Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, Southern Russia

-Supplementary information-

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Materials and Methods:

Description of the study site. The Pre-Caspian Depression is part of the continental or semi-arid desert biome, divided by the River Volga that flows into the Earth's largest enclosed water body, the Caspian Sea. Widespread deep-water salt deposits of lower Permian age are prominent in the up to 23 km thick sediment piles. Redistributed by extensive salt tectonics, these salt deposits form more than 1,000 salt domes. Supplemented by thousands of smaller saline dips, these salt affected soils cover an area of 500,000 km^2 on both side the border between Russia and Kazakhstan. Lake Kasin is a typical hypersaline axial depression whose central part spans about 1,000 m × 200 m, with the lowest point at ~22 m below sea level. The surrounding landscape is flat and often covered with a thin salt layer (sulfate, carbonate, halite). Spare sagebrush-dominated vegetation covers the underlying dune formations, starting 100 m from the center, where salt affected soils turn into sloping sand and clay soils. Few halophytic plant species like *Salicornia* spp. can be found at the edges of the small lake. The region is mainly used for livestock; crop production only occurs in the Volga lowlands. Mean annual temperature for the region is 7.5°C (-10°C to +30°C) and annual rainfall averages around ~300 mm.

At the time of sampling in August 2009 Lake Kasin was partially covered with <10 cm saturated water layer (about 50 × 100 m). The air temperature reached 37°C and soil surface temperature increased up to 44°C. Atmospheric conditions were very dry (<40% humidity) with 50% cloud cover and moderate to strong wind.

Most probable number counts and enrichments of anaerobic Fe(II)-oxidizing and Fe(III)-reducing microorganisms. For MPN counts, 1 g of sediment was suspended in 10 mL salt water medium and the suspension was consecutively diluted 1:5 with medium to obtain a master dilution series of 12 dilutions. With each of these 12 dilutions, seven wells per one column of a 96 well plate (900 µL selective growth medium per well) were inoculated (100 µL inoculum per well). The last well of each column served as negative control. All MPN plates were set up in an anoxic chamber (glovebox) under N_2-atmosphere, sealed with a plastic foil, taken out of the anoxic chamber and immediately put into Anaerocult® bags (Merck) together with wetted O_2-indicator strips (Merck) in order to make sure anoxic conditions were maintained during incubation. After 6 weeks of incubation at 20°C in the dark, microbial growth was determined based on the number of parallels per dilution step. The MPN of cells per gram dry soil was calculated with the program "Most Probable Number Calculator" version 4.04 (Environmental Protection Agency, USA). The MPN counts were corrected after Salama as described in Klee (1993) (3). Criteria used to determine microbial growth in the parallels of the dilution series were as follows: Wells were considered positive for growth of anFeOx if they showed a color change from slight green-blue to nearly black. Growth of FeRed was positive if the medium in the wells had turned from red-brown to nearly black. This was in agreement with a reduction of at least twice as much Fe(III) to Fe(II) as in the average of the wells that served as negative control as determined by ferrozine tests (7). FeRed and anFeOx from selected wells were transferred into fresh media as it had been used in the MPNs every 6 to 8 weeks or as soon as most of the Fe(III) had been reduced or the Fe(II) had been oxidized, respectively. Between 1% and 2% of inoculum were used for the transfers that were carried out under sterile and anoxic conditions. Enrichments were incubated in the dark at 28°C.

Gradient tubes to enumerate microaerophilic Fe(II)-oxidizers. In contrast to Emerson and Floyd (1) who used modified Wolfe's mineral medium (MWMM), salt water medium with 0.5 M NaCl as described in the section "Most probable number studies" was used both in the top layer and in the bottom layer. The initial concentration of the sodium bicarbonate buffer in this medium was 15 mM. The FeS was only washed twice with anoxic H_2O and prepared directly before use. In the bottom layer, 1 mL of a 1:1 mix of FeS and salt water medium was used. The final concentration of FeS in the bottom layer was approximately 0.46 M. After addition of the bottom layer, the headspace was exchanged with N_2/CO_2 (90:10), tubes were capped with butyl rubber stoppers and kept at 4°C for 30 minutes. The medium for the top layer was prepared anoxically by taking the medium out of the autoclave at 80°C and flushing the headspace with N_2/CO_2 for 10 minutes. After the top layer medium had cooled down to 40°C, buffer, vitamins and all other additives were added as listed in the section "Most probable number studies and enrichments of Fe(II)-oxidizing and Fe(III)-reducing microorganisms" of the manuscript. In order to stabilize the top layer, the concentration of low melt agarose was increased to 0.5%. 10 mL of top layer were added anoxically on top of the bottom layer with syringes and needles that had been flushed with N_2/CO_2. In total, 21 gradient tubes were prepared and kept overnight to allow them to solidify before inoculation. For inoculation, tenfold dilutions of Lake Kasin sediment from 0-10 cm in SWM up to a dilution of 10^{-5} were prepared as described in the manuscript. From each dilution, four parallel tubes were inoculated with 100 µL of sediment suspension using 1 mL syringes and needles. Tubes were inoculated sterilely under atmospheric conditions in order to bring some O_2 into the system. Inocula were inserted at about 2/3 depth of the top layer. One gradient tube that served as a negative control was also penetrated with an empty needle down to the same depth. After inoculation, tubes were closed again with butyl rubber stoppers and incubated at 28°C for two months.

DGGE.

The first amplification reactions of the long archaeal 16S rRNA gene fragments were performed in a final volume of 25 µL containing 1× PCR buffer with 1.5 mM $MgCl_2$ final concentration (Promega), 200 µM dNTP mix (New England Biolabs), 200 nM of each primer, 0.625 U Taq DNA-Polymerase (Promega) and 10 ng of DNA extract as a template in a total volume of 25 µL. The thermocycler program was as follows: initial denaturation at 94°C for 2 min; 30 cycles of denaturing (94°C for 30 sec), annealing (58°C for 1 min) and elongation (72°C for 1 min 30 sec) and a final elongation at 72°C for 10 min. The second amplification reactions of the short archaeal and the amplification reactions of the bacterial 16S rRNA gene fragments were performed in a final volume of 50 µL. The reaction mix contained 1× PCR buffer (Promega), 4 mM $MgCl_2$, 200 µM dNTP mix (New England Biolabs), 200 nM of each primer, 1.25 U Taq DNA-Polymerase (Promega) and 10 ng of DNA extract as a template. The thermocycler program included: initial denaturation at 94°C for 2 min; 10 cycles of denaturing (94°C for 1 min), annealing (65°C to 56°C with a temperature decrease of 1°C per cycle for 1 min) and elongation (72°C for 1 min) plus 20 cycles of denaturing (94°C for 1 min), annealing (55°C for 1 min) and elongation (72°C for 1 min) and a final elongation at 72°C for 10 min. The concentrations of the PCR products were estimated by comparing the intensities of the resulting bands on an agarose gel to the band intensity of a marker fragment of defined concentration. Depending on the band intensities of the PCR products, between 5 and 20 µL were mixed with 5 µL of loading buffer consisting of 60% glycerol, 1 mM EDTA pH 8.0, 1% bromophenol blue and 0.5% xylem xylol and applied on a DGGE gel. DGGE gels were prepared in a DGGE unit type V20 HCDC from BioRad Laboratories GmbH, Munich, Germany according to the manufacturer’s suggested protocol. All DGGE gels had a gradient of denaturant (7 M of urea and 40% v/v of formamide) ranging from 35% in the uppermost part of the gel to 60% in the lowest part of the gel. For bacterial 16S rRNA gene amplicons, 6% polyacrylamide gels were used and run for 16 h at 100 V and 60°C. Archaeal 16S rRNA gene amplicons were applied on 8% polyacrylamide gels which were run for 6 h at 100 V and 60°C. Gels were stained with ethidium bromide and DNA bands were visualized under UV.

