

Abundance and ecophysiology of MiDAS 4 family f37-13 filamentous bacteria in Japan possessing the Eikelboom morphotype 1863

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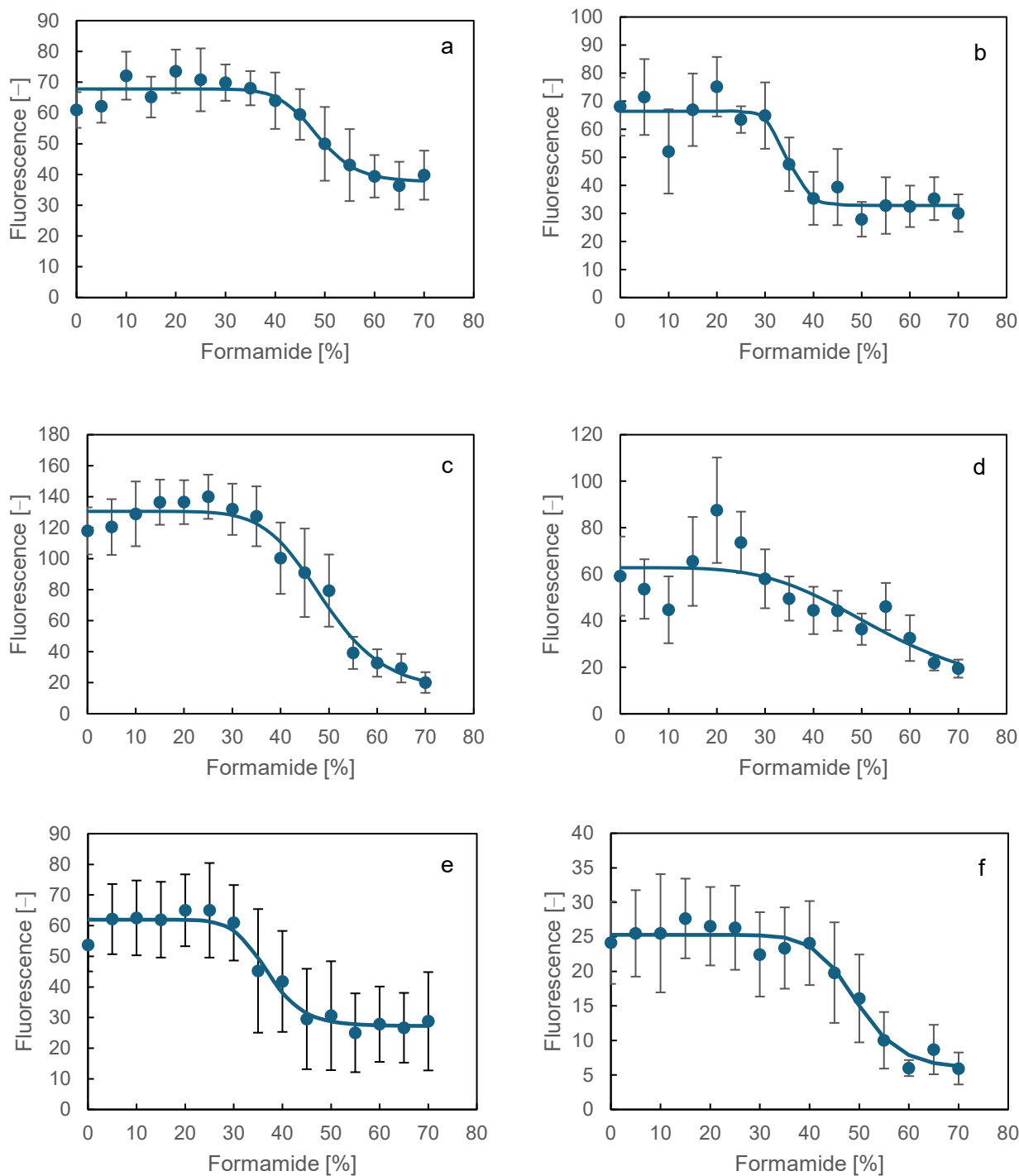


Fig. S1 Dissociation curves determined for the six new oligonucleotide probes designed in this study: a) CHI563, b) CHI564, c) CHI656, d) CHI844, e) CHI1013 and f) CHI1283A against activated sludge samples collected from WWTP E3.

