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Novel microbiota *Mesosutterella faecium* sp. nov. has a protective effect against inflammatory bowel disease

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Running header: *Mesosutterella faecium* sp. nov. protects DSS-induced colitis

Keywords: gut microbiota, *Mesosutterella*, inflammation, inflammatory bowel disease, murine colitis model

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Supplementary Materials
Supplementary Tables

Biochemical analyses	**AGMB02718^T**
Growth temperature (℃)	
15	-
20	-
25	+
30	++
37	+++
40	++
45	+
50	-
Salt tolerance test (NaCl, %)	
0.5	+++
1	++
2	-
3	-
4	-
5	-
Catalase :	-
Oxidase :	-
Respiratory quinones :	MK-6, MMK-6

Supplementary Table S1. Biochemical characterization of strain AGMB02718^T. Growth temperature, salt tolerance, catalase, oxidase, and respiratory quinone analyses. Cells were grown in TSAB for 3 days at 37 ℃ in anaerobic conditions. +++, strongly positive; ++, positive; +, weakly positive; −, negative.

Characteristics	1	2
Acid production (API 20A) from :		
L-tryptophane	-	-
urea	-	-
D-glucose	-	-
D-mannitol	-	-
D-lactose (bovine origin)	-	-
D-saccharose (sucrose)	-	-
D-maltose	-	-
salicin	-	+
D-xylose	-	-
L-arabinose	-	-
gelatin (bovine origin)	-	-
esculin ferric citrate	-	-
glycerol	-	-
D-cellobiose	-	-
D-mannose	-	-
D-melezitose	-	-
D-raffinose	-	+
D-sorbitol	-	w
L-rhamnose	-	-
D-trehalose	-	-

Characteristics	1	2
Enzymatic activity (Rapid ID 32A and ZYM)		
Urease	-	-
Arginine dihydrolase	-	-
α-Galactosidase	-	-
β-Galactosidase	-	-
β-Galactosidase 6-phosphate	-	-
α-Glucosidase	-	-
β-Glucosidase	-	-
α-Arabinosidase	-	-
β-Glucuronidase	-	-
β-N-acetyl-glucosaminidase	-	-
Mannose fermentation	+	+
Raffinose fermentation	+	+
Glutamic acid decarboxylase	-	-
α-Fucosidase	-	-
Nitrate reduction	+	+
Indole production	-	-
Alkaline phosphatase	+	+
Arginine arylamidase	+	+
Proline arylamidase	w	w
Leucyl glycine arylamidase	w	w
Phenylalanine arylamidase	+	+
Leucine arylamidase	+	+
Pyroglutamic acid arylamidase	w	w
Tyrosine arylamidase	+	+
Alanine arylamidase	+	+
Glycine arylamidase	+	+
Histidine arylamidase	+	+
Glutamyl glutamic acid arylamidase	+	w
Serine arylamidase	w	+
Esterase (C4)	-	+
Esterase lipase (C8)	w	w
Lipase (C14)	-	-
Valine arylamidase	-	-
Crystine arylamidase	-	w
Trypsin	-	-
α-chymotrypsin	-	-
Acid phosphatase	+	+
Naphthol-AS-BI-phosphohydrolase	+	w
α-mannosidase	-	-

Supplementary Table S2. API 20A, Rapid ID 32A, and ZYM for AGMB02718^T and the reference strain. Strains: 1, AGMB02718^T; 2, *M. multiformis* DSM 106860^T. All data were obtained in this study, and for duplicates, only one experimental result is listed. Cells were grown in TSAB for 3 days at 37 °C in anaerobic conditions. +, positive; w, weak; −, negative.

Properties	**Values**
Genome assembly	
Assemble method	Flye 2.9.2, SPAdes 3.13.1
Sequencing technology	PacBio Sequel, Illumina NovaSeq
Annotation	NCBI PGAP
Genome features	
Genome size (bp)	2,606,253
Genome coverage	250.0×
G+C content (%)	62.2
No. of contigs	3
rRNA genes	15
tRNA genes	53
Open reading frame	2,278
CDS assigned by COG	1,801
GenBank Accession No.	JAKZJU020000000

Supplementary Table S3. Genomic characteristics of *Mesosutterella faecium* strain AGMB02718^T.

