

Supplementary data

Phenylobacterium ferrooxidans sp. nov., isolated from a sub-surface geothermal aquifer in Iceland

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List of figures:

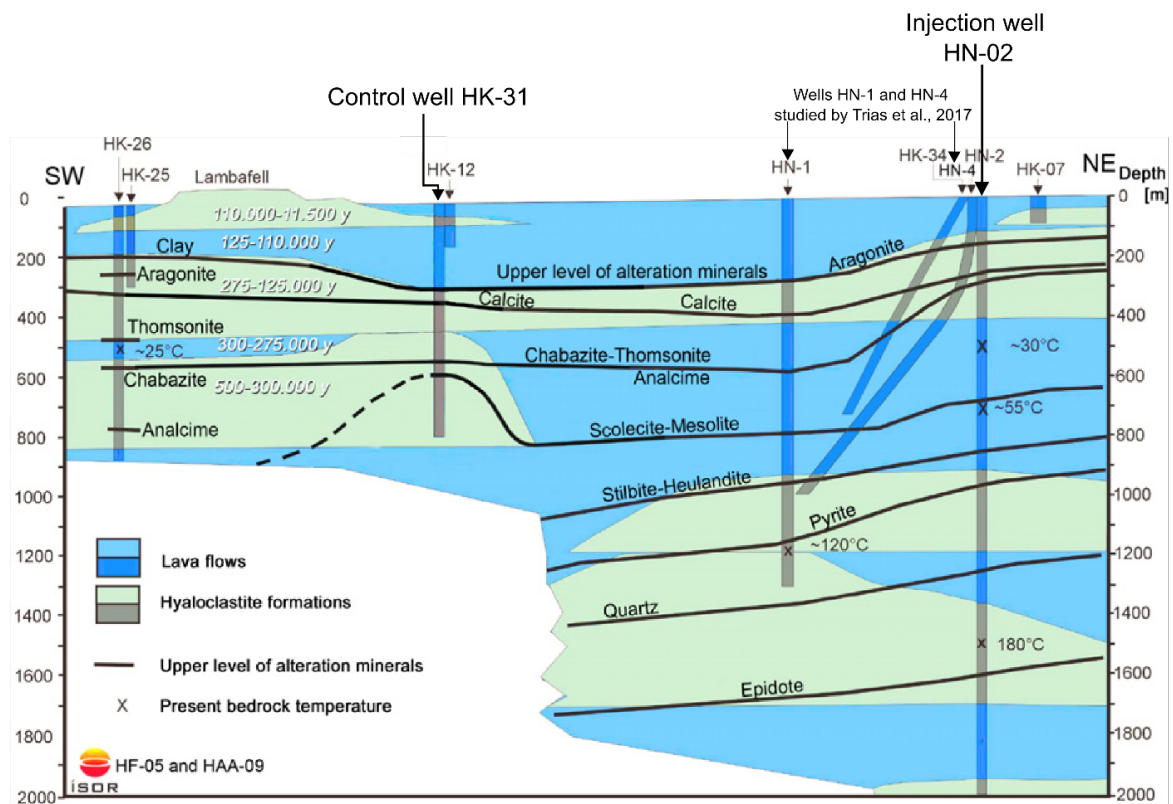


Figure S1: North-South geological section at the CarFix-1 site showing the position of the control well HK-31 targeted in this study and injection wells studied by Trias et al., (2017) (Modified from Matter et al., 2011).

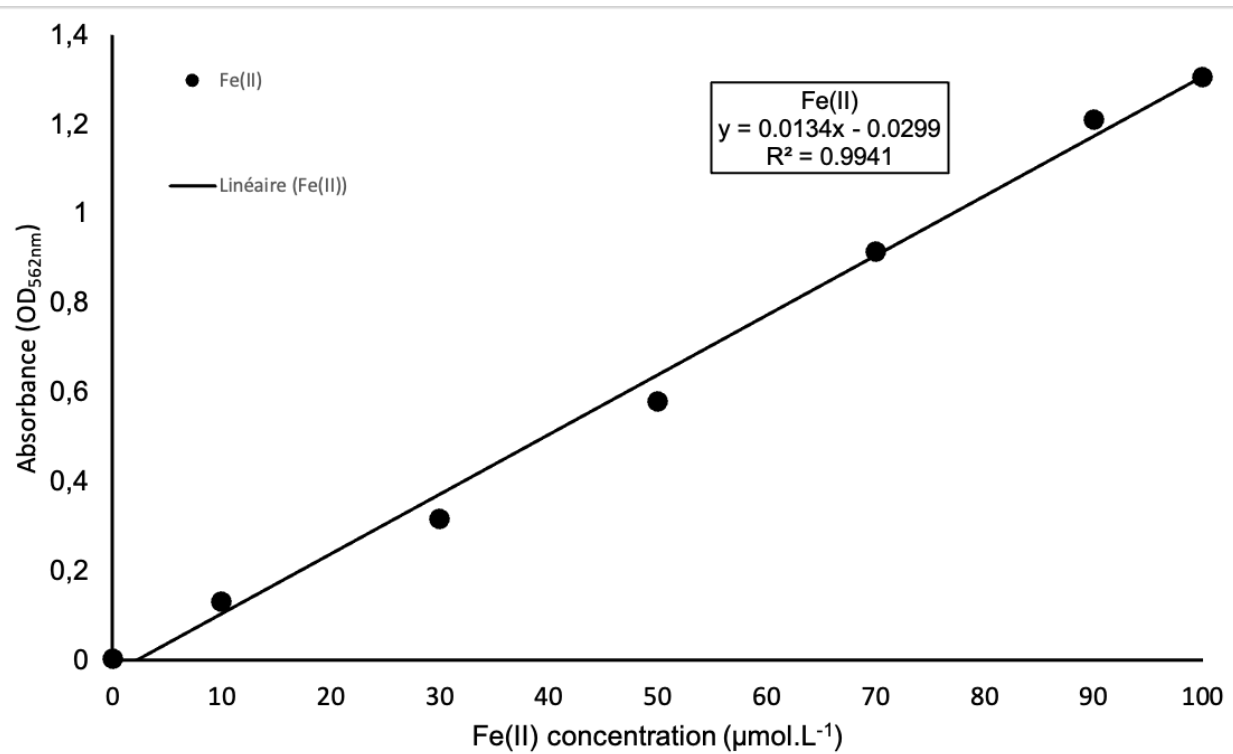


Figure S2. Standard curve used for the determination of Fe(II) concentration in cultures.

