

## Supplementary Material

### Comparative genomics of *Thiohalobacter thiocyanaticus* and *Guyparkeria* sp. SCN-R1, halophilic chemolithoautotrophic sulfur-oxidizing *Gammaproteobacteria* capable of using thiocyanate as energy source

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Vcdig' U30' Hwpevkqpcn' cppqvcvkqp" qh' vj g" i gpqo g" qh" I w{rctngtk" ur 0' UEP/T30

Vcdig' U40' Hwpevkqpcn' cppqvcvkqp" qh' vj g" i gpqo g" qh" Vj kjj crqdcvgt" vj kq{cpcvkewu" J Tj 3<sup>VO</sup>

**Fig. S1.** Maximum likelihood tree of SoeA (sulfite:quinone oxidoreductase subunit A) sequences showing position of a SoeA-like protein encoded in the genome of *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup>.

**Fig. S2.** Dithionite-reduced minus O<sub>2</sub>-oxidized cytochrome spectra of cell membranes from *Thiohalobacter thiocyanaticus* grown at 1 M NaCl.

**Fig. S3.** A fragment of multiple alignment of sequences of the C-subunit of ATP synthases from *Guyparkeria* SCN-R1 and *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> and their homologs.

**Fig. S4.** Comparative SDS-PAGE (5-15%) of soluble (**Sol**) and membrane (**Mb**) fractions from cells of *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> grown either on thiocyanate or thiosulfate as energy source.

**Fig. S5.** A fragment of multiple alignment of thioredoxin-like protein sequences from *Guyparkeria* SCN-R1 and *Thiohalobacter thiocyanaticus* and their homologs.

**Fig. S6.** Posterior probabilities of transmembrane helix predicted by TMHMM2.0 server in the third protein encoded downstream to TcDH.

**Fig. S7.** Genomic comparison between *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> and *Thiohalobacter* sp. strain FOKN1.

**Table S1.** Functional annotation of the genome of *Guyparkeria* sp. SCN-R1.

Gene symbol	Description	Locus tag	Cellular localization	Homolog with known function	Homolog accession number	% identity
<b>FCC</b>						
<i>cytC</i>	cytochrome c class I (monoheme c)	D5687_07270	TM	Cytochrome subunit of sulfide dehydrogenase	P20958	44
<i>fcc</i>	Sulfide dehydrogenase [flavocytochrome C] flavoprotein chain	D5687_07275	CP	Sulfide dehydrogenase [flavocytochrome c] flavoprotein chain	Q06530	51
<b>Sox system</b>						
<i>soxB</i>	Sulfur oxidation protein SoxB	D5687_09750	CP	Sulfate thiohydrolase SoxB	2WDC_A	33
<i>soxD</i>	Sulfite dehydrogenase diheme cytochrome c subunit SoxD	D5687_00820	CP	Sulfane dehydrogenase SoxCD	2XTS_B	34
<i>soxC</i>	Sulfur oxidation molybdopterin SoxC	D5687_00825	CP	Chain A, Sulfane dehydrogenase SoxCD	2XTS_A	46
<i>soxY</i>	Sulfur oxidation protein SoxY	D5687_01755	TM	Sulfur carrier protein Soxy	2NNC_A	52
<i>soxZ</i>	Sulfur oxidation protein SoxZ	D5687_01760	CP	thiosulfate oxidation carrier complex protein SoxZ	C1DB52	40
<i>soxA</i>	sulfur oxidation protein SoxA	D5687_05410	PP	SoxAX cytochrome complex subunit A	Q8KDM7	39
<i>soxX</i>	Sulfur oxidation protein SoxX	D5687_05415	TM	SoxAX	1H31_B	28
<i>soxW</i>	thioredoxin SoxW	D5687_05545	TM	Thiol:disulfide interchange protein DsbD;	Q6D9J6	28
<b>Sulfur transferases/rhodanases</b>						
Tst	Sulfur transferases/rhodanases	D5687_07715	CP	Putative thiosulfate sulfurtransferase	P27477	26
Tst	Sulfur transferases/rhodanases	D5687_08790	TM	rhodanese	OYY31733.1	75
Tst	Sulfur transferases/rhodanases	D5687_10700	PP	Putative thiosulfate sulfurtransferase	P16385	31
<b>Respiratory chain</b>						
<i>ccoN2</i>	Cytochrome c oxidase subunit CcoN 2	D5687_09640	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoN1;	D9IA43	60
<i>ccoO1</i>	Cytochrome c oxidase subunit CcoO 1	D5687_09645	TMM	cbb <sub>3</sub> cytochrome c oxidase CcoO1	3MK7_B	51
<i>ccoP</i>	Cytochrome c oxidase subunit CcoP	D5687_09655	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoP	D3NRE7	39
<i>ccoG</i>	Type cbb3 cytochrome oxidase biogenesis protein CcoG	D5687_09660	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoG	P18396.1	45
<i>ccoI</i>	Type cbb3 cytochrome oxidase biogenesis protein CcoI; Copper-translocating P-type ATPase	D5687_09670	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoI	P32113	33

<i>ccoO2</i>	CcoO 2	D5687_04270	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoO2	3MK7_B	41
<i>ccoN2</i>	CcoN 3	D5687_04275	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoN2	D9IA43	32
<i>ccoS</i>	Type cbb3 cytochrome oxidase biogenesis protein CcoS, involved in heme b insertion	D5687_03455	TM	cbb <sub>3</sub> -type cytochrome oxidase assembly protein CcoS	WP_025367652.1	57
<i>ccoN3</i>	CcoN 1	D5687_09275	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoN3	D9IA43	58
<i>coxB/cyoA</i>	Cytochrome c oxidase polypeptide II (CoxB/CyoA)	D5687_06885	TM	Cytochrome c oxidase subunit 2	P08306	37
<i>coxA/cyoB</i>	Cytochrome c oxidase polypeptide I (CoxA/CyoB)	D5687_06890	TM	cytochrome c oxidase subunit 1	Q1RI42	53
<b>Carbon fixation</b>						
<i>csoS1 A</i>	CsoS1 A	D5687_04005	CM	Major carboxysome shell protein 1B	P45690	83
<i>csoS1 B</i>	CsoS1 B	D5687_04010	CM	Major carboxysome shell protein 1C	P45688	98
<i>csoS1 C</i>	CsoS1 C	D5687_04015	CM	Major carboxysome shell protein 1A	P45689	98
<i>ccmM</i>	carboxysome peptide B	D5687_04020	CM	carboxysome peptide B	WP_019571977.1	75
<i>ccmL</i>	carboxysome peptide A	D5687_04025	CM	Carboxysome shell Protein	2RCF_A	83
<i>csoS3</i>	CsoS3	D5687_04030	CM	carboxysome shell polypeptide	AAC32551.1	51
<i>csoS2</i>	CsoS2	D5687_04035	CM	carboxysomal carbonic anhydrase	2FGY_A	61
<i>rbcM</i>	cbbM	D5687_04040	CM	Ribulose bisphosphate carboxylase small chain	P45686	74
<i>rbcL</i>	cbbL formI (green)	D5687_04045	CM	Ribulose bisphosphate carboxylase large chain;	O85040	95
<i>rbcR</i>	CbbR RuBisCO operon transcriptional regulator	D5687_04050	CM	RuBisCO operon transcriptional regulator	P25544	55
<b>other Calvin cycle enzymes</b>						
Tkt	Transketolase	D5687_10185	CP	Transketolase 1; Short=TK 1	Q9KUP2	70
GAPDH	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	D5687_10190	CP	Glyceraldehyde-3-phosphate dehydrogenase; Short=GAPDH	P52694	77
PGK	Phosphoglycerate kinase	D5687_10195	CP	Phosphoglycerate kinase	Q1GZ23	77
Fba2	Fructose-bisphosphate aldolase class II	D5687_10205	CP	Fructose-bisphosphate aldolase; Short=FBPA;	O87796	73
PRK	Phosphoribulokinase	D5687_01665	CP	Phosphoribulokinase; Short=PRK	P23015	62
rpe	Ribulose-phosphate 3-epimerase	D5687_01950	CP	Ribulose-phosphate 3-epimerase	P44756	72
TPI	Triosephosphate isomerase	D5687_05205	CP	Triosephosphate isomerase; Short=TPI	Q3J827	57
<i>rpiA</i>	Ribose 5-phosphate isomerase A	D5687_05790	TP	Ribose-5-phosphate isomerase A	Q0ACJ4	69
<i>fba1</i>	Fructose-bisphosphate aldolase class I (bifunctional can work as	D5687_06030	CP	Fructose-bisphosphate aldolase class 1	Q8P5Z7	62