COG	Description	Number of Genes	Percentage (%)
J	Translation, ribosomal structure and biogenesis	149	8.27
A	RNA processing and modification	1	0.06
K	Transcription	132	7.33
L	**Replication, recombination and repair**	**180**	**9.99**
N	Cell motility	1	0.06
D	Cell cycle control, cell division, chromosome partitioning	31	1.72
V	Defense mechanisms	25	1.39
T	Signal transduction mechanisms	41	2.28
M	Cell wall/membrane/envelope biogenesis	121	6.72
U	Intracellular trafficking, secretion, and vesicular transport	56	3.11
O	Posttranslational modification, protein turnover, chaperones	66	3.66
C	**Energy production and conversion**	**190**	**10.55**
G	Carbohydrate transport and metabolism	40	2.22
E	**Amino acid transport and metabolism**	**173**	**9.61**
F	Nucleotide transport and metabolism	79	4.39
H	Coenzyme transport and metabolism	101	5.61
I	Lipid transport and metabolism	46	2.55
P	Inorganic ion transport and metabolism	92	5.11
Q	Secondary metabolites biosynthesis, transport and catabolism	13	0.72
S	Function unknown	264	14.66
Total		**1801**	**100**

Supplementary Table S4. Clusters of orthologous group (COGs) functional category analysis of 1,801 genes. The three major parts of the genes were COG categories C (energy production and conversion), E (amino acid transport and metabolism), and L (replication, recombination, and repair).

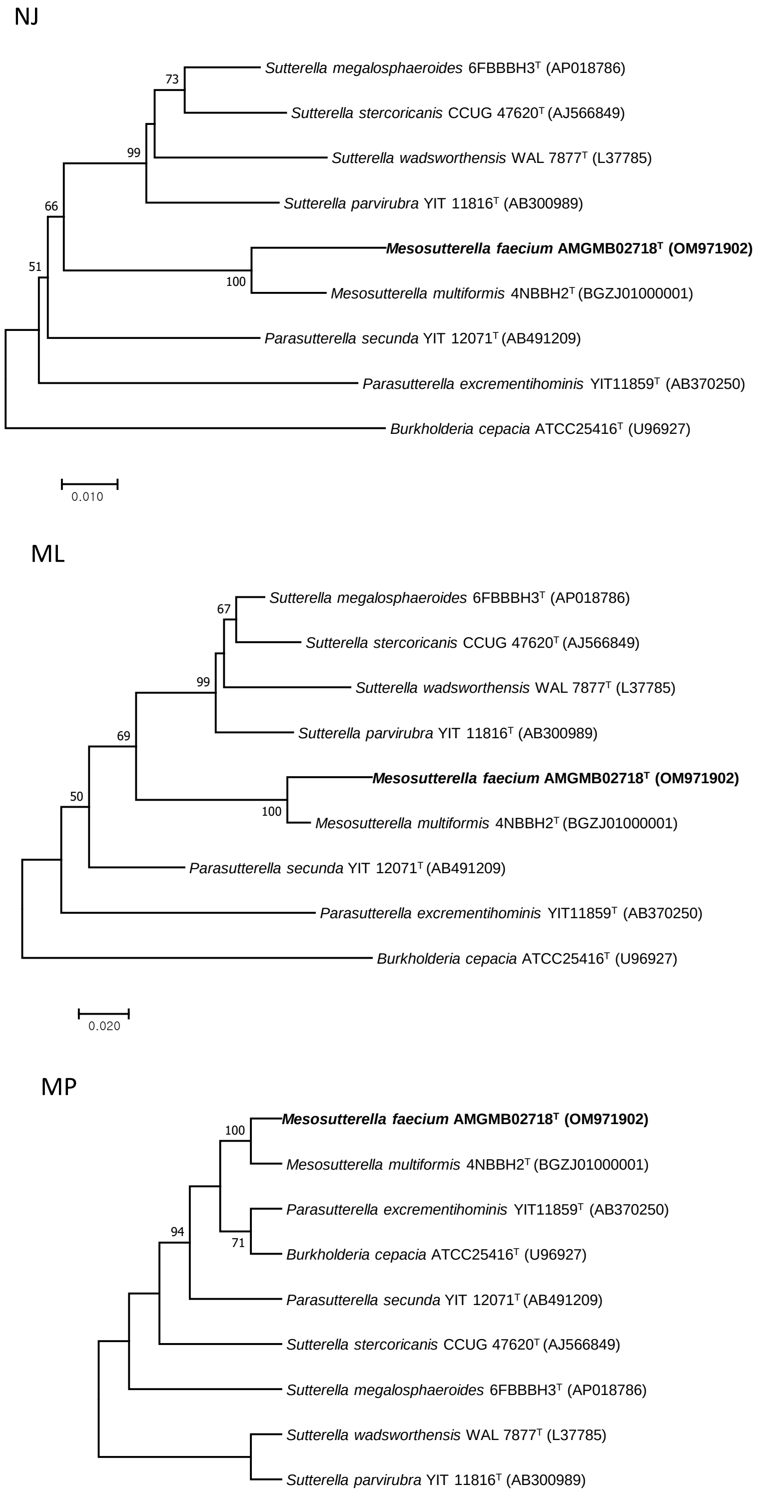
Assembly No.	**Strain**	**ANI**	**orthoANI**	**dDDH**
GCA_003402575.1	*Mesosutterella multiformis* 4NBBH2^T	72.88	72.13	20.9
GCF_003609995.1	*Sutterella megalosphaeroide* 6FBBBH3^T	70.30	69.10	21.5
GCA_003315195.1	*Sutterella wadsworthensis* DSM 14016^T	68.53	68.78	20.8
GCA_000250875.1	*Sutterella parvirubra* YIT 11816^T	70.40	67.07	19.6