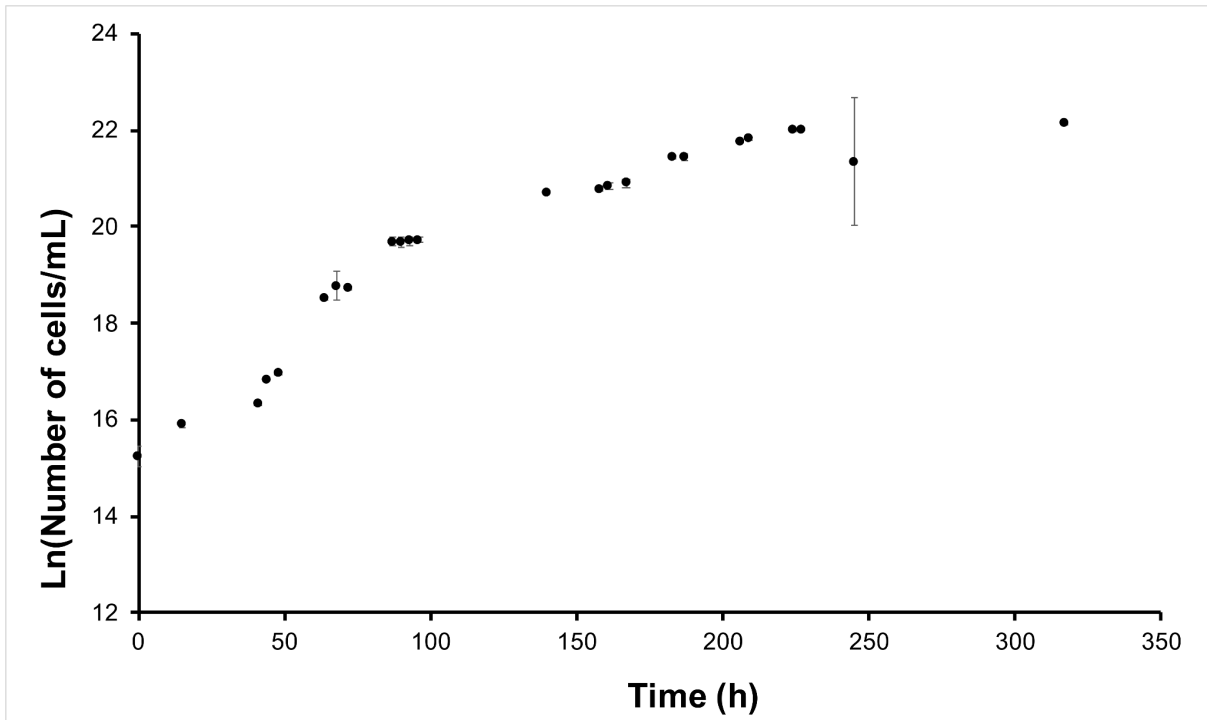


Figure S3. Growth kinetics of strain HK31-G^T under optimal growth conditions. Each value is the mean of three independent replicates. Standard deviation for each mean value is indicated on the graph but is too small to be visible at most points.

PG = Phosphatidylglycerol

GL = Glycolipid

GPL = Glycophospholipid

PL = Phospholipid

L = Lipid

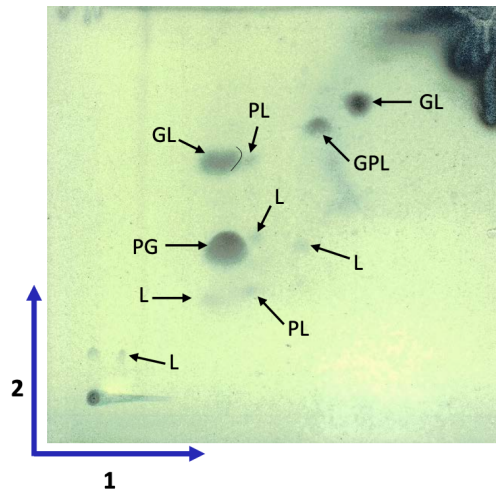


Figure S4. Two-dimensional chromatogram of polar lipids of strain HK31-G^T.

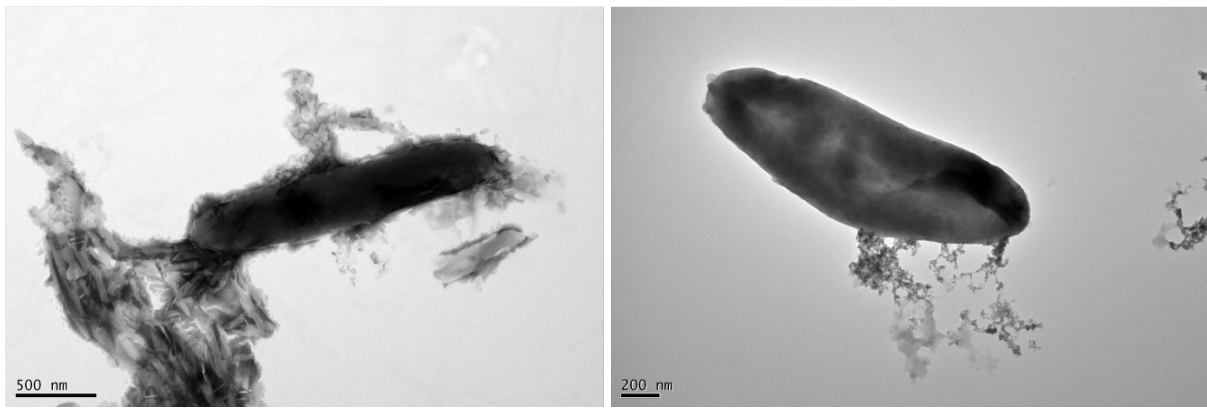


Figure S5. Transmission electron microscopy microphotographs of cells of strain HK31-G^T grown on iron-oxidizing medium at 25 °C under microaerophilic conditions for 5 days, showing potential cell-mineral interactions.

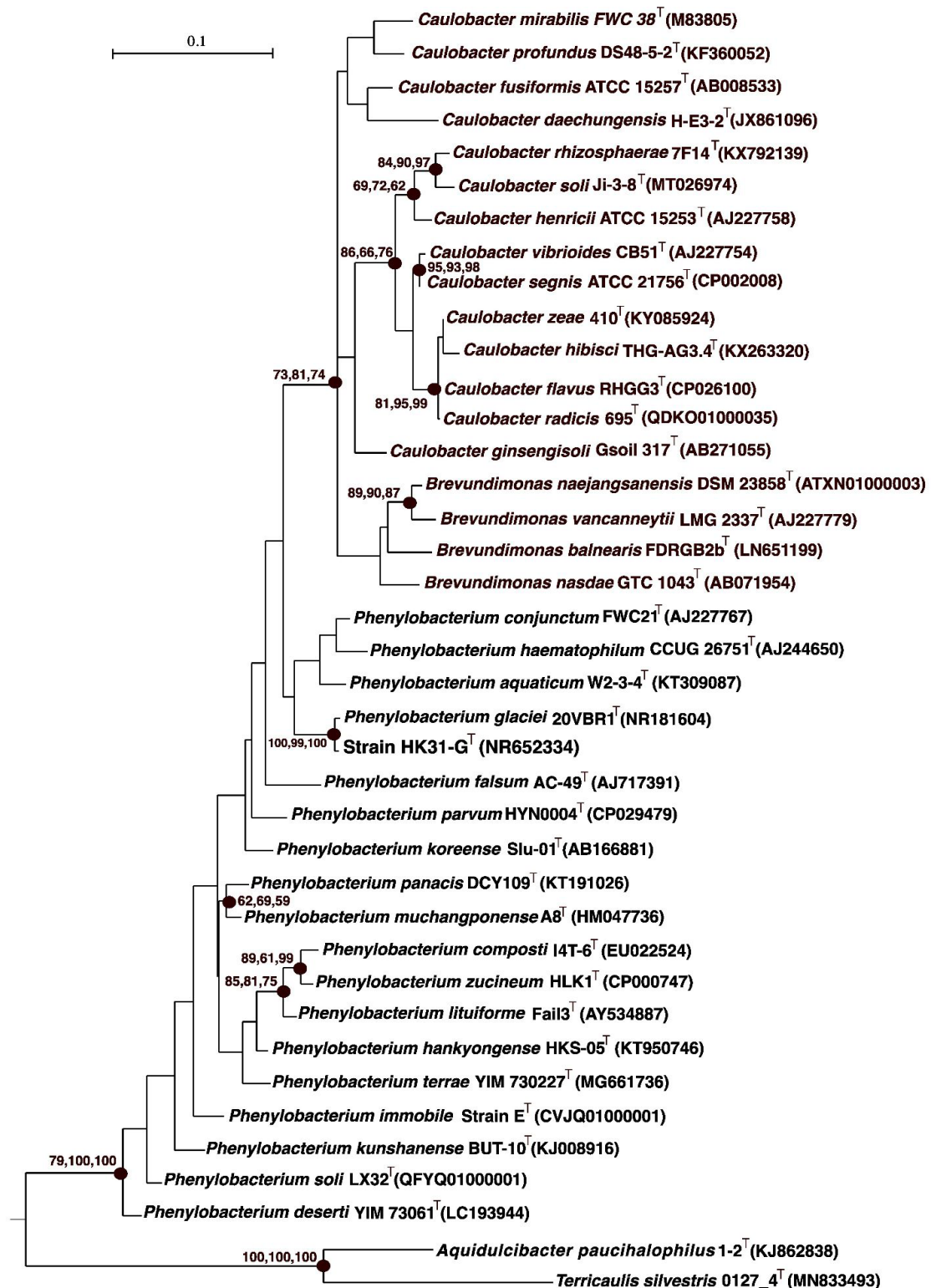


Figure S6. Maximum-likelihood (ML) phylogenetic tree reconstructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain HK31-G^T with the type strains of related species. Filled circles indicate that the corresponding nodes were also recovered using the maximum-parsimony (MP) and neighbor-joining (NJ) algorithms. Numbers at branch nodes indicate bootstrap values (%) as calculated by ML/MP/NJ algorithms. Only values greater than 50% are shown. *Sphingomonas paucimobilis* DSM 1098^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

