shown in Fig. 3. BlastP searches with this insert show no significant hits for any proteins from organisms outside of other Synergistetes, indicating that this insert is a distinctive characteristic of the species of this phylum. Because of its large size, this large insert likely forms a unique domain of the RpoC protein that is only found in the Synergistetes species.

Other indels present in all genome sequenced Synergistetes, and absent in species from other taxonomic groups, are depicted in Supplementary Figs. 1–12 and some characteristics of them are summarized in Table 2 . These indels are present in proteins involved in important cellular processes such as DNA replication (e.g. DNA polymerase I), protein translation (30S ribosomal protein S1) and cell metabolism (2-oxoglutarate synthase). For some of these Synergistetes specific CSIs, protein homologues for one or more Synergistetes species were not detected (Supplementary Figs. 7–13). A 3 aa insert in 2-oxoglutarate synthase (Supplementary Fig. 7) is an example of such an indel. The insert is present in all detected Synergistetes but in this case the homologue for F. fastidiosum was not found in BlastP searches. It is possible that the gene coding for this protein has been lost from this species due to genetic, environmental or physiological factors. However, as fully published genome sequences among the Synergistetes species are available for only T. acidaminovorans, Tv. lienii and Amb. colombiense, the lack of a protein homologue for some of these species could also be due to the fact that their entire genomes have not yet been sequenced and/or annotated. Nevertheless, since these CSIs are only found in the Synergistetes species and not in any other bacteria (0/250; top 250 blast hits), they also provide reliable molecular markers for this group.

The indels identified above are completely specific for the Synergistetes species. However, for a small number of other CSIs discovered in this work, along with their presence in all Synergistetes, these CSIs were also found in a small number (usually 1-2) of species belonging to other taxonomic groups. Two such examples are shown in Fig. 4. The first of these is another 2 aa long insert in the RpoC protein (Fig. 4a). This CSI is found in all Synergistetes in a highly conserved region of the protein, however it is also present in the species Eubacterium yurii from the Clostridia class of the phylum Firmicutes. The CSI is not present in any other organisms, including other Firmicutes species. Different possibilities exist for the presence of the CSI in a single species outside of the phylum. The shared presence of the CSI in E. yurii, a species not considered to be directly related to the Synergistetes (see Fig. 1), might be the result of a lateral gene transfer event wherein the Synergistetes gene containing the indel might have been introduced into E. yurii. Alternatively, it is possible that two separate genetic events led to the presence of similar CSIs in Synergistetes and E. yurii. A second example of such an indel is shown in Fig. 4b. Here a 2 aa insert is present within the 30S ribosomal protein S8 of Synergistetes species and an uncultured Termite group 1 phylotype RS-D17 considered to belong to the phylum Elusimicrobia. As shown, Elusimicrobium minutum itself contains a 1 aa insert in a similar position in the protein. It is possible that the Elusimicrobia are a sister taxon of the Synergistetes and the indel has been passed on to both phyla through a

Table 2 Characteristics of the CS	Is that are	specific for th	le Synergistetes phi	ylum			
Protein name	Gene	GenBank	Fig. no.	Indel	Indel	Species distr	ibution of indel
	name	identifier		size	position"	Synergistete	s ^b Other organisms ^c
DNA-directed RNA polymerase, β' subunit	rpoC	294101621	Fig. 2a	2 aa ins	1197–1241	6/6	1
DNA polymerase III, α subunit	polC	288574785	Fig. 2b	1 aa ins	762-807	6/6	1
DNA-directed RNA polymerase, β' subunit	rpoC	294101621	Fig. 3	313 aa ins	1197–1601	6/6	1
30S ribosomal protein S1	rpsA	282856368	Suppl. Fig. 1	1 aa del	341–386	6/6	1
S-adenosylmethyltransferase MraW	mraW	294101810	Suppl. Fig. 2	1 aa ins	182–220	6/6	1
Ribose phosphate pyrophosphokinase	prsA	294101745	Suppl. Fig. 3	5 aa ins	137–186	6/6	I
UvrD/REP helicase	uvrD	295112231	Suppl. Fig. 4	2–3 aa ins	61–125	6/6	I
GTP-binding protein TypA	typA	288574323	Suppl. Fig. 5	11–13 aa ins	219–287	6/6	I
2-Oxoglutarate synthase	korA	288574831	Suppl. Fig. 6	3 aa ins	126-167	8/8	I
MazG family protein	I	288574801	Suppl. Fig. 7	2 aa ins	154-183	8/8	I
Integrase family protein	I	294102004	Suppl. Fig. 8	1 aa del	264-294	8/8	I
FAD dependent oxidoreductase	I	288574196	Suppl. Fig. 9	2 aa ins	263-290	8/8	I
DNA gyrase, A subunit	gyrA	288573325	Suppl. Fig. 10	1 aa del	562-594	8/8	I
DEAD/DEAH box helicase domain protein	I	294101046	Suppl. Fig. 11	2–3 aa del	278–324	LIL	I
Serine O-acetyltransferase	cysE	294101111	Suppl. Fig. 12	4 aa ins	18-53	6/6	I
DNA directed RNA polymerase, β' subunit	rpoC	288574655	Fig. 4a	2 aa ins	44–93	6/6	Eubacterium yurii
Ribosomal protein S8	Hsqr	294101642	Fig. 4b	2 aa ins	39–86	6/6	Termite group 1 Rs-D17
RecA protein	recA	294101403	Suppl. Fig. 13	2 aa ins	179–228	8/8	Eubacterium yurii
UvrD/REP helicase	uvrD	295112231	Suppl. Fig. 14	3-4 aa ins	88–122	6/6	Ktedonobacter racemifer
DNA polymerase I	polA	260654849	Suppl. Fig. 15	3-4 aa ins	520–552	6/6	Bacteriovorax marinus
DNA-directed RNA polymerase, β subunit	rpoB	282857671	Suppl. Fig. 16	6–9 aa ins	977-1020	6/6	Rubrobacter xylanophilus
Polyribonucleotide nucleotidyltransferase	dud	289523245	Suppl. Fig. 17	2 aa ins ^d	342–390	8/8	Gemmatimonas aurantiaca

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Protein name	Gene	GenBank	Fig. no.	Indel	Indel	Species distribu	tion of indel
	name	identifier		sıze	position"	Synergistetes ^b	Other organisms ^c
Protein of unknown function DUF1385	I	288574776	Suppl. Fig. 18	2 aa del	249–289	LIL	Elusimicrobium minutum, Thermotoga lettingae, Fervidobacterium nodosum
Helicase domain protein	I	288574739	Suppl. Fig. 19	3 aa del	550-604	5/5	Syntrophus aciditrophicus
ABC transporter, periplasmic substrate binding protein	I	289523650	Suppl. Fig. 20	2 aa del	121–164	4/4	Desulfobacterium autotrophicum
3,4-Dihydroxy-2-butanone 4-phosphate synthase	ribB	294101989	Suppl. Fig. 21	3 aa ins	221-270	7/8	I
DEAD/DEAH box helicase domain protein	I	282856966	Suppl. Fig. 22	6–9 aa ins	28–74	7/8	I
DNA repair protein RadA	radA	289523258	Suppl. Fig. 23	2 aa del	129–184	7/8	1
Nicotinate nucleotide adenylyltransferase	nadD	294101840	Suppl. Fig. 24	2 aa ins	94–121	7/8	I
Orotate phosphoribosyltransferase	pyrE	269792697	Suppl. Fig. 25	1 aa del	82-128	8/9	I
Cell division protein FtsA	ftsA	260655410	Suppl. Fig. 26	1–2 aa ins	10-45	7/8	Sutterella wadsworthensis
Putative metal dependent phosphohydrolase	I	260654227	Suppl. Fig. 27	3-4 aa ins	208–250	8/9	Syntrophothermus lipocalidus, Thermotogales bacterium mesGl
^a The indel position provided indic ^b Homologous sequences correspon	cates the re	egion of the pr	otein containing the	e CSI d not he det	tected for som	ie of the eight Sv	neroistetes snecies and this is indicated accordinoly hy

the second of the two numbers

^c BLAST searches were carried out for the top 250 hits. The number of non-Synergistetes organisms, which were observed to contain the CSI, is indicated. Species containing a larger or a shorter CSI than indicated were not included in the total

^d The 2 aa insert indicated here consists of two separate 1 aa inserts in close proximity of each other

