Supplementary Information for

Seeking active RubisCOs from the currently uncultured microbial majority colonizing deep-sea hydrothermal vent environments

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Supplementary Material and Methods

Supplementary Material and Methods S1

Culturing different RubisCO active Proteobacteria. Six genomic fosmid libraries were constructed with DNA isolated from six different proteobacterial strains, namely from the Gammaproteobacterium *Hydrogenovibrio crunogenus* TH-55 (DSMZ no. 12353) (formerly *Thiomicrospira crunogena* (1)), the Betaproteobacterium *Thiobacillus denitrificans* AB7 (DSMZ no. 12475), and the Alphaproteobacteria *Rhodovulum sulfidophilum* W4 (DSMZ no. 1374), *Thioclava pacifica* TL 2 (DSMZ no. 10166), *Rhodobacter capsulatus* SB 1003 and *Rhodobacter sphaeroides* ATH 2.4.1 (DSMZ no. 158). In order to isolate genomic DNA (gDNA) these isolates were grown as described in the following: *H. crunogenus* TH-55 was grown on T-ASW medium at 28°C as described before (2, 3), *T. denitrificans* AB7 was cultivated anaerobically at 30°C on DSMZ medium 113 according to DSMZ's instructions, *R. sulfidophilum* W4 and *R. sphaeroides* ATH 2.4.1 were cultivated anaerobically with light at room temperature on DSMZ medium 27 supplemented with 2.5% NaCl as recommended by the DSMZ, *T. pacifica* TL 2 was grown on Bacto marine broth at 28°C according to DSMZ's recommendation and *R. capsulatus* SB 1003 was cultivated aerobically in the dark with shaking on RVC medium with DL-malate as carbon source as described elsewhere (4, 5).

Supplementary Material and Methods S2

Isolating (meta)genomic DNA suited for (meta)genomic library construction. For isolating gDNA cells were routinely harvested at the end of the exponential growth phase. Cell pellets were washed twice with 1x PBS (phosphate buffered saline) and stored at -20°C until gDNA was isolated. gDNA of *H. crunogenus* TH-55 was extracted using a common phenol-chloroform extraction method (6). For all other strains the UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, CA, USA) was used to isolate gDNA following manufacturer's instructions.

Prior to isolating metagenomic DNA (mDNA) the three chimney samples from Sisters Peak, Mephisto, and Site B were first grinded using mortar and pistil. The fluids of Drachenschlund, Irina II, and Lilliput, which were concentrated on a polycarbonate filter, were directly utilized for mDNA isolation without any pre-treatment. mDNA of Sisters Peak, Mephisto, Drachenschlund, and Site B were isolated by using common phenol-chloroform extraction (6). For mDNA isolation from Irina II and Lilliput the UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, Inc.), following manufacturer's protocol, was used.

Supplementary Material and Methods S3

Treatment of (meta)genomic DNA prior to fosmid library construction. Isolated (meta)genomic DNA was first visualized on a 0.8% TAE-agarose gel in order to estimate the size and exclude that the DNA is sheared. DNA concentrations and purities of isolated (meta)genomic DNA were measured on a NanoDrop2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). DNA

concentrations and purities of genomic DNA used for the construction of the six genomic libraries were as follows: *T. crunogena* TH-55 (87 ng*µl^{-1}, ratio$_{260/280}$: 1.9), *T. denitrificans* AB7 (22 ng*µl^{-1}, ratio$_{260/280}$: 2.0), *R. sulfidophilum* W4 (93 ng*µl^{-1}, ratio$_{260/280}$: 1.9), *T. pacifica* TL 2 (107 ng*µl^{-1}, ratio$_{260/280}$: 1.9), *R. capsulatus* SB1003 (82 ng*µl^{-1}, ratio$_{260/280}$: 2.0) and *R. sphaeroides* ATH 2.4.1 (70 ng*µl^{-1}, ratio$_{260/280}$: 2.0).

Since DNA concentrations and purities of isolated mDNA from all six hydrothermal vent sites did not meet the standards needed for the construction of fosmid libraries multiple displacement amplification (MDA) was applied. For this purpose the REPLI-g Kit with the phi29 DNA polymerase (Qiagen, Hilden, Germany) was used according to the manufacturer's instruction. For each reaction, three parallel samples with 2.5 µl starting material were applied and pooled after MDA was completed. Subsequently the MDA treated, pooled mDNA was purified using phenol/chloroform extraction as described in the following. First of all the volume of each pooled MDA was increased with nuclease free water from 120 µl to 600 µl in order to minimize losses of DNA during purification. Pooled MDA samples were then washed twice with (i) 600 µl chloroform-isoamyl alcohol (v/v, 24:1) followed by (ii) 600 µl chloroform. Precipitation was implemented overnight at -20°C with sodium acetate (0.3 M final concentration) and isopropanol (1 v/v). After pelleting MDA treated DNA via centrifugation (16,100 x g, 20 minutes and 4°C), two washing steps with ethanol (70%) were performed. Dried mDNA was finally resuspended in 30 µl nuclease-free water. Thus, from the low concentrated, impure DNA isolated from (I) Drachenschlund (42 ng*µl^{-1}, ratio$_{260/280}$ 1.46), (II) Sisters Peak (113 ng*µl^{-1}, ratio$_{260/280}$: 1.42), (III) Mephisto (9 ng*µl^{-1}, ratio$_{260/280}$: 1.39), (IV) Site B (5 ng*µl^{-1}, ratio$_{260/280}$: 1.66), (V) Lilliput (11 ng*µl^{-1}, ratio$_{260/280}$: 1.5) and (VI) Irina II (6 ng*µl^{-1}, ratio$_{260/280}$: 1.7) DNA, suited for the construction of metagenomic libraries, were obtained [(I) Drachenschlund (188 ng*µl^{-1}, ratio$_{260/280}$ 1.82), (II) Sisters Peak (840 ng*µl^{-1}, ratio$_{260/280}$: 1.89), (III) Mephisto (774 ng*µl^{-1}, ratio$_{260/280}$: 1.64), (IV) Site B (64 ng*µl^{-1}, ratio$_{260/280}$: 1.91), (V) Lilliput (40 ng*µl^{-1}, ratio$_{260/280}$: 1.33) and (VI) Irina II (72 ng*µl^{-1}, ratio$_{260/280}$: 1.85)].

Supplementary Material and Methods S4

Quality control of (meta)genomic libraries. Quality control of fosmid inserts was randomly performed by exemplarily testing at least 10 fosmid clones. Insert sizes were checked through restriction analyses with three different enzymes (*Bam*H1, *Eco*R1 and *Hind*III). Additionally, fosmid inserts of these clones were sequenced from both insert ends (Eurofins MWG Operon, Ebersberg, Germany) using T7 forward and pCC1FOS reverse sequencing primers (see manual for the CopyControl™ Fosmid Library Production Kit, epicentre®, Madison, USA) to verify the origin of fosmid inserts and exclude contaminations with foreign DNA. The obtained TH-55 genomic library contained 1 152 fosmid clones with average insert sizes of 29 kb ± 5 kb. All tested fosmid clones from the TH-55 library exhibited high DNA similarities (91 to 98%) to the closest relative genome sequence of *H. crunogenus* XCL-2 (7). The *T. denitrificans* AB7 genomic library comprises 1 536 fosmid clones with inserts sizes of 26 kb ± 7 kb and 100% DNA similarity to the *T. denitrificans* AB7 genome sequence (BioProject:

PRJNA169731, NCBI). The *R. sulfidophilum* genomic library consists of 1 344 fosmid clones carrying inserts with 37 kb ± 5 kb in size and DNA similarities of 100 % to the genome sequence of *R. sulfidophilum* W4 (8). The obtained *T. pacifica* TL 2 genomic library comprises 1 344 fosmid clones with insert sizes of 37 kb ± 4 kb and exhibit high DNA similarities (100%) to the *T. pacifica* TL 2 genome sequence (9). The genomic library of *R. capsulatus* SB 1003 was made up of 2 304 fosmid clones with insert sizes of 38 kb ± 4 kb and DNA similarities of 100 % to the genome sequence of *R. capsulatus* SB 1003 (10). The generated *R. sphaeroides* ATH 2.4.1 genome library consists of 1 152 fosmid clones with an average insert size of 26 kb ± 4 kb and highest similarities on DNA level (100%) with the genome sequence of *R. sphaeroides* ATH 2.4.1 (11, 12).