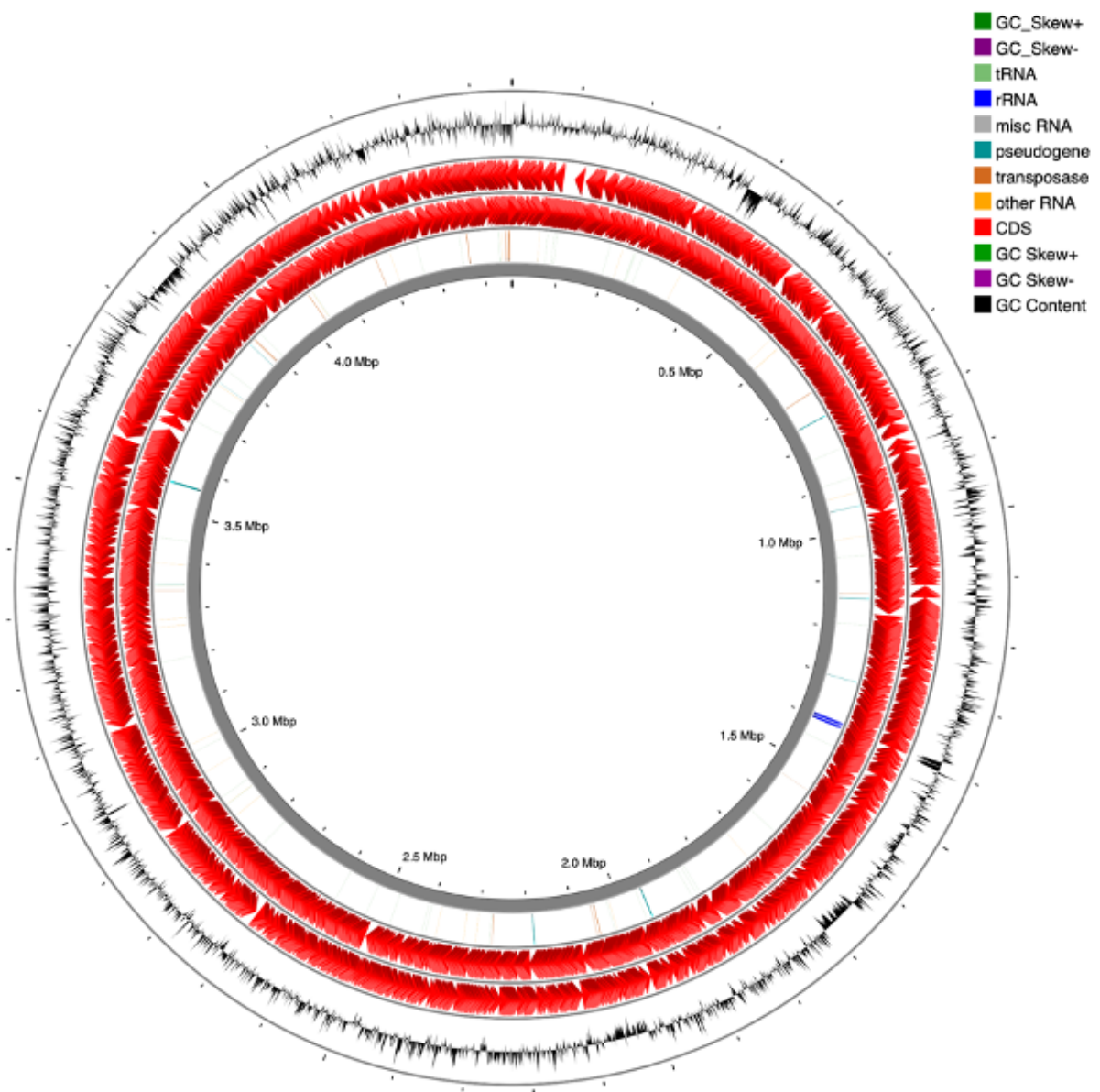


Figure S7. Circular mapping of the genome of strain HK31-G^T (*Phenylobacterium ferrooxidans*) obtained from the Circular Genome Viewer of the MaGe platform.