	sedoheptulose-bisphosphate aldolase)					
<i>fbp1</i>	Fructose-1,6-bisphosphatase, type I (bifunctional can work as sedoheptulose-bisphosphate phosphatase)	D5687_02015	CP	Fructose-1,6-bisphosphatase class 1;	A1WZH0	63
<b>Cyanate metabolism</b>						
<i>cynA</i>	Cyanate ABC transporter, ATP-binding protein	D5687_04980	CP	Bicarbonate transport ATP-binding protein CmpD	Q55463	49
<i>cynB</i>	Cyanate ABC transporter, permease protein	D5687_04985	TM	Nitrate transport permease protein NrtB	P73451	44
<i>cynC</i>	Cyanate ABC transporter, substrate binding	D5687_04990	CP	Nitrate transport ATP-binding protein NrtC	P73450	33
<i>cyn</i>	Cyanate hydratase (cyanase)	D5687_05025	CP	Cyanate hydratase; Short=Cyanase	A6W1Q1	61
ACA	alpha-Carbonic anhydrase	D5687_05030	PP	Carbonic anhydrase alpha	O52538	45
GCA	gamma-carbonic anhydrase	D5687_08965	CP	gamma-class carbonic anhydrase family	P0A9W9	47
<b>F1F0 ATP synthase</b>						
<i>atpC</i>	ATP synthase epsilon chain	D5687_02835	CP	ATP synthase epsilon chain	B8GRB7	42
<i>atpD</i>	ATP synthase beta chain	D5687_02840	CP	ATP synthase subunit beta	B8GRB8	86
<i>atpG</i>	ATP synthase gamma chain	D5687_02845	CP	ATP synthase gamma chain	A6W3S9	57
<i>atpA</i>	ATP synthase alpha chain	D5687_02850	CP	ATP synthase subunit alpha	Q1LHK8	75
<i>atpH</i>	ATP synthase delta chain	D5687_02855	CP	ATP synthase subunit delta	B8GRC1	49
<i>atpF</i>	ATP synthase F0 sector subunit b	D5687_02860	TM	ATP synthase subunit b:	Q31DL6	53
<i>atpE</i>	ATP synthase F0 sector subunit c	D5687_02865	TM	ATP synthase subunit c	Q31DL5	87
<i>atpB</i>	ATP synthase F0 sector subunit a	D5687_02870	TM	ATP synthase subunit a	A8G1X1	54
<b>Na/H antiporter</b>						
<i>shaG</i>	Na(+) H(+) antiporter subunit G	D5687_07980	TM	Probable K(+)/H(+) antiporter subunit G	Q9Z3Q3	38
<i>shaF</i>	Na(+) H(+) antiporter subunit F	D5687_07985	TM	Probable K(+)/H(+) antiporter subunit F	Q52983	44
<i>shaE</i>	Na(+) H(+) antiporter subunit E	D5687_07990	TM	Na(+)/H(+) antiporter subunit E1	P60688	28
<i>shaD</i>	Na(+) H(+) antiporter subunit D	D5687_07995	TM	Na(+)/H(+) antiporter subunit D	Q9RGZ2	35
<i>shaC</i>	Na(+) H(+) antiporter subunit C	D5687_08000	TM	Probable K(+)/H(+) antiporter subunit C	Q52980	53
<i>shaAB</i>	Na(+) H(+) antiporter subunits AB	D5687_08005	TM	Probable K(+)/H(+) antiporter subunit A/B;	Q52978	46
<b>Osmotic adaptation</b>						
<i>opuD</i>	Glycine betaine transporter OpuD	D5687_09280	TM	Glycine betaine transporter OpuD	P54417	46

ectoine synthesis operon ectABC						
<i>ectC</i>	L-ectoine synthase ectC	D5687_03645	CP	L-ectoine synthase	Q9AP33	62
<i>ectB</i>	Diaminobutyrate-pyruvate aminotransferase ectB	D5687_03650	CP	Diaminobutyrate--2-oxoglutarate transaminase	Q7M9K2	54
<i>ectA</i>	L-2,4-diaminobutyric acid acetyltransferase ectA	D5687_03655	CP	L-2,4-diaminobutyric acid acetyltransferaseShort=DABA acetyltransferase	O06059	44
Sucrose-trehalose synthesis						
TPS1	alpha-trehalose-phosphate synthase 1	D5687_07445	CP	Sucrose-6 phosphatase1	1S2O_A	29
TPS2	Alpha,alpha-trehalose-phosphate synthase 2	D5687_02105	CP	Sucrose-6 phosphatase 2	Q3J7M5	46
SPS1F	Sucrose phosphate synthase	D5687_02110	CP	Sucrose-phosphate synthase	P31927	49
INV	neutral invertase	D5687_02115	CP	Alkaline/neutral invertase A, mitochondrial;	Q9FXA8	47
TCDH						
<i>yhaC</i>	Pentapeptide protein	D5687_03355	PP	Fusion of Np275 And Np276, Pentapeptide Repeat Proteins from <i>Nostoc punctiforme</i>	2J8K_A	39
SOD	Superoxide Dismutase	D5687_03345	PP	Superoxide dismutase C	2WWN_A	49
<i>tcdh</i>	Thiocyanate dehydrogenase	D5687_03340	PP	Thiocyanate dehydrogenase from <i>Thioalkalivibrio paradoxus</i> ARh1	5F30_A	37
-	Thioredoxin like protein	D5687_03335	PP	TlpA family protein disulfide reductase	WP_125181026.1	48
-	Sigma 54 transcriptional activator	D5687_03330	CP	Sigm54 Activator	1NY5_A	41
-	Membrane bound histidine kinase	D5687_03325	TM	histidine kinase	OYY62640	40
<i>cusA</i>	CusA	D5687_03320	TM	CusA	3K07_A	32
<i>cusB</i>	CusB-1	D5687_03315	PP	secretion protein HlyD	OJZ16289.1	40
<i>cusB</i>	CusB-2	D5687_03310	PP	HlyD family efflux transporter periplasmic adaptor subunit [ <i>Thiobacillus thioparus</i> ]	WP_018509490.1	43