Supplementary Material and Methods S5

Sampling of hydrothermal vents. Six hydrothermally influenced environmental samples originating from geographically and chemically distinct vent fields along the Mid-Atlantic Ridge were used to construct metagenomic libraries (see Supplementary Figure S1 for location of sampling sites). All these samples were collected within the DFG-SPP 1144 priority program “From Mantle to Ocean: Energy-, Material-, and Life-cycles at Spreading Axes”. Sampling was done by a remote operated vehicle (ROV 6000, GEOMAR, Kiel) during the MSM 10-3 cruise (January/February 2009) with the RV Maria S. Merian and the MAR SUED V cruise (March/April 2009) with the RV Meteor. Two different sample types were examined, namely chimney samples and hydrothermal fluid samples. Chimney samples were taken from (i) Sisters Peak at 4°48’S/12°22’W at a water depth of 2 982 m (20), from (ii) Mephisto at 4°47’S/12°22’W at a water depth of 3 042 m (part of the Red Lion vent field) (20) and from (iii) Site B at 14°45’N/44°58’W at a water depth of 3 047 m (part of the Logatchev vent field) (9 and references therein). Chimney samples were stored immediately at -70°C until further investigations. Hydrothermal fluids were collected from (i) the Nibelungen vent field at 8°18’S/13°30’W at a water depth of 2 915 m (21), from the interface zone between hot fluids emanating from Drachenschlund and ambient seawater (21), from (ii) the Logatchev hydrothermal field at 14°45’N/44°58’W at a water depth of 3 003 m (10), from the mussel bed at Irina II and from (iii) the Lilliput vent field at 9°32’S/13°12’W from the Bathymodiolin mussel assemblages at the Lilliput main site at a water depth of 1 519 m (6). The hydrothermal fluids were routinely concentrated onboard on a 0.2 µm polycarbonate filter and kept at -20°C until further analyses. Detailed sampling procedures and further information on the sampling sites are described elsewhere (6, 9, 10, 22-25).

Supplementary Material and Methods S6

Brief methods description of the used activity-based RubisCO screen. In order to screen the six constructed metagenomic fosmid libraries (approx. 2 600 clones each) for clones harboring RubisCO activity, we used a recently established HPLC (High Performance Liquid Chromatography) based screening procedure (6, 13). Briefly, 24 metagenomic fosmid clones were cultivated together on one LB agar plate (12.5 µg

ml-1 chloramphenicol) and allowed to grow over night at 37°C. Grown colonies were then swamped off with 10 ml LB medium (12.5 µg ml-1 chloramphenicol) and used to inoculate (0.1% v/v) a working culture (200 ml pre-heated LB medium supplemented with chloramphenicol [12.5 µg ml-1] and autoinduction solution [1x final concentration (epicentre®)]. After 18 hours of growth at 28°C, working culture was harvested by centrifugation (9 800 x g, 10 minutes, and 4°C). The cell pellet was washed twice with buffer A (100 mM Tris-HCl (pH 7.8), 10 mM MgCl2, 1 mM EDTA, 25 mM $NaHCO_3$ and 1 mM DTT), followed by cell lysis using the French pressure cell press method. After pelletizing cellular debris via centrifugation (19 580 x g, 20 minutes, and 4 °C), the crude extract was stored over night at 4°C prior to using it as template to perform the RubisCO activity assay. Two assay temperatures were routinely tested (25°C and 55°C). Concentrations of the reactant (RuBP) and the product (3-PGA) of the classical RubisCO catalyzed reaction were quantified over time (t1=0min; t2=30min and t3=120min) using HPLC measurements (12, modified from 27). Here, the LaChrom Elite® system (Hitachi, Tokyo, Japan) with a Lichrospher® 100 RP 18e column (VWR International GmbH, Darmstadt, Germany) was used. Separation was done at 22°C via ion pair chromatography with 0.05 M Tetrabutylammonium hydrogensulfate (Merck, Darmstadt, Germany) in isocratic runs with 10 % acetonitrile (v/v) as eluent and a flow rate of 0.6 ml per minute. Detection was performed at 210 nm. Three different controls were measured in parallel with each activity assay: (i) the genomic fosmid clone Hcrun6F8, whose insert has been demonstrated not to encode any RubisCO genes, serves as a negative control. (ii) The RubisCO active genomic fosmid clone Hcrun6F3 serves as positive control. (iii) A protein free reference sample with 5 mM RuBP and 5 mM 3-PGA dissolved in buffer A was used as an internal standard. If a pool of 24 clones exhibited a decrease of RuBP and/or an increase of 3-PGA, the pool was broken down (e.g. 2 x 12 clone pools, followed by 3 x 4 clone pools, and 4 individual clones) until the one clone responsible for this conversion was identified.

Supplementary Material and Methods S7

Measuring RubisCO activities of single clones. Specific RubisCO activities of 20 genomic and 48 metagenomic fosmid clones were measured using HPLC as described above for fosmid clone pools, but with some modifications with respect to culturing and assay performance. Thus, single clones were pre-cultured in 5 ml LB medium supplemented with chloramphenicol (12.5 µg ml-1) at 37°C over night and used as inoculum for the 200 ml working culture (1:1000). The assays differ in that RubisCO activities of single genomic- and metagenomic fosmid clones were measured at only one temperature (25°C). Additionally, the assay was modified in order to measure RubisCO activities of metagenomic fosmid clones under anoxic conditions at 25°C and 55°C. Aerobically produced crude extracts were allowed to become anoxic by placing them under a N_2/H_2 atmosphere (95%/5%; v/v) at 4°C over night. The assay was performed in an anaerobic chamber under the same atmosphere using anoxic buffer A and anoxic RuBP substrate. Final HPLC measurements of heat inactivated subsamples were then performed under aerobic conditions as described above. At least three biological replicates and three technical replicates were used for the

RubisCO activity assay. Mean values of technical replicates were used to calculate the overall mean. Errors of RubisCO activity measurements were calculated with the Gaussian propagation of error. Standard deviation of technical replicates were propagated forward and are thus entered into the equation. Significant differences were calculated using an unpaired t-test with equal variance and two-tailed distribution. Controls performed for each HPLC run were the same as mentioned above for the activity-based RubisCO screen.

Supplementary Material and Methods S8

Preparation of PCR suited DNA pools. In order to prepare PCR suited DNA pools a copy of the microtiterplate to be examined was prepared. Therefore, each well of the new microtiterplate was filed with 200 µl LB medium supplemented with chloramphenicol (12.5 µg ml^{-1}) and inoculated with cells of the original plate using a stamp with 96 pins. After incubation for 24 hours at 37°C the 96 grown fosmid clones were unified in one single reaction tube and centrifuged (16,100 x g, 45 seconds and 4°C). Cell pellets were then resuspended in 200 µl TE_{DNA} buffer [10 mM Tris-HCl (pH = 8.0) and 0.1 mM EDTA] and heated up for 10 minutes at 95°C to lyse cells. Cell debris were removed by centrifugation (16 100 x g, 10 minutes and 4°C) and supernatants were transferred to a new, nuclease free tube. Finally 1 µl of this PCR suited DNA pool was used as template to screen for RubisCO form I, II and III structural genes.

