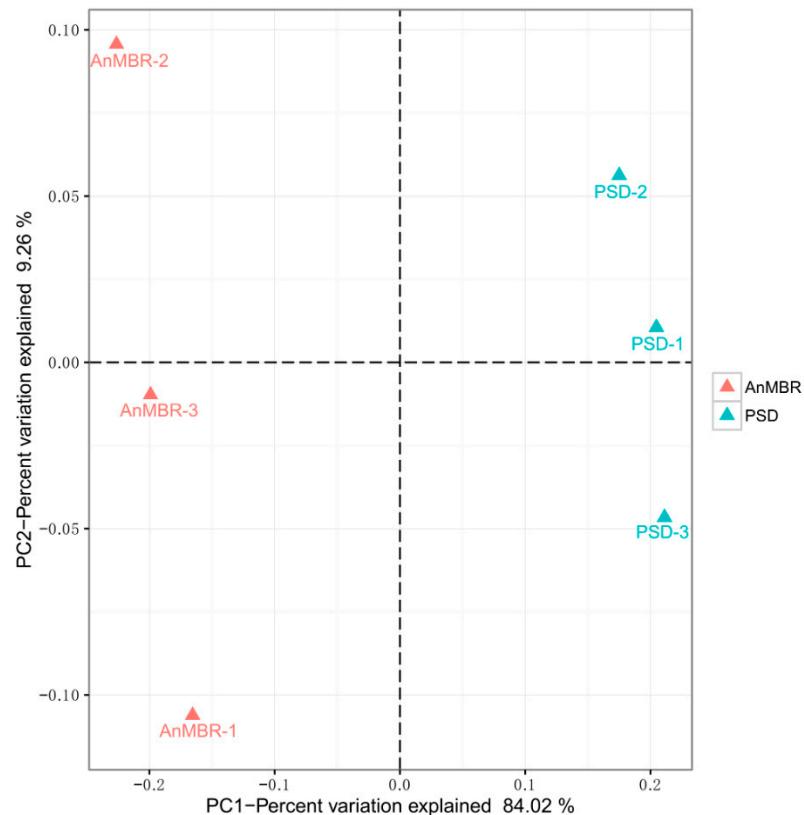
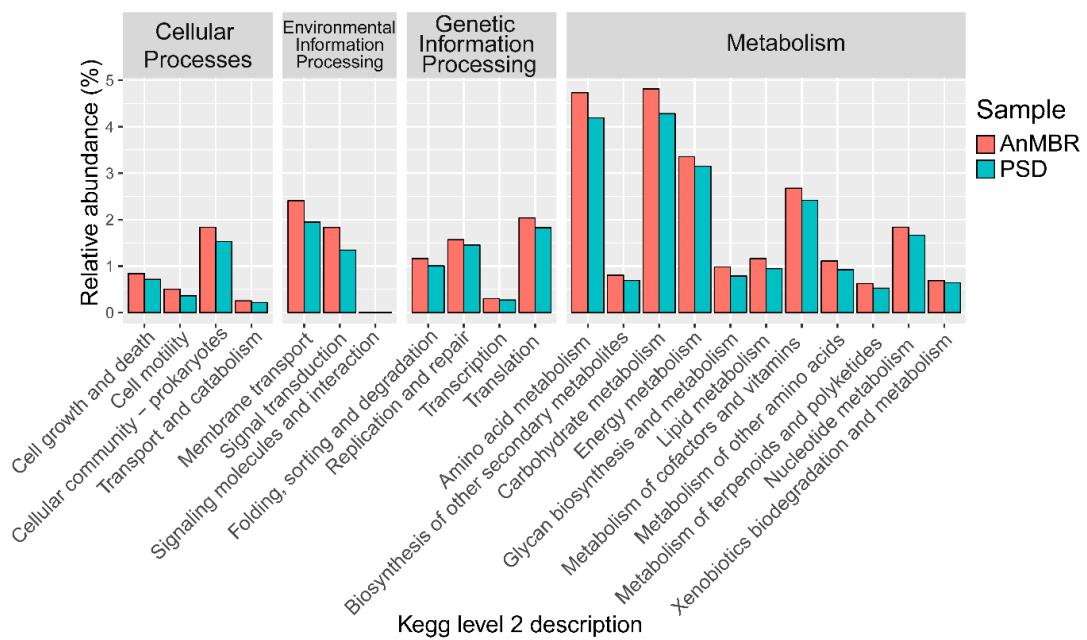


# Genome-Centered Metagenomics Analysis Reveals the Microbial Interactions of a Syntrophic Consortium during Methane Generation in a Decentralized Wastewater Treatment System