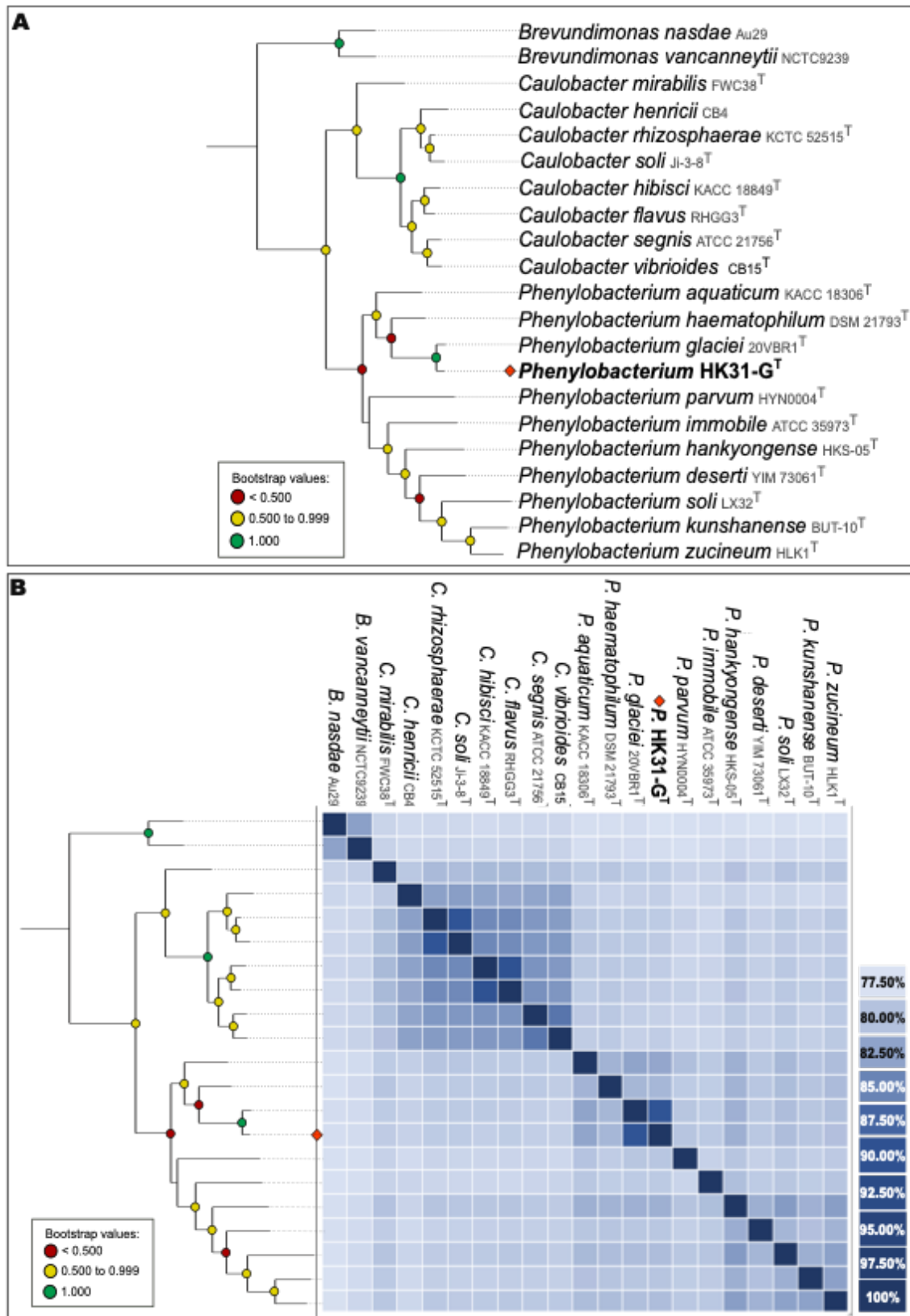


Figure S8. Phylogenomic tree built with anvio 7.1 from a comparative analysis of genomes showing the relationships of strain HK31-G^T with the reference strains of closely related species and (b) heatmap showing FastANI scores between genomes. Bootstrap scores are represented by color circles (below 0.5 in red, between 0.5 and 0.999 in yellow and equal to 1 in green). The genome sequence of *Sphingomonas paucimobilis* ZJSH1 was used as an outgroup. In the heatmap, all FastANI values were greater than 77.5%.



Figure S9. Geographic distribution of *Phenylobacterium* sp030693625 species in the publicly released metagenomes. The sandpiper tool was used to obtain this map (https://sandpiper.qut.edu.au/taxonomy/s_Phenylobacterium%20sp030693625). Note that: Iceland was missing on the map generated by Sandpiper and was added manually.

List of tables:

Table S1. Marker nucleotides in the 16S rDNA genes of strain HK31-G^T and closest relatives. All species are lacking nucleotides 73–89, 199–212, 453–477 and 829–832 (*Escherichia coli* str. K12 subtr. MG1655 numbering). Species: 1, HK31-G^T (data from this study); 2, *P. glaciei* 20VBR1^T; 3, *P. aquaticum* W2-3-4^T; 4, *P. haematophilum* CCUG 26751^T; 5, *P. conjunctum* FWC 21^T; 6, *P. parvum* HYN0004^T; 7, *P. koreense* Slu-01^T.

<i>E. coli</i> no.	<i>Phenylobacterium</i> genus (Abraham <i>et al.</i> , 2008)	1	2	3	4	5	6	7
122	G	G	G	G	G	G	G	G
178	T (<i>P. falsum</i> : C)	T	T	T	T	T	T	T
359	A	A	A	A	A	A	A	A
610	G	G	G	G	G	G	G	G
639	G	G	G	G	G	G	G	G
823	G	G	G	G	G	G	G	G
877	C	C	C	C	C	C	C	C
1145	C	C	C	C	C	C	C	C
1265	T (<i>P. falsum</i> : A)	A	A	T	T	T	T	T
1270	A (<i>P. falsum</i> : T)	T	T	A	A	A	A	A

Table S2. Classification of the coding DNA sequences (CDS) of strain HK31-G^T in clusters of orthologous groups (COG) categories.

Process	Class ID	Description	CDS (nb)	CDS (%)
CELLULAR PROCESSES AND SIGNALING	D	Cell cycle control, cell division, chromosome partitioning	29	0.6203 %
	M	Cell wall/membrane/envelope biogenesis	196	4.1925 %
	N	Cell motility	51	1.0909 %
	O	Post-translational modification, protein turnover, chaperones	161	3.4439 %
	T	Signal transduction mechanisms	204	4.3636 %
	U	Intracellular trafficking, secretion, and vesicular transport	89	1.9037 %
	V	Defense mechanisms	65	1.3904 %
	Z	Cytoskeleton	1	0.0214 %
INFORMATION STORAGE AND PROCESSING	B	Chromatin structure and dynamics	1	0.0214 %
	J	Translation, ribosomal structure and biogenesis	181	3.8717 %
	K	Transcription	237	5.0695 %
	L	Replication, recombination and repair	208	4.4492 %
METABOLISM	C	Energy production and conversion	211	4.5134 %
	E	Amino acid transport and metabolism	214	4.5775 %
	F	Nucleotide transport and metabolism	70	1.4973 %
	G	Carbohydrate transport and metabolism	127	2.7166 %
	H	Coenzyme transport and metabolism	106	2.2674 %
	I	Lipid transport and metabolism	179	3.8289 %
	P	Inorganic ion transport and metabolism	229	4.8984 %
	Q	Secondary metabolites biosynthesis, transport and catabolism	129	2.7594 %
POORLY CHARACTERIZED	S	Function unknown	1228	26.2674 %

Table S3. Summary of Average Nucleotide Identity (ANI) scores calculated between the genome of the strain HK31-G^T and the genomes and MAGs affiliated to *Phenylobacterium* sp. available on NCBI database. In bold, the accession number corresponding to MAGs having an ANI score above the threshold for species delineation and affiliated to species *Phenylobacterium* sp030693625 in GTDB.

Accession number	FastANI value	Accession number	FastANI value	Accession number	FastANI value	Accession number	FastANI value
GCA_030645635 .1	97.9033	GCA_030698145 .1	82.3559	GCA_017991675 .1	81.2341	GCA_030693405 .1	80.592
GCA_030704785 .1	97.1471	GCA_036279815 .1	82.3452	GCA_001425915 .1	81.1474	GCA_030679875 .1	80.5322
GCA_030683775 .1	97.1291	GCA_947379345 .1	82.2768	GCA_001428705 .1	81.1373	GCA_035944335 .1	80.5083
GCA_030693625 .1	97.0431	GCA_022695515 .1	82.2104	GCA_001425305 .1	81.1073	GCA_036502675 .1	80.5061
GCA_030652015 .1	97.0203	GCA_030697925 .1	82.1973	GCA_036567285 .1	81.1065	GCA_024699985 .1	80.4964
GCA_030696765 .1	96.9398	GCA_947371235 .1	82.1591	GCA_019264505 .1	80.9808	GCA_028291325 .1	80.4289
GCA_035428425 .1	96.3998	GCA_947378715 .1	82.097	GCA_030654915 .1	80.9729	GCA_030682345 .1	80.427
GCA_030654475 .1	95.9416	GCA_947377555 .1	82.0873	GCA_040545335 .1	80.9583	GCA_003254475 .1	80.4205
GCA_031425575 .1	93.2717	GCA_001429025 .1	82.0695	GCA_013822795 .1	80.9567	GCA_000017265 .1	80.4135
GCA_030651185 .1	90.0682	GCA_947379565 .1	82.0526	GCA_030646615 .1	80.9366	GCA_035539755 .1	80.4084
GCA_018001015 .1	89.5097	GCA_947372525 .1	82.0106	GCA_014196295 .1	80.9255	GCA_001557235 .1	80.4082
GCA_016772415 .2	89.1632	GCA_031984885 .1	81.872	GCA_030148385 .1	80.9119	GCA_035569475 .1	80.4032
GCA_018061625 .1	89.1479	GCA_018780225 .1	81.8699	GCA_014359675 .1	80.901	GCA_004799545 .1	80.3999
GCA_018058385 .1	89.013	GCA_001724985 .1	81.73	GCA_013911965 .1	80.8698	GCA_030651755 .1	80.3873
GCA_018006855 .1	88.7571	GCA_945952175 .1	81.6429	GCA_945952355 .1	80.8263	GCA_004799515 .1	80.3851
GCA_017999155 .1	88.7545	GCA_031412415 .1	81.5827	GCA_029256285 .1	80.7689	GCA_036723115 .1	80.315
GCA_037968635 .1	88.5732	GCA_035431805 .1	81.5166	GCA_002693985 .1	80.7369	GCA_028698405 .1	80.2998
GCA_030645195 .1	88.5519	GCA_035387405 .1	81.4738	GCA_001825585 .1	80.7296	GCA_034004485 .1	80.2883
GCA_017989235 .1	88.2551	GCA_035423395 .1	81.4056	GCA_030683235 .1	80.7165	GCA_028291005 .1	80.2739
GCA_034366425 .1	86.4913	GCA_003254505 .1	81.3997	GCA_035539035 .1	80.7029	GCA_001557375 .1	80.2531
GCA_031429955 .1	82.7383	GCA_027286305 .1	81.3681	GCA_030681155 .1	80.6874	GCA_028290945 .1	80.2467
GCA_024298925 .1	82.6141	GCA_026411515 .1	81.3651	GCA_030680185 .1	80.6624	GCA_028291215 .1	80.2271
GCA_036676115 .1	82.5374	GCA_017999535 .1	81.3524	GCA_036963605 .1	80.637	GCA_028290925 .1	80.2246
GCA_036677185 .1	82.4977	GCA_040391295 .1	81.2432	GCA_036821595 .1	80.6052	GCA_036567325 .1	80.224