Supplementary Material and Methods S9

Breaking down a RubisCO active 96 clone pool. If a pool of 96 clones was tested positive, the one clone harboring the RubisCO gene needs to be identified. For this purpose another 20 pools were prepared from the positive tested microtiterplate: (i) 12 pools of the columns 1 to 12 (8 clones per pool) and 8 pools of the rows A to H (12 clones per pool). These pools were prepared in the same way as described for 96 clones, but with the exception that cell pellets were resuspended in 100 µl TE_{DNA} buffer. Again 1 µl of these pools were used as template for PCR following the same conditions as mentioned in the main text for pools of 96 clones. By a crossover comparison of the PCR based hits for RubisCO encoding genes in all rows and columns of one microtiterplate, the one fosmid clone harboring the RubisCO encoding gene was identified.

Supplementary Tables

Supplementary Table S1. Physical and chemical parameters specific for the studied hydrothermal vent locations.

sampling sites	sample type	T (°C)	pH (at 25°C)	H_2 (µM)	sulfide (µM)	O_2 (µM)	references
basalt-hosted							
Sisters Peak, 5°S (274 ROV1B, MAR-SÜD V)	chimney	464	6.6 ± 0.1	1 600	82 ± 22	141 ± 20	(14)
Mephisto, 5°S (297 ROV 1, MAR-SÜD V)	chimney	320-348	5.1 ± 1.3	314-350	2 916 ± 1 674	no data	(15)
Lilliput main site, 9°S (324 ROV 6-8, MAR-SÜD V)	diffuse fluids	9	6.3 ± 0.01	0.9	47 ± 7	71 ± 2	(16)
ultramafic-hosted							
Site B, 15°N (313 ROV 1, HYDROMAR VII)	chimney	350	3.8	1 800	1 241	no data	(17)
Irina II, 15°N (300 Rov 19-21, HYDROMAR VII)	diffuse fluids	6.4-11	6.54-6.94	162-327	1-6	208-235	(18)
Drachenschlund, 8°S (314 ROV 7-9, MAR-SÜD V)	crater fluids	90-120	5.6 ± 0.3	17-26	8.2 ± 3.2	56 ± 44	(19)

Supplementary Table S2. Primer-Pairs and PCR conditions used for the sequence-based RubisCO screening of genomic fosmid libraries.

RubisCO form	primer	sequence (5'-3')	PCR conditions	reference
H. crunogenus				
green- type form I	cbbLF	GACTTCACCAAAGACGACGA	95°C for 3 min; 32 cycles: 95°C for 30 s, 46°C for 30 s, 72°C for 45 s;	(20)*
	cbbLR	TCGAACTTGATTTCTTTCCA	final extension, 72°C for 5 min	
green-type form II	RuIIF1	GGHAACAACCARGGYATGGGYGA	*first cycle:* 94°C for 3 min, 66°C for 30 s, and 72°C for 30 s;	(21)
	RuIIR3	CGHAGIGCGTTCATGCCRCC	*35 subsequent cycles:* 94°C for 30 s, 66°C for 30 s, and 72°C for 30 s; final extension, 72 ° C for 7 min.	
T. denitrificans				
green- type form I	RubIgF	GAYTTCACCAARGAYGAYGA	*first cycle:* 94°C for 3 min, 58°C for 1 min, and 72°C for 1 min;	(21)
	RubIgR	TCRAACTTGATYTCYTTCCA	*7 subsequent cycles:* 94 °C for 30 s, 58°C for 20 s, and 72°C for 45 s; *last 28 cycles:* 94°C for 30 s, 45°C for 30 s, and 72 ° C for 30 s; final extension, 72° C for 7 min	
green-type form II	RuIIF1	GGHAACAACCARGGYATGGGYGA	*first cycle:* 94°C for 3 min, 66°C for 30 s, and 72°C for 30 s;	(21)
	RuIIR3	CGHAGIGCGTTCATGCCRCC	*35 subsequent cycles:* 94°C for 30 s, 66°C for 30 s, and 72°C for 30 s; final extension, 72 ° C for 7 min.	
R. sulfidophilum				
green- type form I	cbbLF	GACTTCACCAAAGACGACGA	95°C for 3 min; 32 cycles: 95°C for 30 s, 46°C for 30 s, 72°C for 45 s;	(20)*
	cbbLR	TCGAACTTGATTTCTTTCCA	final extension, 72°C for 5 min	
T. pacifica				
green- type form I	RubIgF	GAYTTCACCAARGAYGAYGA	*first cycle:* 94°C for 3 min, 58°C for 1 min, and 72°C for 1 min;	(21)
	RubIgR	TCRAACTTGATYTCYTTCCA	*7 subsequent cycles:* 94 °C for 30 s, 58°C for 20 s, and 72°C for 45 s; *last 28 cycles:* 94°C for 30 s, 45°C for 30 s, and 72 ° C for 30 s; final extension, 72° C for 7 min	
R. capsulatus				
green- type form I	R.cap_cbbLF	TCATTGCCGAAGATCGAGAC	95°C for 5 min; 32 cycles: 95°C for 45 s, 59°C for 45 s, 72°C for 60 s;	this study
	R.cap_cbbLR	TATGCCTTCATCGCCTATCC	final extension, 72°C for 5 min	
red-type form II	R.cap_cbbMF	CGCCATCCAGGTGACCAAAG	95°C for 5 min; 32 cycles: 95°C for 45 s, 58°C for 45 s, 72°C for 60 s;	this study
	R.cap_cbbMR	CCTGCTATCTGCGCCTGTTC	final extension 72°C for 5 min	
R. sphaeroides				
red-type form I	R.sph_cbbLF	GTCCTGCTCGAAGAAGATCC	95°C for 5 min; 32 cycles: 95°C for 45 s, 57°C for 45 s, 72°C for 55 s;	this study
	R.sph_cbbLR	CTGCTATGTGGCCTATGACC	final extension 72°C for 5 min	
red-type form II	R.sph_cbbMF	TGAGATCGACCCGGAGAAGG	95°C for 5 min; 32 cycles: 95°C for 45 s, 58°C for 45 s, 72°C for 55 s;	this study
	R.sph_cbbMR	CTCGTCGGTCAGCATGTAGG	final extension 72°C for 5 min	

* The annealing temperature of cbbLF and cbbLR was adapted from 48°C to 46°C.

Supplementary Table S3. Predicted insert sizes of identified metagenomic fosmid clones calculated by restriction analyses.

clone	insert size*
IrII31F9	39 ± 13
SB29A9	47 ± 11
SP52C8	14 ± 2
SP82B6	27 ± 1
SP88G2	14 ± 4
SP92E10	30 ± 8
SP96F12	25 ± 3
SP119A5	25 ± 9
SP133E7	22 ± 6
D71C2	37 ± 2

*Mean values and standard deviation were calculated from restriction patterns of three different enzymes (*Bam*H1, *Eco*R1 and *Hind*III).

Supplementary Table S4. Primers used for primer walking on IrII31F9's fosmid insert.

primer description	sequence 5'-3'
sequencing of IrII31F9's Fragment A	
T7 promoter primer[1]	TAATACGACTCACTATAGGG
IrII31F9_T71	GGTGGATACATCACAATCTC
IrII31F9_T7_pw1	CGCTATCAGCATATCAAAGG
IrII31F9_FragAR01	GGATCCTTATGTGCCATCAG
IrII31F9_FragAR02	ACTGGATGCCCTGCCTTATG
IrII31F9_FragAR03	GCGCTTGGTGCAACTTAGAG
IrII31F9_FragAR04	ACCCTATGAACCGCATACTG
IrII31F9_FragAR05	TATCAGATTGGCACCCTTGG
IrII31F9_FragAR06	GCTTTAGCGGGTAACAGTGC
sequencing of IrII31F9's Fragment BC	
pCC1FOS_rev[1]	CTCGTATGTTGTGTGGAATTGTGAGC
IrII31F9_pCCpw1	CTGTTATGACGTCTGCATTC
IrII31F9_56F	TGCCGATATTCGTCAGACCC
IrII31F9_FragCR01	ACAGTTCGCGACGGTGATCC
IrII31F9_FragBR01	ACAGGGTGCTTCTGGTATCC
IrII31F9_FragCF02	TTGTGTACGCCCAGTTTCTC
RuIIR3[2]	CGHAGIGCGTTCATGCCRCC
RuIIF1[2]	GGHAACAACCARGGYATGGGYGA
IrII31F9_FragBF01	GCGATGTAACCACCGTTGTC
IrII31F9_FragBCR02	GGGACAGCAACTCAGGTAAG
IrII31F9_FragBCR03	AATACCTGAGCCCGTCTCAC
IrII31F9_FragDF01	TGCGGACAGGGTTGCTTTGG
IrII31F9_FragBCR04	TCAGCTGCCTTGGGAGAAAC
IrII31F9_103F	TTGTAGCTGCGCCGCCTAAC
IrII31F9_BCR05_SeeMemo	AGGTCGCTCAAGGAATTTGC
IrII31F9_103R	GTTGGGCTGTTATATGTAAG
IrII31F9_FragBCR06	GCGGCAGAATCTTCAACAGG