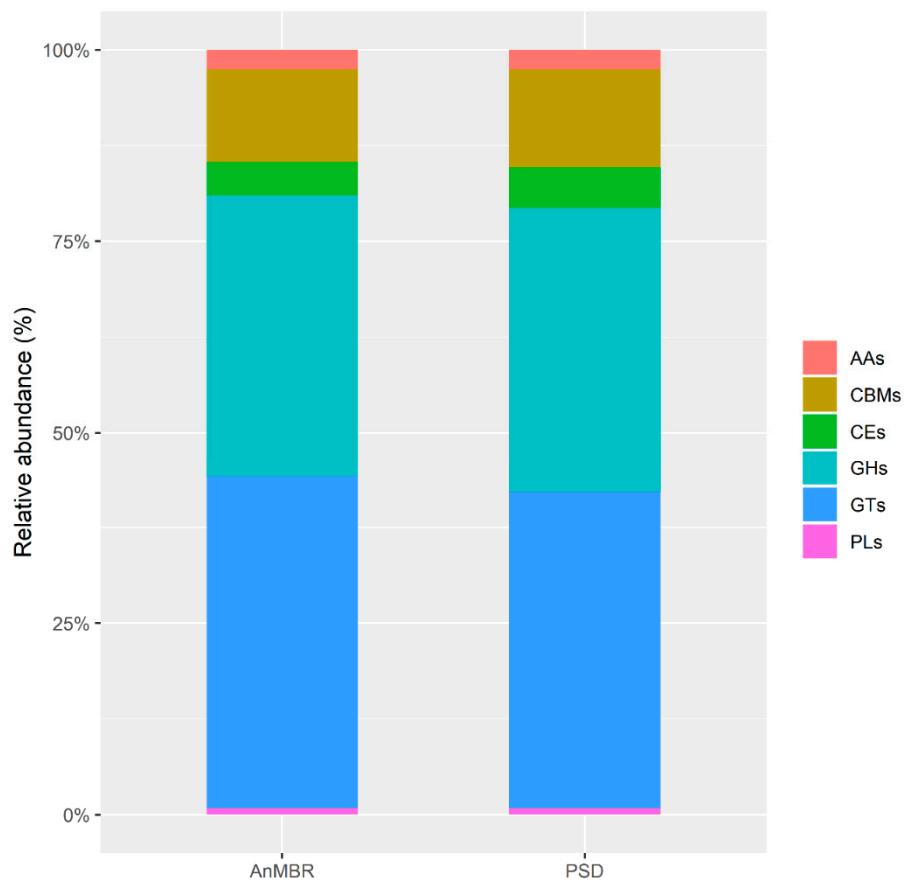
Kun Zhang <sup>1</sup>, Yan-Ling Zhang <sup>2,\*</sup>, Xin Ouyang <sup>1</sup>, Jun-Peng Li <sup>1</sup>, Jun-Jie Liao <sup>1</sup>, Ao You <sup>1</sup>, Xiu Yue <sup>1</sup>, Guang-Jian Xie <sup>1</sup>, Jie-Liang Liang <sup>3</sup> and Jin-Tian Li <sup>3,\*</sup>



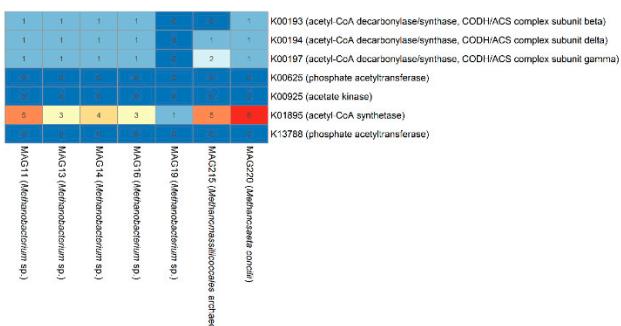
**Figure S1.** Weighted PCoA clustering of the microbial communities in the PSD (blue triangles) and AnMBR (red triangles) samples based on the UniFrac distance.



