

Low Diversity of Thermophilic Cyanobacteria in Nakabusa Hot Springs Supports a Hypothesis of Adaptive Evolution from Lower Temperature

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Conclusion

The diversity of Cyanobacteria in Nakabusa hot springs was extremely low, supporting a hypothesis of the adaptive evolution of thermophilic cyanobacteria from lower temperature relatives.

Materials and Methods

26 thermophilic microbial communities were collected from Nakabusa hot springs (Fig. 1) from temperatures of 87°C to 53°C. 16S rRNA (V4) was sequenced in extracted DNA, and the diversity of major microbes was analyzed.

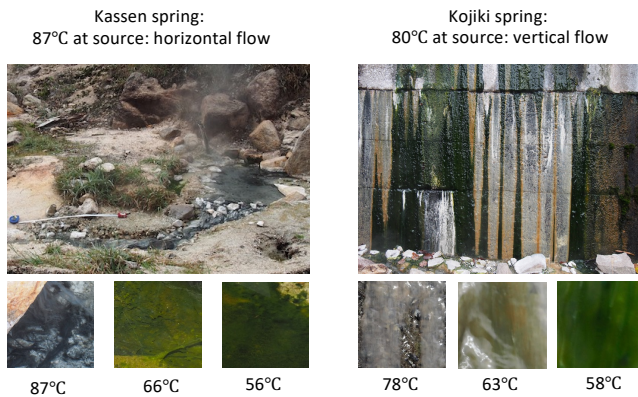


Fig. 1 Hot spring flow and microbial mats and streamers in Nakabusa at two research sites

Results

Four major phyla were detected (Fig. 2); Chloroflexi (21%, anaerobic, photosynthetic), Aquificae (20%, aerobic, chemosynthetic), Cyanobacteria (11%, aerobic, photosynthetic), and Thermodesulfobacteria (8%, anaerobic, chemosynthetic). The species assigned in Cyanobacteria were two, and the abundance of the thermophilic cyanobacterium, *Thermosynechococcus elongatus*, was 99.92% among the two (Fig. 2, third bar). Only one sequence variant was observed in *T. elongatus* (Fig. 3, fifth bar), whereas 3 to 8 variants in more than 1% were detected in 6 abundant species in the other three major phyla (fig. 3, 1st to 4th and 6th to 7th bars).

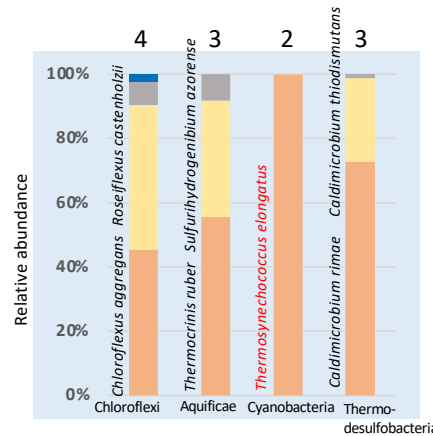


Fig. 2 Number of species (top) and relative abundance in four major phyla

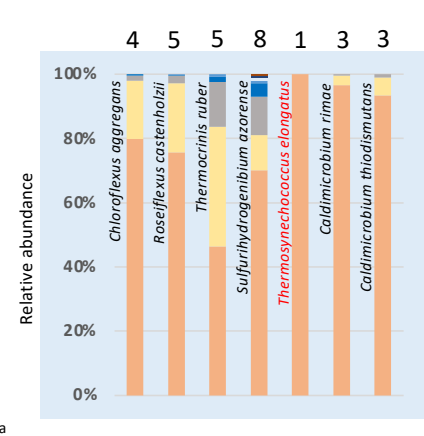


Fig. 3 Number of variants (top) and relative abundance in seven major species

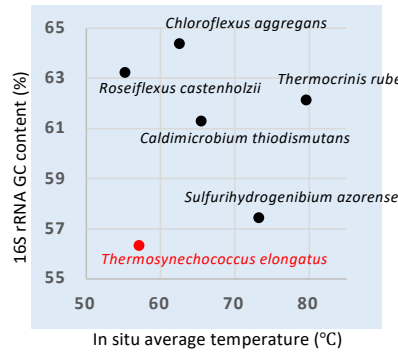


Fig. 4 16S rRNA GC content vs. in situ average temperature of 6 major species

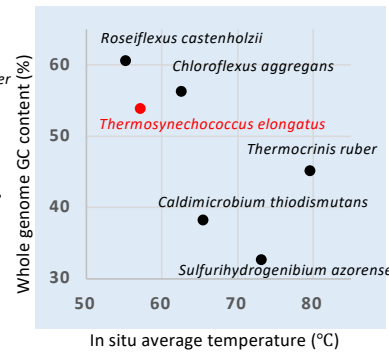


Fig. 5 Whole genome GC content vs. in situ average temperature of 6 major species

Discussion

We propose that low sequence diversity in *Thermosynechococcus* at Nakabusa reflects limited evolutionary time scales and a short history of existence in thermal environments; the mesophilic origin as has been suggested (1,2,3). It is also consistent with the low genomic diversity of *Thermosynechococcus* genomes from various Japanese hot springs (4). This argument is supported by the finding of low GC content in the 16S rRNA region (Fig. 4) but not in the whole genome (Fig. 5); 16S rRNA GC content and growth temperature correlation has been proposed (5,6). In summary, the results support a hypothesis of the adaptive evolution of thermophilic cyanobacteria from lower temperatures.