Table 2 continued

(\mathbf{A})				1203	1241
(\mathbf{A})		Aminobacterium colombiense	294101621	QGVSINNKHIEVILRKVA	PV NRIRVVEEGDTSFVAGDLV
		Thermovirga lienii	357419479		
		Aminomonas paucivorans	312880379	DT	V SP S-E
G		Anaerobaculum hydrogeniforma	ns 289523376		
Synergis	stetes 7	Jonquetella anthropi	260655389		-IVKITDEFA
9/9		Fretibacterium fastidiosum	295112262		-LVLM-
		Pyramidobacter piscolens	282857670		-1 L 1D L
		Detniosulfovibrio peptidovor	ans 288574655		
		Anermanavib. acidaminovorans	269/92801		
		Dictyogiomus thermophilum	206900872	AEDV-QMT	V-IEDPSN-LF-Q
		Paoillus on P14005	106654020		
		lycinibacillus fusiformis	2005/1020	E-GDMV-QML	
		Denitrovibrio acetinhilus	293341920		KK-I-FDPSN-MPNEF-
		Eubacterium ballii	225026545		
		Prochlorococcus marinus	124026589		SKV-IEDAT-IP-E-I
		Deferribacter desulfuricans	291280154	NDT-V-QMM	BKVIIEDPSDYMPNEE-
		Clostridium leptum	160935067		KKVDDIIMPNS
0.1	. /	Burkholderia oklahomensis	167564423	KDV-QMI	R-VQI-DNR-IP-FQ-
Other sp	ecies	Ruminococcus torques	291549813	EDV-QML	KKIE-KE-LP-TM-
0/250	0	Oxalobacter formigenes	237749510	KDV-QML	R-VNDAYIT-EQ-
		Thermosinus carboxydivorans	121534752	EDMV-QML	HKVK-EDPDLLP-EYI
		Ralstonia solanacearum	300702766	KDV-QML	R-VQI-DVK-IP-EQ-
		Rothia mucilaginosa	283457533	E-HDVV-QML	R-VT-I-SDLLP-E
		Nocardioides sp. JS614	119714959	HDI-V-QML	RT-I-SNLLPS
		Acidothermus cellulolyticus	117927509	P-HDI-V-QML	K-VNIL-SE-LP-E
		Marchantia polymorpha	11466681	Q-SI-V-QMT	SKVITL-D-M-NVFLPGEL
		Eggerthella lenta	257790478	DDA-QML	RKVA-MDA-ESD-LP-RQ-
		Hyphomonas neptunium	114798314	PDV-QML	QKVEITDGV-IT-EHI
(B)	D. + 1. 4 -		000574705		
(_)	Thermo	Sulfovibrio peptidovorans	2885/4/85		
	Aminoh)virga ilenil Nactonium colombiones	35/419591	FPVF-55 H	VHL-IL-KHADH-S
		acterium colombiense	294101731	w T PK	R-INE
Synergistetes	Dunami	dobacton niscolone	2000000002	W V PK S K	······································
Syncigisteres	Angong	baculum bydrogoniformans	202000002	BV I E S H	
9/9	Anaero		209525190	GALS- M	V-APM-TLGR-T-G-DL-E
	Therma	anaerovibrio acidaminovorans	269792442	GPL-SS- T	VRM-TLGR-TKNL-YS
,	Fretir	acterium fastidiosum	295112106	N-KASS-	MK
	Thermo	sinus carboxydivorans	121534258	PQL	VMGANEKVGL-IE-C-RL
	Selend	omonas sputigena	260887140	M	-MDTN-KVGV-IELC-RMKI
	Actino	myces urogenitalis	227497604	TPT-YL	QKDNKL-L-LG-C-HMK
	Mycoba	acterium tuberculosis	215403402	N-PA-YGL	V-DDKA-V-LADCLT
	Nocard	lia farcinica	54023768	N-PA-YGL	V-DDKA-V-LSDC-RLT
	Strept	omyces hygroscopicus	302544658	L	VRDDKS-V-LN-CMK
	Clostr	idium thermocellum	125973779	WCY-PVIL-N-	FMGSS-KISQH-CLE
	Syntro	ophomonas wolfei	114567554	PV-YLC-F-S-	VIDNQ-KVVS-IK-C-RLK
Other species {	Desulf	obacterium autotrophicum	224369575	LM	DM-NI-SVVKFID-C-NHE-N
0/250	Geobac	ter lovleyi	189425779	L-SC	DMDST-KVLKSISDC-EQE
-	Strept	comyces coelicolor	21234220	L	V - DD KAG I - LADA L - VT
	Syntro	ophus aciditrophicus	85859457	PVL	EKDNR-KIIK-IHVCKEMAI
	Thermu	is aquaticus	14194701	vPvL-SV	ERHDS-KV-E-I-DA-AL
	Spiroc	chaeta thermophila	307718499	NN	EIGNP-KL-Q-IG-T-AME
	Bacter	oldes capillosus	154499865	F-GIYL	VLDSSEKV-E-IA-CKECSL
	Deterr	IDacter desulturicans	291280374	L-SN	
	THEISSE		323204/84	wPAIMS-	ELUNI-QLKHFYDDU-ANEF

Fig. 2 Partial sequence alignments of conserved region within the **a** RNA polymerase β' subunit (RpoC) and **b** DNA polymerase III α subunit showing two CSIs (*boxed*) that are uniquely present in species from the Synergistetes phylum, but not in any other bacteria. The *dashes* (–) in this and all other alignments indicate identity with the corresponding amino acid on the top line. The numbers in the second column are the GenBank identifier numbers of the particular proteins. The numbers below the taxon identifiers indicate the number of species detected with the indel and the total species of the respective taxon which were detected. Only representative species are shown in the alignments, however, no other species in the indicated number of blast hits contained the indel (0/250). Information for 12 other CSIs in widely distributed proteins that are specifically present in all sequenced species from the Synergistetes phylum is provided in Supplemental Figs. 1–11 and summarized in Table 2

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Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	QGVYRSQGVSINNKHIEVILRKVAPVNRIRVVEEGDTSFVAGDLVWTDEIEDENEAIRRENEKNIMEATRIFS QEVYESQGVSINNKHIEVILRKVAPINRVKITDEGDTSFVAGEFAWTSDVEKEIEEIKASNEKNLTEAVESLK QDVYRSQGVSINDKHIEVILRKVAPVNRVRVIEEGDSSFVAGELVWKEDLEAESRRISEQN-ARFLEEASFLC QKVYRMQGVEIGDKHIEVIVRQMLRKVRVIEAGDTDLLPGSLL
Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	GRVVKSIAASRLTDTILQYQNMPLTEEAIRTLLRPGYLISQMVLEGDEGSDLILVIGEAAFRKRMEGLELIET GKKLSDRGGALKGLLPDGAFDKPVTEDVLRKVLAPGGAVTELYFTDEEGP-LRVVVGEAAFRKEMRGMELVEA GAVVKDAVGSLDGTGITRDEELTMDKLGRLLSPGVGASELICQDREGL-LRVIVGEASFKRELEGLELLSD
Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	FKSEDGKE-IAAGTILTPGQLGIVTSGDPVSICVRDHETIEKLVDSSYLAEDIVVDNEVMAEKDHIFTQAMAA VTKGD-KVVPADEP-LTVAQLSVITQGAPVPHMFRSVEKLRKQQDEGYVAEAVIAESGVLAKADQLLTEDLIE LPVADGCVISAGSR-LTASDASRIVAMGPQPLLVKDLKAMEDMVGEVYLAEDVTVDGRVLSLKDRLFDLEVFQ
Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	ICFEHNIQAVKIWHSVERISVLDALQERLMNNIWGRHLTQAVDSEGNGLTDVSQMVDARIIRGLVDDEISAVD QIRRSSAPSVKIWKTVDTISVRDLLQERLINRIWGRQLKLAIGADGQALSGSVHMVDGSVVKGIVEGQIQGLV ELRSLPVESVRIWRNPERLDLCKDVYEYLIGNYLSQRVLRVITRDGAVTEPLDNRISMEIAEGIRSGEVEAIE
Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	LEG-EILTREKALIELLNTLIYGKVLLEPVVDEKGQILVDSGQEINRAMIDLLVRSQAGEFVVRPLSARHDEK FADDSNTSRETELTEALSSVVSGKVLLEDVKNSDGTVALQAGQEIGKKQLAKIVAADPTILTVRPVLDQTETV LDGNNVVSRERVLKALLTEKVYGKVLLEPVRDVDGNVVVPSGREVSHQVMDQIVAACPGEMVVRPILAQGEHR DIHQ ELRQ
Aminobac. colombiense Jonquetella anthropi Therm. acidaminovorans Bacillus sp. B14905 Prochlorococcus marinus Pseudomonas fluorescens	1617 TLIWDVTFVRKLREGPKCRPFVHGITKAALATESFLSAASFQQTAQVLAGAAVKGEMD QLISRVSFVRRLRLGPQWRPFIHGVTKAALATDSFLSAASFQQTAQVLAGAAVRNQVD RLIQRISFVRRLRELPTWKPVLHGITKAALATDSFLSAASFQQTAQVLASAAVRGEVD FAEANADAVMNGKNPATCRPVILGITKASLETESFLSAASFQETTRVLTDAAIKGKRD VEDTNQAISITGGAPSEFTPVLLGITKASLNTDSFISAASFQETTRVLTEAAIEGKSD VLVENERLSTEDKFVAKFTRVLLGITKASLSTESFISAASFQETTRVLTEAAVTGKRD : *:***:* *:**:******:*:*:*: *:*:

Fig. 3 Partial amino acid sequence alignment of the RpoC protein showing a large insert that is specifically present in all sequenced Synergistetes species. Partial sequence for the neighbouring regions is also shown in the alignment. The *dashes* in this particular alignment represent sequence gaps. The identical and conserved residues in this alignment are indicated by * and

common ancestor. However, this postulation is not supported by the phylogenetic trees (Fig. 1) and it is possible that the CSI in these two taxa occurred independently or by means of LGT. The information for other CSIs where indels found in Synergistetes are also present in one or two species from other taxa is summarized in Table 2 and the sequence semicolons (:), respectively. Blastp searches with the insert sequence (without the flanking region) show no significant hit for any protein except for the RpoC homologs from the Synergistetes species. Sequence information is shown for only a few Synergistetes, but this insert is present in all sequenced species

alignments for these are presented in Supplementary Figs. 13-20.

A further seven CSIs, specific for species of the Synergistetes phylum, were discovered where one species from the phylum was detected to lack the indel. A 3 aa insert in the 3-4-dihydroxy-2-butanone 4-phosphate synthase (Supplementary Fig. 21) is an