Supplementary figure S1:

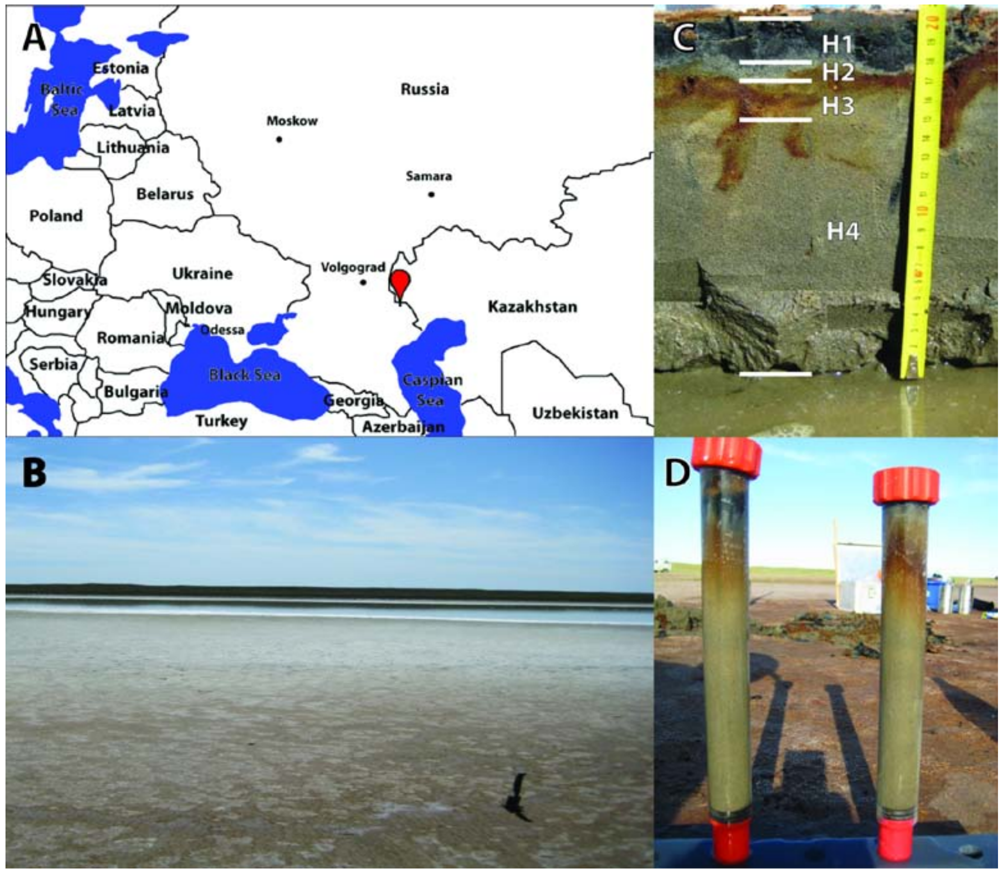

FIGURE S1: Geographic maps showing A) the location of Lake Kasin in Southern Russia and B) the exact sampling position at the lake about 50 m away from the water-covered area. C) Shows the depth profile of the hypersaline sediment as well as the distribution of the four soil horizons H1-H4. The sediment cores were sampled with plastic tubes as illustrated in D).

Supplementary figure S2:

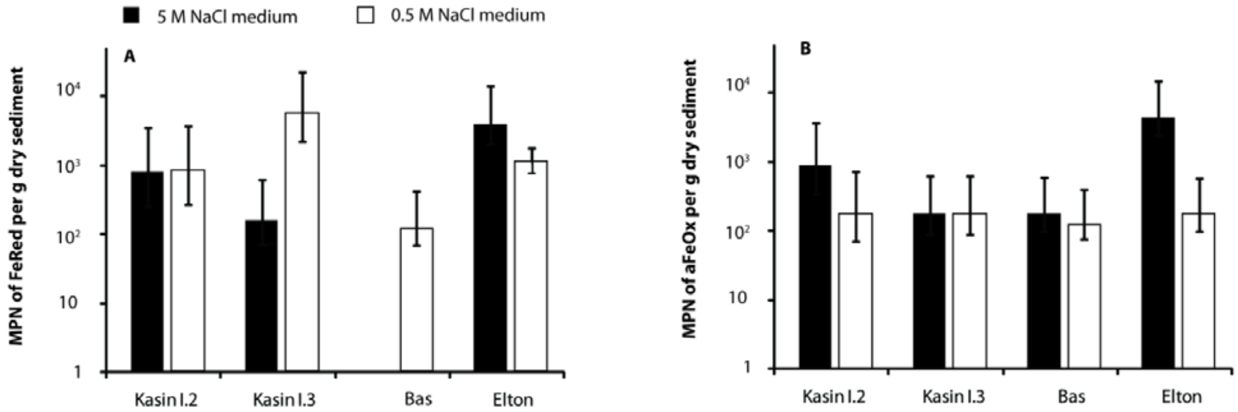

FIGURE S2: Most probable number counts of A) Fe(III)-reducing (FeRed) and B) anaerobic Fe(II)-oxidizing (anFeOx) microorganisms from the top 10 cm of different salt lake sediments in mineral medium with 5 M (black bars) or 0.5 M NaCl (white bars), respectively. Medium for Fe(III)-reducer was supplemented with 0.5 M ferrihydrite as electron acceptor and 0.5 M of each lactate and acetate as electron donors. For the anaerobic Fe(II)-oxidizer, 10 mM $FeCl_2$ was added as electron donor and 0.4 M NO_3^- as electron acceptor. The Fe(II)-oxidizer medium further contained 0.05 M acetate as a carbon source. Error bars denote 95% confidence intervals determined from seven replicate samples according to Klee (1993) (3).