Accession number	FastANI value	Accession number	FastANI value	Accession number	FastANI value	Accession number	FastANI value
GCA_004297125 .1	80.201	GCA_003136395 .1	79.842	GCA_020402055 .1	79.61	GCA_020402445 .1	79.4695
GCA_040508045 .1	80.1999	GCA_004799395 .1	79.8199	GCA_031360265 .1	79.6093	GCA_003243355 .1	79.4665
GCA_001824475 .1	80.1946	GCA_943332615 .1	79.8183	GCA_036402295 .1	79.6	GCA_036496685 .1	79.4649
GCA_028290955 .1	80.1925	GCA_030694925 .1	79.8083	GCA_036403175 .1	79.5834	GCA_001724605 .1	79.4629
GCA_030696615 .1	80.1592	GCA_031181185 .1	79.7934	GCA_030697305 .1	79.5747	GCA_020402305 .1	79.4562
GCA_025351295 .1	80.1573	GCA_004299445 .1	79.7903	GCA_003254705 .1	79.5696	GCA_020402585 .1	79.4551
GCA_030696555 .1	80.1498	GCA_016463085 .1	79.772	GCA_036263695 .1	79.567	GCA_020253625 .1	79.4538
GCA_028290885 .1	80.1266	GCA_036262155 .1	79.7713	GCA_020253885 .1	79.5663	GCA_020402725 .1	79.4485
GCA_036563285 .1	80.1194	GCA_024699945 .1	79.769	GCA_020402145 .1	79.5587	GCA_020402435 .1	79.4484
GCA_029977805 .1	80.0858	GCA_036270195 .1	79.7641	GCA_037189685 .1	79.55	GCA_020402565 .1	79.4474
GCA_035309685 .1	80.078	GCA_036383455 .1	79.7484	GCA_020402065 .1	79.5498	GCA_022402485 .1	79.4452
GCA_028291025 .1	80.0331	GCA_019636135 .1	79.74	GCA_020402425 .1	79.5426	GCA_020402485 .1	79.4446
GCA_036268175 .1	80.0114	GCA_003254525 .1	79.7363	GCA_021298495 .1	79.5416	GCA_036561045 .1	79.4425
GCA_035276905 .1	80.008	GCA_035656795 .1	79.7229	GCA_020402405 .1	79.5239	GCA_020402045 .1	79.4405
GCA_035274295 .1	79.9922	GCA_030680595 .1	79.7207	GCA_020253725 .1	79.5012	GCA_020402625 .1	79.4399
GCA_028291375 .1	79.9727	GCA_003150835 .1	79.6819	GCA_020402505 .1	79.4965	GCA_020402345 .1	79.439
GCA_035656755 .1	79.9507	GCA_016793285 .1	79.6804	GCA_035475775 .1	79.4958	GCA_020401895 .1	79.4388
GCA_036264965 .1	79.9383	GCA_020401885 .1	79.6741	GCA_020285465 .1	79.489	GCA_020402665 .1	79.4384
GCA_036563385 .1	79.9281	GCA_016124325 .1	79.6619	GCA_020402835 .1	79.4858	GCA_020253945 .1	79.4359
GCA_943327825 .2	79.9248	GCA_020402115 .1	79.6583	GCA_020402745 .1	79.4854	GCA_020253665 .1	79.4355
GCA_036501465 .1	79.9221	GCA_020402165 .1	79.6336	GCA_020402325 .1	79.4838	GCA_020402685 .1	79.4321
GCA_034376405 .1	79.9212	GCA_020402525 .1	79.6275	GCA_028291285 .1	79.4742	GCA_020402245 .1	79.4278
GCA_038920875 .1	79.9097	GCA_036268735 .1	79.6197	GCA_020253645 .1	79.4704	GCA_020402205 .1	79.4272
GCA_034665885 .1	79.8832	GCA_020402385 .1	79.6131	GCA_020402285 .1	79.4699	GCA_020253835 .1	79.4269

Accession number	FastANI value	Accession number	FastANI value	Accession number	FastANI value
GCA_020401985 .1	79.426	GCA_020402185 .1	79.3394	GCA_945859865 .1	78.9728
GCA_024508875 .1	79.4173	GCA_020253795 .1	79.3342	GCA_038920955 .1	78.8877
GCA_020402645 .1	79.4158	GCA_020402805 .1	79.3289	GCA_037138395 .1	78.8476
GCA_020401995 .1	79.4154	GCA_020402225 .1	79.3147	GCA_945907165 .1	78.826
GCA_035627295 .1	79.4153	GCA_902826855 .1	79.3123	GCA_945874825 .1	78.8177
GCA_020402545 .1	79.4142	GCA_020402025 .1	79.2931	GCA_025925325 .1	78.7597
GCA_020401865 .1	79.4138	GCA_021300115 .1	79.2871	GCA_023260155 .1	78.6622
GCA_039930425 .1	79.4131	GCA_020402365 .1	79.2848	GCA_036495085 .1	78.6425
GCA_020402695 .1	79.411	GCA_036273595 .1	79.2811	GCA_947380745 .1	77.6672
GCA_020253705 .1	79.4105	GCA_020253785 .1	79.2598	GCA_002221445 .1	77.6254
GCA_020253865 .1	79.4102	GCA_020253825 .1	79.2364	GCA_947377895 .1	77.5927
GCA_030693805 .1	79.4087	GCA_016793225 .1	79.1602	GCA_947378305 .1	77.5481
GCA_020402105 .1	79.3968	GCA_036383735 .1	79.1561	GCA_947375025 .1	77.4164
GCA_020402755 .1	79.3879	GCA_020401955 .1	79.1502		
GCA_020402775 .1	79.3862	GCA_026126275 .1	79.14		
GCA_035694305 .1	79.3646	GCA_040508585 .1	79.1157		
GCA_020253765 .1	79.3627	GCA_025350885 .1	79.1024		
GCA_036788895 .1	79.3609	GCA_028291065 .1	79.099		
GCA_020401945 .1	79.36	GCA_018135625 .1	79.0855		
GCA_020402825 .1	79.3581	GCA_025934275 .1	79.0816		
GCA_020402265 .1	79.3512	GCA_028290985 .1	79.0543		
GCA_020253745 .1	79.3484	GCA_020402595 .1	79.0445		
GCA_020401875 .1	79.3477	GCA_034377185 .1	79.0334		
GCA_020253685 .1	79.342	GCA_945883235 .1	79.0263		

Table S4. MAGs statistics and Overall Genome Relatedness Indices (OGRI) between the genome of strain HK31-G^T and the six MAGs affiliated to the species *Phenylobacterium* sp030693625. Species: 1, HK31-G^T (data from this study); 1a, GCA_030645635; 1b, GCA_030652015; 1c, GCA_030683775; 1d, GCA_030693625; 1e, GCA_030696765; 1f, GCA_030704785 (data from GTDB).