<sup>1</sup>CP, cytoplasmic; PP, periplasmic; TM, transmembrane

**Table S2.** Functional annotation of the genome of *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup>.

Gene symbol	Description	Locus tag	Cellular localization <sup>1</sup>	Homolog with known function	Homolog accession number	% identity
<b>FccAB</b>						
<i>fccA</i>	Sulfide dehydrogenase [flavocytochrome C] flavoprotein	D6C00_05870	CP	Sulfide dehydrogenase [flavocytochrome c] flavoprotein chain	Q06530	52
<i>fccB</i>	cytochrome c class I (monoheme)	D6C00_05875	TM	Cytochrome subunit of sulfide dehydrogenase	Q8KAS5	63
<b>Sox system</b>						
<i>soxH</i>	SoxH protein	D6C00_12485	PP	SoxH protein	GAW75230	43
<i>soxW</i>	thioredoxin SoxW	D6C00_14040	PP	Thioredoxin-fold protein	4FYB_B	26
	Rhodanese domain protein	D6C00_03730	CP	Sulfur-mobilizing rhodanese-like protein	D3RPB9	50
<i>soxX</i>	SoxX	D6C00_03745	CP	SoxX	1H31_B	33
<i>soxY</i>	SoxY	D6C00_03750	TM	SoxY	2NNC_A	44
<i>soxZ</i>	SoxZ	D6C00_03755	CP	SoxZ	<u>2OXH_Z</u>	41
<i>soxA</i>	SoxA	D6C00_03760	PP	SoxA	Q8RLX0	56
<i>soxB</i>	SoxB	D6C00_03765	CP	Sulfate thiohydrolase SoxB	<u>2WDC_A</u>	39
<i>soxH</i>	SoxH protein	D6C00_15065	PP	SoxH-like protein	<u>EGV49958.1</u>	59
<b>rDSR system</b>						
<i>dsrC</i>	DsrC	D6C00_14360	CP	sulfur relay protein DsrC	<u>WP_114278802.1</u>	64
<i>dsrA</i>	dsrA	D6C00_14370	CP	Sulfite reductase, dissimilatory-type subunit alpha	O33998	81
<i>dsrB</i>	dsrB	D6C00_14375	CP	Sulfite reductase, dissimilatory-type subunit beta	Q59110	44
<i>dsrM</i>	DsrMKJOP_DsrM (= HmeC)	D6C00_14400	TM	Hdr-like menaquinol oxidoreductase cytochrome b-like subunit	O29749	35
<i>dsrK</i>	DsrMKJOP_DsrK (=HmeD)	D6C00_14405	CP	Hdr-like menaquinol oxidoreductase iron-sulfur subunit 2	O29748	43
<i>dltD</i>	Protein similar to glutamate synthase [NADPH] small chain, clustered with sulfite reductase	D6C00_14410	CP	Glutamate synthase [NADPH] small chain	P9WN18	31
<i>dsrJ</i>	DsrMKJOP_multiheme protein?? DsrJ (=HmeF) there is only a single heme there	D6C00_14415	CP	Hdr-like menaquinol oxidoreductase cytochrome c subunit	O29747	30
<i>dsrO</i>	DsrMKJOP iron-sulfur protein DsrO (=HmeA)	D6C00_14420	TM	Hdr-like menaquinol oxidoreductase iron-sulfur subunit 1	O29751	39
<i>dsrP</i>	DsrMKJOP_DsrP (= HmeB)	D6C00_14425	TM	Hdr-like menaquinol oxidoreductase	O29750	26

<i>dsrC2/tusE</i>	Dsr gamma - DsrC2/TusE	D6C00_03120	CP	Sulfurtransferase TusE	Q32HT7	42
<i>dsrC3/tusE</i>	DsrC3/TusE	D6C00_05110	CP	Sulfite reductase, dissimilatory-type subunit gamma	P45573	36
<i>dsrC1tusE</i>	putative: Dsr gamma subunit DsrC1/could be sulfur transferase/TusE	D6C00_14490	CP	Sulfurtransferase TusE	Q5PGB9	45
<b>AprABM</b>						
<i>aprA*</i>	Adenylyl-sulfate reductase subunit A	D6C00_01360	CP	Adenylyl-sulfate reductase subunit A	<a href="#">O33997</a>	94
<i>aprB</i>	Adenylyl-sulfate reductase subunit B	D6C00_01365	CP	Adenylyl-sulfate reductase subunit B	T2G899	44
<i>aprM</i>	Adenylyl-sulfate reductase membrane anchor	D6C00_01365	TM	Adenylyl-sulfate reductase membrane anchor ( <i>Allochromatium vinosum</i> )	Q9RB52	62
<b>Sat</b>						
<i>sat</i>	Sulfate adenylyl transferase	D6C00_00180	CP	Sulfate adenylyl transferase	Q5PGB9	45
<b>SoeABC-like complex</b>						
<i>soeA**</i>	Sulfite dehydrogenase SoeA	D6C00_06975	PP	Putative sulfite dehydrogenase subunit A	<a href="#">WP_120795598</a>	75
<i>soeB</i>	Sulfite dehydrogenase SoeB ferridoxine	D6C00_06980	PP	Putative sulfite dehydrogenase subunit B	<a href="#">D3RNN7</a>	79
<i>soeC</i>	Sulfite dehydrogenase SoeC	D6C00_06985	TM	Putative sulfite dehydrogenase subunit C	D3RNN6	57
<i>tst</i>	Thiosulfate sulfurtransferase, rhodanese	D6C00_13690	CP	Putative thiosulfate sulfurtransferase	D4GYM0	31
<b>Hydrogenase complex</b>						
<i>hdrD_5</i>	FeS-binding domain -Hdr	D6C00_13600	CP	CoB--CoM heterodisulfide reductase iron-sulfur subunit D	P96797	28
<i>hupB</i>	Uptake hydrogenase large subunit1	D6C00_13605	CP	Uptake hydrogenase large subunit	P18636	59
<i>hydB</i>	Ni,Fe hydrogenase, subunit beta	D6C00_13615	CP	Sulfhydrogenase 1 subunit beta	Q8U2E5	33
<i>TauD</i>	TauD-aurine dioxygenase	D6C00_14705	CP	Taurine catabolism dioxygenase TauD	<a href="#">WP_012638742</a>	57
<i>hydG</i>	cytochrome-c3 hydrogenase gamma chain	D6C00_13620	CP	Sulfhydrogenase 1 subunit gamma	Q8U2E4	34
<i>hydD</i>		D6C00_13625	CP	Sulfhydrogenase 1 subunit delta	WP_126605181	67
<b>Cyanate metabolism</b>						
Cyn	Cyanate hydratase	D6C00_04640	CP	Cyanate hydratase	A6W1Q1	63
cynA	Cyanate ABC transporter, ATP-binding protein	D6C00_04645	CP	Bicarbonate transport ATP-binding protein CmpD	Q55463	47
GCA	Gamma-carbonic anhydrase	D6C00_08200	CP	Gamma carbonic anhydrase 1	Q9FWR5	43