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			44	03
(A)	Dethiosulfovibrio peptidovorans	288574655		
(A)	Thermovirga lienii	357419479		IS
	Pyramidobacter piscolens	282857670	······································	
	Aminobacterium colombiense	294101621	YII	
Synergistetes	Aminomonas paucivorans	312880379	R-I	
0/0	Thermanaerovibrio acidaminovorans	269792801	V	
212	Anaerobaculum hydrogeniformans	289523376	IVRNIV	SK
	Jonquetella anthropi	260655389	Y	KS
	🗣retibacterium fastidiosum	295112262	R-YTR R	
	Æubacterium yurii	306821196	KKK	SSK
	Anaerococcus lactolyticus	227486351	KDYSRM RYV-EK	KSK
	Cryptobacterium curtum	256826829	RI RIV-E	RAK
	Slackia exigua	269216200	KDWRI RIV-E	RSK
	Corynebacterium diphtheriae	38233061	RDWRV RYIE	KSK
	Micrococcus luteus	289705072		RSKD
	Brevibacterium linens	260905736		RAK
ļ		207004081	KDWSRV RIV-E	KAK
Other species	Acidothermus cellulolyticus	117027500	BDWYBV BIE	3AKD
1/250	Collinealla intestinalis	220815704	K	TAK
1/250	Brochothriv thermosphacta	1/05200		KSK
	Staphylococcus enidermidis	242372761		KSK
	Leuconostoc citreum	170017896	DDYBI BYIV	SSK
	Bacillus subtilis	221307920		BAK
	Enterococcus faecalis	29377681	RI RYIV	RSK
	Pediococcus pentosaceus	116493169	DDYRI RYIV	KSK
	Lactobacillus brevis	116334276	DDWRI RYV	RSK
	Catenibacterium mitsuokai	224542339	V RYV	RSS
(B)			39	86
	Aminobacterium colombiense	294101642	ILKEEGYIRNYKVINDPKK PY AVVRVFLNYGPNKERV	QGLRRISKPG
	Thermovirga lienii	357419500	KRL G-LKLY	
	Fretibacterium fastidiosum	295111409		
Supervistote	Bunamidabaatan niasalana	288574676		
Synergistete	S EVENITIONACIEF DISCOTEUS			/ M
	longuetella anthroni	282857349		/M
9/9	Jonquetella anthropi	282857349 260655369 269792780		/M T-V
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans	282857349 260655369 269792780 289523355		/M T-V IV
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas naucivorans	282857349 260655369 269792780 289523355 312880358		/M T-V IV
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17	282857349 260655369 269792780 289523355 312880358 189485099		/M T-V V I
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum	282857349 260655369 269792780 289523355 312880358 189485099 187251808		
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358	SL	/M
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716		/M T-V V
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654	SL A GTIKMR SF	/N
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans	262657349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654		
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979		
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldieellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220330979 83591262	A GTIK - M	
9/9	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138	A GTIK - M	
9/9 Other specie	Jonquetella antropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83551262 307694138 114567827		
9/9 Other species	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726		
9/9 Other species 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Envylheaillue bervic	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83551262 307694138 114567827 302388726 15612711 236202920		
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalie	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726 15612711 226309822 29374865	A GTIK - M - SN - GTLK - M - GTLKI - S F - N - GTL A - A V H	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 226897654 2268513654 220390979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655	A GTIK - M	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus Lactobacillus brevis	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 527509945	GTIK - M- A GTIK - M- GTIK - M- S-F- N- GTIK - M- GTLKI-S- R S-F- N- GTLKI-S- R GTLKI-S- R S-F- M- GTLKI-S- R GTLKI-S- R M- GTLKI-S- R GTLKI-S- R R M- GTLKI-S- R R R M- GTLKI-S- R R S-L- IH-R- SKGKV-I V P-L-L- L- R S-L- IH-R- TSKGKV-I V P-L- R R S-L- IH-R- TSKGKV-I V P-L- R	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus Lactobacillus brevis	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 227509945 195977222	A GTIK - M - F - N - GTLK - M - F - N - GTLKI - S F - N - GTLKI - N F - N - GTLKI - N F - N - GTLKI - N F - N F - N - F - G - F - G - G - GTI - T	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus Lactobacillus brevis Streptoccocus equi Weissella paramesenteroides	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83551262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 227509945 195977222 241895174	A GTIK - M - SH - N GTIK - M - SH - N GTIK - M - SH - N GTIK - M - SH - N GTLKI - S - R - N GTLKI - S - R - N GTLKI - S - R - N GTLK - M - SH - N GTLKI - S - R - N GTLK - M - SH - N GTLKI - S - R - N V - D - K - S - FIKGIENQ TQ S - L - IH - R - SKGKV - I V - D - K - S - EIKGIENQ TQ S - L - IH - R - SKGKV - I V - TA - AVHNET - GG GI - S - K - T - SKGKV - I V - TA - AVHNET - GG GI - S - K - T - SKGKV - I V F - KD E - E - G - Q GI - S - K - T - SKGKV - I F - KD E - E - G - Q GI - S - K - T - SKGKV - I F - KD E - E - G - Q GI - S - K - T - SKGKV - I F - KD - E - E - G - Q GI - S - K - T - SKGKC - N F - KD - E - G - Q GI - S - K - T - S - SKG	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus Lactobacillus brevis Streptococcus equi Weissella paramesenteroides Thermosinus carboxydivorans	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 227509945 195977222 241895174 121534648	A GTIK - M - S - F - O S - F - O N- GTLK - M - S - F - O S - F - O S - F - O S - F - O S - F - O S - F - O S - F - O S - F - O S - E - C S - E - E - G GI F - KD - E - E - G GI F - KD - E - E - G GI F - KD - E - C - G GI F - KD - E - C - G GI F - KD - E - C - G GI F - KD - E - C - G GI F - KD - E - C - G GI F - KD - E - C - G GI F - G - D - E - G - G GI	
9/9 Other specie: 1/250	Jonquetella anthropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus hevis Streptococcus equi Weissella paramesenteroides Thermostinus carboxydivorans Ilyobacter polytropus	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220330979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 227509945 195977222 241895174 121534648 310779582	GTIK	
9/9 Other specie: 1/250	Jonquetella antropi Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Aminomonas paucivorans Termite group 1 Rs-D17 Elusimicrobium minutum Ammonifex degensii Caldicellulosiruptor bescii Clostridium difficile Desulfotomaculum acetoxidans Halothermothrix orenii Moorella thermoacetica Ruminococcaceae bacterium Syntrophomonas wolfei Thermosediminibacter oceani Bacillus halodurans Brevibacillus brevis Enterococcus faecalis Geobacillus kaustophilus Lactobacillus brevis Streptococcus equi Weissella paramesenteroides Thermosinus carboxydivorans Ilyobacter polytropus Fusobacterium ulcerans	282857349 260655369 269792780 289523355 312880358 189485099 187251808 260893358 222529716 126697654 258513654 220930979 83591262 307694138 114567827 302388726 15612711 226309822 29374865 56418655 527509945 195977222 241895174 121534648 310779582 257470840	GTIK - M- A GTIK - M- A S-F- N- GTIK - M- GTLKI-S- A S-F- N- GTLKI-S- A GTLKI-S- A S-F- N- GTLKI-S- A GTLKI-S- A S-F- GTLKI-S- A GTLKI-S- A A GTLKI-S- A GTLKI-S- A A	

Fig. 4 Partial sequence alignments of RpoC and RpsH proteins showing two CSIs, which in addition to the Synergistetes species are also present in isolated other bacterial species. a Excerpt from RpoC sequence alignment depicting a 2 aa conserved insert which in addition to the Synergistetes is also present in *Eubacterium yurii*. b Sequence alignment of the ribosomal protein S8 (RpsH) showing a 2 aa insert which in

example of such a CSI. The insert is present in all detected species of the Synergistetes except for *P. piscolens*. No species outside of the phylum contain the insert. The information for other such CSIs is

addition to the Synergistetes is also found in an *Uncultured Termite group 1 bacterium phylotype Rs-D17*. A 1 aa insert in this position is also present in *Elusimicrobia minutum*. Sequence information for 8 other CSIs in different protein containing an isolated exception is provided in Supplementary Figs. 13–20 and Table 2

summarized in Table 2 and sequence alignments for them are presented in Supplementary Figs. 21–27. It is possible that these CSIs were also originally introduced in a common ancestor of the Synergistetes phylum but they were lost in some species over time due to ecological/physiological pressures or by mechanisms such as LGT followed by gene loss. In some of the CSIs described above, in addition to the CSIs that were specific for the Synergistetes, indels of different lengths were also present in species from other taxonomic groups. Due to their different lengths, these CSIs have likely originated from independent genetic events.

CSIs that are specific for subgroups of the Synergistetes phylum

All Synergistetes species are currently classified as part of a single class (Synergistia), order (Synergistales) and family (Synergistaceae) (Jumas-Bilak et al. 2009; www.bacterio.cict.fr). The relationships among the species/genera of this phylum are not well understood. In the phylogenetic trees based upon concatenated protein sequences and the 16S rRNA a number of strongly supported relationships among the species within this phylum are observed (Fig. 1). Importantly, in the present work, our analyses of protein sequences from Synergistetes have led to discovery of several CSIs that are commonly shared only by species from this phylum and that are absent in all others. These CSIs independently support a specific evolutionary relationship among these species and they, in conjunction with the results from phylogenetic analyses, can be used for determination of the relationships among the members of the phylum Synergistetes.

In the phylogenetic trees shown in Fig. 1, a clade consisting of D. peptidovorans, J. anthropi and P. piscolens is supported with high statistical support in both the concatenated protein tree and the rRNA tree. In our analysis, we have identified seven indels (Table 3) that are uniquely present in these three species supporting independently that these three species are closely related and form a distinct clade within the Synergistetes phylum. The first of these is a 4 aa deletion in the penicillin-binding protein 1A family protein which is involved in cell wall construction (Fig. 5). This deletion is found only in homologues of the protein from D. peptidovorans, P. piscolens and J. anthropi and all other Synergistetes, as well as non-Synergistetes species, lack this deletion. An additional 6 CSIs specific to these three organisms were discovered in the proteins tRNA modification enzyme TrmE, ribonucleoside diphosphate reductase, putative DEAD/DEAH box helicase, RpoB and the PlsC proteins. Information for these CSIs is summarized in Table 3 and their sequence alignments are presented in Supplementary Figs. 28-33. Among the three organisms which are part of this clade, J. anthropi and P. piscolens were observed as being more closely related to each other than either is to D. peptidovorans. This close association is underscored by a total of 15 CSIs, including an example that is shown

Protein name	Gene name	GenBank identifier	Figure no.	Indel size	Indel position ^a	Other species containing indel ^b
Penicillin binding protein, 1A family	_	288574813	Fig. 5	4 aa del	102-140	_
Ribonucleoside diphosphate reductase	nrdA	260654687	Suppl. Fig. 28	1 aa ins	193–225	-
Putative DEAD/DEAH box helicase	-	260655128	Suppl. Fig. 29	1 aa del	398–457	-
Putative DEAD/DEAH box helicase	-	260655128	Suppl. Fig. 30	6-8 aa ins	437–496	-
DNA directed RNA polymerase, β subunit	rpoB	282857671	Suppl. Fig. 31	13 aa ins	358-407	-
1-Acyl-sn-glycerol-3-phosphate acyltransferase	plsC	282855432	Suppl. Fig. 32	1 aa ins	57–84	P. staleyi, T. mathranii
tRNA modification GTPase TrmE	trmE	260655716	Suppl. Fig. 33	1 aa ins	263–299	E. minutum, Termite group 1 Rs-D17

Table 3 Characteristics of the CSIs that are Specific for a Clade Consisting of J. anthropi, P. piscolens and D. peptidovorans

^a The indel position provided indicates the region of the protein containing the CSI

^b BLAST searches were carried out for the top 250 hits. Organisms, other than *J. anthropi*, *P. piscolens* and *D. peptidovorans*, which were observed to contain the CSI are indicated. Species containing a larger or a shorter CSI than indicated were not included in the total