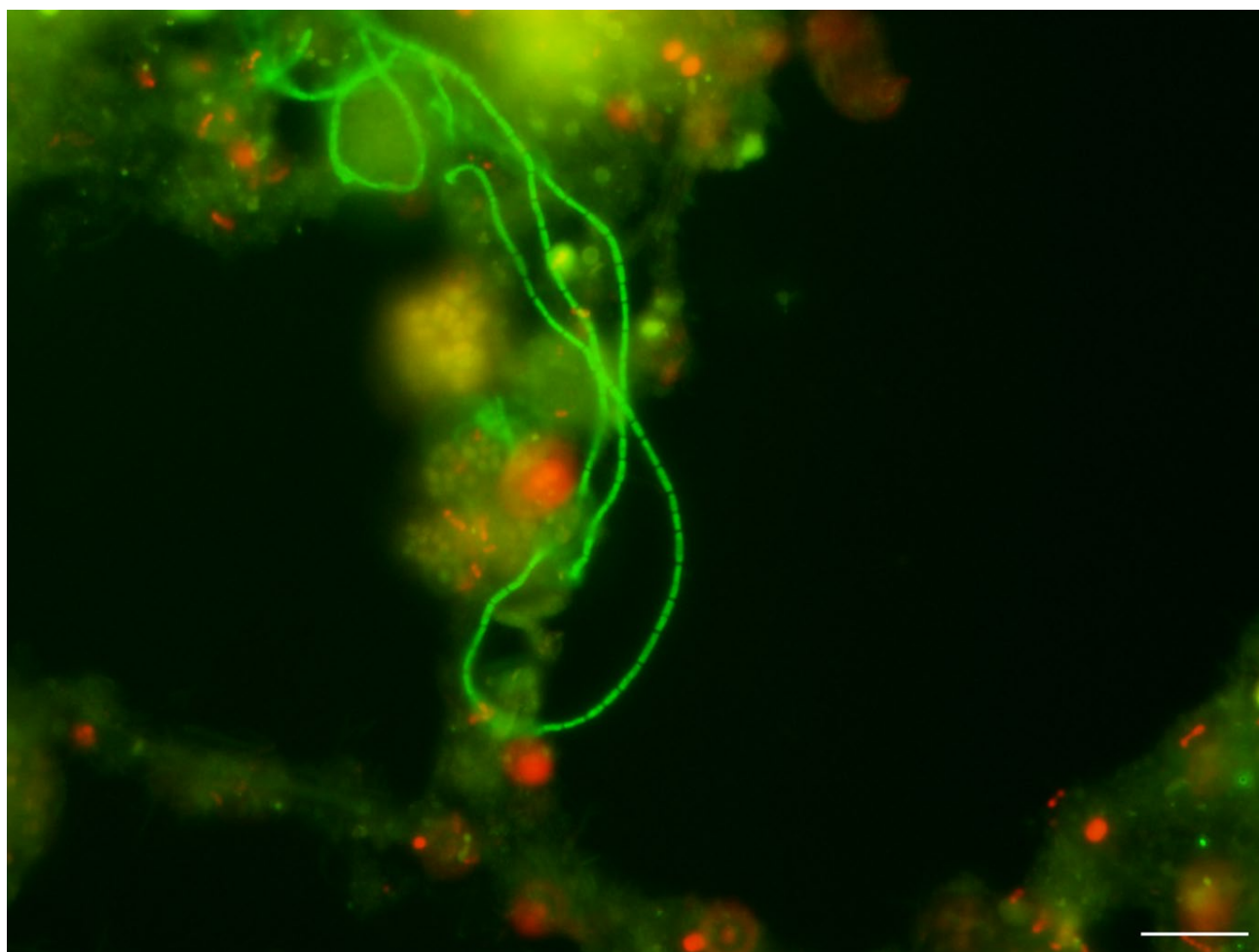


Fig. S2 Composite FISH image of activated sludge biomass after exposure to CHI563+CHI564 (FAM, green) and GAM42a (Cy3, red). FA concentration for FISH analysis was set to 30%. Scale bar shows 10 μm .