Supplementary figure S3:

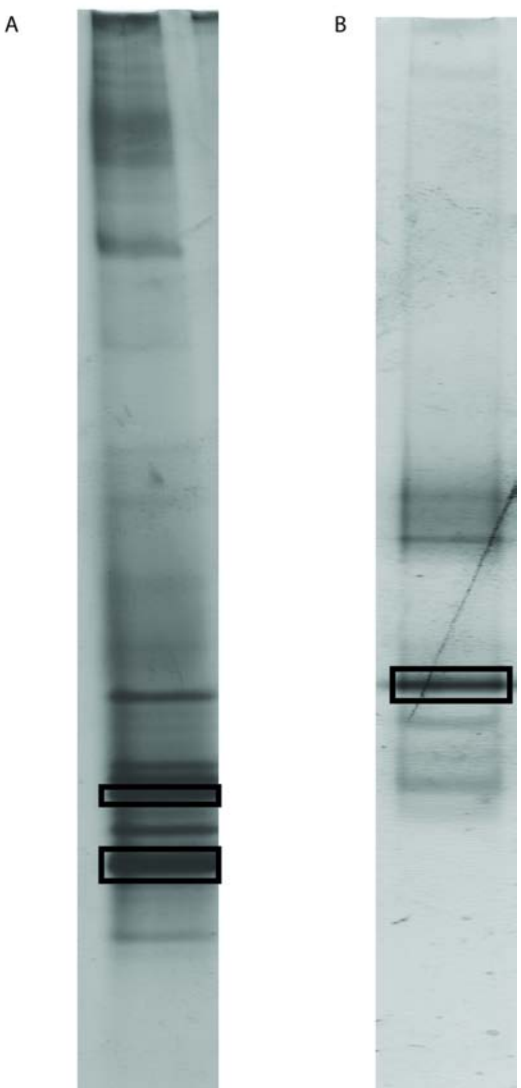

FIGURE S3: DGGE gels of PCR amplicons of partial 16S rRNA gene fragments from enrichments from Lake Kasin sediment (0-10 cm) that reduce Fe(III) over several transfers. A) The banding pattern originating from an archaeal enrichment that reduces Fe(III) at 5 M salinity and B) the banding pattern originating from bacterial enrichment that reduces Fe(III) at 0.5 mM salinity. Dominant bands marked with black rectangles were cut out from ethidium bromide-stained gels, re-amplified, ligated into vector pCR4 (Invitrogen) and transformed into competent *Escherichia coli* cells (DH5α). Three plasmids with inserts originating from each band were sequenced in order to enable classification of the dominant strains in the Fe(III)-reducing enrichment cultures.

TABLE S1: Sequences of qPCR primers applied in this study.

Target	forward Primer (5'-3')	reverse Primer (5'-3')	Target group hits of forward/reverse primer*	References
General *Bacteria*	341F: CCTACGGGAGGCAGCAG	797R: GGACTACCAGGGTATCTAATCCTGTT	-	(4, 5)
General *Archaea*	109F: ACKGCTCAGTAACACGT	915R: GTGCTCCCCCGCCAATTCCT	-	(2, 6)
Desulfosporosinus sp.	Desulfosp.432F: GTACTGTCTTTGGGGAAG	Desulfosp.598R: CCTGATCTTTCACACCGG	84/72	This study
Bacillus sp.	Bacilli354F: GCAGTAGGGAATCTTCCG	Bacilli518R: ATTACCGCGGCTGCTGG	24894/529028	This study, (4)
Halobaculum gomorrense and close relatives	Hgomorr396F: ACTCCGAGTGCGGAGGCA	Hgomorr656R: CCCTTCGAGTCTCCCTGT	7/7	This study

* Based on a 100 % match using the program TestProbe (SILVA probe match/evaluation tool) and the SILVA SSUr108Ref data set (http://www.arb-silva.de/search/testprobe/).

TABLE S2: Thermal profiles and standards used for qPCR in this study. Vectors pCR2.1® and pCR4® were obtained from Invitrogen (Darmstadt, Germany).

Target	**Thermal profile**	**Origin of 16S rRNA gene used as standard**	**Vector**	**Molecular weight vector + standard**
General *Bacteria*	98°C-5s/60°C-12s	*Thiomonas sp.*	pCR2.1®	3.29×10^6 g/mol
General *Archaea*	98°C-5s/52°C-10s/ 72°C-15s	*Halobacterium salinarum*	pCR4®	3.33×10^6 g/mol
Desulfosporosinus spp.	98°C-5s/60°C-12s	DGGE band of Fe(III)-reducing enrichment (HE604952)	pCR4®	2.81×10^6 g/mol
Bacilli	98°C-5s/60°C-12s	Clone library sequence (Kasin-B2-E03; HE604676)	pCR4®	3.28×10^6 g/mol
Halobaculum gomorrense	98°C-5s/60°C-12s	Sequence from Fe(III)-reducing enrichment (HE604942)	pCR4®	3.33×10^6 g/mol

TABLE S3: Mössbauer parameters for Lake Kasin sediment spectra of the Fe-rich sediment layer at 1-3 cm depth.

Site	Mineral	Fe ox. State	δ (mm/s)	ΔE_Q or 2ε (mm/s)	B_{hf} (T)	σ (mm/s or T)	A (%)	Fe(II)/Fe_T
				RT				
D1	Ak/Lp	Fe(III)	0.38	0.55	-	0.2	51	0.11
D2	Akaganéite	Fe(III)	0.37	0.95	-	0.2	20	
D3	Akaganéite	Fe(III)	0.37	1.33	-	0.2	12	
D4	Green rust	Fe(II)	1.05	2.67	-	0.2	3	
D5	Green rust	Fe(II)	0.6	2.2	-	0.2	8	
D6	Green rust	Fe(III)	0.27	0.73	-	0.2	7	
				77 K				
D1	Ak/Lp/GR	Fe(III)	0.46	0.42	-	0.2	24	0.02
D2	Akaganéite	Fe(III)	0.46	0.91	-	0.2	22	
D3	Akaganéite	Fe(III)	0.46	1.51	-	0.2	7	
S1	Akaganéite	Fe(III)	0.45	-0.12	47.7	1.0	7	
S2	Akaganéite	Fe(III)	0.42	-0.41	43.6	1.0	4	
S3	Ak/Lp	Fe(III)	0.47	0.05	0.05	30.5	35	
D4	Green rust	Fe(II)	1.25	2.55	-	0.2	1	
D5	Green rust	Fe(II)	1.27	2.86	-	0.2	1	
				~5 K				
S1	Ak/GR	Fe(III)	0.47	-0.32	48.9	2.2	42	0.05
S2	Akaganéite	Fe(III)	0.48	0.38	48.7	2.3	30	
S3	Ak/Lp	Fe(III)	0.40	0.22	41.6	2.0	11	
D1	Ak/Lp/GR	Fe(III)	0.47	0.55	-	0.3	13	
D2	Green rust	Fe(II)	1.22	2.97	-	0.3	5	

TABLE S4: Phylogenetic affiliation of the 16S rRNA gene sequences from the archaeal and bacterial clone libraries from Lake Kasin sediment including sequences from dominant strains of the Fe(III)-reducing enrichment cultures obtained in this study. “OTU”= operational taxonomic unit; “ACC” = accession number. Sequences that were grouped into one OTU have a 16S rRNA gene sequence similarity of ≥ 97%. ”Similarity” refers to the sequence similarity between a respective OTU and its closest cultivated relative.