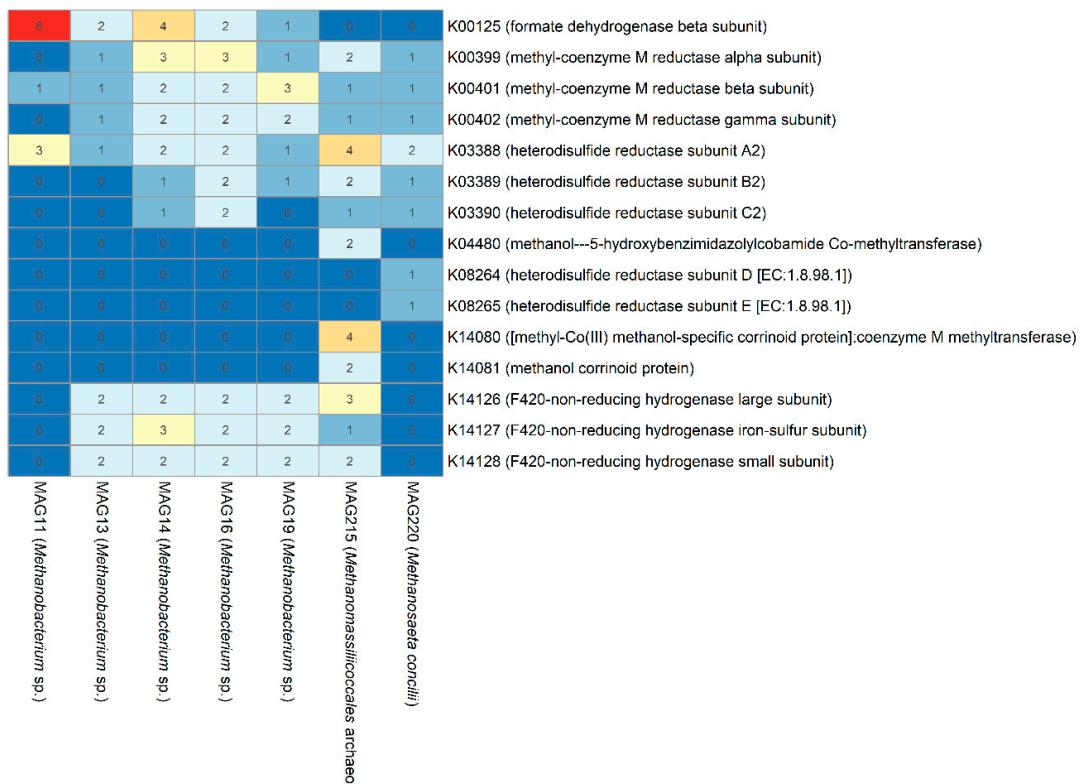
**Figure S2.** Relative abundances of the KEGG categories of functional genes in the PSD and AnMBR metagenomes.



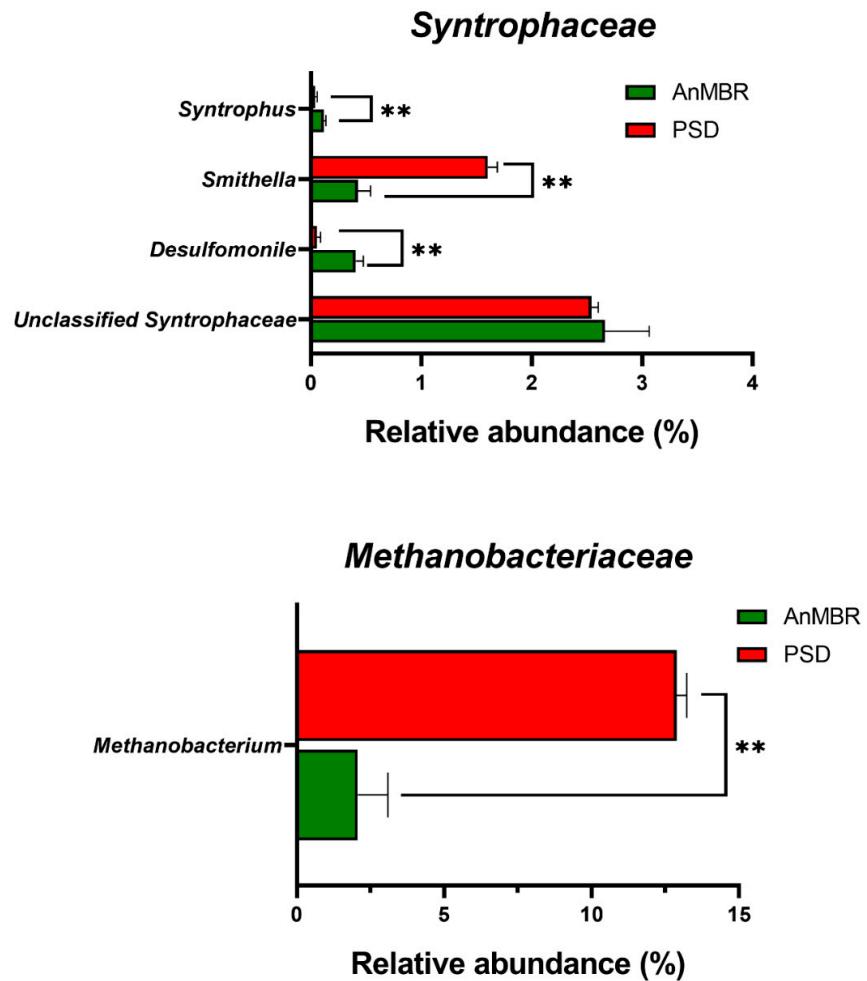
**Figure S3.** Read abundance of CAZyme in the PSD and AnMBR metagenomes.



