

## Supplementary Information

### Reconstructing genomes of carbon monoxide oxidisers in volcanic deposits including members of the class Ktedonobacteria

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#### Supplementary figure legend:

Figure S1. Relative abundance of main phyla based on classification of scaffolds >500 bp. Other phyla include members of Planctomycetes, Gemmatimonadetes, Cyanobacteria, Firmicutes, Armatimonadetes and Patescibacteria.

#### Supplementary table legends:

Table S1. List of strains used for the tree construction.

Table S2. Summary table of other complete cellular functions in the MAGs isolates from sites 1640, 1751 and 1957 retrieved from KEGG analysis. Reference genomes for Ktedonobacteria: DSM45816T [1], DSM44963<sup>T</sup> [2] and NBRC 113551<sup>T</sup> [3], (K: KEGG orthology; M: KEGG Mode). Asterisks indicate the MAGs isolated from the class Ktedonobacteria.

Table S3. Summary of the metagenome-assembled genomes (MAGs) isolated in the present study. Average amino-acid identity (AAI) was calculated by comparing the MAGs with their closest reference genomes identified based on the RAST results. The RefSeq accession number of the reference genomes are provided in parenthesis.

Table S4. Summary of enzymatic functions for CO-, H<sub>2</sub>-, and formate-oxidation in the Ktedonobacteria reference genomes. Carbon monoxide oxidation: K03518 carbon monoxide dehydrogenase small subunit coxS, K03519 carbon monoxide dehydrogenase medium subunit coxM, K03520 carbon monoxide dehydrogenase large subunit coxL. Formate oxidation: K00122 formate dehydrogenase, K00123 formate dehydrogenase major subunit. Hydrogen oxidation: H<sub>2</sub> dehydrogenase: K00436: NAD-reducing hydrogenase large subunit.

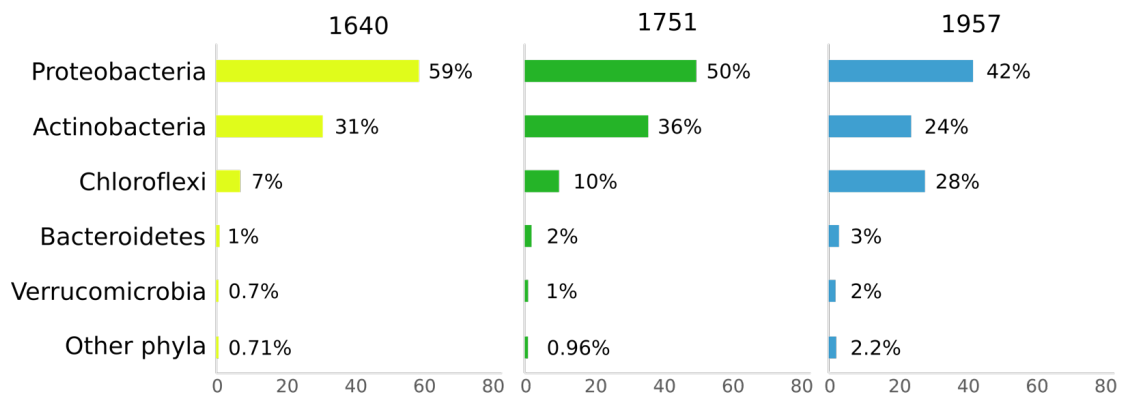


Figure S1. Relative abundance of main phyla based on classification of scaffolds >500 bp. Other phyla include members of Planctomycetes, Gemmatimonadetes, Cyanobacteria, Firmicutes, Armatimonadetes and Patescibacteria.

Table S1. List of strains used for the tree construction.

