

Supplementary Material

Thriving in wetlands: Ecophysiology of the spiral-shaped methanotroph *Methylospira mobilis* as revealed by the complete genome sequence

Igor Y. Oshkin¹, Kirill K. Miroshnikov¹, Olga V. Danilova¹, Anna Hakobyan²,
Werner Liesack², Svetlana N. Dedysh¹

¹Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia;

²Max-Planck-Institut für terrestrische Mikrobiologie, D-35043 Marburg, Germany.

Correspondence: Svetlana N. Dedysh

Tel: 7 (499) 135 0591. Fax: 7 (499) 135 6530. Email: dedysh@mail.ru

Running title: Genome analysis of a spiral-shaped methanotroph.

Supplementary Table S1. Methylotrophy genes identified in the genomes *Methylococcus capsulatus* Bath and *Methylospira mobilis* Shm1
Positive detection of a homologue was based on annotations in Genbank, and on BLAST searches against NCBI and Uniprot databases.

Gene name	Gene symbol	<i>M. capsulatus</i>	Coordinate 1	Coordinate 2	<i>M. mobilis</i>	Coordinate 1	Coordinate 2
Methane oxidation							
particulate methane monooxygenase (pMMO), subunit C	pmoC	MCA1798	1928445	1929227	F6R98_01470	388598	389377
		MCA2855	3048914	3049696	F6R98_16065	3544488	3543709
particulate methane monooxygenase (pMMO), subunit A	pmoA	MCA1797	1927548	1928291	F6R98_01475	389539	390282
		MCA2854	3048017	3048760	F6R98_16060	3543547	3542804
particulate methane monooxygenase (pMMO), subunit B	pmoB	MCA1796	1926197	1927441	F6R98_01480	390434	391681
		MCA2853	3046666	3047910	F6R98_16055	3542652	3541405
soluble methane monooxygenase (sMMO), subunit alpha	<i>mmoX</i>	MCA1194	1252141	1253724	F6R98_10915	2421999	2421999
soluble methane monooxygenase (sMMO), subunit beta	<i>mmoY</i>	MCA1195	1253805	1254974	F6R98_10910	2421925	2420756
soluble methane monooxygenase (sMMO), regulatory component	<i>mmoB</i>	MCA1196	1254991	1255416	F6R98_10905	2420745	2420317
soluble methane monooxygenase (sMMO), subunit gamma	<i>mmoZ</i>	MCA1198	1256767	1257279	F6R98_10900	2420303	2419797
Methanol oxidation							
PQQ-dependent methanol dehydrogenase (MDH), large subunit	<i>mxoF</i>	MCA0779	823278	825083	F6R98_08155	1857763	1859568
periplasmic protein, probable ABC transporter substrate-binding protein	<i>mxoJ</i>	MCA0780	825441	826013	F6R98_08160	1859625	1860473
periplasmic c-type cytochrome, primary electron acceptor from PQQdependent	<i>mxoG</i>	MCA0781	826019	826498	F6R98_08165	1860484	1860963
PQQ-dependent methanol dehydrogenase (MDH), small subunit	<i>mxoI</i>	MCA0782	826540	826824	F6R98_08170	1860991	1861281
ATPase, P-loop NTPase family	<i>mxoR</i>	MCA0783	826988	828007	F6R98_08175	1861343	1862368
a von Willebrand factor type A (vWA) domain involved in methanol oxidation	<i>mxoS</i>	MCA0784	828004	828846	F6R98_08180	1862365	1863210