Phylogenetic affiliation	**OTU**	**Clone(s)**	**ACC**	**Closest cultivated relative**	**ACC**	**Similarity (%)**
Methanomicrobia_Methanosarcina	OTU1	Kasin-A2-C07	HE604512	*Methanohalobium evestigatum Z-7303*	CP002069	92
Methanomicrobia_Methanosarcina	OTU1	Kasin-A3-E09	HE604615	*Methanohalobium evestigatum Z-7303*	CP002069	97
Thermoplasmata_Marine Benthic Group D and DHVEG-1	OTU1	Kasin-A1-H01	HE604477	n/a		
		Kasin-A1-H02	HE604486			
		Kasin-A2-H09	HE604561			
		Kasin-A1-A04	HE604413			
		Kasin-A2-D12	HE604525			
Thermoplasmata_KTK4A	OTU1	Kasin-A3-D11	HE604605	n/a		
		Kasin-A1-H09	HE604483			
Thermoplasmata	OTU1	Kasin-A1-D08	HE604449	n/a		
		Kasin-A1-F09	HE604467			
		Kasin-A1-G08	HE604473			
		Kasin-A1-F10	HE604468			
		Kasin-A1-C07	HE604438			
		Kasin-A1-H05	HE604479			
	OTU1	Kasin-A2-H03	HE604555			
		Kasin-A2-C10	HE604515			
	OTU1	Kasin-A3-A06	HE604570			
	OTU1	Kasin-A2-G04	HE604545			
		Kasin-A1-H07	HE604481			
Thermoplasmata_CCA47	OTU1	Kasin-A2-C05	HE604510	n/a		
		Kasin-A1-B11	HE604430			
Thermoplasmata_SAGMEG	OTU1	Kasin-A3-A04	HE604568	*Methanocaldococcus fervens*	AF056938	79
Thermoplasmata_SAGMEG	OTU1	Kasin-A3-H03	HE604634	*Methanocaldococcus fervens*	AF056938	80
		Kasin-A1-B05	HE604425			
		Kasin-A2-C04	HE604509			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-A01	HE604487	*Halopelagius inordinatus*	EU887284	88-89
		Kasin-A2-A03	HE604488			
		Kasin-A2-A04	HE604489			
		Kasin-A2-A05	HE604490			
		Kasin-A2-A08	HE604492			
		Kasin-A2-A11	HE604494			
		Kasin-A2-B01	HE604496			
		Kasin-A2-B03	HE604497			
		Kasin-A2-B06	HE604500			
		Kasin-A2-B07	HE604501			
		Kasin-A2-B08	HE604502			
		Kasin-A2-B09	HE604503			
		Kasin-A2-B10	HE604504			
		Kasin-A2-B11	HE604505			

Kasin-A2-B12	HE604506
Kasin-A2-C03	HE604508
Kasin-A2-C06	HE604511
Kasin-A2-C11	HE604516
Kasin-A2-D04	HE604519
Kasin-A2-D08	HE604522
Kasin-A2-D11	HE604524
Kasin-A2-E02	HE604527
Kasin-A2-E03	HE604528
Kasin-A2-E06	HE604530
Kasin-A2-E07	HE604531
Kasin-A2-E08	HE604532
Kasin-A2-F02	HE604536
Kasin-A2-F05	HE604538
Kasin-A2-F06	HE604539
Kasin-A2-F10	HE604540
Kasin-A2-F12	HE604542
Kasin-A2-G02	HE604543
Kasin-A2-G03	HE604544
Kasin-A2-G05	HE604546
Kasin-A2-G06	HE604547
Kasin-A2-G07	HE604548
Kasin-A2-G08	HE604549
Kasin-A2-G12	HE604552
Kasin-A2-H01	HE604553
Kasin-A2-H05	HE604557
Kasin-A2-H07	HE604559
Kasin-A2-H10	HE604562
Kasin-A2-H12	HE604564
Kasin-A3-A01	HE604565
Kasin-A3-A05	HE604569
Kasin-A3-A07	HE604571
Kasin-A3-A08	HE604572
Kasin-A3-A09	HE604573
Kasin-A3-A12	HE604575
Kasin-A3-B01	HE604576
Kasin-A3-B02	HE604577
Kasin-A3-B03	HE604578
Kasin-A3-B04	HE604579
Kasin-A3-B07	HE604581
Kasin-A3-B08	HE604582
Kasin-A3-B09	HE604583
Kasin-A3-C01	HE604585
Kasin-A3-C02	HE604586
Kasin-A3-C03	HE604587
Kasin-A3-C07	HE604590
Kasin-A3-C09	HE604591
Kasin-A3-C12	HE604594
Kasin-A3-D01	HE604595

Kasin-A3-D04	HE604598
Kasin-A3-D06	HE604600
Kasin-A3-D07	HE604601
Kasin-A3-D08	HE604602
Kasin-A3-D09	HE604603
Kasin-A3-D10	HE604604
Kasin-A3-E01	HE604607
Kasin-A3-E03	HE604609
Kasin-A3-E04	HE604610
Kasin-A3-E08	HE604614
Kasin-A3-F01	HE604617
Kasin-A3-F04	HE604619
Kasin-A3-F07	HE604621
Kasin-A3-F10	HE604624
Kasin-A3-F11	HE604625
Kasin-A3-F12	HE604626
Kasin-A3-G04	HE604628
Kasin-A3-G06	HE604630
Kasin-A3-G12	HE604633
Kasin-A3-H04	HE604635
Kasin-A3-H06	HE604637
Kasin-A3-H07	HE604638
Kasin-A3-H12	HE604641
Kasin-A1-A03	HE604412
Kasin-A1-A05	HE604414
Kasin-A1-A08	HE604417
Kasin-A1-A11	HE604419
Kasin-A1-A12	HE604420
Kasin-A1-B01	HE604421
Kasin-A1-B02	HE604422
Kasin-A1-B03	HE604423
Kasin-A1-B04	HE604424
Kasin-A1-B06	HE604426
Kasin-A1-B07	HE604427
Kasin-A1-B10	HE604429
Kasin-A1-B12	HE604431
Kasin-A1-C01	HE604432
Kasin-A1-C03	HE604434
Kasin-A1-C04	HE604435
Kasin-A1-C09	HE604440
Kasin-A1-C10	HE604441
Kasin-A1-D02	HE604444
Kasin-A1-D03	HE604445
Kasin-A1-D10	HE604451
Kasin-A1-D11	HE604452
Kasin-A1-E02	HE604455
Kasin-A1-E03	HE604456
Kasin-A1-E08	HE604459
Kasin-A1-E09	HE604460