[1] CopyControl™ Fosmid Library Production Kit (epicentre®)
[2] (21)

Supplementary Table S5. Primers used for primer walking on SB29A9's fosmid insert.

primer description	sequence 5'-3'
sequencing of SB29A9's Fragment A	
T7 promoter primer[1]	TAATACGACTCACTATAGGG
SB29A9_FragAR01	TCGGCAGGTCAACTGGATGG
SB29A9_FragAR02	AGTGGGCGACGCAAACTGAC
SB29A9_AIIF01	AGCAGGATGTCGCGTTCCTC
SB29A9_FragAR03	GACGCAATCGGCTCCGAAAG
SB29A9_FragBF07	AAGCACGACCGAACAGGATG
SB29A9_AIIR04	TTGCGCGACATTCTCGACAC
sequencing of SB29A9's Fragment B	
SB29A9_FragBF06	GCCCGACAAAGGACAACACG
SB29A9_FragAR05	TATCGTGCCGGTATCAACCC
SB29A9_FragBF05	ACCAGGAAAGCGGGATTTCG
SB29A9_FragBF04	CGAAGCACGGTCTGAAACTG
SB29A9_FragBF03	TTGTCGGTGAACGGTTTCTG
SB29A9_FragBF02	CATTACCCTGCGCCAGATGC
SB29A9_FragBF01	TTCACCACCGAGCCCTCTTC
cbbLRpw1	GCGGTCACGTTCAGATAGTGG
cbbLF[2]	GACTTCACCAAAGACGACGA
cbbLR[2]	TCGAACTTGATTTCTTTCCA
cbbLFpw1	TTTCGCTGGAAGCATGTGTC
SB29A9_FragCR01	GGTCAGTTGCGGAAGATACG
sequencing of SB29A9's Fragment C	
pCC1FOS_rev[1]	CTCGTATGTTGTGTGGAATTGTGAGC
SB29A9_pCC85	CTCAAGCAAAGGAACAAATC
SB29A9_FragCF01	ACTACAGTTCCCGTCTGTCC
SB29A9_FragCF02	CTTCGGATGCACGGCGTTTG
SB29A9_FragCR07	AGCATCCGCACCAGCACATC
SB29A9_c112F	CTGGTTCAGGTGGCACAAAG
SB29A9_FragCR08	GGCTGAAGGACTATCTTGAC
SB29A9_FragBR02	CGATGATGAAGGACCTGAAG
SB29A9_FragBR03	CCATCCCGGTTCCTGAAGAC
SB29A9_FragBR04	TGCAGGCGCCGTCAAGATAG
SB29A9_FragBR04checkII	AAGGTGGCATCAAGCGTGTC
SB29A9_FragBR05neu	GTGTTTACCGCCGCACCAAG
SB29A9_FragBR01	AAGCCGACGCCAAAGAATAC
SB29A9_FragBR07	CGACGTGGCCCACAAATAGG

[1] CopyControl™ Fosmid Library Production Kit (epicentre®)
[2] (20)

Supplementary Table S6. Sanger sequencing results of fosmid clones identified via function- and/or sequence-based screening.

clone	function based	sequence based	length [bp]	blastn description	query cover	e-value[1]	identity	accession
IrII31F9 T7 site	+	+	3 765	*Hydrogenovibrio crunogenus* XCL-2	98%	0.0	91%	CP000109.2
IrII31F9 pCC1FOS rev site			6 322	*Hydrogenovibrio crunogenus* XCL-2	99%	0.0	91%	CP000109.2
SB29A9 T7 site	-	+	3 189	*Paracoccus* sp. CBA4604 chromosome, complete genome	15%	9e-61	71%	CP025583.1
SB29A9 cbbL site			4 395	*Thioclava nitratireducens* strain 25B10_4, complete genome	31%	0.0	81%	CP019437.1
SB29A9 pCC1FOS rev site			2 349	*Thioclava nitratireducens* strain 25B10_4, complete genome	82%	0.0	75%	CP019437.1
SP52C8 T7 site	-	+	542	*Aciduliprofundum* sp. MAR08-339, complete genome	76%	1e-106	76%	CP003168.1
SP52C8 arubIII site			1153	*Aciduliprofundum* sp. MAR08-339, complete genome	97%	0.0	77%	CP003168.1
SP52C8 pCC1FOS rev site			529	*Aciduliprofundum* sp. MAR08-339, complete genome	97%	2e-130	81%	CP003168.1
SP82B6 T7 site	-	+	770	no significant similarity found				
SP82B6 pCC1FOS rev site			668	no significant similarity found				
SP88G2 T7 site	-	+	485	*Aciduliprofundum* sp. MAR08-339, complete genome	100%	2e-79	74%	CP003168.1
SP88G2 arubIII site			572	*Aciduliprofundum* sp. MAR08-339, complete genome	99%	4e-130	79%	CP003168.1
SP88G2 pCC1FOS rev site			468	no significant similarity found				
SP92E10 T7 site	-	+	601	*Aciduliprofundum* sp. MAR08-339, complete genome	98%	2e-141	79%	CP003168.1
SP92E10 arubIII site			819	*Aciduliprofundum* sp. MAR08-339, complete genome	99%	6e-173	77%	CP003168.1
SP92E10 pCC1FOS rev site			439	no significant similarity found				
SP96F12 T7 site	-	+	602	*Aciduliprofundum* sp. MAR08-339, complete genome	99%	4e-124	81%	CP003168.1
SP96F12 arubIII site			1154	*Aciduliprofundum* sp. MAR08-339, complete genome	100%	0.0	78%	CP003168.1
SP96F12 pCC1FOS rev site			460	*Aciduliprofundum* sp. MAR08-339, complete genome	98%	2-123	82%	CP003168.1
SP119A5 T7 site	-	+	763	*Aciduliprofundum* sp. MAR08-339, complete genome	53%	2-66	75%	CP003168.1
SP119A5 arubIII site			895	*Aciduliprofundum* sp. MAR08-339, complete genome	99%	0.0	78%	CP003168.1
SP119A5 pCC1FOS rev site			552	*Aciduliprofundum* sp. MAR08-339, complete genome	75%	7e-53	75%	CP003168.1
SP133E7 T7 site	-	+	710	*Aciduliprofundum* sp. MAR08-339, complete genome	84%	2e-60	74%	CP003168.1
SP133E7 arubIII site			775	*Aciduliprofundum* sp. MAR08-339, complete genome	99%	2e-152	76%	CP003168.1
SP133E7 pCC1FOS rev site			673	no significant similarity found				
D71C2 whole insert[2]	+	+	35 198	*Hydrogenovibrio crunogenus* XCL-2	99%	0.0	96%	CP000109.2
D16D10 T7 site[3]	+	+	613	*Hydrogenovibrio crunogenus* XCL-2	99%	0.0	92%	CP000109.2
D16D10 pCC1FOS rev site[3]			612	*Hydrogenovibrio crunogenus* XCL-2	100%	0.0	87%	CP000109.2

[1]Cut-off e-value was set at <1e-05; [2]Fosmid clone D71C2 was listed representatively as one out of twelve different fosmid clones [D74C10; D74E1; D77H1; D77F4; D77D9; D78G5; D78E10; D80G3; D81E1; D81G7; D84G4], whose inserts were fully sequenced and share 100 % similarity to each other (for details see reference 6); [3]Fosmid clone D16D10 was listed in place of twenty-seven fosmid clones over all [D2B3, D2B7, D2F7, D3E5, D6F10, D7D9, D7G5, D8D11, D8F12, D10A9, D10D2, D11C11, D12E1, D13E6, D13E9, D13G12, D14D8, D14D11, D14E6, D15F5, D16B1, D16C10, D16D10, D16E3, D210C1, D210H6, and D211F9], whose insert ends were sequenced and show (i) 100 % similarity to each other and (ii) are 100% identical with D71C2's insert ends, too.

