

Supplementary Material
to “*Criibacterium bergeronii* gen. nov., sp. nov., a new member of the
family *Peptostreptococcaceae*, isolated from human clinical samples”

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Supplementary results:

Research for outer membrane marker proteins in strains CCRI-22567^T and CCRI-24246:

The genomes of strains CCRI-22567^T and CCRI-24246 were assessed for genes encoding 17 outer membrane marker proteins conserved in enterobacteria and diderm *Firmicutes*: early LPS synthesis (LpxACDB), Omp85, POTRA, ExbD, Secretin, TamB, TonB (C-terminal region), and lipid-A synthesis and export (KdsBACD, LptA, LptB, FabZ) [1]. We used HMMER 3.2.1 (<http://hmmer.org/>) [2-4] and included *Escherichia coli* and *Veillonella parvula* genomes as controls. None of the aforementioned marker proteins were detected in strains CCRI-22567^T and CCRI-24246. Conversely, 13 genes coding for marker proteins were detected in *Escherichia coli* (KdsBACD, FabZ, LptA, LpxACB, Omp85, POTRA, Secretin, TamB) and 15 in *Veillonella parvula* (KdsBACD, FabZ, LptA, LptB, LpxACB, ExbD, Omp85, POTRA, Secretin, TamB).

Supplementary Tables:

Table S1. Cellular Fatty Acid Composition of *Criibacterium bergeronii* gen. nov., sp. nov. as compared to selected genera and species in the family *Peptostreptococcaceae*

Fatty acid*	<i>Criibacterium bergeronii</i> CCRI-22567 ^T	<i>Romboutsia ilealis</i> CRIB ^T /CCRI-22875 ^T	<i>Filifactor alocis</i> ATCC 35896 ^{T†}	<i>Intestinibacter bartlettii</i> DSM 16795 ^{T&}	<i>Terrisporobacter glycolicus</i> DSM 1288 ^{T&}	<i>Asaccharospora irregularis</i> DSM 2635 ^{T&}	<i>Peptostreptococcus anaerobius</i> DSM 2949 ^{T&}
Saturated, Straight-Chain							
C _{10:0} (10.000)	2.7	Nd/Nd/Nd**	5.3	Nd	Nd	Nd	Nd
C _{12:0} (12.000)	2.3	1.6/2.2/Nd	5.4	0.2	1.1	1.5	2.2
C _{14:0} (14.000)	2.0	3.8/3.6/4.4	11.0	1.1	2.0	4.0	6.7
C _{15:0} (15.000)	Nd	6.2/7.1/Nd	Nd	1.3	3.2	2.2	0.2
C _{16:0} (16.000)	5.8	31.3/21.9/18.5	15.0	4.2	15.6	26.9	9.6
C _{17:0} (17.000)	Nd	10.0/10.1/Nd	Nd	2.0	7.4	1.4	Nd
C _{18:0} (18.000)	3.3	6.6/4.1/14.3	2.0	1.0	1.6	2.3	0.3
Saturated, branch-chained							
C _{12:0} iso (11.608)	Nd	Nd/Nd/Nd	Nd	0.2	0.4	2.0	4.7
C _{13:0} anteiso (12.703)	Nd	Nd/Nd/Nd	Nd	0.3	0.3	2.4	8.4
C _{14:0} iso (13.618)	Nd	Nd/Nd/Nd	Nd	3.2	2.0	0.7	11.2
C _{13:0} iso 3OH (14.114)	Nd	Nd/Nd/Nd	Nd	Nd	Nd	Nd	3.5