Strain name	Type strain	NCBI Accession number
<i>Acetobacter acetii</i> TMW2.1153	NZ_CP014692.1	
<i>Acetobacter orleanensis</i> JCM 7639	NZ_BAMY01000269.1	
<i>Acetobacter pasteurianus</i> IFO 3283-01	NC_013209.1	
<i>Acetobacter syzygii</i> 9H-2	NZ_BAMZ01000094.1	
<i>Acidipila dinghuensis</i> DHOF10	NZ_SDMK01000001.1	
<i>Acidipila rosea</i> DSM 103428	NZ_SMGK01000001.1	
<i>Acidiphtheria rubrifaciens</i> HS-AP3	NZ_BANB01001239.1	
<i>Acidobacterium capsulatum</i> ATCC51196	NC_012483.1	
<i>Acidocella aminolytica</i> 101	NZ_FQVJ01001009.1	
<i>Actinosynnema mirum</i> DSM 43827	NC_013093.1	
<i>Ammonifex degensii</i> KC4	NC_013385.1	
<i>Asaia bogorensis</i> NBRC 16594	NZ_AP014690.1	
<i>Bacteroides fragilis</i> YCH46	NC_006347.1	
<i>Bacteroides ovatus</i> ATCC 8483	NZ_CP012938.1	
<i>Bacteroides uniformis</i> ATCC 8492	NZ_DS362249.1	
<i>Bacteroides vulgatus</i> ATCC 8482	NC_009614.1	
<i>Bacteroides dorei</i> CLO3T12C01	NZ_CP011531.1	
<i>Beutenbergia covernae</i> DSM 12333	NC_012669.1	
<i>Bryobacter aggregatus</i> MPL3	NZ_JNIF010000001.1	
<i>Candidatus</i> Koribacter versatilis Ellin345	NC_008009.1	
<i>Candidatus</i> Solibacter usitatus Ellin6076	NC_008536.1	
<i>Chloroflexus aurantiacus</i> J-10-fI	NC_010175.1	
<i>Dictyobacter aurantiacus</i> S-27	NZ_BIFQ01000001.1	
<i>Dictyobacter alpinus</i> Uno16	NZ_BIF01000001.1	
<i>Dictyobacter kobayashii</i> Uno11	NZ_BKZW01000001.1	
<i>Dictyobacter vulcani</i> W12	NZ_JQK01000001.1	
<i>Edaphobacter aggregans</i> DSM 19364	NZ_SHKW01000001.1	
<i>Edaphobacter modestus</i> DSM 18101	NGF010000005.1	
<i>Gemmatimonadetes</i> bacterium 2013_60CM_65_52	NC_012489.1	
<i>Gemmatimonas aurantiaca</i> T-27	NZ_CP011454.1	
<i>Gemmatimonas phototrophica</i> AP64	NZ_CP007128.1	
<i>Gemmatirosa kalamazoonesis</i> KBS708	NC_011365.1	
<i>Glucanacetobacter diazotrophicus</i> PAI 5	NZ_NKUF01000001.1	
<i>Glucanacetobacter entanii</i> LTH 4560	NZ_BJVA01000001.1	
<i>Glucanobacter kanchanaburiensis</i> NBRC 103587	NC_019396.1	
<i>Glucanobacter oxydans</i> H24	NZ_LHZP010000023.1	
<i>Glucanobacter roseus</i> LMG 1418	NZ_BJMK01000001.1	
<i>Glucanobacter sphaericus</i> NBRC 12467	NZ_BANH01000191.1	
<i>Glucanobacter thailandicus</i> F149-1	NZ_BJUZ01000001.1	
<i>Glucanobacter wancherniae</i> NBRC 103581	NC_016631.1	
<i>Granulicella mallensis</i> MP5ACTX8	NC_015064.1	
<i>Granulicella tundralica</i> MP5ACTX9	CP000875.1	