Supplementary Table S7. Highest blastx similarity found for each orf identified on the partial fosmid insert sequences of IrII31F9 and SB29A9.

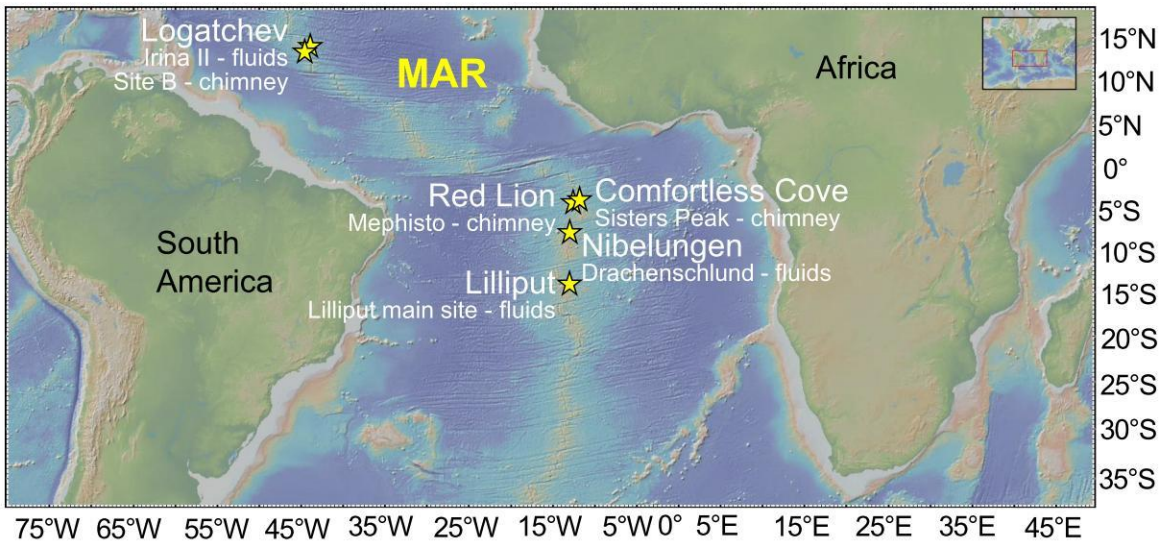
	orf nr.	length	predicted function of gene product	related organism	e-value	identity	accession
Fragment A	*IrII31F9orf01*	partial	*cbbO-m*; von Willebrand factor type A	uncultured bacterium	0.0	99%	AIM47440.1
	IrII31F9orf02	219aa	*CA*; carbonate dehydratase	uncultured bacterium	3e-159	98%	AIM47439.1
	IrII31F9orf03	464aa	*yeeE/yedE*; YeeE/YedE family protein	uncultured bacterium	0.0	94%	AIM47437.1
	IrII31F9orf04	partial	*cbbAs*; RubisCO associated protein, shown to be involved in expressing a fully functional enzyme (homologous to orf06 in 6)	*Hydrogenovibrio crunogenus*	4e-49	84%	WP_011369841.1
Fragment BC	*IrII31F9orf05*	partial	*cbbL*; ribulose-1,5-bisphosphate carboxylase large subunit	uncultured bacterium	2e-116	100%	AIM47445.1
	IrII31F9orf06	309aa	*cbbR1*; transcriptional regulator, LysR family	uncultured bacterium	0.0	99%	AIM47444.1
	IrII31F9orf07	315aa	*cbbR2*; transcriptional regulator, LysR family	*Hydrogenovibrio crunogenus*	0.0	98%	WP_011369846.1
	IrII31F9orf08	460aa	*cbbM*; ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, form II	*Hydrogenovibrio crunogenus*	0.0	100%	WP_011369845.1
	IrII31F9orf09	267aa	*cbbQ-m*; ATPase AAA-type	uncultured bacterium	0.0	99%	AIM47441.1
	IrII31F9orf10	partial	*cbbO-m*; von Willebrand factor type A	uncultured bacterium	7e-128	99%	AIM47440.1
Fragment A	*SB29A9orf01*	partial	*SDR*; SDR family NAD(P)-dependent oxidoreductase	*Pseudovibrio* sp. Tun.PSC04-5.I4	9e-19	67%	WP_093190685.1
	SB29A9orf02	126aa	*SDR*; SDR family NAD(P)-dependent oxidoreductase	*Stappia indica*	2e-60	76%	WP_067221338.1
	SB29A9orf03	71aa	no significant similarity found	-	-	-	-
	SB29A9orf04	799aa	hypothetical protein	*Paracoccus* sp. J55	5e-64	63%	WP_028714378.1
	SB29A9orf05	442aa	hypothetical protein A3D16_22760, putative Zn-dependent peptidase	*Rhodobacterales bacterium*	6e-93	47%	OHC52846.1
	SB29A9orf06	partial	hypothetical protein A3D16_22765, putative Zn-dependent peptidase	*Rhodobacterales bacterium*	4e-27	51%	OHC52847.1
Fragment B	*SB29A9orf07*	428aa	*pqqL*; insulinase family protein [Zn-dependent peptidase]	*Halomonas* sp.	3e-122	55%	WP_078086495.1
	SB29A9orf08	96aa	*ABC*; ABC transporter, ATP-binding protein	*Roseibium* sp. TrichSKD4	2e-30	74%	EFO28650.1
	SB29A9orf09	313aa	*cbbR1*; transcriptional regulator, LysR family	*Rhodobacteraceae bacterium*	2e-135	67%	PCJ73695.1
	SB29A9orf10	473aa	*cbbL*; ribulose-bisphosphate carboxylase large subunit, form I	*Thioclava atlantica*	0.0	90%	WP_038148573.1
	SB29A9orf11	114aa	*cbbS*; ribulose-bisphosphate carboxylase small subunit	*Roseibium* sp. TrichSKD4	2e-69	81%	WP_009760098.1
Fragment C	*SB29A9orf12*	partial	*cbbQ*; CbbQ/NirQ/NorQ/GpvN family protein	*Thioclava pacifica*	9e-79	82%	WP_038078092.1
	SB29A9orf13	192aa	Nitric oxide reductase activation protein NorD	*Gammaproteobacteria*	8e-37	76%	RMG34471.1
	SB29A9orf14	93aa	*cbbQ*; CbbQ/NirQ/NorQ/GpvN family protein	*Gammaproteobacteria*	8e-170	85%	RMG34470.1
	SB29A9orf15	120aa	*cbbS*; ribulose bisphosphate carboxylase small subunit	*Roseibium* sp. TrichSKD4	1e-69	81%	WP_009760098.1

Supplementary Table S8. GenBank accession numbers of RubisCO encoding genes used to generate the phylogenetic tree shown in Figure 2.