**Figure S4.** Numbers of genes involved in the relevant acetotrophic methanogenesis pathway in archaeal MAGs.



**Figure S5.** Numbers of genes involved in the relevant methylotrophic methanogenesis pathway in archaeal MAGs.



**Figure S6.** The relative abundances of *Syntrophaceae* and *Methanobacteriaceae* in PSD and AnMBR.

**Table S1.** Physicochemical characteristics of the influent and effluent.

Sampling Point.	Influent	AnMBR	Influent of PSD	PSD	Effluent
<b>Conductivity (mS/min)</b>	<b>15.02</b>	<b>15.75</b>	<b>17.25</b>	<b>27.7</b>	<b>17.35</b>
pH	8.04	7.11	7.13	7.25	7.42
TS (%)	0.09	4.11	0.7	4.24	
VS (%)	0.06	2.87	0.62	2.87	
COD (mg/L)	712		761	3007	340
N total (mg/L)	165		183	118	92.4
NH <sub>4</sub> -N (mg/L)	98.8		157	104	111
NO <sub>2</sub> -N (mg/L)	<0.2		<0.2	<0.2	<0.2
NO <sub>3</sub> -N (mg/L)	0.877		1.51	2.53	0.795
P total (mg/L)	19.96		30.9	30.9	14
PO <sub>4</sub> -P (mg/L)	9.37		29.9	25.6	14.1
SO <sub>4</sub> <sup>2-</sup> ( mg/L)	34.1		28.9	52.4	2
TC (ppm)	49.5		80.6	321.4	63.2
TIC (ppm)	32.4		17.7	32.4	33.1
TOC (ppm)	17.1		62.9	289	30.1
Alkalinity (mM/L)	11.165				12.924

**Table S2.** Sequence numbers and alpha diversity of 16S rRNA genes in AnMBR and PSD.

Samples	No. sequences	Alpha diversity indices <sup>a</sup>						PD
		Chao 1	Observed OTUs	Shanno n	Ace	Simpson	Goods' coverage	
AnMBR-1	41,310	1349	541	7.387	136 9	0.9875	0.89213	51.9 8
AnMBR-2	22,564	2255	608	6.884	227 6	0.9689	0.85595	55.3 8
AnMBR-3	28,338	772	386	6.765	711	0.9785	0.93668	41.2 7
PSD-1	16,126	1879	491	5.936	187 0	0.9444	0.88141	55.2 9
PSD-2	19,897	2429	523	5.969	228 5	0.9450	0.86901	58.6 5
PSD-3	20,071	2342	546	6.015	231 0	0.9368	0.86365	58.1 6

<sup>a</sup> All samples were subsampled to 16,126 sequences before alpha diversity indices calculation.

**Table S3.** The difference of alpha diversity indices of 16S rRNA genes between PSD and AnMBR samples.

	Chao 1	Observed OTUs	Shanno n	Ace	Simpson	Goods' coverage	PD
Average of AnMBR	1459	512	7.012	1452.15 76	0.97828 01	0.89492	49.5435 4
Average of PSD	2217	520	5.973	2155.08 41	0.94203 21	0.87136	57.3674 43
Significant difference between AnMBR and PSD (P value)	0.178	0.908	<b>0.006</b>	0.213	<b>0.004</b>	0.381	0.149

**Table S4.** Taxonomic profiling of 16S rRNA genes at the family level of in AnMBR and PSD <sup>a</sup>.

Samples.	Taxonomics	
	<i>Methanobacteriaceae</i>	<i>Syntrophaceae</i>
AnMBR-1	2.03%	3.27%
AnMBR-2	3.13%	4.02%
AnMBR-3	1.08%	3.54%
PSD-1	13.37%	4.15%
PSD-2	12.68%	4.34%
PSD-3	12.92%	4.22%

<sup>a</sup> Only showed the taxa with relative abundance larger than 1%.

**Table S5.** The KEGG categories of PSD and AnMBR metagenomes <sup>a,b</sup>.

Level 2	Level 3	Samples	
		PSD	AnMBR
<b>Amino acid metabolism</b>	Alanine, aspartate and glutamate metabolism [PATH:ko00250]	0.77 %	0.94%
Amino acid metabolism	Cysteine and methionine metabolism [PATH:ko00270]	0.75 %	0.85%
Amino acid metabolism	Glycine, serine and threonine metabolism [PATH:ko00260]	0.77 %	0.84%

Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism [PATH:ko00520]	0.75 %	0.86%
Carbohydrate metabolism	Butanoate metabolism [PATH:ko00650]	0.73 %	0.80%
Carbohydrate metabolism	Citrate cycle (TCA cycle) [PATH:ko00020]	0.66 %	0.70%
Carbohydrate metabolism	Glycolysis / Gluconeogenesis [PATH:ko00010]	0.92 %	1.00%
Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism [PATH:ko00630]	0.78 %	0.83%
Carbohydrate metabolism	Pentose phosphate pathway [PATH:ko00030]	0.50 %	0.56%
Carbohydrate metabolism	Propanoate metabolism [PATH:ko00640]	0.55 %	0.63%
Carbohydrate metabolism	Pyruvate metabolism [PATH:ko00620]	1.01 %	1.10%
Cellular community - prokaryotes	Quorum sensing [PATH:ko02024]	1.13 %	1.30%
Energy metabolism	Carbon fixation pathways in prokaryotes [PATH:ko00720]	0.94 %	1.00%
<b>Energy metabolism</b>	<b>Methane metabolism [PATH:ko00680]</b>	<b>1.05 %</b>	<b>0.88%</b>
Energy metabolism	Oxidative phosphorylation [PATH:ko00190]	0.66 %	0.79%
Genetic information processing	Chaperones and folding catalysts [BR:ko03110]	0.60 %	0.75%
Genetic information processing	Chromosome and associated proteins [BR:ko03036]	0.82 %	1.00%
Genetic information processing	DNA repair and recombination proteins [BR:ko03400]	2.17 %	2.46%
Genetic information processing	DNA replication proteins [BR:ko03032]	0.97 %	0.98%
Genetic information processing	Messenger RNA biogenesis [BR:ko03019]	0.55 %	0.62%
Genetic information processing	Mitochondrial biogenesis [BR:ko03029]	0.89 %	1.04%
Genetic information processing	Replication and repair	0.51 %	0.53%
Genetic information processing	Ribosome [BR:ko03011]	0.62 %	0.74%
Genetic information processing	Ribosome biogenesis [BR:ko03009]	0.75 %	0.92%
Genetic information processing	Transcription factors [BR:ko03000]	0.51 %	0.62%
Genetic information processing	Transcription machinery [BR:ko03021]	0.52 %	0.65%
Genetic information processing	Transfer RNA biogenesis [BR:ko03016]	1.78 %	2.01%
Membrane transport	ABC transporters [PATH:ko02010]	1.51 %	1.81%
Metabolism	Amino acid related enzymes [BR:ko01007]	1.28 %	1.51%
Metabolism	Enzymes with EC numbers	1.62 %	1.77%
Metabolism	Peptidases [BR:ko01002]	1.09 %	1.34%
Metabolism	Peptidoglycan biosynthesis and degradation proteins [BR:ko01011]	0.57 %	0.69%
Nucleotide metabolism	Purine metabolism [PATH:ko00230]	1.13 %	1.27%
Nucleotide metabolism	Pyrimidine metabolism [PATH:ko00240]	0.79 %	0.81%

Poorly characterized	Function unknown	1.26 %	1.35%
Replication and repair	DNA replication [PATH:ko03030]	0.57 %	0.53%
Replication and repair	Homologous recombination [PATH:ko03440]	0.58 %	0.69%
Replication and repair	Mismatch repair [PATH:ko03430]	0.56 %	0.63%
Signal transduction	Two-component system [PATH:ko02020]	1.01 %	1.46%
Signaling and cellular processes	Bacterial motility proteins [BR:ko02035]	0.55 %	0.76%
Signaling and cellular processes	Exosome [BR:ko04147]	1.18 %	1.36%
Signaling and cellular processes	Prokaryotic defense system [BR:ko02048]	1.04 %	1.17%
Signaling and cellular processes	Secretion system [BR:ko02044]	0.76 %	0.99%
Signaling and cellular processes	Transporters [BR:ko02000]	4.34 %	5.41%
Translation	Aminoacyl-tRNA biosynthesis [PATH:ko00970]	1.08 %	1.22%
Translation	Ribosome [PATH:ko03010]	0.62 %	0.74%

<sup>a</sup> The e-value cutoff of  $e^{-10}$  and minimum alignment length of 150nt was used as the annotation parameters. <sup>b</sup> Only showed the categories with relative abundance higher than 0.5%.