Supplementary Table S5. ANI, orthoANI (%), and dDDH (%) values between AGMB02718^T and closely related type strains. Data were calculated using ANI calculator, Orthologous Average Nucleotide Identity Tool (OAT) software, and the GGDC web server.

Gene name	Description	EC number	Locus tag	AGMB02718^T
LpxA	UDP-N-acetylglucosamine acyltransferase	EC:2.3.1.129	MUN46_RS06795	+
LpxC	UDP-3-O-acyl-N-acetylglucosamine deacetylase	EC:3.5.1.108	MUN46_RS01520	+
LpxD	UDP-3-O-acyl-glucosamine N-acyltransferase	EC:2.3.1.191	MUN46_RS06785	+
LpxH	UDP-2,3-diacylglucosamine hydrolase	EC:3.6.1.54	MUN46_RS05595	+
LpxB	lipid-A-disaccharide synthase	EC:2.4.1.182	MUN46_RS06800	+
LpxK	tetraacyldisaccharide 4'-kinase	EC:2.7.1.130	MUN46_RS00875	+
WaaA	lipid IV$_A$ 3-deoxy-D-manno-octulosonic acid transferase	EC 2.4.99.12	MUN46_RS03340	+
	(Kdo)-lipid IV$_A$ 3-deoxy-D-manno-octulosonic acid transferase	EC 2.4.99.13		+
LpxL	Kdo$_2$-lipid IV$_A$ lauroyltransferase/acyltransferase	EC 2.3.1.241	MUN46_RS02870	+
LpxM	lauroyl-Kdo$_2$-lipid IV$_A$ myristoyltransferase	EC:2.3.1.243	–	–
LpxJ	Kdo$_2$-lipid IV$_A$ 3' secondary acyltransferase	EC:2.3.1.-	–	–