BCA	Beta-carbonic anhydrase	D6C00_09370	CP	Carbonic anhydrase 2	P9WPJ8	38
CAH	OI-carbonic anhydrase (3)	D6C00_12350	CP	Carbonic anhydrase	<u>5ZTP_A</u>	47
CAH	OI-carbonic anhydrase (1)	D6C00_04625	CP	Carbon disulfide hydrolase	S5FT07	77
<b>RuBisCo</b>						
CsoS1 A	carboxysome shell protein CsoS1 (A)	D6C00_05915	CP	Carbon dioxide-concentrating mechanism protein CcmK	P0A328	97
CsoS1 B	carboxysome shell protein CsoS1 (B)	D6C00_05920	CP	Carbon dioxide-concentrating mechanism protein CcmK	Q7V2D1	95
CsoS1 C	carboxysome shell protein CsoS1 (C)	D6C00_05925	CP	Carbon dioxide-concentrating mechanism protein CcmK	P0A328	92
CcmM	carboxysome peptide B	D6C00_05930	CP	Carboxysome Shell Protein	<u>2RCF_A</u>	48
ccmL	carboxysome peptide A	D6C00_05935	CP	Carboxysome Shell Protein	<u>2RCF_A</u>	70
CsoS3	carboxysome shell protein CsoS3	D6C00_05940	CP	Beta Carbonic Anhydrase	<u>2FGY_A</u>	45
CsoS2	carboxysome shell protein CsoS2	D6C00_05945	CP	carboxysome shell protein CsoS2	<u>EAR23171.1</u>	70
rbcR	RuBisCO operon transcriptional regulator CbbR	D6C00_06940	CP	RuBisCO operon transcriptional regulator	P25544	57
rbcO	Rubisco activation protein CbbO	D6C00_06955	CP	Protein NorD	Q576X0	34
rbcQ	Rubisco activation protein CbbQ	D6C00_06960	CP	Protein CbbQ	Q51858	77
rbcM	cbbM	D6C00_06965	CP	Ribulose biphosphate carboxylase small chain	P24682	83
rbcL	cbbL	D6C00_06970	CP	Ribulose biphosphate carboxylase large chain	Q56259	91
<b>Other Calvin cycle enzyme</b>						
PRK	phosphoribulokinase	D6C00_06745	CP	Phosphoribulokinase	P19923	66
TPI	Triosephosphate isomerase	D6C00_01165	CP	Triosephosphate isomerase	Q3J827	58
Fba1	Fructose-bisphosphate aldolase class I	D6C00_01985	CP	Fructose-bisphosphate aldolase class 1	P0A992	70
Fbp	Fructose-1,6-bisphosphatase, type V, archaeal	D6C00_02820	CP	Fructose-1,6-bisphosphate aldolase/phosphatase	Q72K02	61
Fbp1	Fructose-1,6-bisphosphatase, type I bisphosphate	D6C00_05130	CP	Fructose-1,6-bisphosphatase class 1	Q3SFS6	72
araD	Ribulose-5-phosphate 4-epimerase	D6C00_05625	CP	L-fuculose phosphate aldolase	Q58813	27
Tkt	Transketolase	D6C00_09785	CP	Transketolase 1	Q9KUP2	71
GAPDH	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	D6C00_09790	CP	Glyceraldehyde-3-phosphate dehydrogenase	P52694	80
PGK	Phosphoglycerate kinase	D6C00_09795	CP	Phosphoglycerate kinase	B8GP44	77
Fba2	Fructose-bisphosphate aldolase class II	D6C00_09805	CP	Fructose-bisphosphate aldolase	O87796	78

rpiA	Ribose 5-phosphate isomerase A	D6C00_10225	TM	Ribose-5-phosphate isomerase A	Q0ACJ4	73
<b>ATP synthesis</b>						
atpD	synthase beta chain1	D6C00_02825	CP	ATP synthase subunit beta	A8ZNR6	64
atpC	synthase epsilon chain	D6C00_02830	CP	ATP synthase epsilon chain 2	Q13IW4	37
AtpC	F-type H <sup>+</sup> -ATPase subunit AtpC	D6C00_02835	TM	F-type H <sup>+</sup> -transporting ATPase subunit AtpC	<a href="#">WP_006964569.1</a>	70
NHA	Na <sup>(+)</sup> /H <sup>(+)</sup> antiporter	D6C00_02840	TM	Sodium Proton Antiporter	<a href="#">4CZ9_A</a>	25
atp-1	ATP synthase subunit I	D6C00_02845	TM	ATP synthase subunit I	<a href="#">WP_113862237.1</a>	53
atpB	synthase F0 subunit a1	D6C00_02850	TM	ATP synthase subunit a 2	A8ZNS1	64
atpE	Na <sup>+</sup> -ATP synthase F0 sector subunit c1	D6C00_02855	TM	ATP synthase subunit c 2	A3PS63	65
atpF	synthase F0 sector subunit b2	D6C00_02860	TM	ATP synthase subunit b 2	Q21ZA0	39
atpA	synthase alpha chain2	D6C00_02865	CP	ATP synthase subunit alpha 2	A8ZNS4	63
atpG	synthase gamma chain2	D6C00_02870	CP	ATP synthase gamma chain	B0THN3	30
<b>V-type ATP synthase</b>						
VHA-I	V-type ATP synthase subunit I	D6C00_14700	TM	V-type ATP synthase subunit I	O57721	24
VHA-K	V-type ATP synthase subunit K	D6C00_14705	TM	V-type proton ATPase	Q41773	34
VHA-A	V-type ATP synthase subunit A	D6C00_14720	CP	V-type ATP synthase alpha chain	B8CZG8	49
VHA-B	V-type ATP synthase subunit B F1F0-Na-ATPase	D6C00_14725	CP	V-type ATP synthase beta chain	Q8U4A5	55
ShAB	Na <sup>(+)</sup> H <sup>(+)</sup> antiporter subunit A/B	D6C00_15020	TM	Probable K <sup>(+)</sup> /H <sup>(+)</sup> antiporter subunit A/B	Q52978	52
shaD	Na <sup>(+)</sup> H <sup>(+)</sup> antiporter subunit D	D6C00_15030	TM	Probable K <sup>(+)</sup> /H <sup>(+)</sup> antiporter subunit D	Q52981	42
shaE	Na <sup>(+)</sup> H <sup>(+)</sup> antiporter subunit E	D6C00_15035	TM	Na <sup>(+)</sup> /H <sup>(+)</sup> antiporter subunit E1	P60688	28
shaF	Na <sup>(+)</sup> H <sup>(+)</sup> antiporter subunit F	D6C00_15040	TM	Probable K <sup>(+)</sup> /H <sup>(+)</sup> antiporter subunit F	Q52983	50
shaG	Na <sup>(+)</sup> H <sup>(+)</sup> antiporter subunit G	D6C00_15045	TM	Probable K <sup>(+)</sup> /H <sup>(+)</sup> antiporter subunit G	Q9Z3Q3	45
<b>Osmoprotection</b>						
TPS	Trehalose-6-phosphate synthase	D6C00_02875	CP	Trehalose-phosphate phosphatase	Q49734	40
trePP	Trehalose 6-phosphate phosphorylase	D6C00_02880	CP	Trehalose 6-phosphate phosphorylase	Q9CID5	51
TPS	Trehalose-phosphate synthase	D6C00_10705	CP	Sucrose-phosphatase 1	Q5IH14	54
betC	Choline-sulfatase	D6C00_13525	TM	Arylsulfatase	Q8XNV1	25
ProW	L-proline glycine betaine ABC transport system permease protein	D6C00_06040	TM	Glycine betaine/carnitine transport permease protein GbuB	Q9RR45	52