C _{15:0} iso (14.623)	Nd	Nd/Nd/Nd	Nd	12.2	2.2	2.5	0.5
C _{15:0} anteiso (14.714)	Nd	Nd/Nd/Nd	Nd	7.7	1.2	1.7	7.8
C _{16:0} iso (15.627)	Nd	Nd/Nd/Nd	Nd	4.7	3.2	5.3	6.5
C _{17:0} iso (16.630)	Nd	Nd/Nd/Nd	Nd	3.7	1.5	4.2	0.2
C _{17:0} anteiso (16.723)	Nd	Nd/Nd/Nd	Nd	3.4	1.0	3.0	0.7
Unsaturated, Straight-Chained (ECLs observed)							
C _{14:1} ω5c (13.903)	1.0	Nd/Nd/Nd	Nd	Nd	Nd	Nd	Nd
C _{16:1} ω9c (15.774)	Nd	6.5/7.7/Nd	2.3	1.9	5.1	8.0	Nd
C _{16:1} ω7c (15.818) = Summed Feature 3 [®]	4.0	9.1/9.3/6.7	9.9	2.8	6.9	17.6	0.4
C _{16:1} ω5c (15.909)	Nd	Nd/Nd/Nd	Nd	Nd	0.7	1.5	Nd
C _{17:1} ω6c (16.864)	Nd	3.3/5.8/Nd	Nd	Nd	Nd	Nd	Nd
C _{18:2} ω6,9c (17.723)	3.1	Nd/Nd/Nd	1.9	Nd	Nd	Nd	Nd
C_{18:1} ω9c (17.771)	33.6	3.4/2.9/46.1	3.6	1.0	1.4	2.5	0.2
C _{18:1} ω5c (17.919)	Nd	0.7/1.1/Nd	Nd	Nd	ND	Nd	Nd
Dimethyl Acetal (DMA) or Aldehydes							

C _{11:0} DMA (12.469)	Nd	Nd/Nd/Nd	1.7	Nd	Nd	Nd	Nd
C _{14:0} DMA (14.472)	Nd	Nd/Nd/Nd	Nd	0.9	0.5	Nd	3.5
C _{16:0} ALDE (14.951)	Nd	Nd/Nd/Nd	8.1	1.9	7.1	Nd	3.9
C _{15:0} iso DMA (15.112)	Nd	Nd/Nd/Nd	Nd	3.5	0.3	Nd	0.2
C _{16:1} ω7c DMA (16.285)	0.7	Nd/Nd/Nd	2.6	Nd	Nd	Nd	Nd
C _{16:0} DMA (16.471)	1.0	Nd/Nd/Nd	17.4	10.8	9.3	0.6	9.8
C _{17:0} anteiso DMA (17.196)	Nd	Nd/Nd/Nd	Nd	4.4	0.3	ND	0.3
C _{17:0} DMA (17.469)	Nd	Nd/Nd/Nd	Nd	2.6	2.1	0.2	Nd
C_{18:1}ω9c DMA (18.224)	21.5	Nd/Nd/Nd	Nd	Nd	Nd	Nd	Nd
C _{18:1} ω7c DMA (18.285)	2.7	Nd/Nd/Nd	2.8	Nd	Nd	Nd	Nd
C _{18:0} DMA (18.467)	0.7	Nd/Nd/Nd	1.4	Nd	Nd	Nd	Nd
Cyclopropanes							
C _{19:0} cyclopropane 9,10 (18.870)	Nd	0.8/1.5/ Nd	Nd	Nd	Nd	Nd	Nd
Summed Features®							

Summed feature 4 (14.780)	Nd	1.1/2.7/Nd	3.0	0.5	1.7	0.3	Nd
Summed feature 5 (15.472)	Nd	Nd/Nd/Nd	Nd	2.5	2.7	Nd	0.2
Summed feature 7 (16.765)	5.3	Nd/Nd/Nd	Nd	Nd	0.8	Nd	Nd
Summed feature 8 (16.794)	Nd	5.3/9.0/Nd	1.8	1.8	3.1	1.1	Nd
Summed feature 10 (17.830)	6.4	8.3/7.6/7.5	4.9	1.0	2.9	2.5	Nd
Summed Feature 12 (18.624)	4.1	Nd/Nd/2.6	Nd	Nd	Nd	Nd	Nd
Totals	100&	98.0/96.5&	100&	80.8&	87.6&	94.4&	81&