essential for Ca2+ insertion into MDH	<i>mxmA</i>	MCA0785	829035	829907	F6R98_08185	1863207	1864121
essential for Ca2+ insertion into MDH	<i>mxnC</i>	MCA0786	829904	830887	F6R98_08190	1864118	1865116
essential for Ca2+ insertion into MDH	<i>mxnK</i>	MCA0787	830929	831447	F6R98_08195	1865101	1865667
essential for Ca2+ insertion into MDH	<i>mxnL</i>	MCA0788	831444	832415	F6R98_08200	1865664	1866686
essential for Ca2+ insertion into MDH	<i>mxnD</i>	MCA0789	832434	832958	F6R98_08205	1866683	1867207
unknown	<i>mxnE</i>	MCA1279	1363415	1364404	F6R98_08210	1867211	1868185
transcriptional regulator	<i>mxnB</i>	MCA0777	821027	821674	F6R98_08220	1868579	1869226
coenzyme PQQ biosynthesis	pqqA	MCA1445.1	1538132	1538206	F6R98_04695	1089243	1089317
					F6R98_08215	1868278	1868352
coenzyme PQQ biosynthesis	<i>pqqB</i>	MCA1446	1538293	1539207	F6R98_08475	1932924	1933838
coenzyme PQQ biosynthesis	<i>pqqC</i>	MCA1447	1539216	1539941	F6R98_08480	1933898	1934623
coenzyme PQQ biosynthesis	<i>pqqD</i>	MCA1448	1539947	1540228	F6R98_08485	1934637	1934909
coenzyme PQQ biosynthesis	<i>pqqE</i>	MCA1449	1540197	1541318	F6R98_08490	1934887	1936011
coenzyme PQQ biosynthesis	<i>pqqF</i>	MCA0063	64477	65856	F6R98_11435	2541975	2540590
coenzyme PQQ biosynthesis	<i>pqqG</i>	MCA0064	65846	67171	F6R98_11430	2540600	2539272
xoxF	xoxF	MCA0299	293402	295261	F6R98_11505	2556553	2558418
					F6R98_16780	3702340	3700475
C1 transfer / formaldehyde oxidation							
formaldehyde activating enzyme	fae	MCA2778	2970552	2970911	F6R98_15000	3305441	3304929
		MCA2866	3060088	3060600	F6R98_19005	4173806	4173294
					F6R98_04980	1157466	1156951
methylene-tetrahydromethanopterin (H4MPT) dehydrogenase	mtdB	MCA3018	3201649	3202554	F6R98_14510	3200473	3201384
		MCA3019	3202646	3203527	F6R98_14515	3201733	3202647
formyltransferase/hydrolase complex, alpha subunit	<i>fhcA</i>	MCA2859	3051796	3053463	F6R98_03260	784596	786260
formyltransferase/hydrolase complex, beta subunit	<i>fhcB</i>	MCA2860	3053492	3054763	F6R98_03255	783305	784588
formyltransferase/hydrolase complex, gamma subunit	<i>fhcC</i>	MCA2857	3050056	3050868	F6R98_03270	787178	787996
formyltransferase/hydrolase complex, delta subunit	<i>fhcD</i>	MCA2858	3050903	3051799	F6R98_03265	786257	787153

methylene-H4MPT /methylene-tetrahydrofolate (H4F) dehydrogenase	<i>mtdA</i>	MCA0508	521192	522052	F6R98_09875	2223245	2224111
methenyl-H4F cyclohydrolase	<i>fch</i>	MCA2863	3057182	3058177	F6R98_19020	4176773	4175778
dihydromethanopterin reductase	<i>dmrA</i>	Absent			Absent		
formyl-H4F ligase	<i>ftfL</i>	MCA2219	2396781	2398454	F6R98_03760	889085	890755
2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase	<i>folk</i>	MCA2313	2507044	2507541	F6R98_18580	4092196	4092702
		MCA2987	3161584	3162078			
dihydroneopterin aldolase	<i>folB</i>	MCA2988	3162071	3162436	F6R98_18575	4091843	4092199
dihydroneopterin synthase	<i>folP</i>	MCA2317	2509686	2510570	F6R98_05160	1189782	1190627
GTP cyclohydrolase	<i>folE</i>	MCA1670	1773965	1774522	F6R98_12615	2795380	2795925
dihydrofolate synthase	<i>folC</i>	MCA2492	2673335	2674630	F6R98_08020	1828425	1829702
methenyl-H4MPT cyclohydrolase	<i>mch</i>	MCA2863	3057182	3058177	F6R98_19020	4175778	4176773
dihydrofolate reductase	<i>dfrA</i>	MCA2729	2922594	2923112	F6R98_05325	1223823	1224311
Formate oxidation							
formate dehydrogenase, alpha subunit	<i>fdhA</i>	MCA1210	1271086	1274286	F6R98_12575	2784595	2787438
		MCA1391	1474796	1477639			
		MCA2576	2758784	2761561			
formate dehydrogenase, beta subunit	<i>fdhB</i>	MCA1392	1477696	1479246	F6R98_12580	2787497	2789050
formate dehydrogenase, gamma subunit	<i>fdhC</i>	MCA120	1269579	1270214	F6R98_12585	2789047	2789523
		MCA1393	1479243	1479722			
formate dehydrogenase, delta subunit	<i>fdhD</i>	MCA1389	1473618	1473851	F6R98_12565	2782970	2783179
NAD-dependent formate dehydrogenase	<i>fdh</i>	Absent			F6R98_01730	440807	442012
C1-assimilation: ribulose monophosphate pathway							
3-hexulose-6-phosphate synthase	<i>hxlA</i>	MCA3043	3230263	3230910	F6R98_00315	76334	76993
		MCA3049	3236945	3237592	F6R98_00325	77632	78279
6-phospho-3-hexuloisomerase	<i>hxlB</i>	MCA3044	3230923	3231456	F6R98_00320	76995	77528
		MCA3050	3237605	3238138			
Pyrophosphate-dependent fructose 6-phosphate-1-kinase (EC 2.7.1.90)	<i>pfp</i>	MCA1251	1330927	1332189	F6R98_02840	679990	681249
Fructose-bisphosphate aldolase class II	<i>fbaA</i>	MCA3041	3228215	3229279	F6R98_00290	71340	72404

