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Article

Identification and Characterization of a Novel Species of Genus *Akkermansia* with Metabolic Health Effects in a Diet-Induced Obesity Mouse Model

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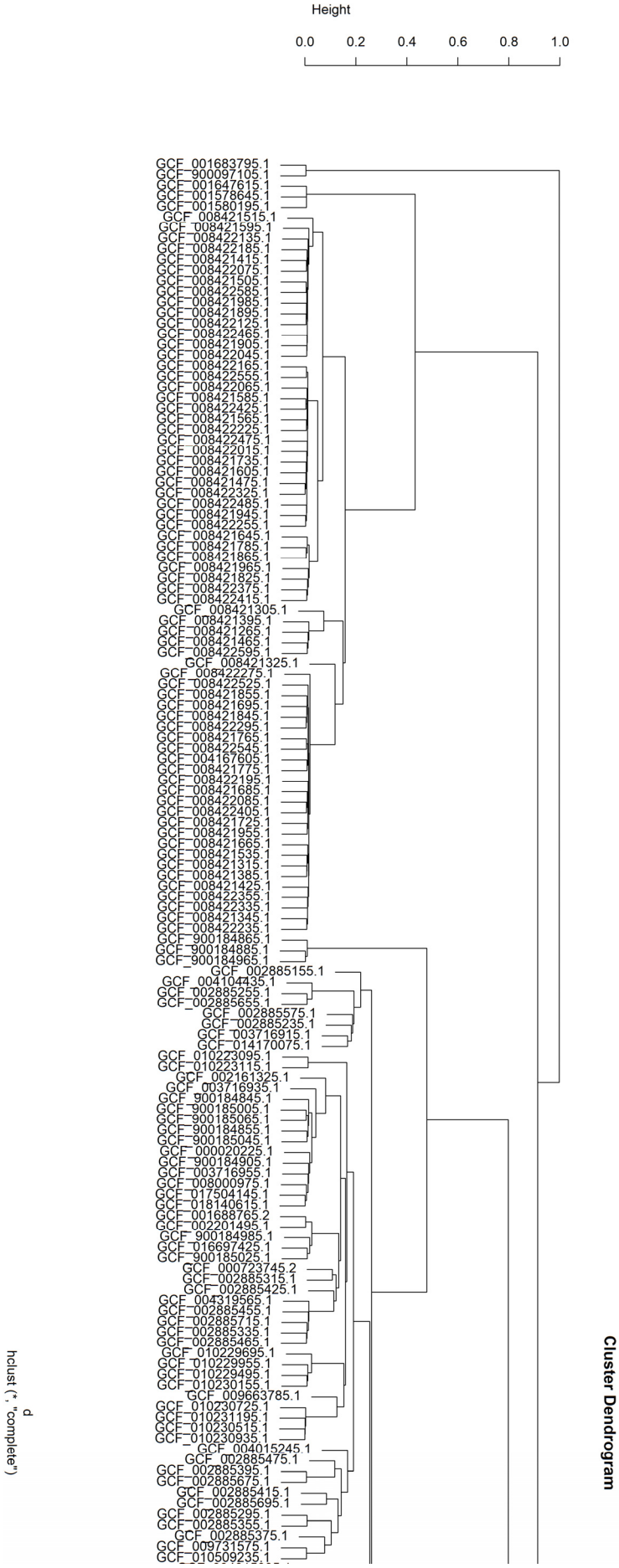
Supplementary Information