abbreviation	strain	gene	accession
A. boonei T469	*Aciduliprofundum boonei* T469	*arubIII*	WP_008086508
Aciduliprofundum sp MAR08-339	*Aciduliprofundum* sp MAR08-339	*arubIII*	WP_015283871
A. ehrlicheii MLHE-1	*Alkalilimnicola ehrlichii* MLHE-1	*cbbL*	WP_011630569
A. ferrooxidans ATCC 23270	*Acidithiobacillus ferrooxidans* ATCC 23270	*cbbL*	WP_009566926
		cbbM	WP_012537012
Anabaena sp. PCC7108	*Anabaena* sp. PCC7108	*cbbL*	WP_016949411
Anabaena sp. WA102	*Anabaena* sp. WA102	*cbbL*	WP_027403993
Candidatus V. okutanii	*Candidatus* Vesicomyosocius okutanii HA	*cbbM*	WP_011930036
D. aromatic RCB	*Dechloromonas aromatica* RCB	*cbbM*	WP_011289362
G. capsiferriformans	*Gallionella capsiferriformans* ES-2	*cbbM*	WP_013292022
H. crunogenus XCL-2	*Hydrogenovibrio crunogenus* XCL-2	*cbbL*	WP_011369848
		cbbM	WP_011369845
H. marinus DSM 11271	*Hydrogenovibrio marinus* DSM 11271	*cbbL*	WP_029912390
		cbbM	WP_029912625
H. halophila DSM 15072	*Hydrogenovibrio halophilus* DSM 15072	*cbbL*	WP_028485359
		cbbM	WP_019895620
M. capsulatus Bath	*Methylococcus capsulatus* str. Bath	*cbbL*	WP_010961949
M. ferroxydans PV-1	*Mariprofundus ferrooxydans* PV-1	*cbbL*	WP_009850044
		cbbM	WP_009851287
M. jannaschii DSM 2661	*Methanocaldococcus jannaschii* DSM 2661	*arubIII*	WP_010870747
N. europaea ATCC 19718	*Nitrosomonas europaea* ATCC 19718	*cbbL*	WP_011112458
N. halophilus Nc4	*Nitrosococcus halophilus* Nc4	*cbbL*1	WP_013034313
		*cbbL*2	WP_013034318
N. hamburgensis X14 chromosom	*Nitrobacter hamburgensis* X14 chromosom	*cbbL*1	WP_011512110
N. hamburgensis X14 plasmid	*Nitrobacter hamburgensis* X14 plasmid	*cbbL*2	WP_011505022
N. multiformis ATCC 25196	*Nitrosospira multiformis* ATCC 25196	*cbbL*	WP_011380043
N. oceani ATCC 19707	*Nitrosococcus oceani* ATCC 19707	*cbbL*	WP_011330306
N. watsonii C-113	*Nitrosococcus watsonii* C-113	*cbbL*	WP_013221603
N. winogradskyi Nb-255	*Nitrobacter winogradskyi* Nb-255	*cbbL*	WP_011316104
P. xenovorans LB400	*Paraburkholderia xenovorans* LB400	*cbbL*	WP_011490903
R. capsulatus SB 1003	*Rhodobacter capsulatus* SB 1003	*cbbL*	WP_013066323
		cbbM	WP_013067553
R. ferrireducens T118	*Rhodoferax ferrireducens* T118	*cbbM*	WP_011463692
R. gelatinosus IL144	*Rubrivivax gelatinosus* IL144	*cbbL*	WP_014429802
R. palustris BisA53	*Rhodopseudomonas palustris* BisA53	*cbbL*	WP_011662799
		cbbM	WP_011664101
R. palustris CGA009	*Rhodopseudomonas palustris* CGA009	*cbbL*	WP_011157118
		cbbM	WP_011160173
R. sphaeroides ATCC 17029	*Rhodobacter sphaeroides* ATCC 17029	*cbbL*	WP_002721829
		cbbM	WP_002723913
R. sulfidophilum DSM 1374	*Rhodovulum sulfidophilum* DSM 1374	*cbbL*	WP_042456572
		cbbM	WP_042462841
Synechococcus sp. PCC7002	*Synechococcus sp.* PCC7002	*cbbL*	WP_012307409
S. elongatus PCC6301	*Synechococcus elongatus* PCC6301	*cbbL*	WP_011242444
T. arctica DSM 13458	*Thiomicrorhabdus arctica* DSM 13458	*cbbL*	WP_019557150
		cbbM	WP_019557147
T. brandeum Hiromi1	*Thiolapillus brandeum* Hiromi1	*cbbM*	WP_041067559
T. denitrificans ATC 25259	*Thiobacillus denitrificans* ATCC 25259	*cbbL*	WP_011313136
		cbbM	WP_011313150
T. nitratireducens strain 25B10_4	*Thioclava nitratireducens strain* 25B10_4	*cbbL*	WP_075774813
T. pacifica DSM 10166	*Thioclava pacifica* DSM 10166	*cbbL*	WP_038078094
uncultured SUP05 cluster bacterium	uncultured SUP05 cluster bacterium	*cbbM*	ACX30513
uncultured bacterium – clone D81G7	metagenomic DNA fragment	*cbbL*	AIM47442
		cbbM	AIM47445
uncultured bacterium – clone IrII31F9	metagenomic DNA fragment	*cbbM*	this study
uncultured bacterium – clone SB29A9	metagenomic DNA fragment	*cbbL*	this study
uncultured bacterium – clone SP52C8	metagenomic DNA fragment	form III	this study
uncultured bacterium – clone SP88G2	metagenomic DNA fragment	form III	this study
uncultured bacterium – clone SP92E10	metagenomic DNA fragment	form III	this study
uncultured bacterium – clone SP96F12	metagenomic DNA fragment	form III	this study
uncultured bacterium – clone SP119A5	metagenomic DNA fragment	form III	this study
uncultured bacterium – clone SP133E7	metagenomic DNA fragment	form III	this study