C1-assimilation: serine cycle							
serine-glyoxylate aminotransferase	<i>sga</i>	MCA1406	1492959	1494146	F6R98_06335	1450866	1452056
hydroxypyruvate reductase	<i>hpr</i>	MCA1407	1494241	1495212	F6R98_01730	440807	442012
glycerate kinase	<i>gck</i>	MCA1408	1495321	1496187	Absent		
enolase	eno	MCA1933	2080068	2081333	F6R98_16530	3645336	3644053
		MCA2515	2693051	2694334			
phosphoenolpyruvate carboxylase	<i>ppcA</i>	Absent			F6R98_13095	2894412	2891602
malate dehydrogenase	<i>mdh</i>	MCA0610	645424	646401	F6R98_13210	2917209	2916232
malate thiokinase, alpha subunit	<i>mtkA</i>	MCA1741	1858803	1859705	F6R98_20180	4432178	4433065
malate thiokinase, beta subunit	<i>mtkB</i>	MCA1740	1857628	1858797	F6R98_20175	4431003	4432175
malyl-CoA lyase	<i>mcl</i>	MCA1739	1856611	1857573	F6R98_20170	4429946	4430911
serine hydroxymethyltransferase	<i>glyA</i>	MCA1660	1762420	1763676	F6R98_12550	2781455	2780199
Calvin cycle							
Phosphoribulokinase	<i>prk</i>	MCA3051	3238358	3239236	F6R98_00300	73273	74169
Ribulose biphosphate carboxylase	<i>cbbl</i>	MCA2743	2934677	2936098	F6R98_15900	3503068	3504447
Phosphoglycerate kinase	<i>pgk</i>	MCA2021	2169482	2170657	F6R98_11375	2528550	2527369
glyceraldehyde 3-phosphate dehydrogenase	<i>gapA</i>	MCA2598	2783826	2784836	F6R98_18320	4036685	4035675
Fructose-bisphosphate aldolase class II	<i>fbaA</i>	MCA3041	3228215	3229279	F6R98_00290	71340	72404
		MCA3047	3234897	3235961			
fructose-1,6-bisphosphatase II	<i>fbp2</i>	Absent			Absent		
Transketolase	<i>tkt</i>	MCA3040	3226183	3228195	F6R98_00285	69185	71191
		MCA3046	3232865	3234877			
fructose-1,6-bisphosphatase I / sedoheptulose-1,7-bisphosphatase	<i>glpX-SEBP</i>	Absent			Absent		
Ribose 5-phosphate isomerase A (EC 5.3.1.6)	<i>rpi</i>	MCA0355	357582	358238	F6R98_00305	74246	74902

Supplementary Table S2. Terminal oxidases encoded in the genomes of strain Shm1, *Mc. capsulatus* Bath and other representative gammaproteobacterial methanotrophs.