Figure S1: The protein sequences from 235 public *Akkermansia* genomes and DSM 33459 genome were clustered at 95% aa identity. The distance between each pair of genomes was estimated using the percent of clusters not shared between them. The clusters by hierarchical clustering were visualized in a dendrogram and a heatmap. The closer the genomes are, the smaller the distance on the dendrogram.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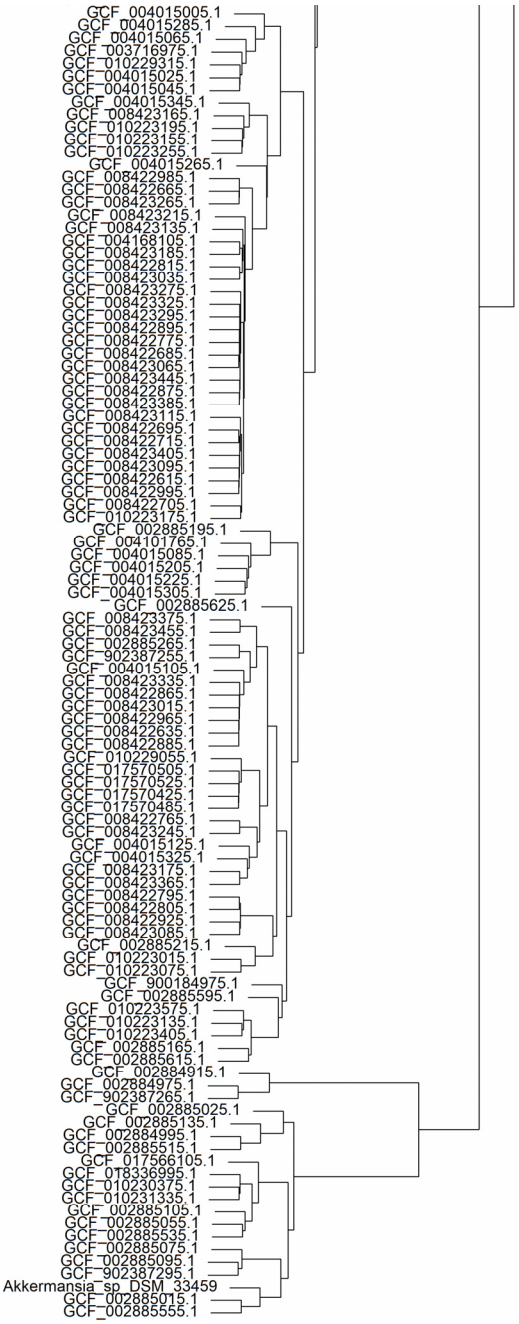
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Table S1. Susceptibility of the quality control strain *Bacteroides fragilis* DSM 2151 (ATCC 25285) to selected antimicrobials using Brucella broth (standard medium). The MIC Quality Control ranges for anaerobes, specifically for *B. fragilis* DSM 2151 (ATCC 25285), are shown in the right-hand column (CLSI standard M100, 31st ed., Table 5E) [68].

Antimicrobial	MIC (μg/mL)	*B. fragilis* DSM 2151 (ATCC 25285) (μg/mL)
Ampicillin	16	-[a]
Ampicillin-sulbactam	1/0.5	0.5/0.25-2/1
Benzylpenicillin	>8	-[a]
Penicillin	8	8-32
Gentamicin	>256	-[a]
Kanamycin	>512	-[a]
Streptomycin	512	-[a]
Clindamycin	1	0.5-2
Tetracycline	≤0.25	-[a]
Ciprofloxacin	2	-[a]
Colistin	>8	-[a]
Fosfomycin*	>128	-[a]
Vancomycin	64	-[a]
Erythromycin	4	-[a]
Chloramphenicol	4	4-16
Imipenem	0.12	0.03-0.25
Meropenem	0.06	0.03-0.25
Metronidazole	0.5	0.25-2
Trimethoprim	8	-[a]

[a] No MIC values for ampicillin, benzylpenicillin, gentamicin, kanamycin, streptomycin, tetracycline, ciprofloxacin, colistin, fosfomycin vancomycin, erythromycin or trimethoprim are available against the quality control strain *Bacteroides fragilis* DSM2151 (ATCC 25285).

*Reinforced medium for Clostridia agar were used

Table S2. Susceptibility of the quality control strain *Bacteroides fragilis* DSM 2151 (ATCC 25285) to selected antimicrobials using YCFAC broth (growth medium for *Akkermansia sp.* DSM 33459). The MIC Quality Control ranges for anaerobes, specifically for *B. fragilis* DSM 2151 (ATCC 25285), are shown in the right-hand column (CLSI standard M100, 31st ed., Table 5E) [68].

Antimicrobial	MIC (μg/mL)	*B. fragilis* DSM 2151 (ATCC 25285) (μg/mL)
Ampicillin	>32	-[a]
Ampicillin-sulbactam	2/1	0.5/0.25-2/1
Benzylpenicillin	>8	-[a]
Penicillin	>16	8-32
Gentamicin	>256	-[a]
Kanamycin	>512	-[a]
Streptomycin	>512	-[a]
Clindamycin	0.5	0.5-2
Tetracycline	0.5	-[a]
Ciprofloxacin	4	-[a]
Colistin	>8	-[a]
Fosfomycin*	>128	-[a]
Vancomycin	64	-[a]
Erythromycin	4	-[a]
Chloramphenicol	4	4-16
Imipenem	>32	0.03-0.25
Meropenem	>32	0.03-0.25
Metronidazole	2	0.25-2
Trimethoprim	>64	-[a]

[a] No MIC values for ampicillin, benzylpenicillin, gentamicin, kanamycin, streptomycin, tetracycline, ciprofloxacin, colistin, fosfomycin vancomycin, erythromycin or trimethoprim are available against the quality control strain *Bacteroides fragilis* DSM2151 (ATCC 25285).