**Table S6.** Number of reads annotated as genes affiliated with CAZyme in AnMBR and PSD metagenomes <sup>a</sup>.

CAZyme	Read counts		
	AnMBR	PSD	
<b>Auxiliary activities</b>			
AA1	4693	6747	
AA3_2	974	1521	
AA5	745	807	
AA0	315	583	
AA3	296	445	
AA6	284	471	
AA10	90	51	
AA1_2	80	28	
AA5_2	28	27	
AA4	26	80	
AA1_1	21	11	
AA1_3	21	20	
AA7	16	32	
AA15	13	17	
AA12	9	2	
AA8	7	0	
AA9	2	2	
AA13	1	0	
AA2	1	2	
AA3_3	1	7	
AA5_1	1	0	
AA14	0	1	
AA3_4	0	4	
<b>Carbohydrate binding modules</b>	CBM50	33718	44803

CBM48	13181	15067
CBM13	4967	5890
CBM5	3939	4396
CBM32	3884	4452
CBM6	3542	6862
CBM51	3533	4142
CBM20	2354	3236
CBM2	2112	2463
CBM57	1414	1976
CBM9	1299	871
CBM35	1296	2088
CBM67	993	2088
CBM41	945	625
CBM34	870	530
CBM38	801	1639
CBM26	764	2225
CBM22	716	637
CBM3	640	612
CBM47	617	1762
CBM54	599	914
CBM12	561	406
CBM62	347	125
CBM66	317	623
CBM4	312	1082
CBM23	278	344
CBM25	239	406
CBM0	223	239
CBM56	210	242
CBM61	158	94
CBM27	153	68
CBM73	138	23
CBM16	83	128
CBM14	82	72
CBM82	74	0
CBM83	71	0
CBM42	69	99
CBM37	66	48
CBM74	66	27
CBM8	56	34
CBM53	53	15
CBM45	40	116
CBM85	37	47
CBM11	24	23
CBM70	24	31
CBM69	19	10
CBM68	18	4
CBM77	17	2
CBM10	16	32
CBM84	16	23
CBM1	12	13
CBM40	12	12
CBM71	12	5

	CBM44	11	22
	CBM59	11	13
	CBM60	9	21
	CBM64	9	25
	CBM18	8	12
	CBM79	8	0
	CBM36	6	2
	CBM43	5	4
	CBM65	5	0
	CBM30	3	5
	CBM58	3	0
	CBM17	2	0
	CBM28	2	0
	CBM21	1	0
	CBM78	1	0
	CBM80	1	0
	CBM46	0	4
	CBM52	0	1
	CBM63	0	1
	CBM72	0	1
	CBM76	0	1
<b>Carbohydrate esterases</b>	CE11	7295	7392
	CE4	5508	5400
	CE9	3661	5188
	CE0	1549	1867
	CE8	1540	2136
	CE12	1355	2343
	CE1	971	2164
	CE14	662	912
	CE15	467	561
	CE3	409	565
	CE6	402	611
	CE7	365	289
	CE2	91	65
	CE16	50	112
	CE5	5	39
	CE13	2	2
<b>Glycoside hydrolases</b>	GH23	21317	27703
	GH2	13089	18476
	GH3	12799	15249
	GH0	11363	15660
	GH13	8249	8494
	GH6	7937	8319
	GH19	7912	11089
	GH13_11	7094	8941
	GH28	6554	7163
	GH78	6129	9117
	GH13_30	5990	7374
	GH13_9	5937	7249
	GH92	5289	5527
	GH133	5230	4912
	GH18	4926	4839

GH77	4885	5897
GH94	4708	4489
GH57	4577	5621
GH31	4080	4305
GH1	4057	2425
GH101	3939	4794
GH43_11	3925	3639
GH36	3341	2098
GH33	3333	3252
GH106	3308	3137
GH73	3009	4121
GH20	2859	3490
GH38	2753	2465
GH65	2525	2703
GH32	2468	2331
GH4	2370	1637
GH29	2358	3721
GH16	2307	3975
GH53	2289	1885
GH37	2057	2295
GH35	2013	2908
GH105	1970	4566
GH97	1892	2812
GH13_26	1848	2338
GH103	1710	1714
GH95	1594	1324
GH72	1443	1469
GH10	1442	2453
GH127	1387	1368
GH51	1369	2691
GH25	1347	1515
GH39	1332	1249
GH17	1316	1163
GH24	1314	4696
GH102	1313	1586
GH55	1224	1600
GH130	1177	1915
GH42	1129	1102
GH109	1060	1447
GH13_8	1048	1131
GH9	1031	2145
GH13_3	1006	616
GH43_18	947	1466
GH27	869	789
GH13_14	815	962
GH50	811	651
GH144	727	1707
GH13_39	721	691
GH26	709	1067
GH15	697	1557
GH163	685	368
GH13_16	651	919