Table S1 Operational parameter data of the AOA process train from 16 sludge samples. BOD: Biochemical oxygen demand; T-N: Total nitrogen; T-P: Total phosphorus; SS: Suspended solids; SVI: Sludge volume index; DO: Dissolved oxygen; MLSS: Mixed liquor suspended solids; SRT: Sludge retention time; HRT: Hydraulic retention time.

	BOD [mg/L]	T-N [mg/L]	T-P [mg/L]	SS [mg/L]	SVI [mL/g]	Temperature [°C]	pH [-]	DO [mg/L]	MLSS [mg/L]	SRT [d]	HRT [h]	Sludge return ratio [%]	SS in return sludge [mg/L]
Range	54-120	20-32	2.0-3.3	22-65	150-330	20.5-28.0	6.4-6.8	1.3-1.8	1300-2500	6.3-10.9	9.0-12.1	41-52	4200-7100
Average	84	27	2.8	35	235	23.9	6.6	1.6	1900	9.0	10.5	45	5906
S.D.	18	3.8	0.37	10	49.4	2.64	0.11	0.12	316.2	1.2	0.900	4.0	867.8

Table S2 Relative abundances of f37-13 members and each genus in 16 activated sludge samples. The SVI values were measured in the aeration tank. Correlation coefficients and *p*-values derived from the correlation analyses between relative abundances and SVI were denoted as *r* and *p*, respectively.

	29/05/2019	10/7/19	7/8/19	4/9/19	18/9/19	2/10/19	17/10/19	30/10/19	13/11/19	27/11/19	11/12/19	08/01/2020	22/1/20	6/2/20	19/2/20	18/3/20	Mean	<i>r</i>	<i>p</i>
midas_g_535	0.611	0.101	0.837	0.573	0.677	0.109	0.524	0.089	0.231	0.380	0.522	0.204	0.335	0.182	0.234	0.287	0.368	0.548	0.028
midas_g_543	0.400	0.262	0.664	0.313	0.000	0.190	0.322	0.124	0.475	0.702	0.619	0.465	0.298	0.340	0.371	0.450	0.375	0.080	0.769
midas_g_1223	0.000	0.000	0.038	0.158	0.161	0.000	0.141	0.054	0.060	0.069	0.102	0.039	0.000	0.000	0.000	0.000	0.051	-0.125	0.644
midas_g_12953	0.039	0.000	0.043	0.000	0.000	0.193	0.050	0.019	0.060	0.049	0.042	0.015	0.032	0.014	0.026	0.000	0.037	-0.378	0.149
f37-13	1.050	0.363	1.583	1.044	0.838	0.492	1.038	0.286	0.826	1.201	1.284	0.722	0.666	0.536	0.631	0.736	0.831	0.329	0.213
SVI [mL/g]	330	280	290	230	280	180	210	150	150	190	260	210	230	260	250	260	235	-	-

Table S3 Relative abundances of putative filamentous bacteria in 16 activated sludge samples. The genera highlighted in orange are those suspected to be partially filamentous. Correlation coefficients and p -values derived from the correlation analyses between relative abundances and SVI were denoted as r and p , respectively.