Microorganism (Genome accession number)	Total number of terminal oxidases			
	Low-affinity		Low-/high-affinity	High-affinity
	<i>aa₃</i>	<i>bo₃</i>	<i>b(o)a₃/ba₃</i>	<i>bd</i>
Strain Shm1 (CP044205)	F6R98_07605- F6R98_07620	none	none	F6R98_00185- F6R98_00195, F6R98_03365- F6R98_03375, F6R98_15065- F6R98_15070
<i>Methylococcus capsulatus</i> Bath (AE017282.2)	MCA0879- MCA0883	none	MCA2396- MCA2397	MCA1105- MCA_RS05455
<i>Methylomonas methanica</i> MC09 (NC_015572.1)	Metme_1607- Metme_1609, Metme_3151- Metme_3155	Metme_1277- Metme_1280	none	Metme_3309- Metme_3311, Metme_4253- Metme_4254
<i>Methylomonas</i> sp. LW13 (NZ_CP033381.1)	U737_RS09955- U737_RS09970	none	U737_RS18060- U737_RS18065	U737_RS02705- U737_RS02710, U737_RS11120- U737_RS11130
<i>Methylobacter tundripaludum</i> SV96 (AEGW00000000.2)	METTU_RS00670- METTU_RS00685, METTU_RS01055- METTU_RS01060	none	none	METTU_RS19325- METTU_RS19330, METTU_RS20835- METTU_RS20840
<i>Methylomicrobium buryatense</i> 5GB1C (NZ_CP035467.1)	EQU24_RS14110- EQU24_RS14130	EQU24_08255- EQU24_RS08270	EQU24_RS09070- EQU24_RS09075	none

Supplementary Table S3. Genes involved in flagella biosynthesis in the genomes of strain Shm1 and their homologs.

Start	End	Length	Locus tag	Predicted product	Nearest homolog	E value	AA % Ident	Accession of homolog
868702	869817	1116	F6R98_03645	flagellar basal body P-ring protein FlgI	flagellar basal body P-ring protein FlgI [<i>Sulfuriferula plumbiphila</i>]	4,00E-156	62.54%	WP_147070396.1
896848	897624	777	F6R98_03780	flagellar biosynthetic protein FliR	flagellar biosynthetic protein FliR [<i>Massilia</i> sp. GV090]	1,00E-93	53.94%	WP_132138946.1
897715	897987	273	F6R98_03785	flagellar biosynthesis protein FliQ	flagellar biosynthesis protein FliQ [<i>Massilia glaciei</i>]	5,00E-37	70.59%	WP_106759030.1
898087	898851	765	F6R98_03790	The bacterial flagellar biogenesis protein FliP forms a type III secretion system (T3SS)-type pore required for flagellar assembly	flagellar type III secretion system pore protein FliP [<i>Yersinia mollaretii</i>]	5,00E-118	74.32%	WP_072079705.1
898841	899203	363	F6R98_03795	flagellar biosynthetic protein FliO	flagellar biogenesis protein FliO [<i>Herbaspirillum frisingense</i> GSF30]	2,00E-24	48.08%	EOA05561.1
899200	899592	393	F6R98_03800	flagellar motor switch protein FliN	flagellar motor switch protein FliN [<i>Noviherbaspirillum</i> sp. UKPF54]	2,00E-56	69.12%	WP_146329939.1
899582	900604	1023	F6R98_03805	flagellar motor switch protein FliM	flagellar motor switch protein FliM [<i>Undibacterium</i> sp. S11R28]	2,00E-143	58.28%	WP_126774426.1
900619	901116	498	F6R98_03810	flagellar basal body-associated protein FliL	flagellar basal body-associated protein FliL [<i>Noviherbaspirillum denitrificans</i>]	2,00E-37	47.33%	OWW18860.1
901474	903438	1965	F6R98_03815	hypothetical protein	TPA: flagellar hook-length control protein FliK [<i>Oxalobacteraceae</i> bacterium]	3,00E-31	46.95%	HCE11017.1
903451	903909	459	F6R98_03820	flagellar export protein FliJ	flagella biosynthesis chaperone FliJ [<i>Herbaspirillum robiniae</i>]	6,00E-34	46.76%	WP_088749909.1
903906	905324	1419	F6R98_03825	flagellar protein export ATPase FliI	flagellar protein export ATPase FliI [<i>Herbaspirillum autotrophicum</i>]	0	71.83%	WP_050462254.1
905321	906031	711	F6R98_03830	flagellar assembly protein FliH	flagellar assembly protein FliH [<i>Sulfuriferula thiophila</i>]	1,00E-47	40.00%	WP_124949960.1
906024	907025	1002	F6R98_03835	flagellar motor switch protein FliG	flagellar motor switch protein FliG [<i>Janthinobacterium</i> sp. 17J80-10]	3,00E-141	60.00%	WP_128901790.1
907022	908776	1755	F6R98_03840	flagellar basal body M-ring protein FliF	flagellar basal body M-ring protein FliF [<i>Noviherbaspirillum</i> sp. 122213-3]	0	55.92%	WP_151636386.1
908943	909308	366	F6R98_03845	flagellar hook-basal body complex protein FliE	flagellar hook-basal body complex protein FliE [<i>Methylothermobacter mobilis</i>]	3,00E-39	57.02%	WP_015831871.1
918452	918799	348	F6R98_03890	flagellar biosynthesis protein	flagellar biosynthesis protein [<i>Solimicrobium silvestre</i>]	1,00E-37	67.74%	WP_105534246.1