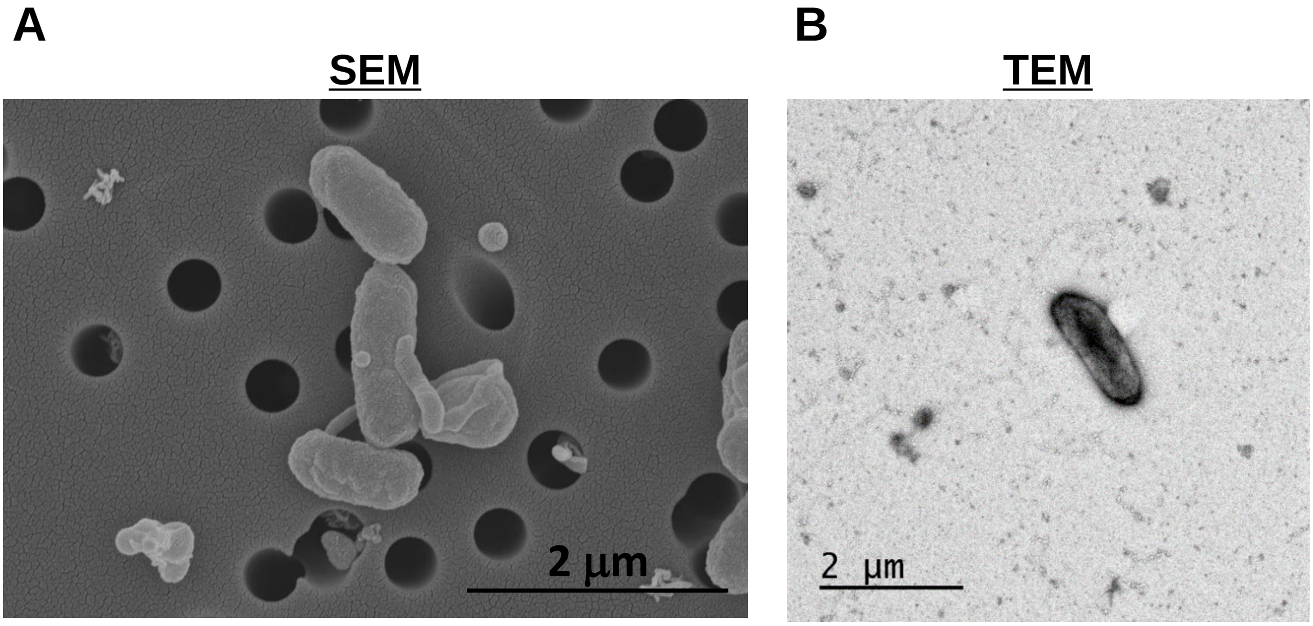
Supplementary Table S6. Genes encoding known Kdo$_2$-lipid A biosynthesis enzymes predicted in the AGMB02718^T genome. +, positive; −, negative.