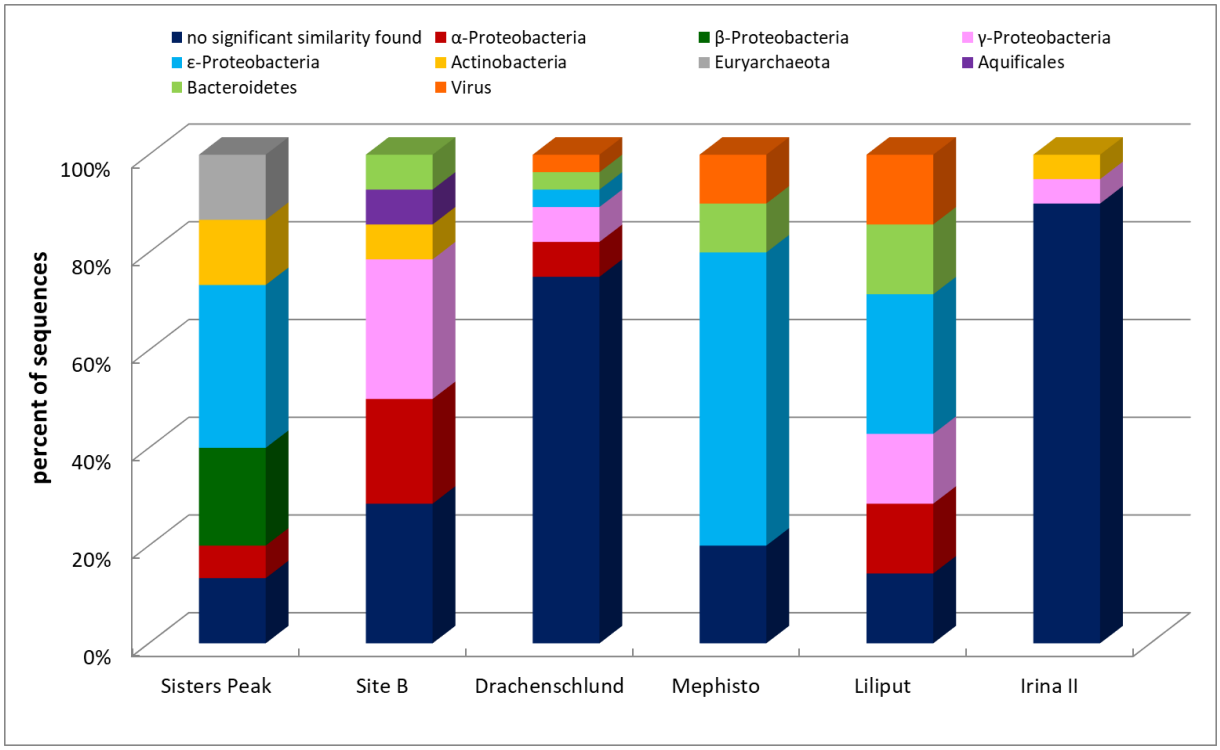
Supplementary Figures

Supplementary Figure S1



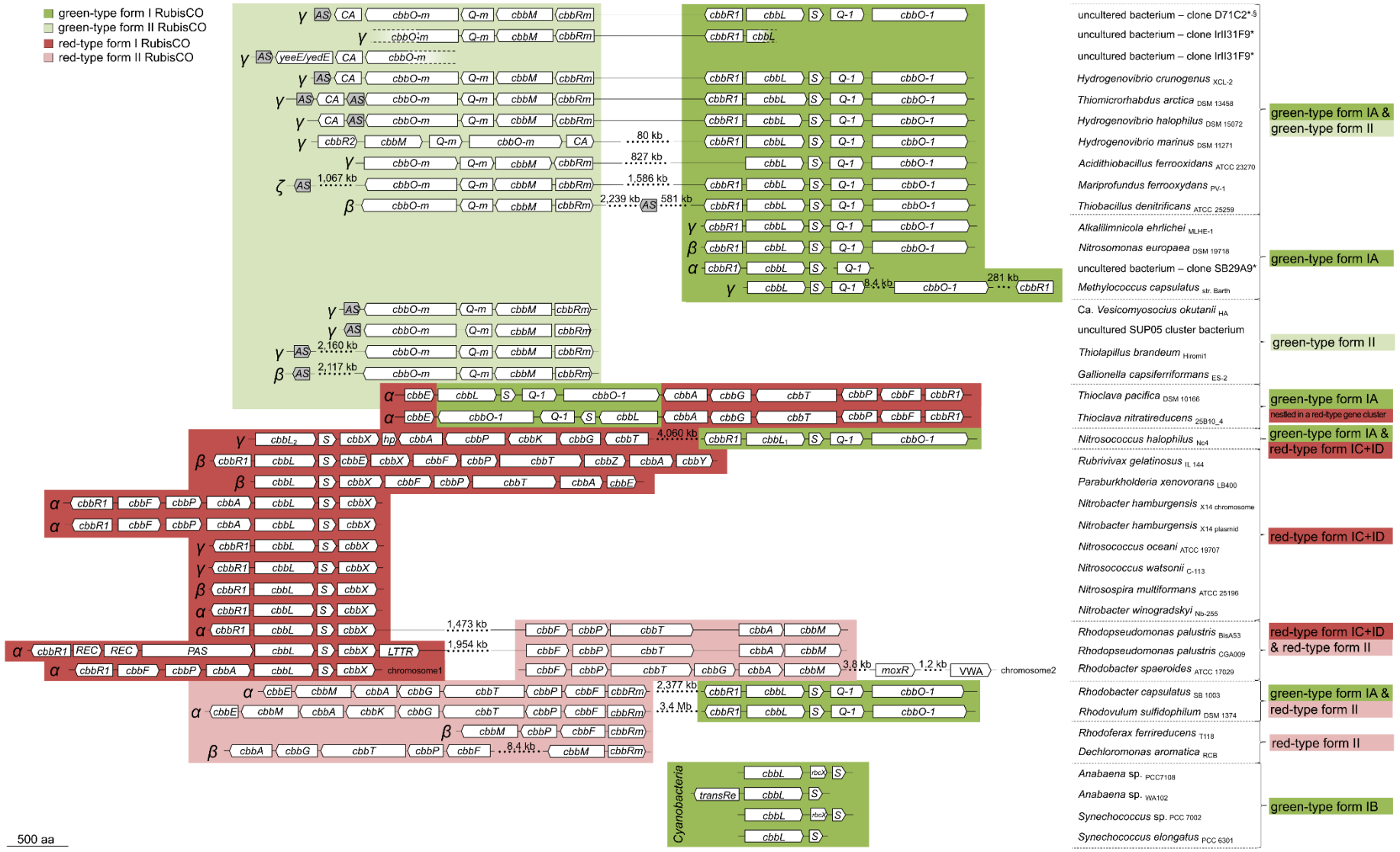
Map of sampled hydrothermal deep-sea vent environments located along the Mid-Atlantic Ridge (MAR). The six vent sites investigated in this study are depicted by yellow stars. The map was created using GeoMapApp software version 3.6.8 (www.geomapapp.org).

Supplementary Figure S2



Affiliation of sequences derived from insert end-sequencing of all six metagenomic libraries. Sequences were edited with Lasergene software (DNA Star, Madison, WI, USA) and annotated using blastn search. These results are only to investigate the quality of the DNA prior to library construction. For statistically relevant data see (14, 18, 22).

Supplementary Figure S3



Gene arrangements in the RubisCO gene cluster among selected *Proteobacteria* and *Cyanobacteria*. RubisCO genes encoded as part of a carboxysome operon were not considered in the overview. Genes encoding enzymes of the CBB cycle are not shown if they are scattered across the genome. Open reading frames (orfs) are indicated as arrows in the direction of transcription. Gene abbreviations are as follows: *CA* - carbonic anhydrase; AS = *cbbAS* - hypothetical protein potentially associated with RubisCO functioning; *cbbQ*-m/*cbbQ*-1 – AAA+ protein, encoding prokaryotic RubisCO activase of green-type RubisCO form IA; *cbbO-m/cbbO-1* - von Willebrand factor type A operating as adaptor between RubisCO and CbbQ; *cbbM* - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, form II; *cbb*R1/*cbb*Rm – LysR type transcriptional RubisCO regulators; LTTR – LysR type transcriptional regulator; *cbbL* - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, form I; *cbbS* - ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit, *cbbA* - fructose-1,6-bisphosphate aldolase; *cbbT* – transketolase; *cbbF* - fructose-1,6-phosphophatase; *cbbZ* - phospoglycolate phosphatase; *cbbG* - glyceraldehyd-3-phosphate dehydrogenase; *cbbP* – phosphoribulokinase; *cbbE* - ribulose-phosphate-3-epimerase; *cbbK* – phosphoglycerate kinase; *cbbX* – AAA+ protein operating as RubisCO activase of red-type RubisCO form IC and ID; PAS - hybrid sensor histidine kinase/response; REC - response regulator. Classification of red-type and green-type form I and II RubisCO genes are based upon the phylogenetic tree shown in Figure 2 of the main manuscript. Gene arrangements of presented species are deduced from genome sequences deposited in the NCBI database (National Center for Biotechnology Information, Bethesda, MD, USA) under following accession numbers: NC_011761.1 - *Acidithiobacillus ferrooxidans* ATCC 23270, NC_008340.1 - *Alkalilimnicola ehrlichii* MLHE-1, NZ_KB235896.1 - *Anabaena* sp. PCC7108, NZ_CP011456.1 - *Anabaena* sp. WA102, NC_009465.1 - Candidatus *Vesicomyosocius okutanii* HA, NC_007298.1 - *Dechloromonas aromatica* RCB, NC_014394.1 - *Gallionella capsiferriformans* ES-2, NC_007520.2 - *Hydrogenovibrio crunogenus* XCL-2, AB122071.1 - *Hydrogenovibrio marinus cbbM* gene cluster, AB122069.1 - *Hydrogenovibrio marinus cbbL* gene cluster, NZ_KB913033.1 - *Hydrogenovibrio halophilus* DSM15072, NZ_DS022294.1 and NZ_DS022295.1 - *Mariprofundus ferrooxydans* PV-1, NC_002977.6 - *Methylococcus capsulatus* str. Bath, NC_007406.1 - *Nitrobacter winogradskyi* Nb-255, NC_013960.1 - *Nitrosococcus halophilus* Nc4, NC_007484.1 - *Nitrosococcus oceani* ATCC 19707, NC_014315.1 - *Nitrosococcus watsonii* C-113, NC_004757.1 - *Nitrosomonas europaea* ATCC 19718, NC_007614.1 - *Nitrosospira multiformis* ATCC 25196, NC_007952.1 - *Paraburkholderia xenovorans* LB400, NC_014034.1 - *Rhodobacter capsulatus* SB 1003, NC_009049.1 - *Rhodobacter sphaeroides* ATCC 17029 Chr.1, NC_009050.1 - *Rhodobacter sphaeroides* ATCC 17029 Chr.2, NC_007908.1 - *Rhodoferax ferrireducens* T118, NC_008435.1 - *Rhodopseudomonas palustris* BisA53, NC_017075.1 - *Rubrivivax gelatinosus* IL144, NC_007404.1 - *Thiobacillus denitrificans* ATCC 25259, NZ_CP019437.1 - *Thioclava nitratireducens strain* 25B10_4, NZ_AUND00000000.1 - *Thioclava pacifica* DSM 10166, AP012273.1 - *Thiolapillus brandeum,* NZ_KB905899.1 - *Thiomicrorhabdus arctica* DSM13458, KJ639815 - uncultured bacterium – clone D71C2, PRJNA34785 - uncultured SUP05 cluster bacterium. *Clones identified within the current study. [§]Uncultured bacterium clone D71C2 was listed representatively for overall 39 Drachenschlund fosmid clones sharing a 100% similarity.