918786	919829	1044	F6R98_03895	flagellar hook-length control protein FliK	flagellar hook-length control protein FliK [<i>Sulfuriferula multivorans</i>]	2,00E-49	44.98%	WP_124704435.1
919856	920302	447	F6R98_03900	flagellar protein FliT	flagellar protein FliT [<i>Burkholderia vietnamiensis</i>]	0,068	32.29%	WP_059462207.1
920185	920676	492	F6R98_03905	flagellar export chaperone FliS	flagellar export chaperone FliS [<i>Undibacterium pigrum</i>]	3,00E-48	59.09%	WP_110253197.1
920713	922191	1479	F6R98_03910	flagellar filament capping protein FliD	flagellar filament capping protein FliD [<i>Tepidiphilus</i> sp. J10]	1,00E-70	36.86%	WP_142804334.1
922211	922627	417	F6R98_03915	flagellar protein FlaG	hypothetical protein CVU31_07740 [<i>Betaproteobacteria</i> bacterium HGW- <i>Betaproteobacteria</i> -4]	7,00E-11	46.97%	PKO47799.1
922719	923924	1206	F6R98_03920	flagellin	flagellin [<i>Xanthomonadaceae</i> bacterium SCN 69-123]	1,00E-67	43.28%	ODU42631.1
1620403	1621341	939	F6R98_07105	flagellar motor protein MotB	flagellar motor protein MotB [<i>Sulfuriferula</i> sp. AH1]	4,00E-124	59.79%	WP_087447141.1
1621358	1622212	855	F6R98_07110	flagellar motor stator protein MotA	flagellar motor stator protein MotA [<i>Herbaspirillum autotrophicum</i>]	2,00E-162	77.82%	WP_050461989.1
1623205	1623612	408	F6R98_07120	flagellar basal body rod protein FlgC	flagellar basal body rod protein FlgC [<i>Herbaspirillum autotrophicum</i>]	3,00E-55	63.16%	WP_050462273.1
1623618	1623983	366	F6R98_07125	flagellar basal body rod protein FlgB	flagellar basal body rod protein FlgB [<i>Sulfuriferula multivorans</i>]	2,00E-41	54.07%	WP_124703299.1
1624140	1624859	720	F6R98_07130	flagellar basal body P-ring formation protein FlgA	flagellar basal body P-ring formation protein FlgA [<i>Herbaspirillum</i> sp. CF444]	3,00E-59	41.95%	WP_007882620.1
1624964	1625293	330	F6R98_07135	flagellar biosynthesis anti-sigma factor FlgM	flagellar biosynthesis anti-sigma factor FlgM [<i>Duganella</i> sp. Leaf126]	7,00E-12	42.59%	WP_056157072.1
1625295	1625789	495	F6R98_07140	flagellar protein FlgN	flagellar protein FlgN [<i>Paucimonas lemoignei</i>]	2,00E-20	36.60%	WP_132256316.1
1625779	1626834	1056	F6R98_07145	OmpA family protein	flagellar motor protein MotD [<i>Sulfuriferula multivorans</i>]	1,00E-70	41.77%	WP_124706240.1
1626845	1627591	747	F6R98_07150	flagellar motor protein	flagellar motor protein [<i>Noviherbaspirillum denitrificans</i>]	2,00E-95	56.15%	WP_088708245.1
1627594	1628307	714	F6R98_07155	RNA polymerase sigma factor FliA	flagellar motor protein [<i>Noviherbaspirillum denitrificans</i>]	2,00E-95	56.15%	WP_088708245.1
1628311	1629093	783	F6R98_07160	MinD/ParA family protein	antiactivator of flagellar biosynthesis FleN protein [<i>Massilia</i> sp. K1S02-61]	1,00E-63	44.84%	WP_119810355.1
1629090	1630367	1278	F6R98_07165	flagellar biosynthesis protein FlhF	flagellar biosynthesis protein FlhF [<i>Sulfuriferula plumbiphila</i>]	5,00E-142	51.92%	WP_147070425.1
1630364	1632448	2085	F6R98_07170	flagellar biosynthesis protein FlhA	TPA: flagellar biosynthesis protein FlhA [<i>Betaproteobacteria</i> bacterium]	0	75.47%	HAN56499.1