Supplementary Figure

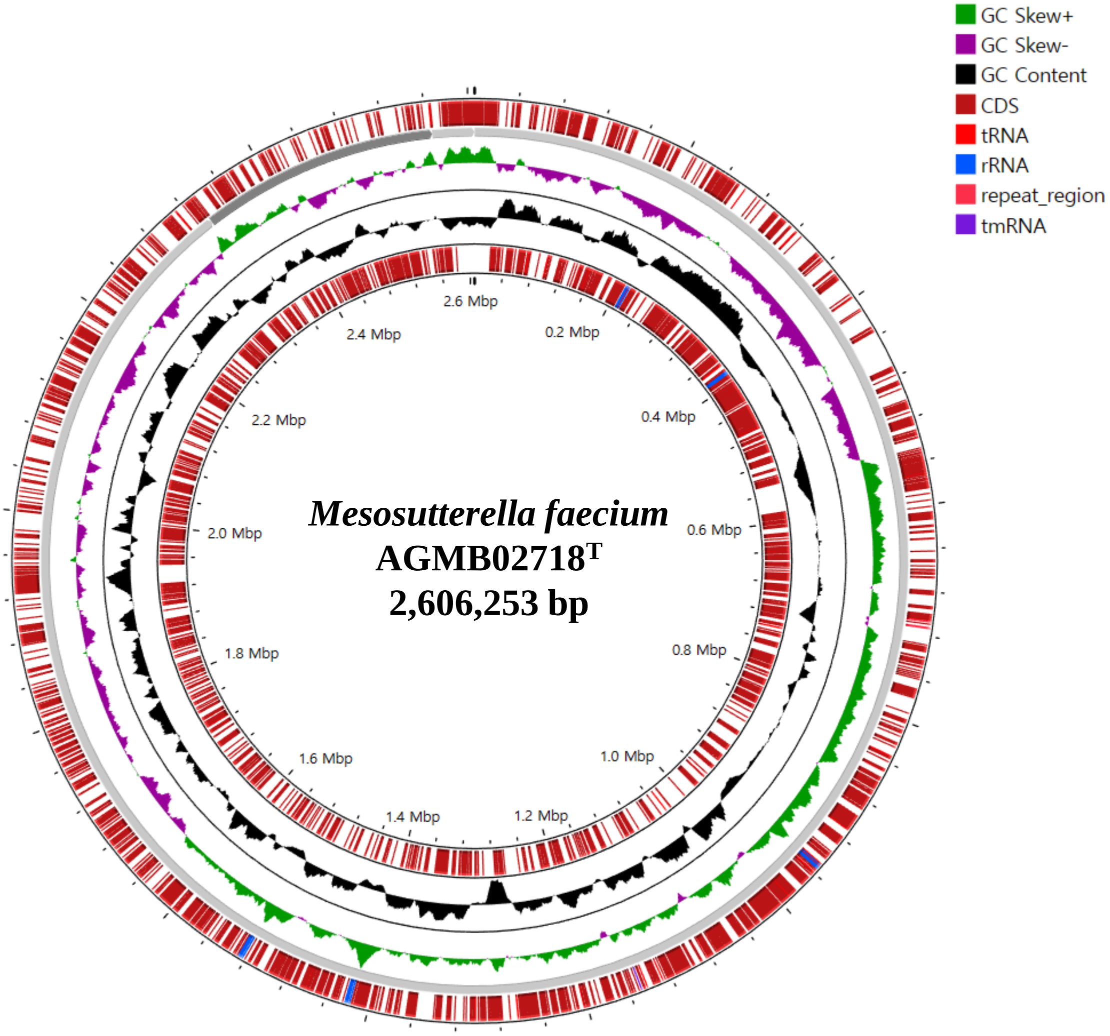


Supplementary Figure S1. Phylogenetic trees based on 16S rRNA gene sequences showing the position of strain AGMB02718^{T}. Bootstrap values (>50%) were calculated using the neighbor-joining (NJ), maximum likelihood (ML), and minimum parsimony (MP) algorithms. Scale bar: 0.010 (NJ), 0.020 (ML) substitutions per nucleotide position.