	1	1a	1b	1c	1d	1e	1f
Number of contigs	104	454	66	220	97	323	73
Size (Mbp)	4.46	3.75	4.29	4.01	4.12	4.05	4.35
Completeness	100	88.25	99.91	99.99	99.96	100	100
Contamination	1.41	3.69	1.64	3.29	1.37	4.81	8.67
G+C content (%)	67.95	68.20	68.06	68.06	68.13	68.18	68.17
Number of CDS	4667	4107	4271	4125	4149	4267	4395
tRNA	48	18	19	19	17	19	19
dDDH (%)	100	83.10	75.30	76.00	76,10	75,70	74,10
OrthoANlu (%)	100	98.12	97.20	97.28	97.22	97.11	97.15
FastANI (%)	100	97.90	97.02	97.13	97.04	96.94	97.14
AAI (%)	100	98.28	97.59	97.98	97.68	97.77	97.83

Table S5. Summary of Average Nucleotide Identity Scores, calculated using FastANI, between the genome of HK31-G^T, the six affiliated to the species *Phenylobacterium* sp030693625 and the most closely related genome (*Phenylobacterium glaciei* 20VBR1^T).

	<i>Phenylobacterium</i> sp. HK31-G ^T	GCA_030645635	GCA_030683775	GCA_030704785	GCA_030696765	GCA_030652015	GCA_030693625	<i>Phenylpbacterium</i> <i>glaciei</i> 20VBR1 ^T
<i>Phenylobacterium</i> sp. HK31-G ^T	100.0%	97.9%	97.2%	97.1%	96.9%	97.0%	97.0%	89.1%
GCA_030645635		100.0%	97.1%	97.4%	97.1%	97.0%	97.2%	88.7%
GCA_030683775			100.0%	98.9%	98.9%	98.7%	98.7%	89.0%
GCA_030704785				100.0%	98.8%	98.6%	98.6%	88.9%
GCA_030696765					100.0%	98.5%	98.6%	88.7%
GCA_030652015						100.0%	100.0%	89.0%
GCA_030693625							100.0%	89.0%
<i>Phenylpbacterium</i> <i>glaciei</i> 20VBR1 ^T								100.0%

Table S6. Relative abundance of the *Phenylobacterium* sp030693625 species in the publicly released metagenomes. Yellow color indicates the matching samples in common with *P. glaciei* 20VBR1^T. In bold, the public metagenomic samples corresponding to the Carbfix project.

Sample ID	Relative abundance (%)	Coverage	Habitat	Release year	Comment
SRR14932480	0.07	1.85	activated sludge	2021	
SRR11674048	0.07	3.03	activated sludge	2021	
SRR14769836	0.07	1.25	activated sludge	2021	
SRR9038830	0.05	0.67	activated sludge	2020	
SRR14769835	0.05	0.75	activated sludge	2021	
SRR14769834	0.04	0.83	activated sludge	2021	
SRR14769837	0.04	0.88	activated sludge	2021	
SRR14932479	0.04	0.86	activated sludge	2021	
SRR11674015	0.03	1.67	activated sludge	2021	
SRR11674009	0.03	1.66	activated sludge	2021	
SRR11674044	0.03	1.14	activated sludge	2021	
SRR11674054	0.02	0.75	activated sludge	2021	
SRR11673976	0.02	0.91	activated sludge	2021	
SRR11673989	0.02	0.74	activated sludge	2021	
SRR12983868	2.35	5.91	aquatic	2021	
SRR12983519	0.49	3.25	aquatic	2021	
ERR3478701	0.43	56.27	aquifer – well HK26	2021	
ERR3478695	0.09	6.77	aquifer – well HK26	2021	subsurface basaltic
ERR3478691	0.06	5.77	aquifer – well HK12	2021	aquifers in
ERR3478705	0.06	6.14	aquifer – well HK31	2021	near carbon
ERR3478707	0.05	4.28	aquifer – well HK31	2021	sequestration
ERR3478710	0.03	3.92	aquifer – well HK31	2021	operations in
ERR4904394	0.02	0.72	aquifer – well HK12	2021	Iceland
SRR9061329	0.31	3.37	freshwater	2019	
SRR10492803	0.1	1.85	freshwater	2019	
SRR10132648	0.04	0.53	freshwater	2019	
ERR2583788	0.06	0.6	freshwater	2018	
SRR14559660	0.08	0.63	freshwater sediment	2021	
SRR14639177	0.07	1.78	freshwater sediment	2021	
SRR5431181	0.04	0.83	freshwater sediment	2017	
SRR13723392	2.37	18.54	groundwater	2021	subsurface weathering of pyrite in shale
SRR15669517	0.16	6.04	groundwater	2021	
SRR12964614	0.1	3.65	groundwater	2021	
SRR12964612	0.09	2.6	groundwater	2021	
SRR12964611	0.08	2.21	groundwater	2021	

SRR12964613	0.07	2.37	groundwater	2021
SRR8797414	0.05	1.22	groundwater	2020
SRR15669520	0.04	1.78	groundwater	2021
ERR3858137	0.03	2.03	groundwater	2021
SRR8863431	0.03	1.04	groundwater	2019
ERR3858140	0.03	1.56	groundwater	2021
SRR8863439	0.02	0.77	groundwater	2019
ERR3858136	0.01	0.71	groundwater	2021
ERR3858138	0.01	0.41	groundwater	2021
SRR6448214	0.02	1.08	microbial mat	2018
SRR5352528	0.51	5.03	mine drainage	2017
SRR5352529	0.26	1.98	mine drainage	2017
SRR13608725	0.06	3.85	riverine environment	2021
SRR13608736	0.06	4.57	riverine environment	2021
SRR13608705	0.05	2.92	riverine environment	2021
SRR13608700	0.05	3.2	riverine environment	2021
SRR13608737	0.03	2.14	riverine environment	2021
SRR13608730	0.01	0.78	riverine environment	2021
SRR13723396	0.04	2.03	rock	2021
SRR13723397	0.03	1.75	rock	2021
SRR13723394	0.03	1.5	rock	2021
SRR13723393	0.02	1.15	rock	2021
ERR635996	0.04	1.35	root	2016
ERR636002	0.04	1.29	root	2016
ERR635998	0.03	1.6	root	2016
ERR635995	0.03	1.05	root	2016
SRR15221269	0.06	1.65	runoff	2021
SRR15221270	0.02	0.62	runoff	2021
SRR3322106	0.14	1.23	sediment	2017
SRR3322355	0.09	0.73	sediment	2017
SRR16092984	0.01	0.72	sediment	2021
SRR3992565	0.06	1.57	soil	2017
SRR12404951	0.04	2.73	wastewater	2020
SRR12762320	0.2	8.83	wastewater	2020
SRR12762315	0.17	13.64	wastewater	2020
SRR12762316	0.17	6.81	wastewater	2020
SRR12712851	0.17	1.48	wastewater	2021
SRR12762302	0.15	5.02	wastewater	2020
ERR712369	0.11	2.69	wastewater	2015
SRR12762291	0.09	3.99	wastewater	2020
SRR11088483	0.06	1.01	wastewater	2021
SRR12762280	0.06	2.35	wastewater	2020
SRR12762305	0.05	2.19	wastewater	2020

SRR12712865	0.05	1.15	wastewater	2021
SRR11088384	0.05	0.88	wastewater	2021
SRR12762306	0.05	1.64	wastewater	2020
ERR4682395	0.04	1.32	wastewater	2021
ERR712368	0.03	1.05	wastewater	2015
ERR712388	0.03	0.88	wastewater	2015
SRR11088411	0.03	0.52	wastewater	2021
SRR12762310	0.03	1.44	wastewater	2020
SRR12762309	0.02	1.14	wastewater	2020
SRR12762300	0.02	1.14	wastewater	2020
SRR12762301	0.02	1.13	wastewater	2020
SRR12762304	0.02	1.16	wastewater	2020
SRR11088468	0.02	0.45	wastewater	2021
SRR12762311	0.01	1.29	wastewater	2020

Table S7. Relative abundance of *Phenylobacterium glaciei* 20BVR1^T in the publicly released metagenomes. Yellow color indicates the matching samples in common with *Phenylobacterium* sp030693625.