1632644	1633783	1140	F6R98_07175	flagellar type III secretion system protein FlhB	flagellar type III secretion system protein FlhB [<i>Massilia</i> sp. Leaf139]	1,00E-151	57.57%	WP_056341970.1
1634869	1636434	1566	F6R98_07185	flagellar hook-basal body complex protein	flagellar hook protein FlgE [<i>Gallionellaceae</i> bacterium]	1,00E-32	31.96%	TNC98404.1
1636704	1637441	738	F6R98_07190	flagellar basal body rod protein FlgF	MULTISPECIES: flagellar basal-body rod protein FlgF [unclassified <i>Herbaspirillum</i>]	2,00E-90	56.91%	WP_141915740.1
1637554	1638342	789	F6R98_07195	flagellar basal-body rod protein FlgG	flagellar basal-body rod protein FlgG [<i>Herbaspirillum</i> autotrophicum]	5,00E-117	64.89%	WP_050462269.1
1638432	1639106	675	F6R98_07200	flagellar basal body L-ring protein FlgH	flagellar basal body L-ring protein FlgH [<i>Thiobacillus</i> sp.]	1,00E-57	45.66%	TXH73323.1
1639115	1640236	1122	F6R98_07205	flagellar basal body P-ring protein FlgI	flagellar basal body P-ring protein FlgI [<i>Sulfuriferula plumbiphila</i>]	2,00E-165	64.21%	WP_147070396.1
1640239	1641111	873	F6R98_07210	flagellar assembly peptidoglycan hydrolase FlgJ	flagellar assembly peptidoglycan hydrolase FlgJ [<i>Sulfuriferula thiophila</i>]	5,00E-102	54.45%	WP_124949226.1
1641185	1643824	2640	F6R98_07215	flagellar hook-associated protein FlgK	flagellar hook-associated protein FlgK [<i>Sulfuriferula plumbiphila</i>]	2,00E-86	38.36%	WP_147070392.1
1643839	1644825	987	F6R98_07220	flagellar hook-associated protein 3	flagellar hook-associated protein 3 [<i>Sulfuriferula multivorans</i>]	4,00E-55	39.33%	WP_124703289.1
3470390	3470785	396	F6R98_15745	hypothetical protein	flagellin domain-containing protein [<i>Hylemonella gracilis</i> ATCC 19624]	4,00E-22	46.72%	EGI78503.1