* Values shown are the percent of CFA from total CFAs detected; data generated from this study for the type strain of *Criibacterium bergeronii*, *Romboutsia ilealis* CCRI-22875^T, and *Filifactor alocis* ATCC 35896^T based on an average from 2 runs of the bacterium. ECL, equivalent chain lengths as described the peak naming table found in Moore 6.0 method; Nd, not detected. No species shown here contained CFAs [name, (ECL)]: C_{18:1}ω₆c (17.864), C_{17:0}cyclopropane (16.889), nor C_{19:0}cyclopropane 11,12 (18.904)

** value1/value2/value3: The first two values are the amount of fatty acids from Gerritsen *et al.*, 2014 and Gerritsen *et al.*, 2018 respectively [5, 6]. The third value has been generated by our analysis of CRIB^T /CCRI-22875^T

@ Summed Features represent two or more CFAs that are grouped together by the MIDI system which have identical or nearly identical Equivalent Chain lengths (ECLs) but chemically differ as described in the Sherlock system using the method Moore 6.0. Summed features detected: Summed Feature 3 (cited in some versions of the library) included C_{16:1}ω₇c (15.820) and C_{16:1} ω₆c; Summed

Feature 4 included C_{15:1}ω8c, C_{15:2} and /or an unknown C_{15:2}; Summed Feature 5 included C_{15:0}DMA and/or C_{14:0}3OH; Summed Feature 7 included C_{17:1}ω9c and/or C_{17:2}; Summed Feature 8 included C_{17:1}ω8c and/or C_{17:2}; Summed Feature 10 includes C_{18:1}ω7c and / or an unknown CFA; Summed Feature 12 included unknown CFA ECL 18.622 and C_{19:0} iso.

from reference Gerritsen *et al.*, 2018 [6].

& Data adapted from Supplemental Table 2 of Gerritsen *et al.*, 2014 [5]. However, data alluded to there as unknown CFA compounds that did not correlate to ECLs in Moore6 Peak naming table, were not included in our table. Data for *R. ilealis* CCRI 22875^T from this study. For strains DSM 16795^T and DSM 2949^T the remaining percentages (~19%) represent CFAs that were absent or different from work done at the DSMZ.

† From testing of *F. alocis* ATCC 35896^T, which had the same results as cited in Moore *et al.*, 1994 [7].

Table S2: Characteristics of *Criobacterium* and other genera from the family *Peptostreptococcaceae*.