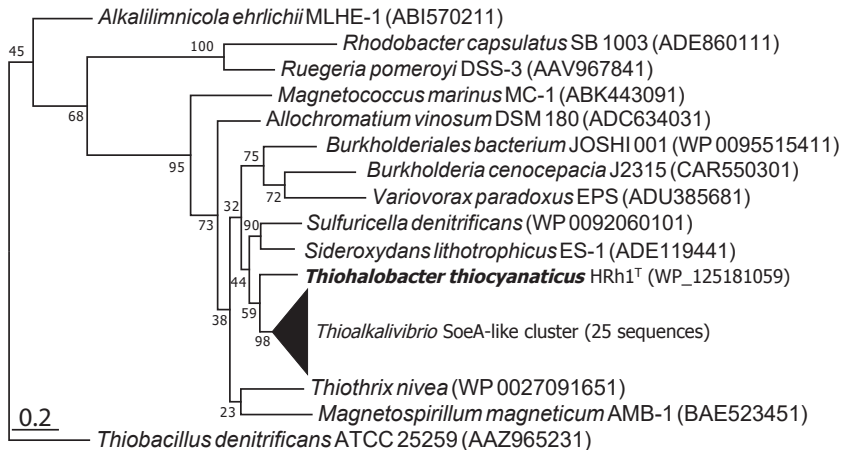
	ProW					
ProV	L-proline glycine betaine ABC transport system permease protein ProV	D6C00_06045	CP	Glycine betaine/choline transport system ATP-binding protein OusV	E0SCY1	58
ProX	L-proline glycine betaine binding ABC transporter protein ProX	D6C00_06035	CP	Glycine betaine/carnitine transport binding protein GbuC	Q9RR44	36
<b>Sucrose biosynthesis</b>						
SUS	Sucrose synthase	D6C00_08230	CP	Sucrose synthase	Q820M5	60
FRK	Fructokinase	D6C00_08240	CP	Probable fructokinase-5	O82616	27
<b>Respiratory cytochromes</b>						
CcoH	CcoH	D6C00_07505	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoH	P18397, RMG59777.1	48
CcoG1	cbb <sub>3</sub> cytochrome oxidase biogenesis protein CcoG	D6C00_07510	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoG	P18396	41
CcoP1	Cytochrome c oxidase subunit CcoP 1	D6C00_07515	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoP	A4VKL4	51
CcoN	Cytochrome c cbb <sub>3</sub> oxidase subunit CcoN	D6C00_09260	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoN	D9IA43	61
CcoO	Cytochrome c oxidase subunit CcoO	D6C00_09265	TM	cbb <sub>3</sub> -type cytochrome oxidase, chain B	<a href="#">3MK7_B</a>	55
CcoP2	Cytochrome c oxidase subunit CcoP 2	D6C00_09275	TM	cbb <sub>3</sub> -type cytochrome c oxidase subunit CcoP	A8HZ17	43
CcoG2	Type cbb <sub>3</sub> cytochrome oxidase biogenesis protein CcoG	D6C00_09280	TM	Protein RdxB	P54932	42
CcoS	Type cbb <sub>3</sub> cytochrome oxidase biogenesis protein CcoS	D6C00_09405	TM	cbb <sub>3</sub> -type cytochrome oxidase assembly protein CcoS	WP_110018494.1	69
<b>Cytochrome c / quinol oxidase ba3</b>						
CoxA/CyoB	Cytochrome c oxidase polypeptide I (a <sub>3</sub> subunit)	D6C00_09220	TM	Probable cytochrome c oxidase subunit 1	Q4ULU5	56
CtaG	Cytochrome oxidase biogenesis protein	D6C00_09225	TM	Cytochrome c oxidase assembly protein CtaG	A4Z2D0	42
Cox2	Cytochrome c oxidase cytochrom b sunbunit polypeptide II	D6C00_09215	TM	Cytochrome c oxidase subunit 2	P08306	39
Cox3	Cytochrome c oxidase polypeptide III Cyt. c oxidase bo <sub>3</sub> ?	D6C00_09230	TM	Cytochrome c oxidase subunit 3	Q9ZZY5	39
CoxB/CyoA	Cytochrome c oxidase (B(O/a) <sub>3</sub> -type) chain II	D6C00_10845	TM	Cytochrome c oxidase subunit 2	P98052	28
Cox1	Cytochrome c oxidase (B(O/a) <sub>3</sub> -type) chain I	D6C00_10850	TM	Cytochrome c oxidase polypeptide 1	P33518	24
<b>TCDH</b>						
<i>yhaC</i>	Pentapeptide protein	D6C00_06780	PP	Pentapeptide Repeat Protein Involved In Heterocyst Differentiation Regulation	<a href="#">3DU1_X</a>	40
-	FMN-binding redoxin-containing protein	D6C00_06785	CP	<a href="#">redoxin domain-containing protein</a>	<a href="#">WP_111384496.1</a>	61

<i>tcdh</i>	Thiocyanate dehydrogenase	D6C00_06790	PP	<a href="#">Thiocyanate Dehydrogenase</a>	<a href="#">5F30_A</a>	34
-	Thioredoxin like protein	D6C00_06795	PP	<a href="#">redoxin domain-containing protein</a>	<a href="#">WP_111384496.1</a>	55
-	Sigma 54 transcriptional activator	D6C00_06800	CP	<a href="#">Sigm54 Activator</a>	<a href="#">1NY5_A</a>	40
-	Membrane bound histidine kinase	D6C00_06805	TM	<a href="#">histidine kinase [Thiobacillus sp. SCN 63-57]</a>	<a href="#">ODU99242.1</a>	41
<a href="#">CusA</a>	CusA	D6C00_06810	TM	<a href="#">CusA</a>	<a href="#">3K07_A</a>	30
<i>CusB</i>	<i>CusB-1</i>	D6C00_06815	PP	secretion protein HlyD [Thiobacillus sp. 63-78]	<a href="#">QJZ16289.1</a>	36
<i>CusB</i>	<i>CusB-2</i>	D6C00_06820	TM	HlyD family efflux transporter periplasmic adaptor subunit	<a href="#">WP_018509490.1</a>	42

