

Supplementary Material

Genome-wide and functional view of proteolytic and lipolytic bacteria for efficient biogas production through enhanced sewage sludge hydrolysis

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Strain	Molasses									Dairy Waste									Malt Extract								
	0.1%			0.5%			1.0%			0.1%			0.5%			1.0%			0.1%			0.5%			1.0%		
	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h
<i>Rummeliibacillus</i> sp. POC 4	0.05	0.10	0.11	0.07	0.14	0.17	0.10	0.18	0.34	0.04	0.10	0.08	0.05	0.27	0.20	0.07	0.38	0.50	0.05	0.05	0.06	0.05	0.06	0.07	0.07	0.07	0.09
<i>Ochrobactrum</i> sp. POC 9	0.06	0.11	0.22	0.05	0.17	0.19	0.06	0.25	0.29	0.05	0.05	0.07	0.06	0.09	0.09	0.07	0.35	0.52	0.05	0.07	0.08	0.05	0.10	0.10	0.06	0.13	0.15
<i>Brevundimonas</i> sp. LFMIX 5	0.05	0.08	0.10	0.05	0.12	0.14	0.06	0.21	0.31	0.05	0.05	0.06	0.05	0.23	0.31	0.07	0.53	0.48	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.09	0.09

OD ₆₀₀ nm	0.01-0.10	0.11-0.20	0.21-0.30	0.31-0.40	0.41-0.50	0.51-0.60
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Figure S1. Bacterial growth on waste substrates. Results of three biological replicate are presented, standard deviation were less than 10% and are omitted for clarity.

Table 1. Genome assembly table.

Strain	N50 [bp]	Essential genes present	Missing essential genes	rRNA copy number	Number of predicted genes	GC content [%]	Number of Scaffolds
<i>Rummeliibacillus</i> sp. POC4	58116	106/111	TIGR00388: glyQ: glycine--tRNA ligase, alpha subunit. TIGR00408: proS_fam_I: proline--tRNA ligase. TIGR00471: pheT_arch: phenylalanine--tRNA ligase, beta subunit. TIGR00775: NhaD: Na+/H+ antiporter, NhaD family. TIGR02387: rpoC1_cyan: DNA-directed RNA polymerase, gamma subunit.	1	3652	34.47	164
<i>Ochrobactrum</i> sp. POC9	182332	105/111	TIGR00389: glyS_dimeric: glycine--tRNA ligase. TIGR00408: proS_fam_I: proline--tRNA ligase. TIGR00471: pheT_arch: phenylalanine--tRNA ligase, beta subunit. TIGR00775: NhaD: Na+/H+ antiporter, NhaD family. TIGR01030: rpmH_bact: ribosomal protein L34 TIGR02387: rpoC1_cyan: DNA-directed RNA polymerase, gamma subunit.	1	4728	55.97	255
<i>Brevundimonas</i> sp. LPMIX5	224500	105/111	TIGR00389: glyS_dimeric: glycine--tRNA ligase. TIGR00408: proS_fam_I: proline--tRNA ligase. TIGR00471: pheT_arch: phenylalanine--tRNA ligase, beta subunit. TIGR00775: NhaD: Na+/H+ antiporter, NhaD family. TIGR01030: rpmH_bact: ribosomal protein L34. TIGR02387: rpoC1_cyan: DNA-directed RNA polymerase, gamma subunit.	1	2939	66.88	97

Table S2. Average aminoacids identity (AAI) values computed using MIGA webserver.

Strain	Reference strain	AAI [%]	Fraction of genome shared [%]
<i>Rummeliibacillus sp.</i> POC4	<i>Rummeliibacillus stabekisii</i> NZ CP014806	72,81	<70
	<i>Kurthia sp.</i> 11kri321 NZ CP013217	65,96	<70
	<i>Kurthia zopfii</i> NZ LR134474	64,62	<70
	<i>Lysinibacillus fusiformis</i> NZ CP010820	61,54	<70
	<i>Lysinibacillus sp.</i> 2017 NZ CP029002	59,97	<70
<i>Ochrobactrum sp.</i> POC9	<i>Ochrobactrum anthropi</i> NZ CP008820	94,35	81,03
	<i>Ochrobactrum anthropi</i> ATCC 49188 NC 009667	93,97	79,8
	<i>Brucella melitensis</i> NZ CP018492	84,98	82,55
	<i>Ochrobactrum pseudogrignonense</i> NZ CP015775	81,83	70,11
	<i>Ochrobactrum sp.</i> A44 NZ CP022604	81,54	72,14
<i>Brevundimonas sp.</i> LPMIX5	<i>Brevundimonas naejangsanensis</i> NZ CP015614	93,67	84,46
	<i>Brevundimonas diminuta</i> NZ CP021995	89,78	84,93
	<i>Brevundimonas naejangsanensis</i> NZ CP032707	86,7	80,23
	<i>Brevundimonas sp.</i> NZ CP015511	66,51	<70
	<i>Brevundimonas sp.</i> LM2 NZ CP019508	62,73	<70

Table S3. Average nucleotide identity (ANI) values. With minimal threshold set at 80% identity, there were no ANI hits for *Rummeliibacillus sp.* POC4 strain.