Property	Organisms																
	<i>Criobacterium</i>	<i>Acetanaerobium</i> [8]	<i>Asaccharospora</i> [5]	<i>Clostridioides</i> [8, 9]	<i>Filifactor</i> * [8, 9]	<i>Intestinibacter</i> [8]	<i>Paenilosiridium</i> [8]	<i>Paraclostridium</i> [8]	<i>Peptacetobacter</i> [10]	<i>Peptonanaerobacter</i> [11]	<i>Peptoclostridium</i> [8]	<i>Peptostreptococcus</i> [8, 9]	<i>Proteocella</i> [8]	<i>Romboutsia</i> [5, 6, 8]	<i>Sporacetigenium</i> [8]	<i>Tepidibacter</i> [8, 9, 12]	<i>Terrisporobacter</i> [5, 9]
GenBank accession No. (16S rRNA)	MF547661.1	GU562448.1	AB971797.1	HM245939.1	AF537211.1	AY438672.1	AB542933.1	HM245932.1	MK144347.1	HM120213.1	X77845.1	D14150.1	AF450134.1	JN381505.1	AY682207.1	AY158079.1	HM245942.1
Genome size, Mb (type species)	2.18	2.1	2.62	4.2	1.93	2.9	3.51	3.5	2.14	2.5	3	2.11	2.4	2.58	ND	2.2	3.21
G+C (mol%)	32.9-33.8	33.3-35.7	30.3	28.8	28-35.4	29.8	30.2	27-30.2	31.1-32.8	30.0-30.07	41.3-44.0	28-46	39.5	27-28.1	53.9-54.3	24-29.8	25.6-29
Cell size, Width μm (type species)	0.63	0.8	0.8-1.6	0.5-1.9	0.4-0.7	1.0-1.5	0.5-1.5	0.5-0.9	0.7-1	0.4-0.8	0.5-1.5	0.5-1.8	0.7-0.8	1.0-2.0	0.9-1.0	0.7-1.6	0.3-1.3
Cell size, Length μm (type species)	1.75	1-5	3.5-12.6	3-16.9	1.5-7.0	5.0-50	2-10	2-4	3.2-8	1.2-2.5	2-8	0.5-1.8	3.0-5.0	1.0-5.3	3.6-7.3	2.3-6.0	1.8-15.4
Cell shape	rods	rods	rods	rods	rods	rods	rods	rods	rods	rods	rods	cocci	rods	rods	rods	rods	rods
Gram staining	variable	-	+	+	variable	variable	+	+	+/-variable	+	variable	+	+	+/-variable	+	+	+/-variable
Oxygen requirement	anaerobe	anaerobe	anaerobe	obligate anaerobe	obligate anaerobe	obligate anaerobe	obligate anaerobe	obligate anaerobe	anaerobe	obligate anaerobe	obligate anaerobe	obligate anaerobe	obligate anaerobe	anaerobe	anaerobe	anaerobe	anaerobe
Spore formation	-	-	+	+	variable	+	+	+	-	-	variable	-	+	+	+	+	+
Motility	+	+	+	+	+/-	-	+	+	-	+	+	-	+	-	+	+	+
Temperature range (C°)	30-42 (42)	37	37	30-37	37	37	20-45	30-35	25-45	30-42 (37)	15-40	30-39 (37)	29-37 (29)	30-45 (37)	20-42 (37)	28-60	15-45
Predominant fatty acids	C18:1 ω 9c and C18:1 ω 9c DMA	ND	C16:0 C16:1 ω 7c	C16:0, C17:1 ISO I/C16:0 DMA	C _{16:0} DMA	iso-C15 :0 and C16 :0 DMA	C12 to C18	C16 :0	C18:1 ω 9c C18:2 ω 6 9c C18:0 iso-C17:0 iso-C15:0	C14:0	ND	C16:0 C18:1 C18:1 aldehyde	C14:0 C16:0	C16 :0	C14:0	iso-C15 :0, iso-C14 :0, C16 :0 iso-C13:0	C16 :0

ND= Not Determined

*Some data are not for the type species *Filifactor villosus* but for *Filifactor alocis*

Supplementary figures:

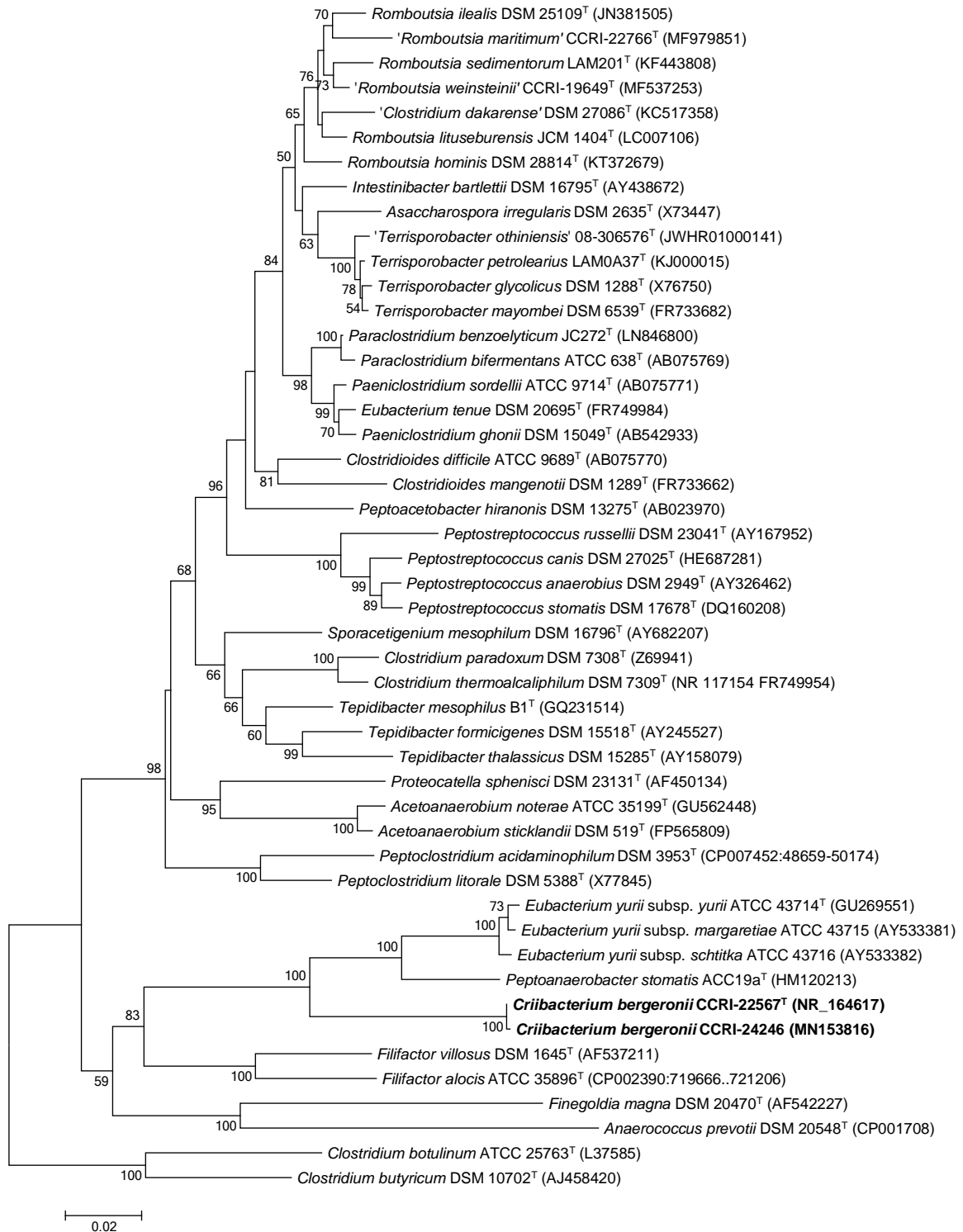


Fig. S1. Neighbor joining phylogenetic tree showing the relationship of *Crüibacterium bergeronii* within the family *Peptostreptococcaceae*. The 16S RNA sequences were aligned using MUSCLE software [13]. The phylogenetic tree was reconstructed using neighbor joining method on 1262 sites with the Maximum Composite Likelihood substitution model implemented in MEGA software version 6.06 [14]. Only bootstrap values >50% based on 1000 replications are shown at branching points. Sequences from type strains are indicated with a superscript T. Organisms with names not validly published are presented with quotation marks. GenBank accession numbers for each 16S rRNA gene sequence are given in parentheses. Each bar indicates 0.02 substitutions per position. *Clostridium botulinum* ATCC 25763^T and *Clostridium butyricum* DSM 10702^T (*Clostridium sensu stricto*) were used as an outgroup.