\*The AprA is annotated in GenBank as frame-shifted, the gene consisted in two separate frames. The recovered protein is the following:

```
<MAYETIVEDNIDILVCGAGLGGTGAWEARYWGQDKKIVIAEKANIDRSGAVAQGLYAINCYMGTRFGENNPEDHVRYPARIDLGMVREDLAFDMARHVDSAVHQFEEWGLPLMRDPKTGAYQREGRWQIMIHGESYKPIVAEA
AKKSADKVFNRICVTHLLMDESKENRIAGAVGFNVRTGNYHVFKSKTVIVAAGGASNIYKPRSVGEGARRVWYAPWSSGSAYGLLISAGAKMTQMENRIVLARFKDGYGYPVGAYPLHLKTYTQNCGLGEEYESKWWPQLQEMVGKE
YLDPEASHRTHRP IPTCLRNHALISEVNAGRGP IHMVTMEAFQDPHLEEI GWHNFLGMTVGQAVLWAATDVPKNENPELTTSEPYVMGSHATGCGAWCSGPEDVSPPEYFWGYNRMTTVEGLFGAGDAVGGT PHAFSSGSFTEG
RLAAKAACKYIDDGKAEGIVVSQKQIEDRRKEIYKPLEHYRIYRNEITAGSVNPNYINPRQGLDRLQKLMDEYAGGASVNYMTNEKLLHIGLKKLLEEDFEKIAAEDIHELLRAWELKHRI LSSEAVMQHTLFRKETRWPGYY
YRGDFLKVDDENWHVLTVSRRDPKTGEYTMKAPCYHLVEDTE>
```

\*\*Based on phylogenetic reconstruction of D6C00\_06975



**Fig.S1.** Maximum likelihood tree of SoeA (sulfite:quinone oxidoreductase subunit A) sequences showing position of a SoeA-like protein encoded in the genome of *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup>. Sequences of SorA were used as outgroup and pruned from the tree. The scale bar represents sequence difference. Bootstrap values (from 500 replicates) are shown on the nodes.

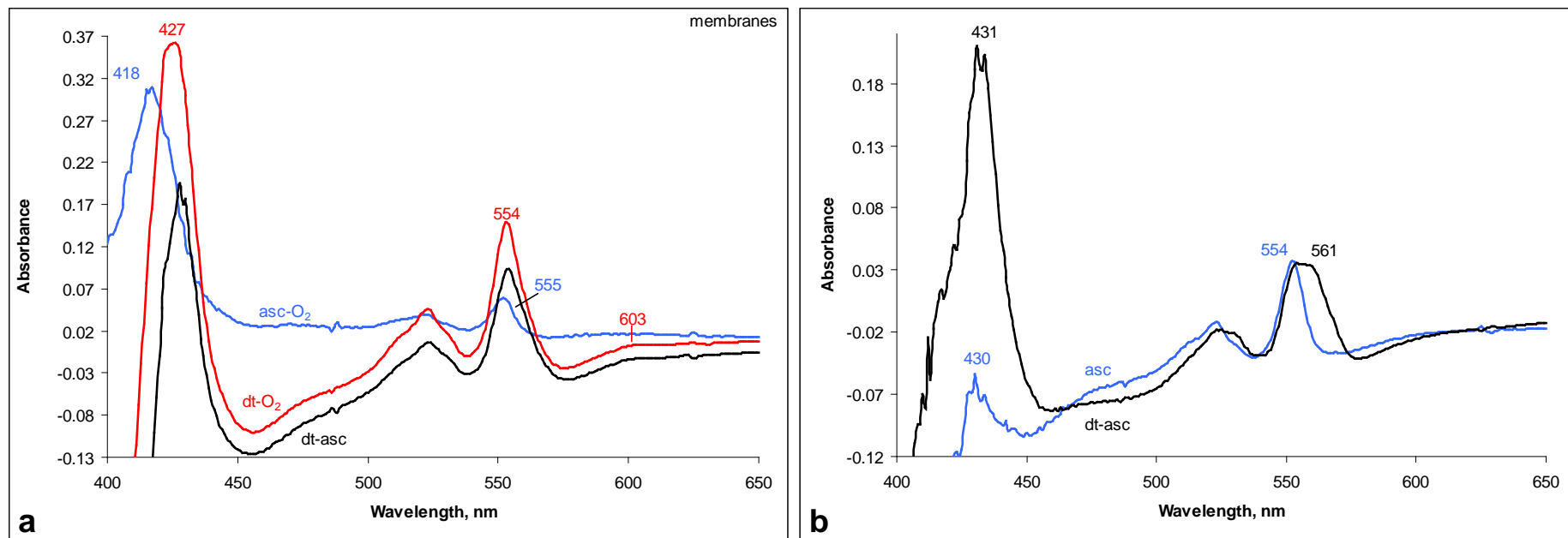


Fig. S2. Dithionite-reduced minus O<sub>2</sub>-oxidized cytochrome spectra of cell membranes from *Thiohalobacter thiocyanaticus* grown at 1 M NaCl with either thiosulfate (a) or thiocyanate (b) as electron donor. In the thiosulfate grown cells 3 types of hemes are present: *c*, *b* and *a*<sub>3</sub> with a domination of heme *c*, while in the thiocyanate-grown cells only *c* and *b* hemes are detectable with a higher proportion of the heme *b* type.