Sample ID	Relative abundance (%)	Coverage	Habitat	Geographic location	Release year
SRR12983868	0.56	1.4	aquatic	-	2021
SRR12983519	0.19	1.27	aquatic	-	2021
SRR11700407	0.07	1.09	freshwater	Nepal: south lake nuptse	2021
SRR5431181	0.05	0.87	freshwater sediment	USA: Lake Washington	2017
SRR15669517	0.08	2.91	groundwater	China: Shaanxi	2021
SRR17221334	0.1	3.43	gut	China: Yunnan	2021
SRR1298748	0.24	0.52	Homo sapiens	Denmark	2014
SRR10968271	0.08	1.85	peat	USA: Alaska	2020
ERR4837138	0.19	3.71	soil	Sweden	2020
SRR13312985	0.04	1.95	soil	USA: Wyoming	2020
SRR12404951	0.03	2.02	wastewater	South Korea: Hwasun-gun	2020

-: missing information

Table S8. Table of genes (names, EC numbers, locus_tag and KEGG reactions) present in the genome of strain HK31-G^T and potentially involved in the reverse oxidative TCA (ro TCA) cycle according to the PGAP and MaGe annotations.

Genes	EC number	PGAP locus_tag	MaGe Locus_tag	KEGG reactions
citrate synthase - "gltA"	EC: 2.3.3.16	OCL97_00660 OCL97_13220	PHEHK31G_v1_10131 PHEHK31G_v1_240022	R00351:acetyl-CoA:oxaloacetate C-acetyltransferase (thioester-hydrolysing)
2-oxoacid oxidoreductase : 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit beta <i>or pyruvate synthase ; 2-oxoglutarate synthase</i>	EC: 1.2.7.11 (<i>or EC: 1.2.7.1 ; EC: 1.2.7.3</i>)	OCL97_19040 OCL97_19045	PHEHK31G_v1_450022 PHEHK31G_v1_450023	R01196: pyruvate:ferredoxin 2-oxidoreductase (CoA-acetylating) R01197: 2-oxoglutarate:ferredoxin oxidoreductase (decarboxylating)
pyruvate, phosphate dikinase - "ppdK"	EC: 2.7.9.1	OCL97_13545	PHEHK31G_v1_250021 - ppdK	R00206: ATP:pyruvate, phosphate phosphotransferase
phosphoenolpyruvate carboxylase - "ppc"	EC: 4.1.1.31	OCL97_09215	HEHK31G_v1_140075 - ppc	R00345: phosphate:oxaloacetate carboxylase (adding phosphate;phosphoenolpyruvate-forming); Orthophosphate:oxaloacetate carboxylase (phosphorylating)
malate dehydrogenase – "mdh"	EC: 1.1.1.37	OCL97_04825	PHEHK31G_v1_60044 - mdh	R00342: (S)-malate:NAD ⁺ oxidoreductase
Fumarase, "fumC"	EC 4.2.1.2	OCL97_21255	PHEHK31G_v1_610008 - fumC	R01082: (S)-malate hydro-lyase (fumarate-forming)
succinate dehydrogenase, "sdhA"	EC: 1.3.5.1	OCL97_14740 OCL97_14745 OCL97_14750 OCL97_14755	PHEHK31G_v1_290012 PHEHK31G_v1_290013 PHEHK31G_v1_290014 - sdhA PHEHK31G_v1_290015 - sdhB	R02164: succinate:quinone oxidoreductase

succinyl-CoA synthetase - "sucC" "sucD"	EC: 6.2.1.5	OCL97_04855 OCL97_04860	PHEHK31G_v1_60050 - sucC PHEHK31G_v1_60051 - sucD	R00405: Succinate:CoA ligase (ADP-forming)
isocitrate dehydrogenase (NADP+) - "icd"	EC: 1.1.1.42	OCL97_03620	PHEHK31G_v1_40096 - icd	R00267: Isocitrate:NADP+ oxidoreductase (decarboxylating)
aconitate hydratase - "acnA"	EC: 4.2.1.3	OCL97_04770	PHEHK31G_v1_60032 - acnA	R01325: citrate hydro-lyase (cis- aconitate-forming) R01900: isocitrate hydro-lyase (cis- aconitate-forming)

Table S9. Table of locus_tags of genes of known function in strain HK31-G^T involved in nitrogen metabolism

PGAP: Product - gene	Locus_tag
Nitrate reductase subunit alpha - <i>narG</i>	OLC97_05710
Nitrate reductase subunit beta - <i>narH</i>	OLC97_05715
Nitrate reductase subunit gamma - <i>narI</i>	OLC97_05720
Nitrate reductase molybdenum cofactor assembly chaperone - <i>narJ</i>	OLC97_05725
Family nitrate transporter - <i>narK</i>	OLC97_05700
NADH-quinone oxidoreductase subunit A - <i>nuoA</i>	OLC97_00445
NADH-quinone oxidoreductase subunit B - <i>nuoB</i>	OLC97_00450
NADH-quinone oxidoreductase subunit C - <i>nuoC</i>	OLC97_00455
NADH-quinone oxidoreductase subunit D - <i>nuoD</i>	OLC97_00460
NADH-quinone oxidoreductase subunit E - <i>nuoE</i>	OLC97_00470
NADH-quinone oxidoreductase subunit F - <i>nuoF</i>	OLC97_00480
NADH-quinone oxidoreductase subunit G - <i>nuoG</i>	OLC97_00485
NADH-quinone oxidoreductase subunit H - <i>nuoH</i>	OLC97_00490
NADH-quinone oxidoreductase subunit I - <i>nuoI</i>	OLC97_00500
NADH-quinone oxidoreductase subunit J - <i>nuoJ</i>	OLC97_00505
NADH-quinone oxidoreductase subunit K - <i>nuoK</i>	OLC97_00510
NADH-quinone oxidoreductase subunit L - <i>nuoL</i>	OLC97_00515
NADH-quinone oxidoreductase subunit M - <i>nuoM</i>	OLC97_00520
NADH-quinone oxidoreductase subunit N - <i>nuoN</i>	OLC97_00525

Table S10. List of genes involved in iron metabolism predicted by PGAP and FeGenie tools. “-” indicates that the genes have not been detected thanks to the prediction tool.

