

## Supplementary Material

			Average	Average		Average	
Genome or	Illumina	SMRT	length of	length of	Nanopore	length of	
metagenome	reads	reads	Illumina	SMRT reads,	reads	Nanopore	
			reads, bp	bp		reads, bp	
Thiothrix unzii A1 <sup>T</sup>							
(ATCC 49747)	4165564	NA	268	NA	141232	7361	
(isolate genome)							
Thiothrix sp. AS	6779709	NA	243	NI A	538091	7606	
(isolate genome)	0728708			INA		/000	
Thiothrix sp. A52	1260800	ΝA	769	NT A	702267	0649	
(metagenome)*	4209800	NA	208	INA	102307	9048	
Thiothrix sp. Ku-5	NT A	264116	NLA	10652	ΝA	NT A	
(isolate genome)	NA	204110	NA	10032	NA	INA	
Thiothrix							
$fructosivorans Q^{T}$	NT A	240026	NT A	2250	156027	10010	
(ATCC 49748)	NA	249020	INA	3332	130937	10210	
(isolate genome)							

Supplementary Table S1. Sequencing statistics.

\*, number of metagenomics reads representing this MAG; NA, not applicable

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dD	DH, %	Genome	$BL^{T}$	AS	Q <sup>T</sup>	Ku-5	G1 <sup>T</sup>	A1 <sup>T</sup>	A52	RT	JP2 <sup>T</sup>	SSD2
	100	$\mathbf{BL}^{\mathrm{T}}$	100.0	56.2	28.8	27.7	28.0	27.8	27.7	24.9	23.9	22.4
	36	AS	56.2	100.0	23.7	28.5	28.6	23.1	27.5	25.3	24.6	24.6
	22	$Q^{T}$	28.8	23.7	100.0	34.7	32.7	31.8	23.7	27.8	23.7	22.2
		Ku-5	27.7	28.5	34.7	100.0	38.5	36.6	35.9	27.9	23.6	22.4
		$\mathbf{G1}^{\mathrm{T}}$	28.0	28.6	32.7	38.5	100.0	37.2	35.0	30.2	23.8	22.7
		$A1^{T}$	27.8	23.1	31.8	36.6	37.2	100.0	46.9	30.5	23.4	22.9
		A52	27.7	27.5	23.7	35.9	35.0	46.9	100.0	32.3	24.6	21.3
		RT	24.9	25.3	27.8	27.9	30.2	30.5	32.3	100.0	25.6	24.9
		JP2 <sup>T</sup>	23.9	24.6	23.7	23.6	23.8	23.4	24.6	25.6	100.0	25.6
		SSD2	22.4	24.6	22.2	22.4	22.7	22.9	21.3	24.9	25.6	100.0

**Supplementary Figure S1.** The heat map of dDDH pairwise values for assembled *Thiothrix* genomes. *T. lacustris* BL<sup>T</sup>, (GCF\_000621325.1); *Thiothrix* sp. AS (GCF\_017901135.1); *T. fructosivorans* Q<sup>T</sup> (GCA\_017349355.1); *Thiothrix* sp. Ku-5 (GCF\_016772315.1); *T. caldifontis* G1<sup>T</sup> (GCF\_900107695.1); *T. unzii* A1<sup>T</sup> (GCA\_017901175.1); MAG of *Thiothrix* sp. A52 (GCF\_017901155.1); *'Ca.* Thiothrix moscowensis' RT (GCA\_016292235.1); *T. nivea* DSM 5205<sup>T</sup> (GCF\_000260135.1); *'Ca.* Thiothrix singaporensis' SSD2 (GCA\_013693955.1).

Fatty acid	ECL*	<i>Thiothrix</i> sp. Ku-5	<i>Thiothrix</i> sp. AS	$T. unzii A1^{T}$	<i>T. lacustris</i> BL <sup>T</sup>	T. caldifontis G1 <sup>T</sup>
8-oxo C <sub>9:0</sub>	9.68	_	2.2	_	_	6.1
C <sub>16:1</sub> ω7	15.80	55.3	62.1	67.1	55.8	45.1
$C_{16:1}\omega 6$	15.85	_	_	_	3.9	_
C <sub>16:0</sub>	16.00	21.0	15.9	19.4	21.2	25.0
C <sub>18:1</sub> ω7	17.81	23.7	19.8	13.5	19.1	23.8

**Supplementary Table S2.** Comparison of the cellular fatty acid contents of *Thiothrix* strains. Values are percentages of the total peak area.

\*ECL, equivalent chain length (HP-5MS column)

\*\* The identification is tentative; mass spectrum is shown in Supplementary Figure S2 (Note that the molecular ion with the m/z=186 corresponds to the formula of  $C_{10}H_{18}O_3$ )

-, not detected

Supplementary Figure S2. Mass spectra of the detected methylated compounds.