			102	140
	Dethiosulfovibrio peptidovorans	288574813	EDSSFYSHHGIRPLAILRSIFS	GDGGHGASTITQQLARN
	Pyramidobacter piscolens	282855808	QKL	-EQ
	Jonquetella anthropi	260655943	DKFLAT	EK-Q
Synergistetes.	Thermovirga lienii	357419625	EK-K-VDFSI-AFWK	IVTS -RVEQ
2/0	Aminomonas paucivorans	312880037	EE-A-LAALWI D	LSH QRARQ-G
3/8	Thermanavib. acidaminovorans	269792666	DE-Q-VS-TAVLV D	LIH RGARQ-GS
	Aminobacterium colombiense	294101764	Q-GVTG-ALMV D	ILH RGARQ-G
	Anaerobaculum hydrogeniformans	289523163	DNN-RDIKG-I-AAWV N	ILTK KGTFQ-G
	Neillonella dispar	238019116	RD-VGAVWV N	IIVH SGVSE-G
	Alteromonas macleodii	239996582	RED-IG-M-AAV-	VLT -EKRQL-MG
	Enterobacter cloacae	311277669	REVD-VG-F-AASV A	LFS -HASQ
	Escherichia coli	209756604	REVD-AG-F-AASV A	LFS -HASQ
	Haemophilus influenzae	16272388	RDLD-IG-A-AL-V	VSN -GASQ
	Klebsiella pneumoniae	206577457	REVD-VG-F-AASV A	MFS -HASQ
	Pasteurella dagmatis	260912704	ARHVD-IG-A-A-KV A	ISK -GASQ
	Salmonella enterica	160867288	REVD-VG-F-AASV A	LFS -HASQ
	Shewanella amazonensis	119776506	ARE-QD-IG-I-AA-V	AAT -EKKQV
Other species	Sodalis glossinidius	229258577	REVD-VGAASI A	LLS -NASQ
office species	Vibrio cholerae	121727599	RY-E-Y-FD-IG-T-AA-A V	LAS -SASQ
0/250	Xenorhabdus bovienii	290473153	REVD-IGVI-AVSV	IMTS -HASQ
	Yersinia pestis	262364265	RDVD-VGAVSI A	MLS -RASQ
	Hydrogenobacter thermophilus	288817433	RNFD-I-VALIA N	IIRE REITQ
	Veillonella atypica	303228365	RD-IGA-WV	IVVH SGVSE-G
	Cylindrospermopsis raciborskii	282899343	RY-W-F-VDGAV-I	ITQSVQQVS
	Raphidiopsis brookii	282896024	RY-W-F-VDGVAV-I	ITQSVQQVS
	Bacillus subtilis	321311701	ARED-VR-GGALVA	IFKD -F-AE-GVVK-
	Listeria monocytogenes	254852796	ARE-DD-IRLGGAVIA	ILTD -F-AELSIIKM
	VFusobacterium gonidiaformans	257466898	KRED-RGLAV-V	ILRS -HARQSK-

Fig. 5 Partial sequence alignment of a family 1A penicillinbinding protein containing a 4 aa deletion that is specific for *D. peptidovorans*, *P. piscolens* and *J. anthropi*. Sequence information for five other CSIs that are specific for this clade of species is presented in Table 3 and Supplementary Figs. 28–33

Jonquetella-			169	208
Pyramidobacter_	-Pyramidobacter piscolens	282857671	AKIIPERGAWVEFV	MAGDSLSVNIDSRRKLPATLLLKAFG
2/2	-Jonquetella anthropi	260655390	L	TK-MVK
212	Aminobacterium colombiense	294101620	BLS	L SPVN-KI
	Dethiosulfovibrio peptidovorans	288574654	DLA	M GSA-YN-KG
	Anaerobaculum hydrogeniformans	289523377	DLD	L TPVNVA
Other Synergistetes ≺	Thermovirga lienii	357419478	VDV-LD	L TP-EVV-NKKL-MY-
0/7	Aminomonas paucivorans	312880380	VDLD	L TPLKN-K-I-V-M
	Thermanaerovibrio acidaminovorans	269792802	LDLD	L TPSEFKN-K-I-V-MFL-
	Fretibacterium fastidiosum	295112261	LDLD-S	M ASIVNV
	Actinomyces coleocanis	227494537	SLE	I DKR VG - R RK Q - V - HF L -
	Corynebacterium diphtheriae	38233060	V-VSLD	V DKR - TVG - R RK - RQ - V - V L -
	Cryptobacterium curtum	256826828	VSLE	T DKR - I R RK Q R - L -
	Gordonia neofelifaecis	326382974	V-VGLD	V DKR-TVG-RRK-RQ-V-IL-
	Mycobacterium tuberculosis	167967486	V-VSLD	V DKR-TVG-RRK-RQ-V-VL-
	Nocardia farcinica	54026619	-RVSLD	V DKR - T - G - R RK - RQ - V - V L -
	Alkalilimnicola ehrlichii	114319612	-RVYS-LD-E	F DPK-NIY-RRIR-L-
	Coxiella burnetii	224493307	-RVYS-LD-E	F DPK-C-F-RRVI-R-L-
	Nitrococcus mobilis	88812764	-RVYS-LD-E	F DPK-AIF-RRIR-L-
Other species (Pseudomonas mendocina	330505214	-RYS-LD-E	F DPK-AVF-RRSVR-L-
0/250	Vibrio vulnificus	302347128	-RYS-LD-E	F DPK-N-Y-RRS-II-R-L-
0/250	Desulfovibrio aespoeensis	317153964	SR M S - LD - D	F DHK-I-Y-RRMIM-
	Geobacter sulfurreducens	39997954	-RVYS-LD-E	F DHK-I-F-RRMVL-
	Myxococcus xanthus	89142894	-RYS-ID-E	F DHK-L-Y-RRV-IR-L-
	Pelobacter carbinolicus	77918308	-RVYS-LD-D	F DHK-L-Y-RRVL-
	Thermomicrobium roseum	221632985	LTLE	T SNR-VKRKI-V-VR-V-
	Natranaerobius thermophilus	188584828	GT N L E	M N-QNVIRV-RTV-V-IR-L-
	Burkholderiales bacterium	303256441	-RYS-LD-E	F D-K-I-HFRRM-G-IML-
	Flexistipes sinusarabici	336322745	-RYS-ID-E	F D-KNVMY-RKKI-V-VL-
	Neorickettsia risticii	41017780	-RYS-LD-E	ED-K-V-YFRKKVR-L-

Fig. 6 Excerpts from sequence alignment for RNA polymerase β subunit (RpoB) showing a 1 aa deletion that is specifically present in *J. anthropi* and *P. piscolens*. The region contains a 1

aa deletion specific for the three species. Sequence information for 14 other CSIs that are specific for these two species is presented in Table 4 and Supplementary Figs. 34–47

Protein name	Gene name	GenBank identifier	Figure no.	Indel size	Indel position ^a	Other species containing indel ^b
DNA-directed RNA polymerase, β subunit	rpoB	282857671	Fig. 6	1 aa del	169–190	_
Chlorohydrolase family protein	-	260654152	Suppl. Fig. 34	1 aa ins	241-272	-
Phospho- <i>N</i> -acetylmuramoyl- pentapeptide-transferase	mraY	260655416	Suppl. Fig. 35	1 aa ins	218-266	-
Recombination protein RecR	recR	260654559	Suppl. Fig. 36	1 aa ins	63-122	_
Transcriptional regulator NrdR	nrdR	260655521	Suppl. Fig. 37	1 aa del	84–133	-
Lipid A biosynthesis acyltransferase	-	282855413	Suppl. Fig. 38	1 aa ins	262-284	_
Glutamate synthase	gltB	282858093	Suppl. Fig. 39	6 aa ins	334–383	-
Acetate kinase	ackA	282856654	Suppl. Fig. 40	2 aa del	238-279	-
AcrB/D/F family transporter	_	260654486	Suppl. Fig. 41	1 aa del	111-154	-
Transcriptional regulator IclR	iclR	282857025	Suppl. Fig. 42	10–13 aa ins	139–202	-
Ribose phosphate pyrophosphokinase	kprS	282858157	Suppl. Fig. 43	1 aa ins	204-246	Dichelobacter nodosus
Phosphoribosyl-formylglycinamidine synthase II	purL	260655584	Suppl. Fig. 44	4 aa del	102–150	<i>Hydrogenobaculum</i> sp. Y04AAS1
MazG family protein	-	282858161	Suppl. Fig. 45	3–4 aa ins	33–75	Clostridium phytofermentans
Glutamate synthase	gltB	282858093	Suppl. Fig. 46	8 aa ins	84–130	Desulfovibrio africanus
S-adenosylmethyltransferase MraW ^c	mraW	289522914	Suppl. Fig. 47	1 aa ins	153-196	-

Table 4 Characteristics of CSIs that are specific for J. anthropi and P. piscolens

^a The indel position provided indicates the region of the protein containing the CSI

^b BLAST searches were carried out for the top 250 hits. Organisms, other than *J. anthropi* or *P. piscolens*, which were observed to contain the CSI are indicated. Species containing a larger or a shorter CSI than indicated were not included in the total

^c All Synergistetes species were observed to contain the indel except *J. anthropi* and *P. piscolens*, thus, differentiating these two species from the rest of the phylum

in Fig. 6, a 1 aa deletion in a conserved region of the enzyme RNA polymerase β subunit. Other indels that provide similar molecular evidence for the observed close relationships between these two genera are presented in Supplementary Figs. 34–47 and information for them is summarized in Table 4. The fidelity of these molecular markers can be tested on cultured but unsequenced members of the phylum Synergistetes and as more species belonging to these genera are sequenced, the identified CSIs should provide molecular markers for their induction into the clade formed by this sub-group of the phylum.