References

1. Boden R, Scott KM, Williams J, Russel S, Antonen K, Rae AW, et al. An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*: reclassification of four species of *Thiomicrospira* to each *Thiomicrorhabdus* gen. nov and *Hydrogenovibrio*, and reclassification of all four species of *Thioalkalimicrobium* to *Thiomicrospira*. *International Journal of Systematic and Evolutionary Microbiology*. 2017;67(5):1140-51.

2. Jannasch HW, Wirsen CO, Nelson DC, Robertson LA. *Thiomicrospira crunogena* sp. nov. a colorless, sulfur-oxidizing bacterium from a deep-sea hydrothermal vent. *International Journal of Systematic Bacteriology*. 1985;35(4):422-4.

3. Dobrinski KP, Longo DL, Scott KM. The carbon concentrating mechanism of the hydrothermal vent chemolithoautotroph *Thiomicrospira crunogena*. *Journal of Bacteriology*. 2005;187(16):5761-6.

4. Weaver PF, Wall JD, Gest H. Characterization of *Rhodopseudomonas capsulata*. *Archives of Microbiology*. 1975;105(3):207-16.

5. Huber H, Gallenberger M, Jahn U, Eylert E, Berg IA, Kockelkorn D, et al. A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic archaeum *Ignicoccus hospitalis*. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105(22):7851-6.

6. Böhnke S, Perner M. A function-based screen for seeking RubisCO active clones from metagenomes: novel enzymes influencing RubisCO activity. *The ISME Journal*. 2015;9(3):735-45.

7. Scott KM, Sievert SM, Abril FN, Ball LA, Barrett CJ, Blake RA, et al. The genome of deep-sea vent chemolithoautotroph *Thiomicrospira crunogena* XCL-2. *PLOS Biology*. 2006;4(12):2196-212.

8. Masuda S, Hori K, Maruyama F, Ren S, Sugimoto S, Yamamoto N, et al. Whole-Genome Sequence of the Purple Photosynthetic Bacterium *Rhodovulum sulfidophilum* Strain W4. *Genome Announcements*. 2013;1(4).

9. Sorokin DY, Tourova TP, Spiridonova EM, Rainey FA, Mulyzer G. *Thioclava pacifica* gen. nov., sp nov., a novel facultatively autotrophic, marine, sulfur-oxidizing bacterium from a near-shore sulfidic hydrothermal area. *International Journal of Systematic and Evolutionary Microbiology*. 2005;55:1069-75.

10. Strnad H, Lapidus A, Paces J, Ulbrich P, Vlcek C, Paces V, et al. Complete genome sequence of the photosynthetic purple nonsulfur bacterium *Rhodobacter capsulatus* SB 1003. *Journal of Bacteriology*. 2010;192(13):3545-6.

11. Choudhary M, Zanhua X, Fu YX, Kaplan S. Genome analyses of three strains of *Rhodobacter sphaeroides*: evidence of rapid evolution of chromosome II. *Journal of Bacteriology*. 2007;189(5):1914-21.

12. Kontur WS, Schackwitz WS, Ivanova N, Martin J, Labutti K, Deshpande S, et al. Revised sequence and annotation of the *Rhodobacter sphaeroides* 2.4.1 genome. *Journal of Bacteriology*. 2012;194(24):7016-7.

13. Böhnke S, Perner M. Unraveling RubisCO form I and form II regulation in an uncultured organism from a deep-Sea hydrothermal vent via metagenomic and mutagenesis studies. *Frontiers in Microbiology*. 2017;8:1303.

14. Perner M, Gonnella G, Kurtz S, LaRoche J. Handling temperature bursts reaching 464°C: different microbial strategies in the Sisters Peak hydrothermal chimney. *Applied and Environmental Microbiology*. 2014.

15. Garbe-Schönberg D, Klevenz V, Meißner D, Strauss H, Breuer C. Mid-Atlantic Expedition 2009, FS METEOR Cruise No. 78, Leg 2, Mantle to ocean on the southern Mid-Atlantic Ridge (5°S - 11°S) (MAR-SÜD V). *Cruise Report M78/2*. 2009:44-7.

16. Perner M, Hentscher M, Rychlik N, Seifert R, Strauss H, Bach W. Driving forces behind the biotope structures in two low-temperature hydrothermal venting sites on the southern Mid-Atlantic Ridge. *Environmental Microbiology Reports*. 2011;3(6):727-37.

17. Perner M, Kuever J, Seifert R, Pape T, Koschinsky A, Schmidt K, et al. The influence of ultramafic rocks on microbial communities at the Logatchev hydrothermal field, located 15 degrees N on the Mid-Atlantic Ridge. *FEMS Microbiology Ecology*. 2007;61(1):97-109.

18. Perner M, Gonnella G, Hourdez S, Böhnke S, Kurtz S, Girguis P. In situ chemistry and microbial community compositions in five deep-sea hydrothermal fluid samples from Irina II in the Logatchev field. *Environmental Microbiology*. 2013;15(5):1551-60.

19. Perner M, Hansen M, Seifert R, Strauss H, Koschinsky A, Petersen S. Linking geology, fluid chemistry, and microbial activity of basalt- and ultramafic-hosted deep-sea hydrothermal vent environments. *Geobiology*. 2013;11:340-55.

20. Campbell BJ, Cary SC. Abundance of reverse tricarboxylic acid cycle genes in free-living microorganisms at deep-sea hydrothermal vents. *Applied and Environmental Microbiology*. 2004;70(10):6282-9.

21. Spiridonova EM, Berg IA, Kolganova TV, Ivanovsky RN, Kuznetsov BB, Tourova TP. An oligonucleotide primer system for amplification of the ribulose-1,5-bisphosphate carboxylase/oxygenase genes of bacteria of various taxonomic groups. *Microbiology*. 2004;73(3):316-25.

22. Gonnella G, Böhnke S, Indenbirken D, Garbe-Schönberg D, Seifert R, Mertens C, et al. Endemic hydrothermal vent species identified in the open ocean seed bank. *Nature Microbiology*. 2016;1(8):16086.