		Kasin-A1-F01	HE604464			
		Kasin-A1-F03	HE604466			
		Kasin-A1-G01	HE604470			
		Kasin-A1-G02	HE604471			
		Kasin-A1-G11	HE604475			
		Kasin-A1-G12	HE604476			
		Kasin-A1-H04	HE604478			
		Kasin-A1-H06	HE604480			
Halobacteriales_Halobacteriaceae	OTU1	Fe(III)-red. (5M NaCl) clone 1	HE604940	*Halogranum rubrum*	EU887283	89
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-E05	HE604458	*Halopelagius inordinatus*	EU887284	85
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-C04	HE604588	*Halopelagius inordinatus*	EU887284	89
		Kasin-A3-G01	HE604627			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-A07	HE604491	*Halogranum rubrum*	EU887283	92-93
		Kasin-A3-B10	HE604584			
		Kasin-A1-D04	HE604446			
		Kasin-A3-F08	HE604622			
		Kasin-A2-D10	HE604523			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-D05	HE604599	*Halalkalicoccus jeotgali* B3	CP002062	89
		Kasin-A3-G05	HE604629			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-H04	HE604556	*Halobaculum gomorrense*	AB477983	97-98
		Kasin-A2-D06	HE604521			
		Kasin-A3-C05	HE604589			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-H08	HE604560	*Halobaculum gomorrense*	AB477983	98-99
		Kasin-A3-E02	HE604608			
		Kasin-A1-C05	HE604436			
Halobacteriales_Halobacteriaceae	OTU1	Fe(III)-red. (5M NaCl) clone 8	HE604947	*Halobaculum gomorrense*	AB477982	97-98
		Fe(III)-red. (5M NaCl) clone 7	HE604946			
		Fe(III)-red. (5M NaCl) clone 5	HE604944			
		Fe(III)-red. (5M NaCl) clone 4	HE604943			
		Fe(III)-red. (5M NaCl) clone 3	HE604942			
		Fe(III)-red. (5M NaCl) clone 9	HE604948			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-C11	HE604593	*Halobaculum gomorrense*	AB477982	89
		Kasin-A3-F05	HE604620			
		Kasin-A3-E06	HE604612			
		Kasin-A1-H08	HE604482			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-H02	HE604554	*Halobaculum gomorrense*	AB477982	89-90
		Kasin-A1-D12	HE604453			
Halobacteriales_Halobacteriaceae	OTU1	Fe(III)-red. (5M NaCl) clone 2	HE604941	*Halomicrobium katesii*	EF533994	85
Halobacteriales_Halobacteriaceae	OTU1	Fe(III)-red. (5M NaCl) clone 10	HE604949	*Halomicrobium katesii*	EF533994	85

Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-A02	HE604566	*Halomicrobium katesii*	EF533994	88
		Kasin-A1-A06	HE604415			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-D09	HE604450	*Halomicrobium katesii*	EF533994	86-89
		Kasin-A2-D02	HE604518			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-E10	HE604461	*Halorhabdus utahensis*	AF071880	93
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-G07	HE604631	*Halorhabdus utahensis*	AF071880	94
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-C09	HE604514	*Halorhabdus utahensis*	AF071880	96
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-F01	HE604535	*Halomicrobium mukohataei*	CP001688	89-90
		Kasin-A3-E11	HE604616			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-C11	HE604442	*Halomicrobium mukohataei*	CP001688	90
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A2-C08	HE604513	*Halomicrobium mukohataei*	CP001688	84
		Kasin-A2-D05	HE604520			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-D12	HE604606	*Halobacterium noricense*	AJ548827	98
		Kasin-A3-H10	HE604640			
Halobacteriales_Halobacteriaceae	OTU1	Fe(III)-red. (5M NaCl) clone 6	HE604945	*Halobacterium noricense*	AJ548827	94
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-E04	HE604457	*Halobacterium noricense*	AJ548827	94
		Kasin-A2-E01	HE604526			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-C02	HE604433	*Halobacterium noricense*	AJ548827	92-93
		Kasin-A1-E01	HE604453			
		Kasin-A1-E12	HE604463			
		Kasin-A1-E11	HE604462			
		Kasin-A1-G07	HE604472			
		Kasin-A1-F11	HE604469			
		Kasin-A1-G10	HE604474			
		Kasin-A3-A03	HE604567			
		Kasin-A3-B06	HE604580			
		Kasin-A3-F02	HE604618			
		Kasin-A2-F03	HE604537			
		Kasin-A2-E09	HE604533			
		Kasin-A2-D01	HE604517			
		Kasin-A2-H11	HE604563			
		Kasin-A3-A10	HE604574			
		Kasin-A2-A09	HE604493			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-E07	HE604613	*Halobacterium noricense*	AJ548827	92-93
		Kasin-A3-F09	HE604623			
		Kasin-A3-G10	HE604632			
		Kasin-A2-A12	HE604495			
		Kasin-A1-D05	HE604447			
		Kasin-A1-F02	HE604465			
		Kasin-A2-H06	HE604558			
		Kasin-A3-D02	HE604596			
		Kasin-A2-B05	HE604499			
		Kasin-A3-D03	HE604597			
		Kasin-A2-G10	HE604550			
		Kasin-A1-H10	HE604484			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A3-C10	HE604592	*Halobacterium noricense*	AJ548827	93
		Kasin-A3-H08	HE604639			
		Kasin-A3-H05	HE604636			

Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-C08	HE604439	*Haloarcula japonica*	EF645685	96
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-H12	HE604485	*Natronomonas pharaonis DSM 2160*	CR936257	92
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-A07	HE604416	*Halorubrum xinjiangense*	AY510707	99
		Kasin-A1-A10	HE604418			
Halobacteriales_Halobacteriaceae	OTU1	Kasin-A1-A02	HE604411	*Halonotius pteroides*	AY498641	95
		Kasin-A1-C06	HE604437			
		Kasin-A1-D01	HE604443			
		Kasin-A2-E11	HE604534			
		Kasin-A3-E05	HE604611			
		Kasin-A2-F11	HE604541			
		Kasin-A2-G11	HE604551			
Crenarchaeota_Marine Benthic Group A	OTU1	Kasin-A2-C02	HE604507	*Staphylothermus marinus*	X99560	79
Miscellaneous Crenarchaeota Group	OTU1	Kasin-A1-D07	HE604448	*Staphylothermus marinus*	X99560	81
Miscellaneous Crenarchaeota Group	OTU1	Kasin-A2-B04	HE604498	*Staphylothermus marinus*	X99560	82
		Kasin-A2-E04	HE604529			
Firmicutes_Bacilli_Bacillaceae	OTU1	Kasin-B5-C03	HE604893	*Virgibacillus necropolis*	AJ315056	98-99
		Kasin-B5-D01	HE604898			
		Kasin-B5-B04	HE604886			
		Kasin-B1-C09	HE604779			
		Kasin-B1-D10	HE604786			
		Kasin-B3-D07	HE604731			
		Kasin-B5-C08	HE604896			
		Kasin-B5-H08	HE604935			
		Kasin-B5-A09	HE604879			
		Kasin-B5-F11	HE604923			
		Kasin-B4-H06	HE604870			
		Kasin-B4-A11	HE604828			
Firmicutes_Bacilli_Bacillaceae	OTU1	Kasin-B4-F11	HE604860	*Paraliobacillus quinghaiensis*	EU135728	95-98
		Kasin-B4-G11	HE604867			
		Kasin-B2-C05	HE604660			
Firmicutes_Bacilli_Bacillaceae	OTU1	Kasin-B1-G04	HE604800	*Pontibacillus chungwhensis*	AY553296	84-96
		Kasin-B1-G11	HE604804			
		Kasin-B4-G04	HE604862			
		Kasin-B1-F03	HE604793			
		Kasin-B1-G07	HE604801			
		Kasin-B2-E03	HE604676			
		Kasin-B3-B08	HE604715			
		Kasin-B2-D02	HE604666			
		Kasin-B3-C02	HE604719			
		Kasin-B1-F04	HE604794			
		Kasin-B1-C01	HE604776			
Firmicutes_Bacilli_Bacillaceae	OTU2	Kasin-B5-E04	HE604910	*Pontibacillus chungwhensis*	AY553296	98
		Kasin-B5-B03	HE604885			
Firmicutes_Bacilli_Bacillaceae	OTU3	Kasin-B1-A01	HE604814	*Pontibacillus chungwhensis*	AY553296	97
Firmicutes_Bacilli_Bacillaceae	OTU1	Kasin-B2-B05	HE604654	*Bacillus neizhouensis*	EU925618	98
Firmicutes_Bacilli_Lactobacillaceae	OTU1	Fe(III)-red. (0.5M NaCl) clone 2	HE604951	*Lactobacillus fabifermentans*	AM905388	99
Firmicutes_Clostridia_Peptococcaceae	OTU1	Fe(III)-red. (0.5M NaCl) clone 1	HE604950	*Dehalobacter restrictus*	U84497	97

Firmicutes_Clostridia_Peptococcaceae	OTU1	Fe(III)-red. (0.5M NaCl) clone 3	HE604952	*Desulfosporosinus lacus*	AJ582757	97
Firmicutes_Clostridia_Peptococcaceae	OTU1	Kasin-B2-D08	HE604671	*Desulfitibacter alkalitolerans*	AY538171	86
	OTU1	Kasin-B1-B12	HE604775	*Halanaerobium fermentans*	AB023308	88
		Kasin-B2-F08	HE604688			
		Kasin-B3-E02	HE604735			
Firmicutes_Clostridia_Halanaerobiaceae	OTU1	Kasin-B3-F04	HE604746	*Halocella cellulosilytica*	X89072	94
Deferribacteres	OTU1	Kasin-B5-D03	HE604900	*Caldithrix abyssi*	AJ430587	78
	OTU1	Kasin-B3-C09	HE604724	*Caldithrix abyssi*	AJ430587	81-82
		Kasin-B4-G05	HE604863			
Chloroflexi_Anaerolineaceae	OTU1	Kasin-B3-E07	HE604738	n/a		
		Kasin-B3-F12	HE604751			
Chloroflexi_Anaerolineaceae	OTU2	Kasin-B3-A04	HE604705	n/a		
Chloroflexi_Anaerolineaceae	OTU3	Kasin-B4-B06	HE604832	n/a		
Chloroflexi_Anaerolineaceae	OTU4	Kasin-B5-A05	HE604877	n/a		
		Kasin-B5-D11	HE604908			
Chloroflexi_Anaerolineaceae	OTU5	Kasin-B1-E05	HE604789	n/a		
		Kasin-B2-H09	HE604703			
		Kasin-B3-B05	HE604712			
Chloroflexi_Anaerolineaceae	OTU6	Kasin-B1-C07	HE604816	n/a		
		Kasin-B2-C10	HE604663			
		Kasin-B4-A03	HE604821			
Chloroflexi_Anaerolineaceae	OTU7	Kasin-B5-F09	HE604922	n/a		
		Kasin-B2-B07	HE604656			
Candidate division OP11	OTU1	Kasin-B2-C02	HE604658	n/a		
	OTU2	Kasin-B3-F01	HE604744	n/a		
	OTU3	Kasin-B5-D10	HE604907	n/a		
	OTU4	Kasin-B1-D12	HE604788	n/a		
Candidate division OD1	OTU1	Kasin-B5-F03	HE604917	n/a		
		Kasin-B5-G05	HE604928			
Candidate division OD1	OTU2	Kasin-B3-D09	HE604732	n/a		
Candidate division OD1	OTU3	Kasin-B5-G03	HE604926	n/a		
		Kasin-B5-H01	HE604931			
Candidate division OD1	OTU4	Kasin-B5-G01	HE604924	n/a		
Candidate division OD1	OTU5	Kasin-B2-D09	HE604672	n/a		
Candidate division OD1	OTU6	Kasin-B1-G09	HE604802	n/a		
Candidate division OD1	OTU7	Kasin-B5-A01	HE604874	n/a		
		Kasin-B5-B01	HE604883			
Candidate division OD1	OTU8	Kasin-B5-A12	HE604882	n/a		
		Kasin-B2-D11	HE604674			
Candidate division TM7	OTU1	Kasin-B2-B04	HE604653	n/a		
		Kasin-B4-E10	HE604851			
Deionococcus-Thermus	OTU1	Kasin-B1-H11	HE604812	*Truepera radiovictrix*	DQ022076	89
		Kasin-B2-F07	HE604687			
Planctomycetes	OTU1	Kasin-B5-D09	HE604906	n/a		
Planctomycetes	OTU2	Kasin-B5-F01	HE604915	n/a		
Planctomycetes_Brocadiaceae	OTU1	Kasin-B1-B10	HE604774	*n/a*		
TAO6	OTU2	Kasin-B1-D05	HE604783	n/a		
TAO6	OTU3	Kasin-B5-D07	HE604904	n/a		