<i>Herpetosiphon aurantiacus</i> DSM 785		
continuation...		
<i>Komagataeibacter europaeus</i> SRCM101446	NZ_CP021467.1	
<i>Komagataeibacter hansenii</i> ATCC 23769	NZ_CV000920.1	
<i>Komagataeibacter oboediensis</i> 174Bp2	NZ_CADT01000200.1	
<i>Komagataeibacter xylini</i> E25	NZ_CP004360.1	
<i>Ktedonobacter racemifer</i> DSM 44963	NZ_ADVG01000010.1	
<i>Ktedonosporobacter rubrifoli</i> SCAM5-G2	NZ_CP035758.1	
<i>Mycobacterium tuberculosis</i> H37Rv	NC_00962.3	
<i>Mycobacterium vanbaalenii</i> PVY-1	NC_008726.1	
<i>Nocardia farcinica</i> NCTC11134	NZ_LN868938.1	
<i>Nocardioopsis dassonvillei</i> DSM 43111	NC_014210.1	
<i>Prostheco bacter fusiformis</i> ATCC 25309	NZ_SOCA01000001.1	
<i>Prostheco bacter debontii</i> ATCC 700200	NZ_FUYE01000055.1	
<i>Rhodococcus jostii</i> RHA1	NC_008268.1	
<i>Roseiflexus castenholzii</i> DSM 13941	NC_009767.1	
<i>Roseimicrobium gellanilyticum</i> DSM 25532	NZ_QNRR01000001.1	
<i>Roseomonas gilardii</i> U14-5	NZ_CP015583.1	
<i>Saccharomonospora viridis</i> DSM 43017	NC_013159.1	
<i>Saccharopolyspora erythraea</i> NRRL2338	NC_009142.1	
<i>Salinispora arenicola</i> CNS-205	NC_009953.1	
<i>Salinispora tropica</i> CNB-440	NC_009380.1	
<i>Sphaerobacter thermophilus</i> DSM 20745	NC_013523.1	
<i>Streptomyces avermitilis</i> MA-4680	NC_003155.5	
<i>Streptomyces coelicolor</i> A3(2)	NC_003888.3	
<i>Streptomyces griseus</i> subsp. griseus NBRC 13350	NC_010572.1	
<i>Streptomyces scabiei</i> 87.22	NC_013929.1	
<i>Streptosporangium roseum</i> DSM 43021	NC_013595.1	
<i>Tengyonobacter tsumagaensis</i> Uno3	NZ_BIFR01000001.1	
<i>Terriglobus albidus</i> ORNL	NZ_CP042806.1	
<i>Terriglobus roseus</i> DSM 18391	NC_018014.1	
<i>Terriglobus saanensis</i> SP1PR4	NC_014963.1	
<i>Terriglobus</i> sp. TAA 43	NZ_JUGR01000001.1	
<i>Thermobifida fusca</i> YX	NC_007333.1	
<i>Thermogemmatimonas carboxidivorans</i> PM5	NZ_JNIM01000001.1	
<i>Thermogemmatimonas tikiterensis</i> T81	NZ_MCF01000002.1	
<i>Thermogemmatimonas onikobensis</i> NBRC 111776	NZ_BKZV01000001.1	
<i>Thermomicrobium aurantia</i> A1-2	NC_011959.1	
<i>Thermomicrobium roseum</i> DSM 5159	NC_013510.1	
<i>Thermomonospora curvata</i> DSM 43183	NZ_BIFX01000001.1	
<i>Thermosporothrix hazakensis</i> SK20-1	NZ_BIFX01000001.1	
<i>Verrucocomicrobium spinosum</i> DSM 4136	NZ_ABIZ01000001.1	
<i>Verrucocomicrobium</i> sp. GAS474	LT629781	