Strain	Reference strain	ANI [%]	Fraction of genome shared [%]
<i>Ochrobactrum sp.</i> POC9	<i>Ochrobactrum anthropi</i> NZ CP008820	90,89	80,07
	<i>Ochrobactrum anthropi</i> ATCC 49188 NC 009667	90,87	74,48
	<i>Brucella melitensis</i> NZ CP018492	82,74	69,17
	<i>Ochrobactrum pseudogrignonense</i> NZ CP015775	80,91	47,5
	<i>Ochrobactrum sp.</i> A44 NZ CP022604	80,5	41,38
<i>Brevundimonas sp.</i> LPMIX5	<i>Brevundimonas naejangsanensis</i> NZ CP015614	93,4	82,63
	<i>Brevundimonas diminuta</i> NZ CP021995	90,59	73,94
	<i>Brevundimonas naejangsanensis</i> NZ CP032707	89,22	75,9

Table S4. Heavy metal resistance genes identified within genomes of the *Brevundimonas* sp. LPMIX5, *Rummeliibacillus* sp. POC4 and *Ochrobactrum* sp. POC9 strains.

Gene Name	Predicted Protein Function	Localization (Genbank acc. Numbers)		
		<i>Brevundimonas</i> sp. LPMIX5	<i>Rummeliibacillus</i> sp. POC4	<i>Ochrobactrum</i> sp. POC9
chrA	Export of Cr(VI)	-	1) QWUA01000024.1 - locus_tag: D1606_09265 2) QWUA01000024.1 - locus_tag: D1606_09270 3) QWUA01000012.1 - locus_tag: D1606_06200	1) QGST01000005.1 - locus_tag: DK867_07700
chrR	Cr(VI) reductase	1) QWTZ01000005.1 - locus_tag D1604_06960	1) QWUA01000028.1 - locus_tag: D1606_10430 2) QWUA01000009.1 - locus_tag: D1606_04965 3) QWUA01000055.1 - locus_tag: D1606_13695	1) QGST01000003.1 - locus_tag: DK867_05445 2) QGST01000032.1 - locus_tag: DK867_21290 3) QGST01000049.1 - locus_tag: DK867_23150
czcD	Export of Cd(II), Co(II), and Zn(II)	1) QWTZ01000005.1 - locus_tag: D1604_07150 2) QWTZ01000011.1 - locus_tag: D1604_11025	1) QWUA01000008.1 - locus_tag: D1606_04560	1) QGST01000031.1 - locus_tag: DK867_21070
fieF	Export of Cd(II), Co(II), Fe(II), Ni(II), and Zn(II)	1) QWTZ01000004.1 - locus_tag: D1604_06130	1) QWUA01000006.1 - locus_tag: D1606_03085	1) QGST01000005.1 - locus_tag: DK867_07870
cadA	Export of Cd(II), Co(II) and Zn(II)	-	-	1) QGST01000025.1 - locus_tag: DK867_19895
zntA	Export of Cd(II), Co(II), Cu(II), Ni(II), Pb(II), and Zn(II)	1) QWTZ01000005.1 - locus_tag: D1604_07430	-	1) QGST01000032.1 - locus_tag: DK867_21330
zntA	Export of Cd(II), Co(II), Cu(II), Ni(II), Pb(II), and Zn(II)	-	-	1) QGST01000034.1 - locus_tag: DK867_21555
zntA	Export of Cd(II), Co(II), Cu(II), Ni(II), Pb(II), and Zn(II)	-	-	1) QGST01000065.1 - locus_tag: DK867_23595
zntA	Export of Cd(II), Co(II), Cu(II), Ni(II), Pb(II), and Zn(II)	-	-	1) QGST01000001.1 - locus_tag: DK867_00450
cadA	Export of Cd(II), Co(II) and Zn(II)	-	-	1) QGST01000004.1 - locus_tag: DK867_06090
yieF	Cr(VI) reductase	1) QWTZ01000002.1 - locus_tag: D1604_04585	1) QWUA01000001.1 - locus_tag: D1606_00205 2) QWUA01000055.1 - locus_tag: D1606_13695	1) QGST01000004.1 - locus_tag: DK867_06700
copA	Cu(II) binding and sequestration	1) QWTZ01000002.1 - locus_tag: D1604_04040	1) QWUA01000029.1 - locus_tag: D1606_10605	1) QGST01000001.1 - locus_tag: DK867_01360
cnrA/nccA	Protein participating in forming of CnrABC transmembrane complex exporting Co(II) Ni(II)	1) QWTZ01000002.1 - locus_tag: D1604_04795 2) QWTZ01000005.1 - locus_tag: D1604_07170	-	-
cnrB/nccB	Protein participating in forming of CnrABC transmembrane complex exporting Co(II) Ni(II)	1) QWTZ01000005.1 - locus_tag: D1604_07165	-	-
cnrC/nccC	Protein participating in forming of CnrABC transmembrane complex exporting Co(II) Ni(II)	1) QWTZ01000005.1 - locus_tag: D1604_07160 2) QWTZ01000002.1 - locus_tag: D1604_04785	-	-
cnrH	RNA polymerase sigma factor activating cnr/ncc genes expression	1) QWTZ01000001.1 - locus_tag: D1604_00685	-	-
nccH	RNA polymerase sigma factor activating cnr/ncc genes expression	1) QWTZ01000004.1 - locus_tag: D1604_05255	-	-
nccN	Component of the NCC cation-efflux system (nccYXHCBAN) that confers resistance to Ni(II), Co(II) and Cd(II). Ion export through a transmembrane tunnel.	1) QWTZ01000005.1 - locus_tag: D1604_07180 2) QWTZ01000002.1 - locus_tag: D1604_04195	-	-
pbrA	Component of the prbABC system, conferring resistance to lead	1) QWTZ01000005.1 - locus_tag: D1604_07185	-	-
pbrB/C	Component of the prbABC system, conferring resistance to lead	1) QWTZ01000007.1 - locus_tag: D1604_08845	-	-