The phylogenetic trees also support a cladal relationship among two other species, *Amm. paucivorans* and *T. acidaminovorans*, which branch as sister organisms with high statistical support (Fig. 1). The clade harbouring these genera has been proposed to form a higher-level taxon within the phylum (Jumas-Bilak et al. 2009). In the present work, we have identified 7 CSIs that differentiate the species representing these two genera from all other species and support a specific grouping of the genera Thermanaerovibrio and Aminomonas (Table 5). Among these CSIs is a 2 aa insert in enzyme S-adenosyl-methionine isomerase (Fig. 7). The information for 6 other CSIs supporting a specific relationship among these two species is provided in Table 5 and their sequence alignments are depicted in Supplementary Figs. 48-53. Two other CSIs identified in the present work, which include a 1-2 aa deletion in the ribosomal protein L13 (Supplementary Fig. 54) and 2 aa deletion in DNA gyrase B (Supplementary Fig. 55), are present in all detected Synergistetes species except Thermanaerovibrio and Aminomonas. The absence of these CSIs in the two species suggests that this clade may have diverged from the common Synergistetes ancestor before the other species of the phylum and the two indels may have been introduced after the divergence of this clade from the common Synergistetes ancestor. A loss of this signature from this clade after its divergence from other Synergistetes can also explain the

Table 5 Characteristics of the CSIs that are specific for a clade consisting of T. acidaminovorans and Amm. paucivorans

Protein name	Gene name	GenBank identifier	Figure no.	Indel size	Indel position ^a	Other species containing the indel ^b
S-adenosylmethionine/tRNA- ribosyltransferase-isomerase	queA	269792529	Fig. 7	2 aa ins	156–194	-
RecA protein	recA	269793250	Suppl. Fig. 48	1 aa ins	143–184	-
Glu/Leu/Phe/Val dehydrogenase	-	269791934	Suppl. Fig. 49	1 aa del	318-361	-
Uracil phosphoribosyltransferase ^c	upp	312880140	Suppl. Fig. 50	4–5 aa ins	133–184	-
Methyltransferase GidB	gidB	269791772	Suppl. Fig. 51	2 aa ins	75–123	Sorghum bicolor
Xanthine/uracil/vitamin C permease	-	269792033	Suppl. Fig. 52	5 aa ins	390–443	Mesembryanthemum crystallinum
Electron transport complex, RnfABCDGE type, C subunit	-	312880739	Suppl. Fig. 53	1 aa ins	166–213	Saccharophagus degradans, marine gamma proteobacterium, Eubacterium cellulosolvens
Ribosomal protein L13 ^d	rplM	294101309	Suppl. Fig. 54	1–2 aa del	108–138	_
DNA gyrase subunit B ^d	gyrB	294102629	Suppl. Fig. 55	2 aa del	191–234	Acidaminococcus fermentans, Acetonema longum, Seinonella peptonophila
Hypothetical protein Taci_0455 ^e	-	269792069	Suppl. Fig. 56	2 aa ins	205–256	-

^a The indel position provided indicates the region of the protein containing the CSI

^b BLAST searches were carried out for the top 250 hits. The number of non-Synergistetes organisms, which were observed to contain the CSI, is indicated. Species containing a larger or a shorter CSI than indicated were not included in the total

^c BLAST searches were carried out for the top 250 hits. However, in the indicated case, no species outside of the Synergistetes phylum contained the protein homolog or the conserved region corresponding to the sequences flanking the indel

^d All Synergistetes species were observed to contain the indel except *Amm. paucivorans* and *T. acidaminovorans*, thus, differentiating these two species from the rest of the phylum

^e The CSI is also present in *Thermovirga lienii* species from the Synergistetes phylum

observation. These indels also support a close relationship among the genera *Thermanaerovibrio–Aminomonas* and information for them is also summarized in Table 5. These two species were observed to branch in a weakly supported clade with the *Tv. lienii* and *An. hydrogeniformans* (Fig. 1). However, only 1 CSI supporting the three-species-clade with *Tv. lienii* was identified in a protein of unknown function (Supplementary Fig. 56) and no CSI specific for all four organisms was discovered.

In the phylogenetic trees (Fig. 1), the species *Amb. colombiense* and *F. fastidiosum* were observed to branch with *J. anthropi*, *P. piscolens* and *D.*

peptidovorans. A specific relationship among these species is also supported by two of the identified CSIs. The first of these CSIs consists of a 1 aa del in GyrB that is uniquely present in all five of these species (Fig. 8a). Another CSI in orotidine 5'-phosphate decarboxylase, also consisting of a 1 aa deletion, is commonly shared by *Amb. Colombiense*, *J. anthropi*, *P. piscolens* and *D. peptidovorans* (Fig. 8b). A homologue for this protein was not detected for *F. fastidiosum*, whose genome has not been fully sequenced. Thus, it is likely that this CSI will also be present in this species and could provide an additional molecular marker for this clade.

Aminomonas-			156	_ 194
Therman derowibrio	Thermanaerovibrio acidaminovorans	269792529	PLPPYIENRQVDGERYQTVYSD PS	KELSVASPTAGLHFT
	🛏 Aminomonas paucivorans	312880164	R -D	R-G-A-A
2/2	Pyramidobacter piscolens	282855627	AK	DPR-A-A
	Jonquetella anthropi	260654964	AK	DAN - A - A
Other Synergistetes	Dethiosulfovibrio peptidovorans	288574883	FAN	-VG-A-A
Other Synergisteres	Thermovirga lienii	357419866	IFAE	RLG-A-A
0/6	Anaerobaculum hydrogeniformans	289523146	RGNGRAS - Y AR	VDG A F
	Aminobacterium colombiense	294101858	- F KSTTATALD FAK	RNG-S-AS
	Alkaliphilus oremlandii	158320752	K	NQG - A - A
	Anaerococcus hydrogenalis	212696227	KEKLK-KK	NPGA
	Clostridium botulinum	148381020	KEKLD-K-MK	E-G-A-A
	Finegoldia magna	169824511	KEKLN-KK	HNG - A - A
	Ruminococcus obeum	295109865	THKLK - KN AK	NDG - A - A
	Thermoanaerobacter ethanolicus	326390116	- V KKKLK - K AK	Y-G-A-A
	Bacillus subtilis	296331683	KEQLD-RK	EIG-A-A
	Enterococcus faecalis	257081115	KE - LD - PD AE	ENG-A-A
	Listeria monocytogenes	313608657	KEQLA - QD AK	ENG-A-A
	Staphylococcus aureus	258424065	KE - LD - PD AK	ESG-A-A
Other species <	Streptococcus pneumoniae	15903316	AK	ESG-A-A
0/250	Bulleidia extructa	283769037	KKKLD - S AK	IDG-A-A
0,200	Erysipelothrix rhusiopathiae	323343219	R	V-G-A-A
	Selenomonas sputigena	260887834	CR	E-G-A-A
	Mitsuokella multacida	255658191	NR	E-G-A-A
	Acidaminococcus fermentans	284048860	NR	ERG-A-A
	Thermosipho africanus	217076403	K-QK-PFK	ETGA
	Microcoleus vaginatus	334118005	A-	RLGAA
	Leptotrichia hofstadii	260890428	AK	EGEA
	Sebaldella termitidis	269121052	AK	EGE A
	Haloplasma contractile	335428870	K	NVG-A-A

Fig. 7 Partial sequence alignment of S-adenosylmethionine/ tRNA-ribosyltransferase-isomerase protein showing a 2 aa insert in a conserved region that is specific for T.

CSIs that are commonly shared by species of the Synergistetes phylum with other taxonomic groups

The Synergistetes is a taxonomic group that has only recently been identified as a separate phylum within the bacterial domain. Though it branches distinctly in the 16S rRNA trees with long branches separating it from other bacterial groups (Fig. 1; Jumas-Bilak et al. 2009), species from the phylum had previously been classified as part of *Syntrophomonadaceae* family in the Firmicutes (Baena et al. 1998, 1999a; Diaz et al. 2004) and misclassified as *Selenomonas* (Guangsheng et al. 1992). The presence or absence of CSIs that associate these groups with the Synergistetes should prove helpful in determining whether any link exists between the Synergistetes and these other groups of bacteria.

In our analysis we have identified some CSIs that, along with being present in some or all the Synergistetes species, were present in other groups of organisms. Two examples of such indels are presented in Fig. 9. Figure 9a shows a 1 aa insert in the MiaBfamily RNA modification enzyme that is uniquely *acidaminovorans* and *Amm. paucivorans* species. Nine other CSIs that are specific for these two species have also been identified (Table 4 and Supplementary Figs. 34–47)

present in all detected Synergistetes as well as various species from the phylum Chloroflexi. All other bacteria lack this insert. Similarly, in the DNA polymerase III α -subunit, a 1 aa insert is present in all detected Synergistetes and also in various Fusobacteria, an Opitutaceae species as well as in Thermomicrobium (Fig. 9b). In phylogenetic trees constructed from these protein sequences, the Synergistetes species do not branch with species from these taxa (unpublished results) indicating that the shared presence of these CSIs is not due to their being sister taxa of Synergistetes or LGTs. The CSIs in these groups have thus likely originated independently. Other CSIs that the Synergistetes share with species from other taxonomic groups are listed in Table 6 and sequence information for them is provided in Supplementary Figs. 57-74. These other taxa include the *Fusobacteria* (Supplementary Figs. 57-61), Elusimicrobia (Supplementary Figs. 61, 62), class Negativicutes (Supplementary Figs. 63–66), Acidobacteria (Supplementary Fig. 67), Proteobacteria (Supplementary Figs. 68-70), Aquificae (Supplementary Fig. 71), Erysipelotrichi (Supplementary Fig. 72), Actinobacteria (Supplementary Fig. 73) and order Lactobacillales (Supplementary Fig. 74). The Synergistetes share the greatest number