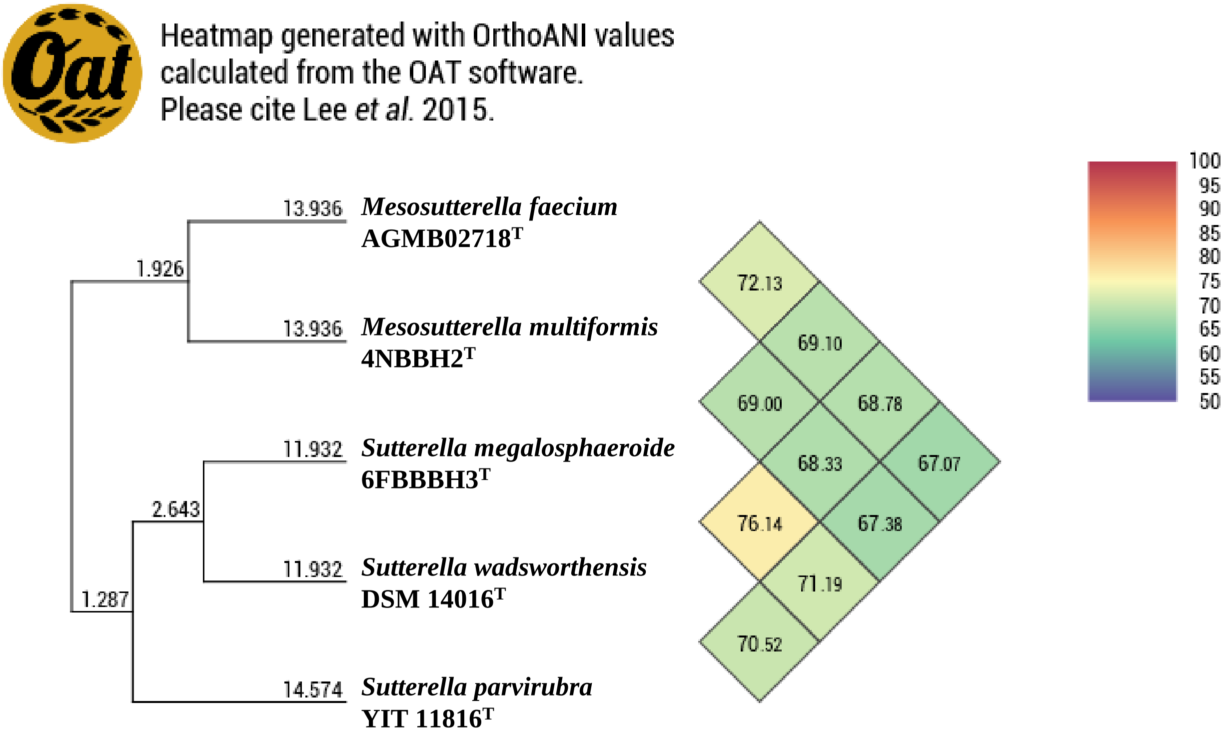
Supplementary Figures



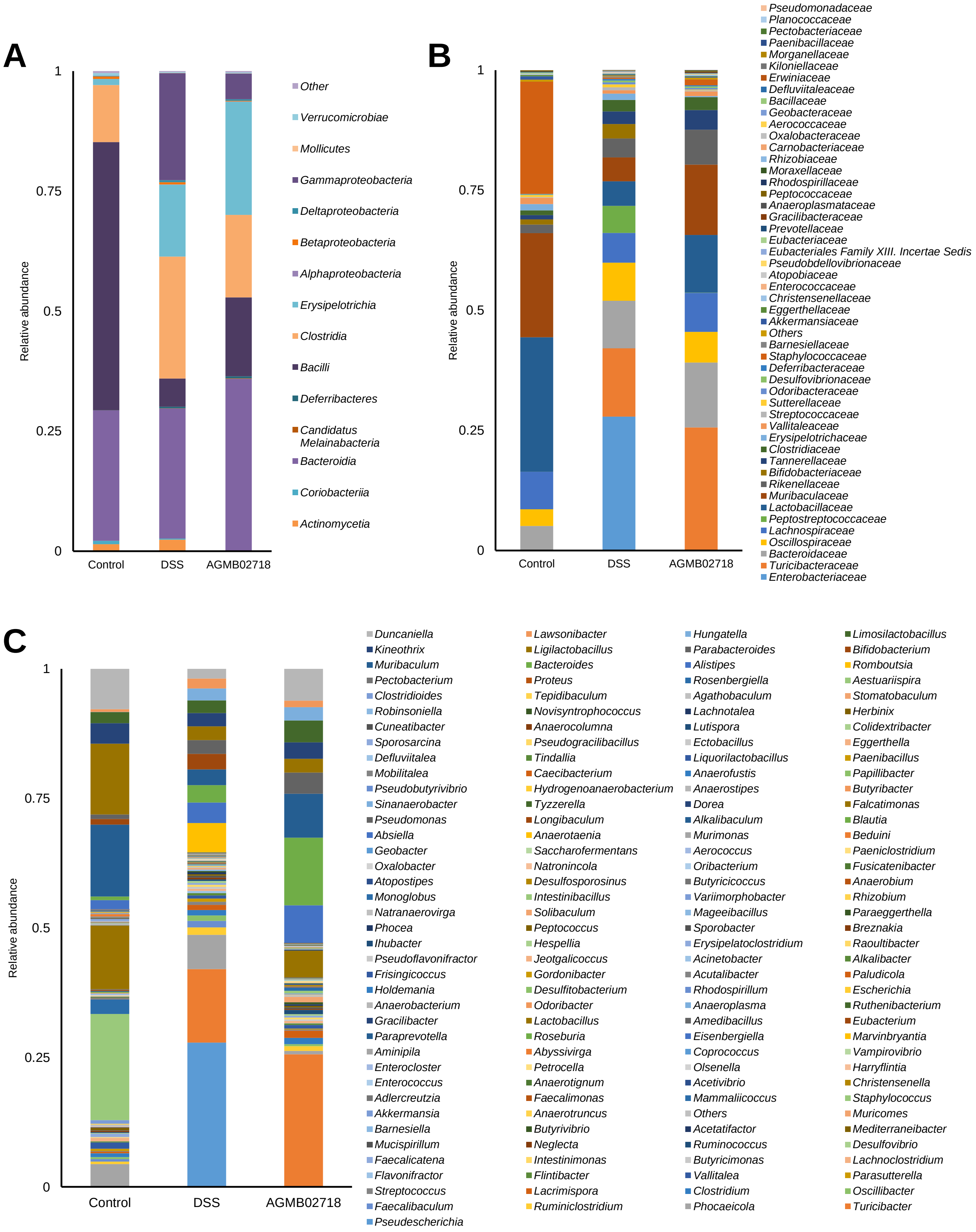
Supplementary Figure S2. Cell morphology of strain AGMB02718^T. Cells were grown in TSAB plates at 37 ℃ for 3 days in anaerobic conditions. Cells were analyzed using **(A)** scanning electron microscopy and **(B)** transmission electron microscopy. Bar indicates 2 μm.



Supplementary Figure S3. Map of the AGMB02718^T genome generated with CGView. From outside to the center: region coding genes (wine), GC skew (green/mauve), GC content (black), tRNA genes (red), rRNA genes (blue), and tmRNA genes (lightmauve).