GH13_38	637	889
GH30_3	636	623
GH13_31	636	169
GH108	625	3409
GH116	611	813
GH13_20	606	158
GH99	595	821
GH13_18	591	177
GH8	552	284
GH141	550	1117
GH146	549	1288
GH43_10	514	1189
GH5	513	612
GH43	481	887
GH43_26	478	426
GH63	475	191
GH13_29	465	64
GH13_10	462	844
GH43_3	432	1282
GH5_38	430	354
GH114	427	256
GH88	426	240
GH13_23	416	858
GH125	402	802
GH115	383	1198
GH153	382	238
GH120	381	170
GH148	346	324
GH43_24	336	937
GH154	335	623
GH13_4	324	997
GH43_4	292	1064
GH165	291	119
GH13_5	288	78
GH140	283	715
GH5_7	270	150
GH43_12	270	295
GH117	265	586
GH43_29	262	787
GH43_34	251	617
GH104	250	380
GH89	241	234
GH47	234	86
GH13_19	228	211
GH13_32	208	198
GH136	205	368
GH81	202	232
GH13_28	201	162
GH112	194	42
GH5_4	192	32
GH145	192	485
GH5_13	187	437

GH43_9	184	43
GH156	184	63
GH43_17	182	185
GH67	181	804
GH84	178	147
GH76	163	869
GH137	158	464
GH110	153	217
GH142	150	431
GH123	148	110
GH74	147	151
GH43_2	146	167
GH12	146	199
GH5_1	142	316
GH13_13	142	167
GH43_35	140	96
GH119	134	149
GH64	130	123
GH13_21	130	130
GH85	129	33
GH71	127	41
GH43_1	126	748
GH139	126	281
GH135	126	220
GH147	121	149
GH151	120	71
GH43_28	119	662
GH5_46	118	429
GH13_41	113	5
GH11	111	471
GH49	109	20
GH43_5	103	86
GH138	95	375
GH149	94	36
GH43_16	93	109
GH5_2	92	432
GH93	89	113
GH44	89	65
GH5_45	88	13
GH143	88	281
GH13_36	87	56
GH70	86	5
GH66	84	6
GH91	83	48
GH30	83	163
GH5_18	80	53
GH30_1	79	29
GH13_37	75	24
GH13_33	75	102
GH43_22	70	46
GH159	70	40
GH43_19	66	41

GH5_36	64	300
GH43_27	64	10
GH113	63	50
GH128	61	166
GH43_31	60	480
GH62	59	49
GH129	59	45
GH13_27	57	65
GH87	54	43
GH13_12	54	7
GH86	51	62
GH161	47	18
GH30_4	46	32
GH5_25	45	412
GH5_48	43	47
GH5_5	41	52
GH30_2	41	48
GH13_7	41	19
GH121	41	14
GH48	40	52
GH43_7	36	29
GH58	34	174
GH54	33	139
GH43_33	33	15
GH43_32	32	36
GH158	29	58
GH13_42	27	49
GH30_5	26	18
GH5_41	25	29
GH5_44	24	5
GH46	24	37
GH5_21	23	15
GH59	21	20
GH68	20	2
GH5_8	20	5
GH107	20	31
GH5_54	19	0
GH13_6	17	22
GH5_10	16	6
GH43_8	16	135
GH152	16	23
GH5_28	15	4
GH164	14	1
GH90	13	168
GH5_39	12	8
GH5_34	12	35
GH5_11	12	19
GH52	10	12
GH96	9	3
GH79	9	13
GH5_37	9	2
GH5_26	8	10

	GH14	8	18
	GH5_55	7	29
	GH43_15	6	6
	GH131	6	6
	GH111	6	7
	GH160	5	5
	GH13_2	5	21
	GH100	5	1
	GH13_1	4	12
	GH98	3	27
	GH5_9	3	10
	GH5_12	3	18
	GH150	3	38
	GH5_29	2	6
	GH5_22	2	3
	GH43_37	2	2
	GH30_8	2	2
	GH30_6	2	0
	GH162	2	4
	GH13_22	2	0
	GH82	1	0
	GH75	1	1
	GH5_52	1	0
	GH5_50	1	0
	GH5_17	1	0
	GH45	1	13
	GH43_30	1	4
	GH43_23	1	2
	GH22	1	200
	GH13_40	1	7
	GH126	1	0
	GH5_47	0	2
	GH5_42	0	3
	GH5_35	0	3
	GH43_20	0	1
	GH13_25	0	1
	GH13_17	0	2
<b>Glycosyl transferases</b>			
	GT2	98165	119782
	GT4	84059	97803
	GT51	18982	19175
	GT28	10686	12551
	GT35	10643	12206
	GT0	10176	13096
	GT83	7724	8201
	GT5	6784	8020
	GT9	6710	6495
	GT84	6029	6026
	GT30	5484	5684
	GT13	4600	5236
	GT26	4280	4794
	GT19	3685	4134
	GT8	3425	4048