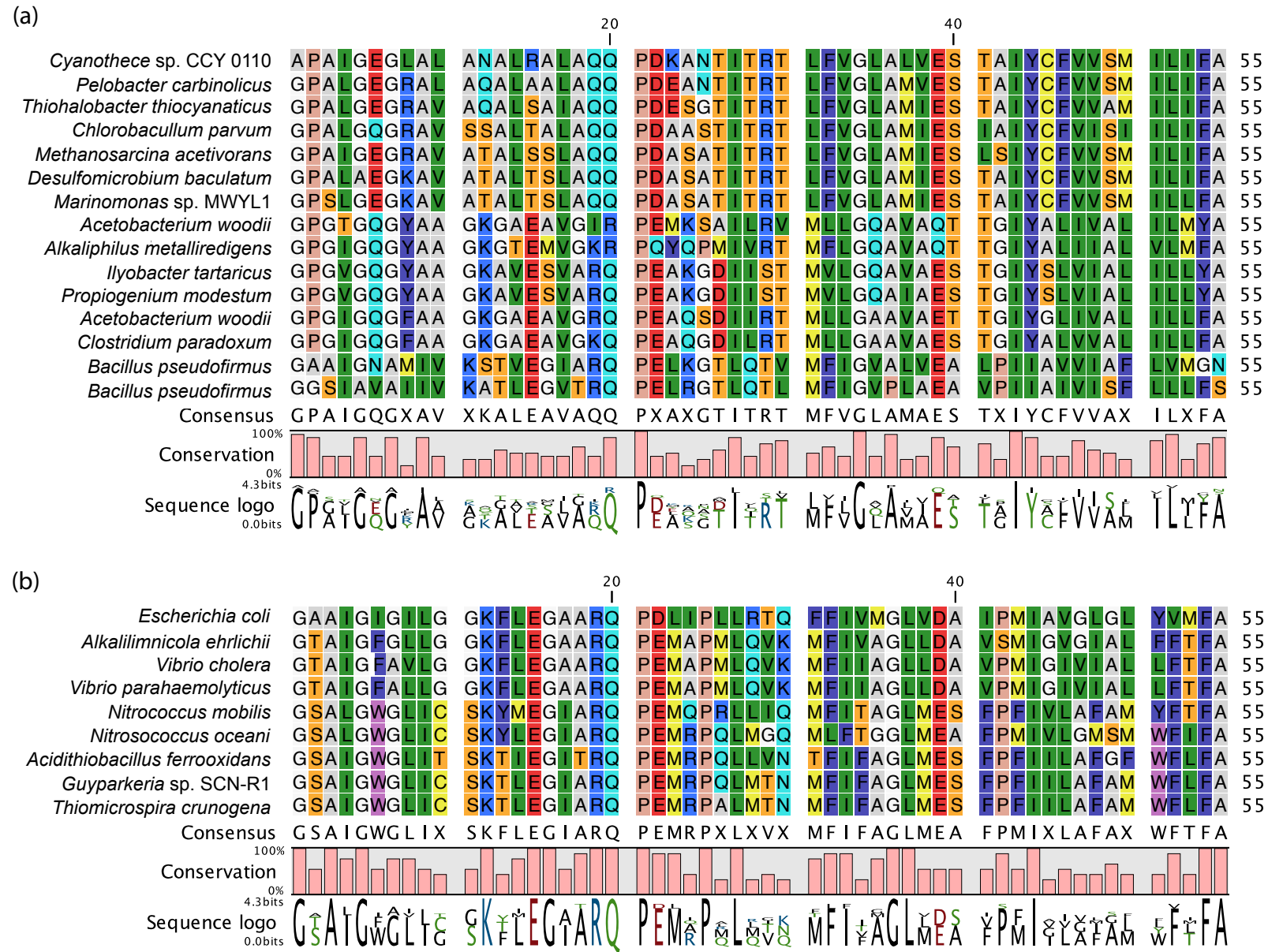


Fig. S3. A fragment of multiple alignment of sequences of the C-subunit of ATP synthases from *Guyparkeria* SCN-R1 and *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> and their homologs (used to construct the phylogenetic tree (Fig. 3)).

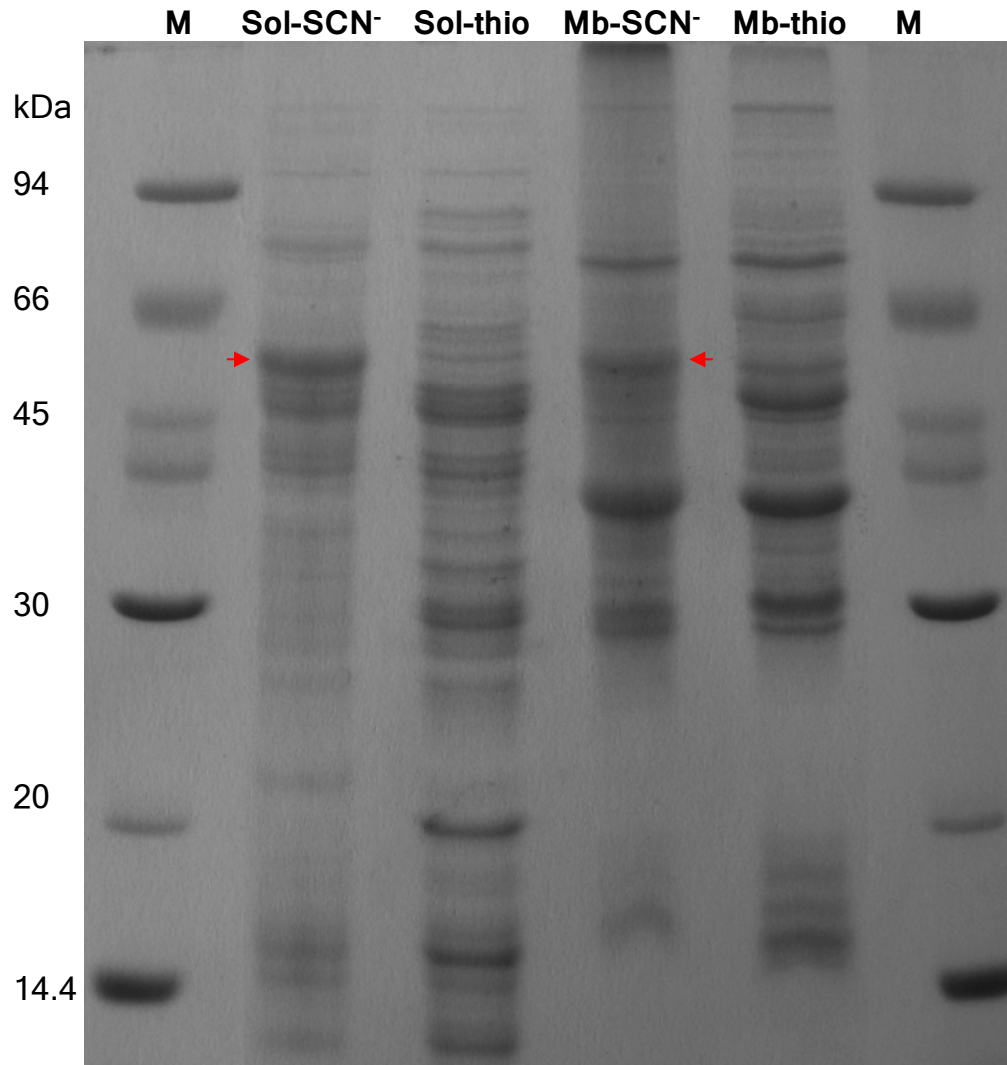


Fig. **S4**. Comparative SDS-PAGE (5-15%) of soluble (Sol) and membrane (Mb) fractions from cells of *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> grown either on thiocyanate or thiosulfate as energy source. The arrows indicate a polypeptide differentially overexpressed during growth with thiocyanate. Protein load - 20  $\mu\text{g ml}^{-1}$ .