Table S3. Summary of the metagenome-assembled genomes (MAGs) isolated in the present study. Average amino-acid identity (AAI) was calculated by comparing the MAGs with their closest reference genomes identified based on the RAST results. The RefSeq accession number of the reference genomes are provided in parenthesis.

MAG	Completeness (%)	Contamination (%)	GC (%)	N50	Size (bp)	Phyla	Class/Order	Reference genome	AAI (%)
MAG-1640-1.1	80.57	1.536	69.1	8867	5073303	Actinobacteria	Actinomycetales	<i>Actinosynnema mirum</i> DSM 43827 <sup>T</sup> (NC_013093.1)	58.65
MAG-1640-2.1	85.36	3.738	66.8	11294	5751495	Proteobacteria	Rhodospirillales	<i>Roseomonas gilardii</i> U14-5 (NZ_CP015583.1)	49.1
MAG-1751-1.1	70.3	3.1	70.8	7414	4752807	Acidobacteria	Bryobacteriales	<i>Candidatus</i> Koribacter versatilis Ellin345 (NC_008009.1)	40.96
MAG-1957-1.1	85.8	3.48	58.3	67512	4938955	Acidobacteria	Bryobacteriales	<i>Bryobacter aggregatus</i> MPL3 <sup>T</sup> (NZ_JNIF01000001.1)	49.77
MAG-1957-2.1	96.2	3.96	53.4	119701	6327585	Chloroflexi	Ktedonobacteria	<i>Ktedonosporobacter rubrisol</i> SCAWS-G2 <sup>T</sup> (NZ_CP035758.1)	59.01
MAG-1957-3.1	72.22	3.47	51.7	13375	6142618	Chloroflexi	Ktedonobacteria	<i>Dictyobacter vulcani</i> W12 <sup>T</sup> (NZ_BKZW01000001.1)	63.43
MAG-1957-4.1	72.64	0.1	67.2	5165	3007516	Firmicutes	Thermoanaerobacteriales	<i>Ammonifex degensii</i> KC4 <sup>T</sup> (NC_013385.1)	41.36
MAG-1957-5.1	98.05	0.9	71.4	79391	5837494	Actinobacteria	Actinomycetales	<i>Actinosynnema mirum</i> DSM 43827 <sup>T</sup> (NC_013093.1)	55.92
MAG-1957-6.1	80.36	1.98	54.8	22264	5685492	Chloroflexi	Ktedonobacteria	<i>Ktedonosporobacter rubrisol</i> SCAWS-G2 <sup>T</sup> (NZ_CP035758.1)	58.66
MAG-1957-7.1	94.02	1.71	58.9	137749	4659534	Acidobacteria	Bryobacteriales	<i>Candidatus</i> Koribacter versatilis Ellin345 (NC_008009.1)	47.98
MAG-1957-8.1	70.49	3.3	72.5	10863	4227183	Gemmatimonadetes	Gemmatimonadales	<i>Gemmatirosa kalamazoonesis</i> KBS708 <sup>T</sup> (NZ_CP007128.1)	60.46
MAG-1957-9.1	90.97	4.94	71.3	26163	6509599	Actinobacteria	Actinomycetales	<i>Streptosporangium roseum</i> DSM 43021 <sup>T</sup> (NC_013595.1)	52.26
MAG-1957-10.1	72.61	1.07	71.4	17599	4220629	Actinobacteria	Actinomycetales	<i>Streptosporangium roseum</i> DSM 43021 <sup>T</sup> (NC_013595.1)	52.93
MAG-1957-11.1	89.03	1.72	60.9	88061	3399414	Acidobacteria	Acidobacteriales	<i>Acidipila rosea</i> DSM 103428 (NZ_SMGK01000001.1)	54.36
MAG-1957-12.1	73.41	1.72	58.9	22191	4793286	Acidobacteria	Acidobacteriales	<i>Acidobacterium capsulatum</i> ATCC 51196 <sup>T</sup> (NC_012483.1)	57.18
MAG-1957-13.1	95.6	4.09	60.5	13562	5255071	Verrucomicrobia	Verrucomicrobiales	<i>Prostheco bacter debontii</i> ATCC 700200 <sup>T</sup> (NZ_FUYE01000055.1)	44.57
MAG-1957-14.1	96.79	3.19	61.9	29422	6536060	Proteobacteria	Rhodospirillales	<i>Acidisphaera rubrifaciens</i> HS-AP3 <sup>T</sup> (NZ_BANB01001239.1)	61.34
MAG-1957-15.1	94.61	3.54	61.5	71986	3914044	Acidobacteria	Acidobacteriales	<i>Acidipila rosea</i> DSM 103428 (NZ_SMGK01000001.1)	57.33
MAG-1957-16.1	99.14	0.86	65.4	109666	3669710	Acidobacteria	Acidobacteriales	<i>Acidipila rosea</i> DSM 103428 (NZ_SMGK01000001.1)	57.44

Table S4. Summary of enzymatic functions for CO<sub>2</sub>-, H<sub>2</sub>-, and formate-oxidation in the Ktedonobacteria reference genomes. Carbon monoxide oxidation: K03518 carbon monoxide dehydrogenase small subunit coxS, K03519 carbon monoxide dehydrogenase medium subunit coxM, K03520 carbon monoxide dehydrogenase large subunit coxL. Formate oxidation: K00122 formate dehydrogenase, K00123 formate dehydrogenase major subunit. Hydrogen oxidation: H<sub>2</sub> dehydrogenase: K00436: NAD-reducing hydrogenase large subunit.