Gene category	PGAP tag	PGAP gene product	FeGenie gene product	Prokka gene product	
Iron aquisition - Iron(II)/(III) transport	OCL97_13350	dipeptide ABC transporter ATP-binding protein	FbpC-family-ATPase	Glutathione import ATP-binding protein GsiA	
	OCL97_13360	ABC transporter ATP-binding protein	YfeB-family-membrane-proteins	Putative ABC transporter ATP-binding protein YxIF	
	OCL97_15185	ferrous iron transporter B	FeoB-family-iron-transporter	Fe ²⁺ transporter FeoB	
	OCL97_15190	ferrous iron transport protein A	FeoA-family-iron-transporter	Hypothetical protein	
	OCL97_15185	ferrous iron transporter B	FeoB-family-iron-transporter iron_aquisition-iron_transport	Fe ²⁺ transporter FeoB	
	OCL97_15190	ferrous iron transport protein A	FeoA-family-iron-transporter iron_aquisition-iron_transport	Hypothetical protein	
	OCL97_07540	FTR1 family protein	-	Ferrous iron permease EfeU	
	-	-	-	Ferrous iron permease EfeU	
	Iron aquisition - Siderophore transport	OCL97_09815	iron ABC transporter permease	FpvE-family-permease	Hemin transport system permease protein HmuU
		OCL97_09820	ABC transporter substrate-binding protein	HatD-family-substrate-binding-protein	Hypothetical protein
		OCL97_03805	efflux RND transporter periplasmic adaptor subunit	PvdR-family-siderophore-export	Multidrug resistance protein MdtA
		OCL97_03810	ABC transporter ATP-binding protein	PvdT-family-siderophore-export	Putative ABC transporter ATP-binding protein YknY
		OCL97_03815	ABC transporter permease	PvdT-family-siderophore-export	Macrolide export ATP-binding/permease protein MacB
OCL97_05930		TonB-dependent receptor	PirA-family-siderophore-receptor	Vitamin B ₁₂ transporter BtuB	
-		-	LbtU-family-siderophore receptor	Vitamin B ₁₂ transporter BtuB	
OCL97_18855		TonB-dependent receptor	PirA-family-siderophore-receptor	Vitamin B ₁₂ - transporter BtuB	
OCL97_09385		TonB-dependent receptor	PirA-family-siderophore-receptor	Putative TonB-dependent receptor	
OCL97_09820		ABC transporter substrate-binding protein	HatD-family-substrate-binding-protein	Hypothetical protein	
OCL97_18855		TonB-dependent receptor	PirA-family-siderophore-receptor	Vitamin B ₁₂ - transporter BtuB	
OCL97_11255		protein TolQ	ExbB-family	Biopolymer transport protein ExbB	
OCL97_11260		ExbD/TolR family protein	ExbD-family	Tol-Pal system protein TolR	
-		-	ExbD-family	Biopolymer transport protein ExbD	
OCL97_11495		MotA/TolQ/ExbB proton channel family protein	ExbB-family	Tol-Pal system protein TolQ	
OCL97_11500		energy transducer TonB	TonB-family	Hypothetical protein	
-		-	TonB-family	Hypothetical protein	
OCL97_09390		MotA/TolQ/ExbB proton channel family protein	ExbB-family	Biopolymer transport protein ExbB	
OCL97_09395		biopolymer transporter ExbD	ExbD-family	Biopolymer transport protein ExbD	

OCL97_09400	energy transducer TonB	TonB-family	Hypothetical protein
OCL97_00360	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_02245	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_02390	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_02520	TonB-dependent receptor	-	Hypothetical protein
OCL97_02730	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_03605	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
-	-	-	Vitamin B ₁₂ transporter BtuB
OCL97_05495	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
-	-	-	Vitamin B ₁₂ transporter BtuB
OCL97_05800	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_05825	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_05935	TonB-dependent receptor	-	-
OCL97_09005	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_09385	TonB-dependent receptor	PirA-family-siderophore-receptor	Putative TonB-dependent receptor
OCL97_09495	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_10675	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_16215	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_17745	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_19150	TonB-dependent receptor	-	Ferric aerobactin receptor
OCL97_19355	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_19465	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
OCL97_19945	TonB-dependent receptor	-	Vitamin B ₁₂ transporter BtuB
Iron gene regulation			
-	-	Sid_PvdS_regulator_Paeruginosa_PA2426_180620	ECF RNA polymerase sigma factor SigR
OCL97_15730	RNA polymerase sigma factor	Sid_PvdS_regulator_Paeruginosa_PA2426_180620	ECF RNA polymerase sigma factor SigE
OCL97_04690	PadR family transcriptional regulator	Sid_YqjI_regulator_for_YqjH_P64588_Escherichia_coli_180606	Hypothetical protein
OCL97_02745	helix-turn-helix domain-containing protein	Sid_PchR_pyochelin_regulator_Pseudomonas_aeruginosa_PA4227_180623	HTH-type transcriptional regulator CdhR
OCL97_01890	transcriptional repressor	PF01475-Iron_dependent_repressor_family	Zinc uptake regulation protein
OCL97_02175	FecR family protein	PF04773_FecR	Hypothetical protein
OCL97_05900	transcriptional regulator FtrA	Sid_PchR_pyochelin_regulator_Pseudomonas_aeruginosa_PA4227_180623	HTH-type transcriptional regulator CdhR
OCL97_05940	FecR domain-containing protein	PF04773_FecR	Protein FecR
OCL97_05945	Sigma-70 family RNA polymerase sigma factor	Sid_PvdS_regulator_Paeruginosa_PA2426_180620	Hypothetical protein

	OCL97_05290	helix-turn-helix domain-containing protein	Sid_PchR_pyochelin_regulator_Pseudomonas_aeruginosa_PA4227_180623	Hypothetical protein
	OCL97_12495	FecR domain-containing protein	PF04773_FecR	Protein FecR
	OCL97_12500	RNA polymerase sigma factor	Sid_PvdS_regulator_Paeruginosa_PA2426_180620	Putative ECF RNA polymerase sigma factor SigI
	OCL97_12725	transcriptional repressor	Iron_dependent_repressor-fur_family	Ferric uptake regulation protein
	OCL97_20500	FecR domain-containing protein	PF04773_FecR	Hypothetical protein
	OCL97_21580	AraC family transcriptional regulator	Sid_PchR_pyochelin_regulator_Pseudomonas_aeruginosa_PA4227_180623	HTH-type transcriptional activator RhaS
Iron Storage	OCL97_15400	bacterioferritin	PF00210-Ferritin like domain	Bacterioferritin
Iron transport - Heme transport	OCL97_09810	ABC transporter ATP-binding protein	-	Hemin import ATP-binding protein HmuV
Unclassified	OCL97_00775	VIT family protein	-	Hypothetical protein
	OCL97_10360	FTR1 family protein	-	-
	-	-	-	Ferric aerobactin receptor
	OCL97_13230	Cation diffusion facilitator family transporter	-	Ferrous-iron efflux pump FieF
	OCL97_10245	ferric reductase-like transmembrane domain-containing protein	-	Protein-methionine-sulfoxide reductase heme-binding subunit MsrQ

Table S11. Summary of genes detected in the genome of strain HK31-G^T using HMMER, with HMM profiles of genes involved in iron oxidation available in FeGenie database.

Profile_HMM	Matches	E-value	BLAST (UniProt)	Organism	Percentage of identity
Cyc1	Cbb3-type cytochrome c oxidase subunit FixP	0.0017			
	Cytochrome c6	0.0032			
	Cytochrome c6	0.013			
	Hypothetical protein	0.049	Cytochrome c	<i>P.glaciei</i>	80.60%
Cyc2_rep1	ND				
Cyc2_rep2	ND				
Cyc2_rep3	ND				
FoxA	Cytochrome c oxidase subunit 1	1.20E-20	Cytochrome c oxidase SU 1 <i>ctaD</i>	<i>P.glaciei</i>	92.60%
			Cytochrome c oxidase SU 1 <i>coxA</i>	<i>Caulobacter vibrioides</i>	86.80%
FoxB	Hypothetical protein	0.00093	Cytochrome c oxidase SU 2 <i>coxB</i>	<i>P.glaciei</i>	92.20%
			Cytochrome c oxidase SU 2 <i>ctaC</i>	<i>Brevundimonas</i> sp.	67.10%
FoxC	ND				
FoxE	Hypothetical protein	0.06	carotenoid 1.2-hydratase	<i>P.glaciei</i>	86.60%
FoxY	Outer membrane protein assembly factor BamB	1.90E-06			
	Quinohemoprotein alcohol dehydrogenase ADH IIB	5.10E-06			
FoxZ	Quinoprotein glucose dehydrogenase	0.22			
	Putative inner membrane transporter YedA	5.40E-46			
	Putative amino-acid metabolite efflux pump	1.00E-07			
MtoA	ND				
MtrB	Vitamin B12 transporter BtuB	0.045			

ND, no hit detected

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