Date	29/05/2019	10/7/19	7/8/19	4/9/19	18/9/19	2/10/19	17/10/19	30/10/19	13/11/19	27/11/19	11/12/19	08/01/2020	22/1/20	6/2/20	19/2/20	18/3/20	Mean	r	p
<i>Kouleoethrix</i>	6.48	6.38	8.93	8.07	4.89	2.77	7.10	9.84	4.64	6.42	6.54	8.32	6.93	6.62	5.64	4.47	6.50	0.005	0.986
<i>Tetrasphaera</i>	0.66	0.44	0.52	0.48	0.50	0.11	0.13	0.41	0.24	0.52	0.92	1.79	1.79	3.52	2.65	1.91	1.04	N.A.	N.A.
<i>Flavobacterium</i>	0.62	0.84	1.19	0.65	0.22	0.00	1.14	0.88	1.39	1.55	1.54	0.94	0.62	0.61	0.59	1.44	0.89	-0.143	0.597
<i>Mycobacterium</i>	0.61	0.69	0.21	0.29	0.71	0.21	0.32	1.04	0.34	0.34	0.33	0.47	0.62	0.97	0.33	0.50	0.50	0.072	0.791
<i>Runella</i>	0.35	0.31	0.11	0.26	0.84	0.34	0.51	0.75	0.32	0.42	0.67	0.58	0.65	0.62	0.51	0.26	0.47	-0.147	0.588
<i>Thiothrix</i>	0.12	0.00	0.00	0.04	1.47	1.02	0.07	0.05	0.03	0.04	0.08	0.15	0.56	0.90	2.22	0.62	0.46	0.157	0.562
<i>Rhodoferrax</i>	0.51	0.26	0.36	0.45	1.23	0.59	0.15	0.20	0.60	0.21	0.62	0.45	0.54	0.45	0.45	0.21	0.46	N.A.	N.A.
<i>Sphaerotilus</i>	0.32	0.31	0.46	0.00	0.70	0.00	1.09	0.74	0.56	0.59	0.32	0.19	0.05	0.05	0.78	0.69	0.43	-0.120	0.658
<i>Streptococcus</i>	0.36	0.26	0.39	0.63	0.62	0.55	0.27	0.27	0.30	0.16	0.44	0.51	0.45	0.43	0.57	0.15	0.40	0.157	0.561
<i>Thermomonas</i>	0.50	0.54	0.06	0.16	0.00	0.00	0.13	0.23	0.15	0.16	0.26	0.50	0.54	0.44	0.83	0.74	0.33	0.326	0.218
midas_g_4871	0.24	0.09	0.31	0.36	0.35	0.39	0.26	0.13	0.14	0.23	0.33	0.23	0.24	0.16	0.30	0.17	0.24	0.155	0.567
<i>Arcobacter</i>	0.16	0.11	0.24	0.27	0.00	0.24	0.11	0.06	0.28	0.29	0.47	0.23	0.19	0.21	0.25	0.36	0.22	-0.032	0.905
midas_g_550	0.27	0.21	0.04	0.08	0.00	0.51	0.24	0.25	0.18	0.15	0.28	0.33	0.31	0.23	0.13	0.06	0.21	-0.346	0.190
<i>Haliscomenobacter</i>	0.17	0.28	0.07	0.23	0.00	0.32	0.16	0.14	0.25	0.34	0.18	0.08	0.00	0.26	0.38	0.06	0.18	-0.246	0.358
<i>Blastocatella</i>	0.12	0.39	0.23	0.17	0.00	0.15	0.20	0.31	0.17	0.17	0.20	0.15	0.17	0.12	0.06	0.08	0.17	-0.207	0.441
midas_g_1668	0.27	0.12	0.20	0.13	0.32	0.11	0.24	0.11	0.07	0.08	0.10	0.16	0.15	0.25	0.15	0.08	0.16	0.572	0.021
<i>Acinetobacter</i>	0.00	0.11	0.08	0.15	0.49	0.16	0.15	0.17	0.12	0.12	0.11	0.12	0.24	0.15	0.15	0.09	0.15	N.A.	N.A.
<i>Trichococcus</i>	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.08	0.11	0.12	0.27	0.37	0.64	0.50	0.20	0.15	N.A.	N.A.
midas_g_119	0.02	0.15	0.14	0.57	0.19	0.32	0.26	0.53	0.05	0.02	0.06	0.00	0.00	0.00	0.00	0.00	0.14	N.A.	N.A.
<i>Cloacibacterium</i>	0.06	0.14	0.20	0.04	0.00	0.00	0.35	0.28	0.12	0.06	0.15	0.07	0.06	0.21	0.09	0.00	0.11	-0.183	0.497

N.A.: Not analysed. This was due to the non-normal distribution of the relative abundance.