(A)			384	413
(11)	Dethiosulfovibrio peptidovorans	288574018	AREAAKKARELVR	KTAMTGLNLPGKLADCS
	Jonquetella anthropi	260655480		- S S S
	Aminobacterium colombiense	294102629	D	M
a	Pyramidobacter piscolens	282855958		-S-LSS
Synergistetes	Fretibacterium fastidiosum	295111747	K	-GM
5/9	Thermovirga lienii	357419158		RLAD
	Thermanaerovibrio acidaminovorans	269791748	D D	R - S S- MD
	Aminomonas paucivorans	312878944	D D	R - S S D
	Anaerobaculum hydrogeniformans	289523899	D	R - S- FG D
	Clostridium difficile	109675347	T-	R - SVLESTSA
	Syntrophomonas wolfei	114565581	T-	R -N-LESTA
	Thermocrinis albus	289549166	K	R RSPLEDTT
	Aquifex aeolicus	15606321	K	R - SPLEEGV
	Bacteroides intestinalis	189464548	VRS-Q	R - SP-S-GGM
	Flexibacter litoralis	9971369	MRM-Q	RLS-IG
	Rhodothermus marinus	268318257	VRQ	R - N- LN-SSA
	Staphylococcus aureus	293497972	VVT-	R -S-LDVAS
~~ · /	Leptospira biflexa	183219432	RRD-I-	RVLE-GG
Other species	llyobacter polytropus	310/7/811	M	R - S- LEVGS
0/250	Fusobacterium ulcerans	257470425	L	R - SVLEVGS
	Erysipelothrix rhusiopathiae	323342250	VRI-	R -G-LEVSS
	Chlamydophila pneumoniae	15618195	TL	R -S-LDSARIL
	Leptospirillum ferriphilum	209863973	RKD-AK	R - NVLE-SQ
	Chlorobium phaeobacteroides	189499015	S-DRKD-I-	R - S-LESSG
	Brachyspira murdochii	296127762	RD-A-	R - N- LESDS
	Microcystis aeruginosa	159028965	-A	R - SVLESSP
	Iricnodesmium erythraeum	113477398	- A KK D	R - SVLESSP
	Actdobacterium sp. MP5ACTX8	299136177	KD-I-	R -G-LD-GG
(B)			180	206
(B)	Aminobacterium colombiense	294102579	180 PGIRPSATG I	206 DDQARTATPKGAIIAGAD
(B)	Aminobacterium colombiense Jonquetella anthropi	294102579 260654639	180 PGIRPSATG I V-LA-A-	206 DDQARTATPKGAIIAGAD T - V D
(B)	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans	294102579 260654639 288575022	180 PGIRPSATG I V-LA-A- V-LTSL-	206 DDQARTATPKGAIIAGAD T-VD T-ICQKN
(B)	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens	294102579 260654639 288575022 282856738	180 PGIRPSATG I V-LA-A- V-LTSL- V-LV-G	206 DDQARTATPKGAIIAGAD T-VD T-ICQKN S-VAD-FRN
(B) Synergistetes	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans	294102579 260654639 288575022 282856738 289523224	180 PGIRPSATG I V-LA-A- V-LTSL- V-LV-G- V-EG-S K	206 DDQARTATPKGAIIAGAD T-VD T-ICQKN S-VAD-FRN IMGQ-KKK
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans	294102579 260654639 288575022 282856738 289523224 312879980	180 PGIRPSATG I V-LA-A- V-LTSL- V-LV-G- V-EG-S K LPGD- T (0)	206 DDQARTATPKGAIIAGAD T-VD T-ICQKN S-VAD-FRN IMGQ-KKK QVDAA-MGR
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans	294102579 260654639 288575022 282856738 289523224 312879980 269792698	180 PGIRPSATG I V-LA-A- V-LTSL- V-LV-G- V-EG-S K LPGD- T (FQGGE V)	206 DDQARTATPKGAIIAGAD T-VD S-VAD-FRN IMGQ-KKK QVD-AA-MGR HVMG-RE-VASS
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930	180 PGIRPSATG I V-LA-A- V-LTSL- V-EG-S K LPGD- T FQGGE V KDFV NK	206 DDQARTATPKGAIIAGAD T-VD S-VAD-FRN IMGQ-KKK QVDAA-MGR HVMG-RE-VASS K-VAE-ARS
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055	180 PGIRPSATG I V-LA-A- V-LTSL- V-EG-S K FQGD- T (FQGE V KDFV NK AGSA Q	206 DDQARTATPKGAIIAGAD T-VD S-VAD-FRN S-VAD-FRN IMGQ-KKK QVD-AA-MGR HVMG-RE-VASS K-VAE-ARS R-ILRQ-LD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101	180 PGIRPSATG I - V-LA-A- - V-LTSL- - V-EG-S K LPGD- T FQGGE V KDFV NK AGSA Q EGSD F	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - V-EG-S K FQGD T FQGGE V KDFV NK EGSD F EGSD F	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141	180 PGIRPSATG I - V-LA-A- - V-LTSL- - V-LV-G- - V-EG-S K FQGGE V FQGGE V KGFV NK EGSD F EGSD F AGSE A	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - VEG-S K FQGGE V FQGEV KGFV NK EGSD F EGSD F EGSD A	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875	180 PGIRPSATG F V-LA-A- V-LTSL- V-LV-G- V-EG-S K FQGGE V FQGGE V FQGSD F EGSD F EGSD A EGSE A	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382	180 PGIRPSATG F V-LA-A- V-LTSL- V-LV-G- V-EG-S K FQGGE V FQGGE V FQGA Q EGSD F EGSD A EGSD A EGSE A EGSE A EGSE A EGSE A	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - VEG-S K FQGGE V FQGGE V FQSD F EGSD F EGSD A EGSD A EGSD A EGSD A EGSD A EGSD A 	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - VEG-S K FQGGE V FQGGE V FQSD F EGSD F EGSD F EGSD A EGSD A EGSA Q EGSA Q 	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - VEG-S K FQGGE V FQGGE V FQSD F EGSD F EGSD F EGSD A EGSD A EGSD A EGSD A EGSD A EGSD A EGSD A EGSD A EGSA Q EGSA Q EGSA Q EGSA A EGSA A EGSA A EGSA A EGSA A EGSA A EGSA A EGSA A EGSA A 	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675	180 PGIRPSATG F - V-LA-A- - V-LTSL- - V-LV-G- - V-EG-S K LPGD- T FQGGE V FQGGE V FGSD F EGSD F EGSD F EGSD A EGSD A EGSA Q EGSA Q 	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238653382 330685500 149005807 161503187 220904675 189424925	180 PGIRPSATG	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421	180 PGIRPSATG	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068	180 PGIRPSATG -V-LA-A- -V-LTSL -V-LPGD- T Graduation -V-EG-S K -V-EG-S -V-EG-S FQGGE	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231	180 PGIRPSATG -V-LA-A- -V-LTSL- -V-LPGD- T Graduation -V-EG-S K -V-EG-S -V-EG-S FQGGE	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden Nostoc punctiforme	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231 186683999	180 PGIRPSATG -V-LA-A- -V-LTSL- -V-LQ-G- -V-EG-S K -V-EG-S -V-FQGD-T G -FQGGE V FQGGE	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden Nostoc punctiforme Acaryochloris marina	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231 186683999 158337488	180 PGIRPSATG -V-LA-A- -V-LTSL- -V-LQ-G- -V-EQ-S K	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden Nostoc punctiforme Acaryochloris marina Fusobacterium gonidiaformans	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231 186683999 158337488 257466503	180 PGIRPSATG -V-LA-A- -V-LTSL -V-LQ-G- -V-EQ-S K -V-EG-S K -V-EG-S K -V-EG-S K -V-EG-S K -V-EGSS K FQGE V	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden Nostoc punctiforme Acaryochloris marina Fusobacterium gonidiaformans Erysipelothrix rhusiopathiae	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231 186683999 158337488 257466503 323342222	180 PGIRPSATG -V-LA-A- -V-LTSL- -V-LQG- -V-FQGE -V-FAA -V-FAA <th>206 DDQARTATPKGAIIAGAD </th>	206 DDQARTATPKGAIIAGAD
(B) Synergistetes 4/8 Other species 0/250	Aminobacterium colombiense Jonquetella anthropi Dethiosulfovibrio peptidovorans Pyramidobacter piscolens Anaerobaculum hydrogeniformans Aminomonas paucivorans Thermanaerovib. acidaminovorans Thermovirga lienii Pseudomonas brassicacearum Haemophilus parasuis Actinobacillus minor Dickeya dadantii Pseudoalteromonas haloplanktis Shewanella amazonensis Lactobacillus gasseri Staphylococcus epidermidis Streptococcus pneumoniae Salmonella enterica Desulfovibrio desulfuricans Geobacter lovleyi Brachyspira murdochii Acetobacter pomorum Magnetospirillum gryphiswalden Nostoc punctiforme Acaryochloris marina Fusobacterium gonidiaformans Erysipelothrix rhusiopathiae Syntrophomonas wolfei	294102579 260654639 288575022 282856738 289523224 312879980 269792698 357419930 330811055 167855101 240949272 242239141 332534575 119774875 238853382 330685500 149005807 161503187 220904675 189424925 296127421 329114068 144899231 186683999 158337488 257466503 323342222 114566804	180 PGIRPSATG -V-LA-A- -V-LTSL- -V-LQG- -V-FQGS K -V-EG-S K -V-FQGE -V-FQGE -V-FQGE -V-FQGE -V-FQGE -V-FQGE -V-FQGE -V-FQGE -V-FQGE FQGSD FQSD	206 DDQARTATPKGAIIAGAD

Fig. 8 Partial sequence alignments of **a** DNA Gyrase subunit B and **b** orotidine 5'-phosphate decarboxylase showing two CSIs in conserved regions that are specific for the species

D. peptidovorans, J. anthropi, Amb. colombiense, P. piscolens, F. fastidiosum, which form or define a higher clade within the Synergistetes group of species