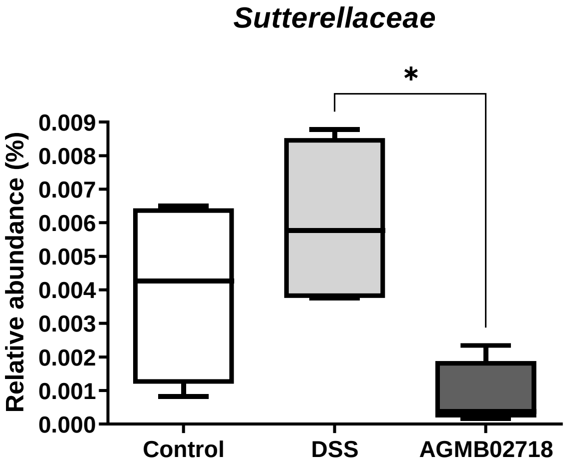


Supplementary Figure S4. Heatmap generated with OrthoANI. ANI value calculated using the OAT software, comparing strain AGMB02718^T and closely related strains.



Supplementary Figure S5. Relative abundance of taxa at the **(A)** class, **(B)** family, and **(C)** genus levels among the three groups.

Supplementary Figure



Supplementary Figure S6. Relative abundance of *Sutterellaceae* ratio among three groups. Data are shown as mean ± SEM (*n*=5). *P<0.05 by One-way ANOVA with Tukey's multiple comparisons.