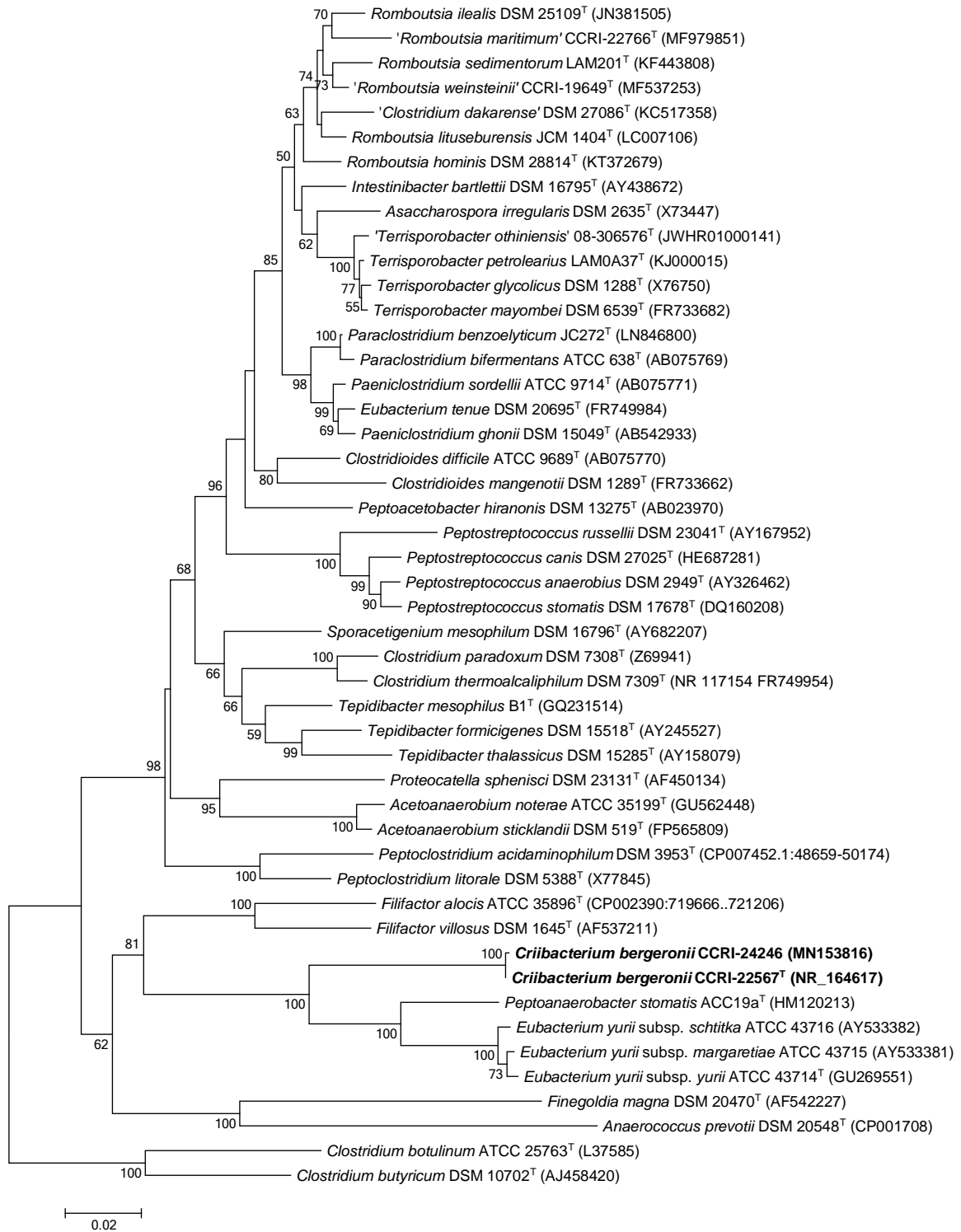


Fig. S2. Minimum evolution phylogenetic tree showing the relationship of *Crüibacterium bergeronii* within the family *Peptostreptococcaceae*. The 16S RNA sequences were aligned using MUSCLE software [13]. The phylogenetic tree was reconstructed using the minimum evolution method on 1262 sites with the Maximum Composite Likelihood substitution model implemented in MEGA software version 6.06 [14]. Only bootstrap values >50% based on 1000 replications are shown at branching points. Sequences from type strains are indicated with a superscript T. Organisms with names not validly published were presented with quotation marks. GenBank accession numbers for each 16S rRNA gene sequence are given in parentheses. Each bar indicates 0.02 substitutions per position. *Clostridium botulinum* ATCC 25763^T and *Clostridium butyricum* DSM 10702^T (*Clostridium sensu stricto*) were used as an outgroup.

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