Table 6 Conserved it	idels common to Syi	nergistetes and shared with	other groups			
Protein	Beta-ketoacyl- X; acyl- carrier protein	aa-Pro dipeptidase A	TP-dependent F rotease La c	Mosphoribosyl- ormylglycin-amidine ·ycloligase	Translation elongation factor 1A	GTP-binding protein Era
	synthase II					
GenBank identifier	260654633 26	50654796 2 ₁	50654937 2	94102156	312880374	294101756
Accession no.	ZP_05860123 ZI	P_05860284 Z	P_05860425	7P_003554014	ZP_07740174	$YP_{-}003553614$
Indel/size	2 aa del 1	aa ins 1	aa ins 1	aa del	1 aa del	1 aa ins
Indel position ^a	273–312 22	20–260 6.	25-672 2	53–299	231–269	75-121
Figure no.	Suppl. Fig. 57 Su	appl. Fig. 58 S.	uppl. Fig. 59 S	suppl. Fig. 60	Suppl. Fig. 61	Suppl. Fig. 62
Synergistetes species containing indel	J. anthropi and D. P. piscolens ₁	: peptidovorans, P. J. piscolens and J. anthropi	<i>anthropi</i> and <i>P. A piscolens</i>	All detected Synergistetes	Amm. paucivorans, T. acidaminovorans, D. peptidovorans	All detected Synergistetes
Other species with indel	Epsilon- Sc proteobacteria	ome Fusobacteria F	usobacteria and Treponema pallidum	Γwo δ-proteobac. and Fusobacteria	Fusobacteria, Proteobacteria, two Elusimicrobia	Some Clostridia species, Termite group 1 Rs-D17
Protein	NADH dehydrogens	ase Translation elongation factor Tu	Penicillin-binding protein 2	Ribonuclease, Rne/Rng family	DNA-directed RNA polymerase, subunit B	Ribosomal RNA large subunit methyltransferase J
GenBank identifier	312880263	294101321	288574639	269792810	288574654	289522985
Accession no.	ZP_07740063	YP_003553179	ZP_06392996	YP_003317714	ZP_06393011	ZP_06439839
Indel/size	1 aa del	1 aa ins	3-4 aa ins	1 aa ins	1 aa ins	1 aa ins
Indel position ^a	31-86	228-270	171-205	14-47	412-457	174-233
Figure no.	Suppl. Fig. 63	Suppl. Fig. 64	Suppl. Fig. 65	Suppl. Fig. 66	Suppl. Fig. 67	Suppl. Fig. 68
Synergistetes species containing indel	All detected Synergistetes	Amb. colombiense, Tv. lienii and An. hydrogeniformans	P. piscolens, D. peptidovorans	A. paucivorans, T. acidamino-vorans	All detected Synergistetes	All detected Synergistetes
Other species with indel	Negativicutes	Negativicutes	Veillonella species	Veillonella species	Acidobacteria and a few clostridi	a Many alpha proteobacteria
Protein	GTP-binding protein Era	Orotidine 5'-phospha decarboxylase	e Ribosomal protein S	5 Homoserine kinase	1-Hydroxy-2-methyl-2- (E)-butenyl 4-diphosphate synthas	3-Oxoacid CoA-transferase, e subunit B
GenBank identifier	294101756	289523224	260655366	294101575	294101868	288573369
Accession no.	YP_003553614	ZP_06440078	ZP_05860854	YP_003553433	YP_003553726	ZP_06391726
Indel/size	4 aa ins	1 aa del	1 aa ins	1 aa ins	1 aa ins	2 aa del
Indel position ^a	213–259	149–177	105-144	222–258	171–218	43–83
Figure no.	Suppl. Fig. 69	Suppl. Fig. 70	Suppl. Fig. 71	Suppl. Fig. 72	Suppl. Fig. 73	Suppl. Fig. 74
Synergistetes species containing indel	All detected Synergistetes	Some Synergistetes	All detected Synergistetes	All detected Synergistetes	Most Synergistetes	All detected Synergistetes
Other species with indel	Anaeromyxobacter, Prochlorococcus m	Pseudomonas species tarinus and A. caldus	Hydrogenothermacea species	e Erysipelotrichi and A. viridans	Most detected Actinobacteria	Few Streptococci
^a The indel position prov	ided indicates the regional	on of the protein containing the	: CSI			

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(five) of these CSIs with the Fusobacteria and they share only 1–2 indels with most other taxonomic groups. In many cases where the Synergistetes share CSIs with other taxa, only some species from the Synergistetes or the other taxa contain the indel. The CSIs in these other groups may have arisen independently through separate genetic events or it is also plausible that their shared presence in some of these cases is due to LGTs.

Discussion and concluding remarks

The Synergistetes are a relatively unknown group of species living ubiquitously in anaerobic environments. Though characteristics for the isolated Synergistetes are known, such as their gram-negative morphology and their ability to ferment amino acids, no single molecular, morphological or physiological characteristic is known that distinguishes them as a group from other bacterial organisms. Utilizing the available genomic data for this group of organisms, we report here identification of over 60 novel CSIs specific for the species of the Synergistetes phylum. Of the various discovered CSIs, 32 were identified to be specific for all or most Synergistetes species (maximum of three exceptions unrelated to each other). These CSIs are present in widely distributed proteins with important cellular functions and they are rarely present in protein homologues of species outside of the phylum. As they are present in most or all Synergistetes and absent in bacteria from all other taxonomic groups, they provide strong evidence that species of the Synergistetes phylum constitute a monophyletic group that is distinct from all other prokaryotic taxa. These CSIs also provide novel molecular means for identification and circumscription of species from this phylum.

The bacteria belonging to the Synergistetes have been classified into 12 different genera (and a candidate genus) within the phylum. Despite the recognition of numerous species and genera, due to the lack of reliable biological characteristics that can identify the interrelationships among these bacteria, all genera are presently grouped into a single class, order and family. Numerous CSIs were discovered during the course of the study that were present in only certain clades of species within the Synergistetes phylum and absent from others. The group specificities of these CSIs are summarized in Fig. 10. Explicitly, 7 CSI were detected to be specifically found in only the J. anthropi, P. piscolens and D. peptidovorans species; 15 CSIs were identified that are specific for the J. anthropi and P. piscolens species (or differentiate them from other Synergistetes) and 9 other CSIs differentiated the T. acidaminovorans and Amm. paucivorans from other members of the phyla. In addition, two of the discovered CSIs also supported a grouping together of the J. anthropi, P. piscolens, D. peptidovorans, Amb. colombiense and F. fastidiosum species. These relationships are also consistently observed in phylogenetic trees created for the Synergistetes group and the identified CSIs provide valuable markers that consolidate these relationships. Furthermore, it should be noted that in contrast to the CSIs supporting these relationships, very few, if any, CSIs that supported alternative relationships among these species were detected. Thus, the identified CSIs provide independent evidence for the existence of these clades and provide molecular means to demarcate and circumscribe these clades. The evidence based upon identified CSIs supports the division of the phylum Synergistetes into a number of distinct families (or other higher taxonomic groupings) and a formal proposal in this regard will be made in future work. Though the branching and interrelationships of several species within the phylum is well supported by multiple CSIs, the relationships of Tv. lienii and An. hydrogeniformans, and also to some extent Amb. colombiense and F. fastidiosum to other Synergistetes species were not resolved by the identified CSIs. This problem may be addressed as genome sequences for additional Synergistetes species become available.

As previously mentioned, the Synergistetes have often been misclassified as a lower ranked taxonomic group with bacteria belonging to other phylogenetic divisions. In our analysis, some CSIs were also discovered that were shared by Synergistetes species along with species from other taxonomic groups. Some of the organisms sharing such indels included species from the Fusobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Aquificae and Firmicutes phyla. Most of these groups shared no more than 1-3 CSIs and in many cases only a few species within the groups contained the indels. Geissinger et al. (2009) presented a study suggesting a shared common ancestor for Elusimicrobium and Synergistetes and a recent study by Gupta (2011) also suggested that the Negativicutes, Fusobacteria, Elusimicrobia and