GT1	3377	5799
GT47	2818	3021
GT20	2612	4338
GT66	2269	4842
GT68	2058	2541
GT3	2029	1503
GT22	1703	1612
GT81	1416	2373
GT41	1116	1074
GT32	1051	1024
GT27	842	1080
GT7	622	1261
GT29	615	760
GT61	610	759
GT25	574	431
GT104	422	106
GT11	391	567
GT14	300	612
GT95	297	551
GT107	257	315
GT49	254	311
GT21	228	401
GT10	194	333
GT80	149	85
GT87	102	98
GT102	93	159
GT56	92	30
GT39	91	114
GT55	88	102
GT103	86	143
GT96	85	233
GT62	82	28
GT60	78	102
GT89	72	68
GT90	71	99
GT99	71	40
GT77	67	23
GT17	62	356
GT105	53	32
GT64	51	25
GT101	45	27
GT6	40	100
GT44	36	2
GT24	34	61
GT45	34	70
GT57	33	172
GT73	33	7
GT70	29	37
GT74	29	89
GT31	25	34
GT23	22	165
GT82	15	16

	GT34	14	8
	GT53	10	91
	GT100	9	7
	GT76	9	32
	GT48	8	2
	GT71	8	12
	GT106	6	6
	GT88	6	10
	GT52	4	0
	GT93	4	19
	GT12	3	26
	GT40	2	0
	GT75	2	7
	GT85	2	18
	GT92	2	0
	GT94	2	6
	GT97	2	1
	GT18	1	2
	GT37	1	0
	GT38	1	1
	GT43	1	0
	GT15	0	1
	GT63	0	1
<b>Polysaccharide lyases</b>			
	PL0	1300	1295
	PL1	1076	491
	PL1_2	407	2166
	PL12	329	425
	PL35	265	191
	PL11_1	244	817
	PL8	239	116
	PL10_1	181	100
	PL9_1	170	181
	PL9_2	138	132
	PL33_1	133	95
	PL11	87	368
	PL6	63	31
	PL33_2	62	83
	PL22_2	62	21
	PL22	59	25
	PL26	54	10
	PL15	53	78
	PL10_2	47	10
	PL17	46	40
	PL9	42	91
	PL17_1	37	25
	PL8_2	35	17
	PL5_1	33	0
	PL22_1	32	13
	PL10_3	31	23
	PL4_2	29	4
	PL14	28	83
	PL21	27	21

PL8_3	25	19
PL17_2	24	3
PL1_5	23	47
PL6_1	20	33
PL12_1	19	7
PL7	18	21
PL27	18	4
PL11_2	18	78
PL10	18	24
PL7_5	17	14
PL1_12	17	22
PL7_1	16	0
PL2_2	16	2
PL2_1	16	2
PL7_3	13	5
PL37	12	1
PL9_3	11	13
PL13	11	10
PL5	10	21
PL12_2	10	6
PL4	9	26
PL29	9	9
PL21_1	9	31
PL15_1	8	15
PL30	6	0
PL12_3	6	6
PL1_1	5	2
PL6_3	4	0
PL4_1	4	14
PL14_3	4	4
PL6_2	2	7
PL4_3	2	6
PL15_2	2	0
PL1_8	2	0
PL1_6	2	14
PL9_4	1	13
PL25	1	1
PL34	0	1
PL31	0	6
PL3_5	0	1
PL3_1	0	1
PL2	0	4
PL16	0	11
PL1_13	0	1

<sup>a</sup> The e-value cutoff of e<sup>-10</sup> and minimum alignment length of 150nt was used as the annotation parameters

**Table S7.** The rate of ORFs annotated by KEGG and CAZy database <sup>a</sup>.

ID	Total ORFs	KEGG annotation		CAZy annotation	
		No. ORFs	%	No. ORFs	%
<b>MAG11</b>	2,223	1,328	59.74	27	1.21
MAG220	2,833	1,241	43.81	45	1.59
MAG13	2,081	1,047	50.31	35	1.68
MAG14	2,361	1,159	49.09	33	1.40
MAG16	1,658	1,029	62.06	30	1.81
MAG19	3,587	1,581	44.08	27	0.75
MAG215	2,197	1,301	59.22	32	1.46
MAG59	2,679	1,240	46.29	269	10.04
MAG228	2,981	1,691	56.73	96	3.22
MAG42	1,935	982	50.75	49	2.53
MAG83	1,880	1,198	63.72	70	3.72
MAG158	2,680	1,293	48.25	232	8.66
MAG217	3,130	1,364	43.58	103	3.29
MAG142	3,526	1,469	41.66	26	0.74
MAG159	2,753	1,001	36.36	26	0.94
MAG100	1,825	985	53.97	47	2.58
MAG55	1,982	1,059	53.43	15	0.76
MAG68	4,635	1,820	39.27	51	1.10
MAG161	2,939	1,645	55.97	45	1.53
MAG200	2,789	1,607	57.62	29	1.04
MAG74	2,924	1,321	45.18	36	1.23

<sup>a</sup> The e-value cutoff of  $e^{-10}$  and minimum alignment length of 150nt was used as the annotation parameters.