Species	Strain	Isolation source	RefSeq	Collection	Reference	K03518	K03519	K03520	K00122	K00123	K00436
<i>Ktedonobacter racemifer</i>	SOSP1-21 <sup>T</sup>	European soils	NZ_ADVG00000000.1	DSM 44963	[2]	x	x	x	x	x	x
<i>Dicytobacter aurantiacus</i>	S-27 <sup>T</sup>	paddy soils	NZ_BIFQ00000000.1	ASM396751v1	[4]	x	x	x	x	x	x
<i>Dicytobacter vulcani</i>	W12 <sup>T</sup>	volcanic soils	NZ_BKZW00000000.1	NBRC113551T	[3]	x	x	x	x	x	x
<i>Thermosporothrix hazakensis</i>	SK20-1 <sup>T</sup>	ripe compost	NZ_BIFX00000000.1	ATCC BAA-1881	[5]	x	x	x	x	x	x
<i>Ktedonosporobacter rubrisoli</i>	SCAWS-G2 <sup>T</sup>	red soils	GCF_004208415.1	DSM:105258	[6]	x	x	x	x	x	x
<i>Tenguaobacter tsumagoiensis</i>	Uno3 <sup>T</sup>	soil-like granular mass	NZ_BIFR00000000.1	ASM396753v1	[7]	x	x	x	x	x	x
<i>Dicytobacter kobayashii</i>	Uno11 <sup>T</sup>	soil-like granular mass	NZ_BIFS00000000.1;	ASM396755v1	[7]	x	x	x	x	x	x
<i>Dicytobacter alpinus</i>	Uno16 <sup>T</sup>	soil-like granular mass	NZ_BIFT00000000.1	ASM396757v1	[7]	x	x	x	x	x	x
<i>Thermogemmatispora aurantia</i>	A1-2 <sup>T</sup>	geothermal soil	NZ_BKZV00000000.1	ASM897428v1	[8]	x	x	x	x	x	x
<i>Thermogemmatispora onikobensis</i>	NBRC 111776	geothermal soil	NZ_BDGT00000000.1	ASM174828v1	unpublished	x	x	x	x	x	x
<i>Thermogemmatispora carboxidivorans</i>	PM5 <sup>T</sup>	biofilm	NZ_JNIM00000000.1;	DSM45816T	[1]	x	x	x	x	x	x

## Supplementary references

1. King, C.E.; King, G.M. Description of *Thermogemmatispora carboxidivorans* sp. Nov., a carbon-monoxide-oxidizing member of the class Ktedonobacteria isolated from a geothermally heated biofilm, and analysis of carbon monoxide oxidation by members of the class Ktedonobacteria. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 1244-1251.
2. Chang, Y.J.; Land, M.; Hauser, L.; Chertkov, O.; Del Rio T.G.; Nolan, M.; et al. Non-contiguous finished genome sequence and contextual data of the filamentous soil bacterium *Ktedonobacter racemifer* type strain (SOSP1-21). *Stand. Genomic. Sci.* **2011**, *5*, 97-111.
3. Zheng, Y.; Wang, C.M.; Sakai, Y.; Abe, K.; Yokota, A.; Yabe, S. *Dictyobacter vulcani* sp. nov., belonging to the class Ktedonobacteria, isolated from soil of the Mt Zao volcano. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 1805-1813.
4. Yabe, S.; Sakai, Y.; Abe, K.; Yokota, A.; Také, A.; Matsumoto, A.; et al. *Dictyobacter aurantiacus* gen. nov., sp. nov., a member of the family Ktedonobacteraceae, isolated from soil, and emended description of the genus *Thermosporothrix*. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 2615-2621.
5. Yabe, S.; Aiba, Y.; Sakai, Y.; Hazaka, M.; Yokota, A. *Thermosporothrix hazakensis* gen. nov., sp. nov., isolated from compost and description of Thermosporotrichaceae fam. nov. within the class Ktedonobacteria. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 1794-1801.
6. Yan, B.; Guo, X.; Liu, M.; Huang, Y. *Ktedonosporobacter rubrisoli* gen. nov., sp. nov., a novel representative of the class Ktedonobacteria, isolated from red soil, and proposal of Ktedonosporobacteraceae fam. nov. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 1015-1025.
7. Wang, C.M.; Zheng, Y.; Sakai, Y.; Toyoda, A. Minakuchi, Y.; Abe, K.; et al. *Tengunoibacter tsumagoiensis* gen. nov., sp. nov., *Dictyobacter kobayashii* sp. nov., *Dictyobacter alpinus* sp. nov., and description of Dictyobacteraceae fam. nov. within the order Ktedonobacterales isolated from Tengu-no-mugimeshi, a soil-like granular mass of microorganisms, and emended descriptions of the genera *Ktedonobacter* and *Dictyobacter*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 1910-1918.
8. Zheng, Y.; Wang, C.M.; Sakai, Y.; Abe, K.; Yokota, A.; et al. *Thermogemmatispora aurantia* sp. nov. and *Thermogemmatispora argillosa* sp. nov., within the class Ktedonobacteria, and emended description of the genus *Thermogemmatispora*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 1744-1750.