(A)			189	224
(11)	Jonquetella anthropi	260654222	YGADLYKKRSLPKLLTELEK	LPQSVWLRLFYLHPS
	Pyramidobacter piscolens	282855982	SFGRPRD E	RG
	Dethiosulfovibrio peptidovorans	288573284	SS-RGSGDAM-A E	EDI
Synergistetes≺	Aminobacterium colombiense	294101399	R-V-G-PIADS-TA S	ELA
7/7	Anaerobaculum hydrogeniformans	289524260	M-WDGSSH-VEDLH	V-DGM-I-PL
	Aminomonas paucivorans	312879348	L-RGGRETDDAP S	GD-FL
	Thermanavib. acidaminovorans	269793254	E- LGTD -MEDQM-A -	VRGHGVLT
	Sphaerobacter thermophilus	269837471	GI-NGGRMIAE E	V-DLPML-IY
	Roseiflexus castenholzii	156741270	RGL-DG-AID-ICA V	ENVM-AY-
Chloroflexi≺	Roseiflexus sp. RS-1	148654700	RGL-DG-ALDCA V	KDR-VM-AY-
6/6	Chloroflexus aggregans	219848510	RGLRDG-AIDCQ V	T-SDI-IM-AY-
0/0		103047415		V H C T M AV
	Thermotoga manitima	15644605		
	Clostridium botulinum	187033300		
	Fubacterium vurii	306819660		IEGIR-I-VL-OV-
	Roseburia intestinalis	240144123	VGEKHBDN-	IKDIF-I-IM-CY-
	Syntrophomonas wolfei	114566788	H-ISPQSATBS-	-DGLF-I-M
	Thermoanaerobacter italicus	289578370	I - I FM Q K SI	I-NIK-II-AY-
	Fusobacterium varium	253583638	I EK AR-MK V-	IDGLKTY-MF-
	Ilvobacter polytropus	310779080	I KA DVMKA-S-	VEGIE-I-TY-MF-N
Other species	Bacteroides finegoldii	255693887	VQME-IERISD	I-GVE-I-H-AY-A
0/250	Psychroflexus torquis	91215250	LN-AERV-	VEGIE-IH-AF-T
0/250	Bacillus tusciae	295696216	LGR-RDKA-ND	VDELR-IH-AY-
	Brachyspira murdochii	296125911	H-IRLADKS-	IEGIE - I - VL - QN -
	Aquifex aeolicus	15606200	KEYK-VEEG	VEGIK-ILY-T
	Thermocrinis albus	289547853	KHRKA-VQKKE	-EGIE-ILY-T
	Prochlorococcus marinus	123967655	Q-I-G-PANS-	VSIP-I-IH-AY-T
	Leptospirillum rubarum	124514462	SEDGEGRE-IDR	IGRIP-VL-AY-T
	Dialister microaerophilus	313891928	QRDGTILKQ-V-	I - EVK - I Y - T
				074
(B)	en alla and ferrit and a same station and	000574705		274
	Dethiosulfovibrio peptidovorans	288574785	FYLRSAEEMWQIFG D DVPEAL	LENTLKIAERG
	Thopmovingo lionii	257410501		
	Thermovirga lienii	357419591	PAKEL	-TVN
	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense	357419591 260655562 294101731	K EL P NF A ED	- T VN - T VE - D A
Svnergistetes⊀	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens	357419591 260655562 294101731 282856832	P	- T VN - T VE - D A B
Synergistetes≺ ₀/₀	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans	357419591 260655562 294101731 282856832 269792442	PNFAEKEL PNFAED QGYSEA-DS- 	- T VN - T VE - D A R - B I V
Synergistetes≺ 9/9	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans	357419591 260655562 294101731 282856832 269792442 312880004	PAK EL PNFA ED QGYS EA-DS- TPSLLG E	- T VN - T VE - D A - R R - R LV - R 0F D
Synergistetes⊀ 9/9	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans	357419591 260655562 294101731 282856832 269792442 312880004 289523190	P	- T VN - T VE - D A - R R - R LV - R QE - D - H VD - S
Synergistetes⊀ 9/9	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106	P	- T VN - T VE - D A - R R - R LV - R QE - D - - H VD - S - D VA
Synergistetes⊀ 9/9	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3 1 5R	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286	P	-T VN -T VE -DA -R LV -R QE D - -H VD - S -D VA -Q - SVE K
Synergistetes 9/9 Fusobacteria	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium periodonticum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954	P	- T VN - T VE - D A - R LV - R LV - R QE - D - - H VD - S - D VA Q - SVE K - I NY SL -
Synergistetes≺ 9/9 Fusobacteria≺	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium periodonticum Fusobacterium gonidiaformans	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914	PAK K EL PDSLA EL-D FQGYSL	-T VN -T VE -D A -R LV -R QE D - -H VD - S -D VA -Q - SVE K - I NY - SL - -Q - SVE K -
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539	PA K EL PDSL A ED FQGY S EA-DS- FPSL A EL-D LK-YYAVL- E QVQ LK-KYAVL- E QVQ LK-K-N	-T VN -T VE -D A -R LV -R QE - D - -H VD - S -D VA -Q - SVE K - I NY - SL - -Q - SVE - K - I ANH - DL -
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584	PAK K EL PDSLA EL-D FQGYSL	-T VN -T VE -D A -R LV -R QE - D - -H VD - S -D VA -Q - SVE K - I NY - SL - -Q - SVE - K - I AV - M
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 225163584 221632747	PNF K EL PDSL A ED PDSL A EL-D	-T VN -T VE -D -R LV -R LV -R QE - D -H VD - S -D VA -Q - SVE K I ANH - DL - -T AV M -L VR
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium genidiaformans Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 225163584 221632747 269837330	PNF K EL PDSL A EL-D FQGY S EA-DS FPSL G E FPSL A EL-D LK-Y A EL-D LK-Y A EL-D LK-Y	-T VN -T VE -D A -R LV -R QE D -D VA -Q - SVE K I NY - SL - -Q - SVE K - I ANH - DL - -T AV - M - -L VR IR
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Amarobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium geniddaformans Fusobacterium geniddaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 22763584 221632747 269837330 224282597	PNF K EL PDSL A EL-D PSL G E PSL G E	-T VN -T VE -D A -R LV -R LV -R QE - D - -D - VA -D - VA -Q - SVE K - I NY - SL - -Q - SVE K - I ANH - DL - -T AV - M - -L VR CD E
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium genidiaformans Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium minutum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629	PNF K EL PDSL A EL-D FQGY S EA-DS FPSL G E FPSL A EL-D LK-YYAVL- E QYQ LK-KDKRFL- E KFHK-1 LK-KDKRFL- E KFEK-1 L-FK-PRL A E Y-IKREL-K A L-FK-QAEL-S YI YI	-T VN -T VE -D A -R LV -R LV -R QE - D - -D - VA -Q - SVE K - I NY - SL - -Q - SVE K - I ANH - DL - -T AV - M -L - VR CD E VS EV - K -
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium sp. 3_1_5R Fusobacterium geriodonticum Fusobacterium geriodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835	PNF K EL PDSL A EL-D PDSL A EL-D	-T VN - T VE - D A - R LV - R LV - R QE - D - - H VD - S - Q - SVE K - I NY - SL - - Q - SVE - K - I ANH - DL - - T AV - M - L - VR CD E VS EV - K - CD L Q -
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium ganidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648	PA K EL PDSL A EL-D FQGY S EA-DS- FPSL A EL-D FPSL A EL-D PVSL A EL-D PVSL A EL-D ELSD - L	-T VN - T VE - R A - R LV - R QE - D - H VD - S - D VA - Q - SVE K - I NY - SL - - Q - SVE K - I ANH - DL - - T AV - M - L VR IR CD E VS EV K - CD L Q - I N
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium ganidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum Clostridium perfringens	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648 110803674	PA K EL PDSL A EL-D PDSL A EL-D	-T VN -T VE -R LV -R LV -R QE - D -H VD - S -D - VA -Q - SVE K - I NY - SL - -Q - SVE K - I ANH - DL - -T AV - M -L - VR IR CD E VS EV - K - CD L Q - I N
Synergistetes≺ 9/9 Fusobacteria≺ 4/4	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium ganidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum Clostridium perfringens Peptostreptococcus stomatis	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648 110803674 307243299	PAK K EL PDSL A EL-D PDSL A EL-D	-T VN -T VE -D A -R LV -R QE - D - -H VD - S -D VA -Q - SVE K - I NY - SL - -Q - SVE - K - I ANH - DL - -T AV - M -L VR -CD E VS EV K - CD L Q - I N
Synergistetes≺ 9/9 Fusobacteria≺ 4/4 Other species	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium ganidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum Clostridium perfringens Peptostreptococcus stomatis Desulfotomaculum acetoxidans	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648 110803674 307243299 258514118	PAK K EL PDSL A ED PDSL A EL-D	-T VN -T VE -D A -R LV -R QE - D - -H VD - S -D VA -Q - SVE K - I NY - SL - -Q - SVE - K - I ANH - DL - -T AV - M -L - VR CD E VS EV - K - CD L Q - I N
Synergistetes≺ 9/9 Fusobacteria≺ 4/4 Other species{ 2/250	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Aminomonas paucivorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium ganidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum Clostridium perfringens Peptostreptococcus stomatis Desulfotomaculum acetoxidans Symbiobacterium thermophilum Bactoridae apnillowu	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648 310803674 307243299 258514118 51891797	PAK K EL PDSL	-T VN -T VE -D A -R LV -R LV -R QE - D - -H - VD - S -D - VA -Q - SVE K - I NY - SL - -Q - SVE K - I NY SL - -Q - SVE K - I NH - DL - -T AV - M -L - VR -T CD E VS EV K - CD L Q - I N E -
Synergistetes≺ 9/9 Fusobacteria≺ 4/4 Other species{ 2/250	Thermovirga lienii Jonquetella anthropi Aminobacterium colombiense Pyramidobacter piscolens Thermanaerovibrio acidaminovorans Anaerobaculum hydrogeniformans Fretibacterium fastidiosum Fusobacterium gonidiaformans Fusobacterium periodonticum Fusobacterium gonidiaformans Fusobacterium nucleatum Opitutaceae bacterium Thermomicrobium roseum Sphaerobacter thermophilus Bifidobacterium bifidum Elusimicrobium minutum Kribbella flavida Eubacterium limosum Clostridium perfringens Peptostreptococcus stomatis Desulfotomaculum acetoxidans Symbiobacterium thermophilum Bacteroides capillosus Coabacten aufurgativesed	357419591 260655562 294101731 282856832 269792442 312880004 289523190 295112106 257452286 262066954 257465914 34763539 225163584 221632747 269837330 224282597 187250629 284030835 310828648 110803674 307243299 258514118 51891797 154499865	PNF K EL PNF A ED PNF A EL-D	-T VN -T VE -D A -R LV -R LV -R QE - D - -H - VD - S -D - VA -Q - SVE K - I NY - SL - -Q - SVE K - I NH - DL - -T AV - M -L - VR CD E VS EV K CD L Q I N E - VS E - VS E - V S - A I VRV - K - I - VR K -
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Fig. 9 Examples of CSIs that are commonly shared by Synergistetes species and other groups of bacteria. **a** A CSI consisting of 1 aa insert in the MiaB-family of RNA modification enzyme that is commonly shared by different Synergistetes and Chloroflexi. **b** A 1 aa insert in a conserved region of DNA polymerase III, α subunit shared by all Synergistetes and Fusobacteria as well as two other bacteria belonging to the Chloroflexi and Verrucomicrobia phyla

Synergistetes phyla might be closely related to each other based on their cell membrane structure and shared indels in their DnaK and GroEL proteins (Geissinger et al. 2009; Gupta 2011). Though the *Elusimicrobia* and *Fusobacteria* share some CSIs with the Synergistetes species, no CSI was found that was

specifically shared by all detected Synergistetes and species from these taxa. Furthermore, the branching of these phyla in the protein trees (Fig. 1) does not support their close relationship with the Synergistetes. Hence, based upon these results, at present no clear relationship of the Synergistetes species to other bacteria phyla can be inferred. These results provide further evidence supporting the placement of Synergistetes species into a distinct phylum.

Due to their specificity, Synergistetes-specific CSIs provide interesting prospects for future research. Since these CSIs are present in conserved regions of various proteins, degenerate primers utilizing the conserved regions can be designed for use as a means for



Fig. 10 A summary diagram portraying the species distribution of various identified Synergistetes-specific CSIs and the evolutionary stages where the genetic changes responsible for them have occurred

identification of various species of the phylum in different environments (Gao and Gupta 2005). This might prove to be especially useful, as it is surmised that universal primers utilized in detection of organisms is metagenomic studies may not efficiently identify some Synergistetes species (Hamady and Knight 2009). As molecular markers, the phylumspecific CSIs can be useful as identification tool for detection of known and unknown species in metagenomics experiments. These CSI can also assist in the classification of newly discovered bacteria into the phylum Synergistetes and its sub-groups.

Finally, some species of Synergistetes have also been notoriously difficult to culture/isolate (Vartoukian et al. 2010) and, for others, their biological nuances have just begun to be understood. It has been suggested that Synergistetes act in concert with other oral bacteria to degrade proteinaceous compounds in periodontitis lesions (Homer and Beighton 1992; Wei et al. 1999; Vartoukian et al. 2007). Prior functional studies on taxa-specific CSIs have shown that such indels are usually present in peripheral regions of proteins and they tend to be essential for the function of the proteins in the organisms where the CSIs occur (Itzhaki et al. 2006; Akiva et al. 2008; Hormozdiari et al. 2009; Singh and Gupta 2009). Hence, agents that bind to these CSIs and inhibit their cellular functions could provide novel therapeutics, which are specifically directed against this group of bacteria. Lastly, the molecular markers discovered in this study, due to their specificity for Synergistetes species provide novel and valuable means for understanding the contribution of this group of bacteria to the environment and to the microbial communities that they inhabit. Thus, analyses devoted to the understanding of the function of these CSIs should provide important insights into the biochemical and physiological properties that define the Synergistetes and their roles in different environments.

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