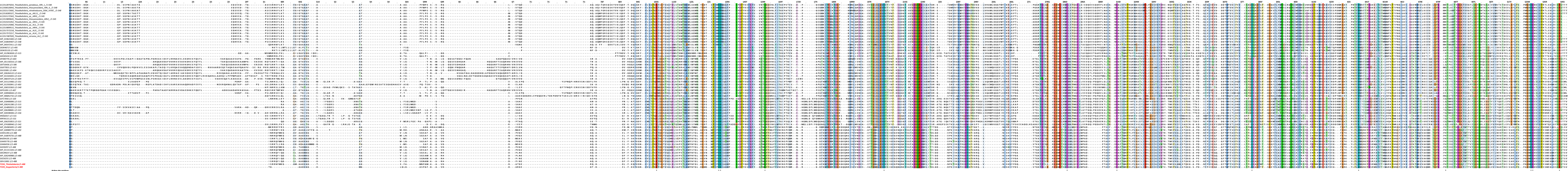
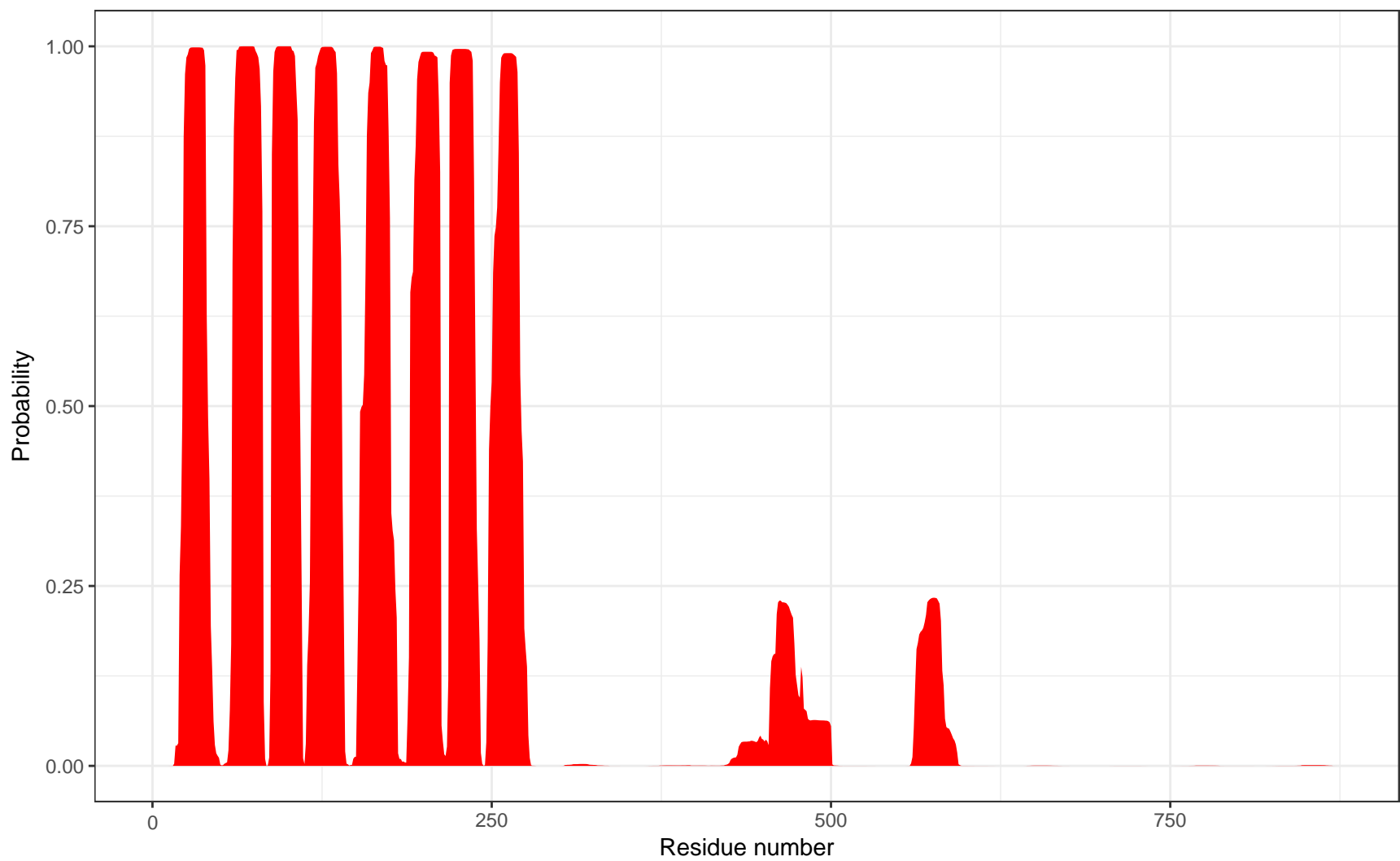
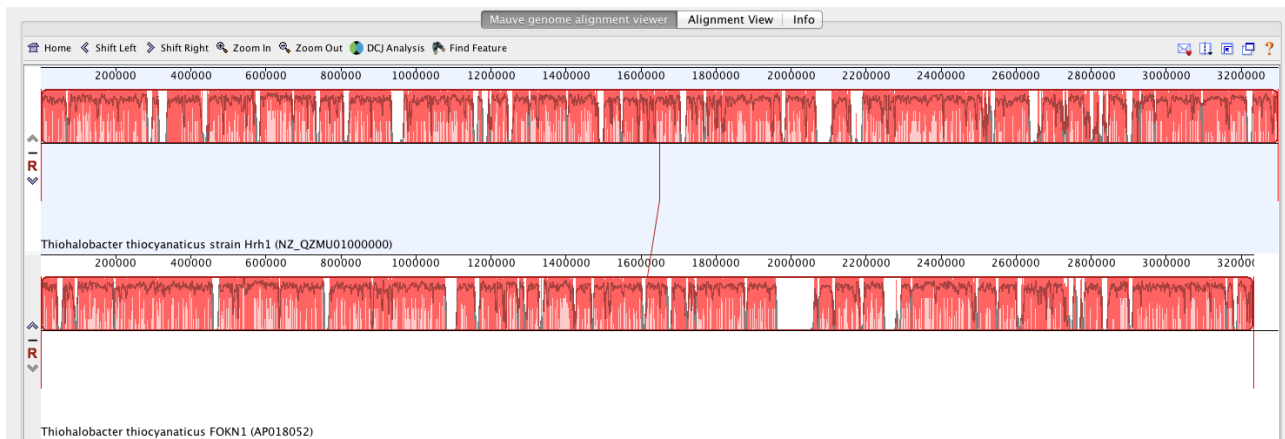


Fig. S5. A fragment of multiple alignment of thiooxidin-like protein sequences from *Gyuaparkeria* SCN-RT1 and *Thiohalobacter thiocyanatus* HRH1 and their homologs. In four most distant homologs a CXXC motif appears and the

Fig. S6. Posterior probabilities of transmembrane helix predicted by TMHMM2.0 server in the third protein encoded downstream to TcDH.





	HRh1 <sup>T</sup>	FOKN1
<b>ANiB</b> [and aligned percentage]		
<i>Thiohalobacter thiocyanaticus</i> HRh1	*	85.79 [70.14]
<i>Thiohalobacter sp.</i> strain FOKN1	86.01 [71.72]	*
<b>ANIm</b> [and aligned percentage]		
<i>Thiohalobacter thiocyanaticus</i> HRh1	*	88.06 [69.74]
<i>Thiohalobacter sp.</i> strain FOKN1	88.06 [71.18]	*
<b>GGDC (DDH formula 2)</b>		
<i>Thiohalobacter thiocyanaticus</i> HRh1	*	31.40 [29-33.9]
<i>Thiohalobacter sp.</i> strain FOKN1	31.40 [29-33.9]	*

Fig. S7. Genomic comparison between *Thiohalobacter thiocyanaticus* HRh1<sup>T</sup> and *Thiohalobacter sp.* strain FOKN1.