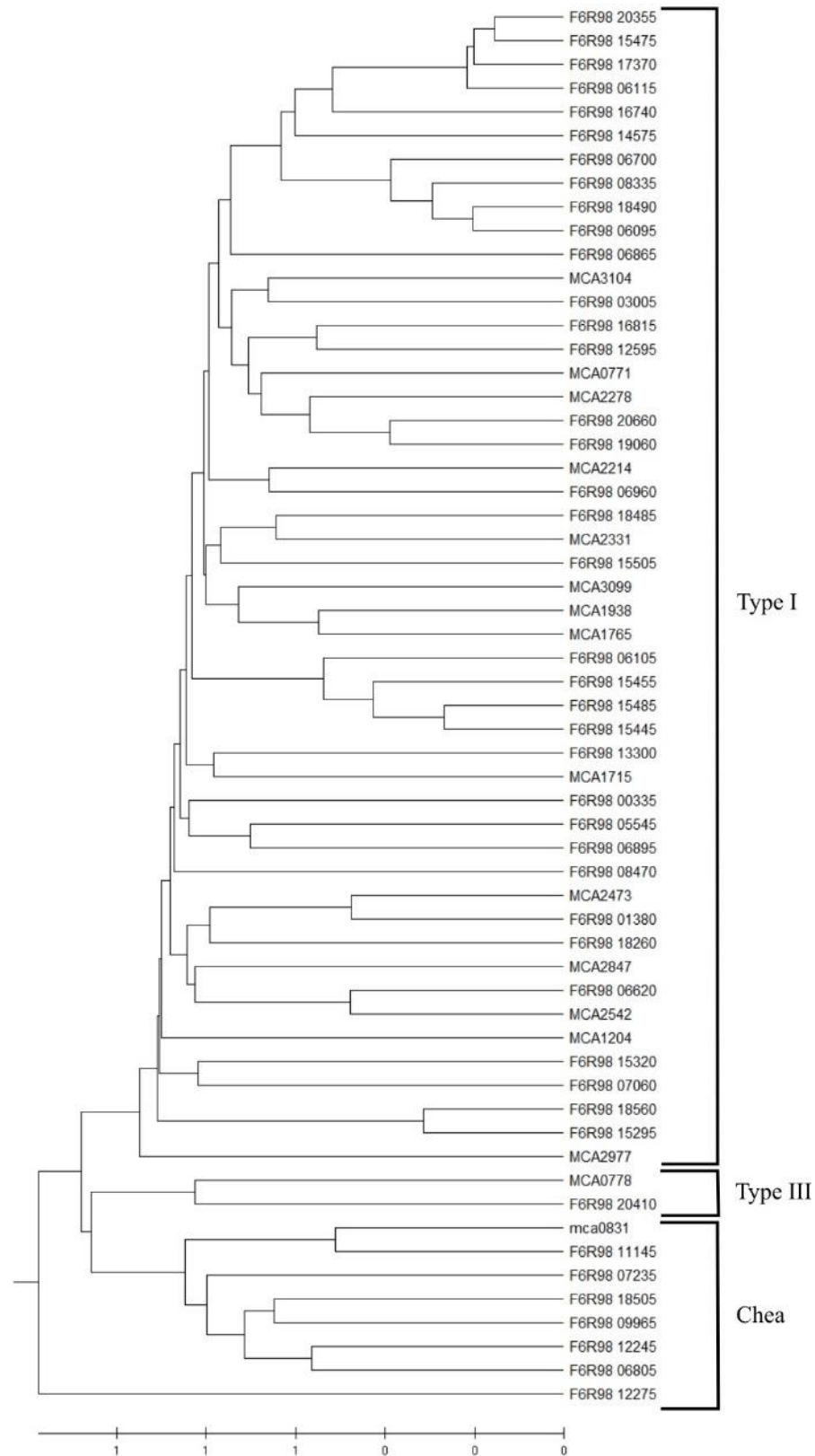


Figure S1. Relationship between the deduced amino acid sequences of HK genes retrieved from the genomes of *M. capsulatus* Bath and strain Shm1. The tree was constructed using MEGAX with UPGMA as hierarchical cluster method. The scale indicates genetic distance between amino acid sequences.

Tree scale: 1



Figure S2. Phylogenetic position of the FlaB protein from strain Shm1. The tree was constructed based on 235 amino acid residues of flagellin-encoded proteins using the Fast Tree package and approximately maximum-likelihood method. Bar, 1 substitutions per amino acid position.