TAO6	OTU4	Kasin-B2-F05	HE604685	n/a		
Gemmatimonadetes	OTU1	Kasin-B3-G03	HE604752	n/a		
		Kasin-B3-G08	HE604756			
		Kasin-B5-C07	HE604895			
		Kasin-B5-G12	HE604930			
		Kasin-B5-A11	HE604881			
Gemmatimonadetes	OTU2	Kasin-B4-A02	HE604820	n/a		
Gemmatimonadetes	OTU3	Kasin-B4-F08	HE604858	n/a		
Gemmatimonadetes	OTU4	Kasin-B4-F12	HE604861	n/a		
Acidobacteria_RB25	OTU1	Kasin-B5-G02	HE604925	n/a		
		Kasin-B5-H03	HE604933			
		Kasin-B5-D06	HE604903			
		Kasin-B2-D06	HE604669			
		Kasin-B5-F05	HE604919			
Nitrospirae_Nitrospiraceae	OTU1	Kasin-B4-D09	HE604844	*Thermodesulfovibrio hydrogeniphilus*	EF081294	87
Nitrospirae_OPB 95	OTU1	Kasin-B4-B12	HE604835	n/a		
		Kasin-B4-F02	HE604853			
Alphaproteobacteria_Rhodobacteriaceae	OTU1	Kasin-B2-A10	HE604649	*Rubrimonas cliftonensis*	D85834	89
		Kasin-B2-F09	HE604689			
Deltaproteobacteria_Deferribacteriaceae	OTU1	Kasin-B5-D02	HE604899	*Flexistipes sinusarabici*	M59231	99
Deltaproteobacteria_Desulfobacteraceae	OTU1	Kasin-B1-D01	HE604781	*Desulfosalsimonas propionicica*	DQ067422	92-96
		Kasin-B4-C03	HE604837			
Deltaproteobacteria_Desulfobacteraceae	OTU2	Kasin-B5-A04	HE604876	*Desulfosalsimonas propionicica*	DQ067422	96
		Kasin-B5-C01	HE604891			
Deltaproteobacteria_Desulfobacteraceae		Kasin-B1-D08	HE604785	*Desulfotignum phosphitoxidans*	AF420288	96
Deltaproteobacteria_Desulfohalobiaceae	OTU1	Kasin-B5-B09	HE604888	*Desulfohalobium utahense*	DQ067421	83
Deltaproteobacteria_Desulfohalobiaceae	OTU2	Kasin-B3-A12	HE604710	*Desulfohalobium utahense*	DQ067421	90
Deltaproteobacteria_Desulfohalobiaceae	OTU3	Kasin-B5-A10	HE604880	*Desulfohalobium utahense*	DQ067421	88-91
		Kasin-B1-H08	HE604809			
Deltaproteobacteria_DTB120	OTU1	Kasin-B4-F09	HE604859	n/a		
Deltaproteobacteria	OTU1	Kasin-B3-F03	HE604745	*n/a*		
Gammaproteobacteria	OTU1	Kasin-B4-C05	HE604839	*Idiomarina ramblicola*	AY526862	98-100
		Kasin-B3-B07	HE604714			
		Kasin-B1-G02	HE604799			
		Kasin-B2-C11	HE604664			
		Kasin-B3-E09	HE604740			
		Kasin-B2-G12	HE604696			
Gammaproteobacteria_Halothiobacillaceae	OTU1	Kasin-B4-E05	HE604849	*Halothiobacillus halophilus*	U58020	97-100
		Kasin-B4-H08	HE604871			
		Kasin-B4-F04	HE604855			
		Kasin-B4-B02	HE604829			
		Kasin-B4-A04	HE604822			
		Kasin-B4-E09	HE604850			
		Kasin-B4-F07	HE604857			
		Kasin-B4-F03	HE604854			
		Kasin-B2-E01	HE604675			
		Kasin-B2-G03	HE604692			
		Kasin-B3-E12	HE604743			
		Kasin-B1-F06	HE604795			

Kasin-B4-H09	HE604872
Kasin-B4-C04	HE604838
Kasin-B4-B04	HE604830
Kasin-B4-B09	HE604834
Kasin-B2-D03	HE604667
Kasin-B4-B07	HE604833
Kasin-B4-D01	HE604841
Kasin-B4-H01	HE604868
Kasin-B4-D07	HE604843
Kasin-B4-D12	HE604846
Kasin-B1-A08	HE604766
Kasin-B1-B08	HE604772
Kasin-B2-H03	HE604699
Kasin-B2-H02	HE604698
Kasin-B2-E07	HE604677
Kasin-B3-C01	HE604718
Kasin-B1-G01	HE604798
Kasin-B3-D06	HE604730
Kasin-B3-B04	HE604711
Kasin-B2-E09	HE604678
Kasin-B3-F11	HE604750
Kasin-B3-H05	HE604761
Kasin-B1-H12	HE604813
Kasin-B3-F10	HE604749
Kasin-B2-D04	HE604668
Kasin-B1-H09	HE604810
Kasin-B2-H01	HE604697
Kasin-B1-C10	HE604780
Kasin-B3-C07	HE604723
Kasin-B1-E08	HE604790
Kasin-B3-H02	HE604759
Kasin-B1-F12	HE604797
Kasin-B2-H08	HE604702
Kasin-B1-F01	HE604792
Kasin-B2-D07	HE604670
Kasin-B1-G12	HE604805
Kasin-B2-G01	HE604691
Kasin-B2-C12	HE604665
Kasin-B3-H07	HE604762
Kasin-B1-D02	HE604782
Kasin-B3-H03	HE604760
Kasin-B3-C05	HE604721
Kasin-B3-C12	HE604727
Kasin-B2-D10	HE604673
Kasin-B5-B12	HE604890
Kasin-B5-E01	HE604909
Kasin-B4-G08	HE604865
Kasin-B1-C12	HE604817
Kasin-B2-B06	HE604655