*Reinforced medium for Clostridia agar were used

Table S3: Genome comparison between *Akkermansia* sp. DSM 33459 and *A. muciniphila* ATCC BAA-835 or the presence of Corrin ring biosynthesis gene cluster.

Gene	***Akkermansia sp.* DSM 33459**	***A. muciniphilia* ATCC BAA-835**	**ECnumber**	**product**
cbiA	1	0	6.3.5.11	Cobyrinate a,c-diamide synthase
cbiC	1	0	5.4.99.60	Cobalt-precorrin-8 methylmutase
cbiD	1	0	2.1.1.195	Cobalt-precorrin-5B C(1)-methyltransferase
cbiET	1	0	unknownEC	Cobalamin biosynthesis bifunctional protein CbiET
cbiF	1	0	2.1.1.271	Cobalt-precorrin-4 C(11)-methyltransferase
cbiKp	1	0	4.99.1.3	Sirohydrochlorin cobaltochelatase CbiKP
cbiL	1	0	2.1.1.151	Cobalt-precorrin-2 C(20)-methyltransferase
chiA1	1	0	3.2.1.14	Chitinase A1
cobO	1	0	2.5.1.17	Cob(I)yrinic acid a,c-diamide adenosyltransferase

Table S4: Global Liver proteomics analysis: Significant proteins are distinctly regulated in DIO mice in Akk^{Gly} and Liraglutide groups.

Peroxisomal proteins	Gene	AkkGly*	Liraglutide*	AkkGly/Vehicle#	Liraglutide/Vehicle#
Peroxisomal bifunctional enzyme	Ehhadh	-2.29	-4.89	1.17	1.35
Peroxisomal coenzyme A diphosphatase NUDT7	Nudt7	-2.43	-3.39	1.26	1.29
Peroxisomal membrane protein 4	Pxmp4	0.49	-3.38	0.86	1.98
Peroxisome assembly factor 2	Pex6	-3.56	-3.34	1.67	1.57
3-ketoacyl-CoA thiolase A, peroxisomal	Acaa1a	-0.87	-3.34	1.06	1.35
Peroxisomal targeting signal 1 receptor	Pex5	-2.3	-3.29	1.21	1.36
Peroxisomal carnitine O-octanoyltransferase	Crot	-0.2	-2.9	0.94	1.32
Glutathione peroxidase 1	Gpx1	0.1	-2.9	1.06	1.26
Peroxisomal membrane protein 2	Pxmp2	-2.62	-2.54	1.19	1.20
Copper chaperone for superoxide dismutase	Ccs	-0.55	-2.52	1.03	1.23
Peroxisomal membrane protein 11A	Pex11a	-2.19	-1.95	1.20	1.15
Peroxiredoxin-5, mitochondrial	Prdx5	-2.45	-0.8	1.15	1.07
Peroxisomal membrane protein PEX14	Pex14	-0.28	2.00	0.97	0.71
Peroxisomal multifunctional enzyme type 2	Hsd17b4	0.33	2.12	0.96	0.85
3-ketoacyl-CoA thiolase B, peroxisomal	Acaa1b	1.91	2.12	0.81	0.72
Peroxisomal sarcosine oxidase	Pipox	1.22	2.45	0.97	0.92
Glutathione peroxidase 3	Gpx3	0.51	2.61	0.96	0.73
Peroxisomal trans-2-enoyl-CoA reductase	Pecr	-0.48	2.74	1.02	0.88
Peroxiredoxin-2	Prdx2	-0.38	2.74	1.01	0.88
Peroxisomal 2,4-dienoyl-CoA reductase	Decr2	0.50	3.02	0.82	0.66
Thioredoxin-dependent peroxide reductase, mitochondrial	Prdx3	-0.04	3.22	0.98	0.75
Peroxisomal acyl-coenzyme A oxidase 1	Acox1	1.90	3.64	0.85	0.75
Peroxisomal membrane protein PEX16	Pex16	1.41	3.66	0.92	0.79
Peroxisomal biogenesis factor 19	Pex19	3.18	3.77	0.48	0.14
Peroxisomal membrane protein PMP34	Slc25a17	1.61	3.86	0.85	0.56
Peroxisome biogenesis factor 1	Pex1	-1.64	3.91	1.03	0.87
Peroxisomal biogenesis factor 3	Pex3	1.15	6.12	0.89	0.58
Peroxisomal membrane protein PEX13	Pex13	0.72	6.28	0.77	0.14
Peroxisomal membrane protein 11C	Pex11g	1.12	7.95	0.95	0.66
			*Z-scores		#Fold