Kasin-B2-C03	HE604659
Kasin-B3-E01	HE604734
Kasin-B2-F11	HE604690
Kasin-B3-G10	HE604757
Kasin-B2-F02	HE604682
Kasin-B2-A07	HE604646
Kasin-B3-C10	HE604725
Kasin-B2-B03	HE604652
Kasin-B5-H11	HE604938
Kasin-B2-C08	HE604662
Kasin-B1-A04	HE604765
Kasin-B3-D01	HE604728
Kasin-B3-B06	HE604713
Kasin-B3-D03	HE604729
Kasin-B3-B09	HE604716
Kasin-B1-D11	HE604787
Kasin-B1-H06	HE604808
Kasin-B3-A10	HE604708
Kasin-B5-H09	HE604936
Kasin-B5-E08	HE604913
Kasin-B5-E05	HE604911
Kasin-B5-A03	HE604875
Kasin-B5-C09	HE604897
Kasin-B5-A08	HE604878
Kasin-B4-A01	HE604819
Kasin-B4-C06	HE604840
Kasin-B4-A06	HE604824
Kasin-B3-E08	HE604739
Kasin-B3-G12	HE604758
Kasin-B3-C11	HE604726
Kasin-B1-B09	HE604773
Kasin-B4-E04	HE604848
Kasin-B3-A11	HE604709
Kasin-B3-C06	HE604722
Kasin-B3-D12	HE604733
Kasin-B2-C07	HE604661
Kasin-B5-D08	HE604905
Kasin-B5-G04	HE604927
Kasin-B5-E11	HE604914
Kasin-B5-F02	HE604916
Kasin-B5-D04	HE604901
Kasin-B5-H07	HE604934
Kasin-B5-H12	HE604939
Kasin-B5-B06	HE604887
Kasin-B4-G09	HE604866
Kasin-B1-E11	HE604791
Kasin-B4-D06	HE604842
Kasin-B4-A07	HE604825
Kasin-B5-C05	HE604894

		Kasin-B5-F04	HE604918			
		Kasin-B3-E10	HE604741			
		Kasin-B4-D11	HE604845			
Gammaproteobacteria _Halomonadaceae	OTU1	Kasin-B5-D05	HE604902	*Halomonas andesensis*	EF622233	95
		Kasin-B4-E02	HE604847			
Gammaproteobacteria _Halomonadaceae	OTU2	Kasin-B1-D09	HE604818	*Halomonas sulfidaeris*	AF212204	96-98
		Kasin-B2-A08	HE604647			
		Kasin-B5-E06	HE604912			
		Kasin-B5-F06	HE604920			
		Kasin-B1-C04	HE604778			
		Kasin-B1-F10	HE604796			
		Kasin-B1-G10	HE604803			
Gammaproteobacteria _Halomonadaceae	OTU3	Kasin-B5-B11	HE604889	*Halomonas kenyensis*	AY962237	94
Gammaproteobacteria _Halomonadaceae	OTU4	Kasin-B2-H07	HE604701	*Halomonas cerina*	EF613112	97
Gammaproteobacteria _Halomonadaceae	OTU5	Kasin-B1-A06	HE604815			
		Kasin-B1-H01	HE604806	*Halomonas cerina*	EF613112	95-96
Gammaproteobacteria _Halomonadaceae	OTU1	Kasin-B1-B05	HE604770	*Chromohalobacter nigrandesensis*	AJ277205	99
Gammaproteobacteria _Alteromonadaceae	OTU1	Kasin-B1-B02	HE604768	*Marinobacter flavimaris*	AY517632	98
		Kasin-B1-B01	HE604767			
		Kasin-B4-F06	HE604856			
		Kasin-B2-E10	HE604679			
		Kasin-B2-F01	HE604681			
		Kasin-B4-A10	HE604827			
Gammaproteobacteria _Alteromonadaceae	OTU2	Kasin-B2-A05	HE604645	*Marinobacter guineae*	AM503093	95-96
		Kasin-B4-G06	HE604864			
		Kasin-B2-B02	HE604651			
		Kasin-B2-A12	HE604650			
Gammaproteobacteria _Alteromonadaceae	OTU3	Kasin-B2-H04	HE604700	*Marinobacter guineae*	AM503093	96-97
		Kasin-B4-C02	HE604836			
		Kasin-B3-E06	HE604737			
		Kasin-B1-B04	HE604769			
		Kasin-B3-B12	HE604717			
Gammaproteobacteria _Alteromonadaceae	OTU4	Kasin-B2-G10	HE604694	*Marinobacter salicampi*	EF486354	96-97
		Kasin-B3-H10	HE604763			
		Kasin-B3-G04	HE604753			
		Kasin-B1-C03	HE604777			
		Kasin-B3-E11	HE604742			
Gammaproteobacteria _Alteromonadaceae	OTU5	Kasin-B2-F06	HE604686	*Marinobacter lacisalsi*	EU047505	99
		Kasin-B1-D07	HE604784	*Halospina dentitrificans*	DQ072719	97-98
		Kasin-B4-B05	HE604831			
		Kasin-B4-A09	HE604826			
		Kasin-B4-E11	HE604852			
		Kasin-B3-E05	HE604736			
		Kasin-B3-G05	HE604754			
		Kasin-B3-G06	HE604755			
		Kasin-B2-A03	HE604643			
		Kasin-B3-C03	HE604720			
		Kasin-B1-H10	HE604811			
		Kasin-B4-H10	HE604873			

		Kasin-B2-A02	HE604642			
		Kasin-B3-F09	HE604748			
		Kasin-B3-H11	HE604764			
		Kasin-B1-H04	HE604807			
		Kasin-B5-B02	HE604884	*Mariprofundus ferrooxydans*	EF493243	83
		Kasin-B5-C02	HE604892			
Candidate division EM19	OTU1	Kasin-B2-C01	HE604657	n/a		
		Kasin-B3-A05	HE604706			
		Kasin-B5-F07	HE604921			
Candidate division EM19	OTU2	Kasin-B5-H02	HE604932	n/a		
		Kasin-B3-F05	HE604747			
		Kasin-B2-F04	HE604684			
Candidate division EM19	OTU3	Kasin-B2-F03	HE604683	n/a		
Candidate division EM19	OTU4	Kasin-B5-H10	HE604937	n/a		
Candidate division EM19	OTU5	Kasin-B4-A05	HE604823	n/a		
		Kasin-B4-H02	HE604869			
Candidate division EM19	OTU6	Kasin-B5-G06	HE604929	n/a		
Candidate division EM19	OTU7	Kasin-B2-G04	HE604693	n/a		
Candidate division EM19	OTU8	Kasin-B3-A09	HE604707	n/a		
Candidate division EM19	OTU9	Kasin-B2-A09	HE604648	n/a		
		Kasin-B2-H11	HE604704			
Spirochaetes_Spirochaetaceae	OTU1	Kasin-B2-A04	HE604644	*Spirochaeta halophila*	M88722	89
Spirochaetes_Spirochaetaceae	OTU2	Kasin-B2-E11	HE604680	*Spirochaeta halophila*	M88722	86
		Kasin-B2-G11	HE604695			
		Kasin-B1-B07	HE604771			

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