References

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Table S1 16S rRNA (V4) amplicon read numbers of variants in 4 major phyla in 26 communities from 87 to 53°C normalized to 100,000 in each community. Dark orange >10%, light orange >1%.

Phylum	nearest sp.	Temperature °C	87	85	79	79	79	78	77	68	67	67	66	66	65	63	60	55	59	59	57	57	56	56	55	53	53	Total			
Aquificae	<i>Thermococcus ruber</i>	Identity (%)	96.44	47782	15168	21738	10810	28127	10275	5076	398	392	686	1694	189	0	30	139	0	145	55	218	75	47	209	99	40	0	5124		
	<i>Thermococcus ruber</i>	96.84	21445	6047	26482	9684	29003	17479	3583	0	0	0	627	645	0	0	0	0	118	0	0	61	129	123	79	89	24	4132			
	<i>Thermococcus ruber</i>	96.05	0	1120	1870	1308	490	1050	3167	44	0	0	1142	7580	17613	47	218	3593	0	0	19	20	3270	107	0	329	41	0	1538		
	<i>Thermococcus ruber</i>	96.44	1386	646	1060	503	1778	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	192		
	<i>Thermococcus ruber</i>	96.05	470	505	580	256	576	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85	
	<i>Hydrogenobacter subterraneus</i>	98.44	0	40	26	158	45	0	0	256	0	0	1652	7727	4643	101	140	2243	696	17	833	0	62	7890	651	16	768	33	260	1012	
	<i>Hydrogenobacter subterraneus</i>	99.00	0	229	289	216	63	209	934	443	47	1013	384	3418	131	1431	352	954	203	1154	294	130	2435	1567	75	823	163	296	624		
	<i>Sulfurihydrogenibium azorense</i>	100.00	37	256	2206	1239	456	39953	43957	8310	574	3354	19488	5143	4660	5281	149	547	687	981	563	1119	1532	140	119	572	262	92	5084		
	<i>Sulfurihydrogenibium azorense</i>	99.60	0	0	168	0	0	4646	4668	0	0	206	5760	757	4421	224	0	0	0	0	0	0	1803	0	0	0	0	0	0	810	
	<i>Sulfurihydrogenibium azorense</i>	98.42	0	0	0	0	0	6322	17131	0	0	0	0	162	298	0	0	0	0	0	0	0	0	0	0	0	0	0	0	855	
	<i>Sulfurihydrogenibium azorense</i>	99.60	0	0	0	0	0	2453	2878	0	0	0	488	1070	260	0	0	0	0	0	0	0	1139	0	0	0	0	0	0	305	
	<i>Sulfurihydrogenibium azorense</i>	99.60	0	0	0	0	0	0	0	0	0	0	1202	540	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	
	<i>Sulfurihydrogenibium azorense</i>	99.60	0	0	0	0	0	656	205	0	0	228	0	254	144	0	0	64	149	64	129	0	0	0	0	0	0	0	0	68	
	<i>Sulfurihydrogenibium azorense</i>	99.60	0	0	0	0	0	0	0	0	0	598	0	317	0	0	0	186	0	0	0	0	0	0	0	0	0	0	0	39	
	<i>Sulfurihydrogenibium azorense</i>	99.21	0	0	0	0	0	0	0	0	0	843	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	
Chloroflexi	<i>Chloroflexus aggregans</i>	100.00	12	25	3	0	0	0	0	16044	28031	16019	1716	4151	18312	15156	7373	2492	14357	26215	10713	13625	12029	9035	7872	4947	1131	606	7602		
	<i>Chloroflexus aggregans</i>	99.60	0	0	0	0	0	0	0	1970	449	6075	138	344	2031	2773	4647	4605	1409	1475	681	1032	1621	1974	1254	6755	1213	1571	1738		
	<i>Chloroflexus aggregans</i>	99.60	0	0	0	0	0	0	0	830	0	0	0	67	2169	940	0	0	0	0	0	0	0	0	0	0	0	0	0	149	
	<i>Chloroflexus aggregans</i>	99.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	295	120	0	0	0	0	0	0	0	0	0	0	0	41	
	<i>Chloroflexus aurantiacus</i>	100.00	0	14	0	0	0	0	0	0	0	123	172	83	0	886	4116	93	21	15	56	17	441	145	7	546	49	298	459		
	<i>Oscillochloris trichoides</i>	92.09	0	0	3	0	0	0	0	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>Roseiflexus castenholzii</i>	100.00	0	7	0	0	15	0	0	69	5	42	0	0	0	0	424	340	28365	14874	13569	6802	12841	465	17439	28616	18964	22481	31899	7157	
	<i>Roseiflexus castenholzii</i>	99.60	0	0	0	0	0	0	0	0	0	0	0	0	0	140	196	7120	3511	4099	1658	3438	120	8002	9344	5895	4495	6596	2023		
	<i>Roseiflexus castenholzii</i>	99.21	0	0	0	0	0	0	0	0	0	0	0	0	0	52	478	340	0	0	175	0	1091	1492	289	903	1309	0	0		
	<i>Roseiflexus castenholzii</i>	99.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	240	0	0	0	197	0	125	120	0	25	
	<i>Roseiflexus castenholzii</i>	99.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	
	Cyanobacteria	<i>Thermosynechococcus elongatus</i>	100.00	0	83	19	68	37	0	0	4122	101	10224	12520	0	742	6887	31698	610	36696	14144	34084	15729	23952	28377	16088	25727	31834	403	11313	
		<i>Thermoleptolyngbya sichuanensis</i>	99.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		<i>Caldimicrobium rimae</i>	98.81	24559	33151	27388	21924	26931	11269	9659	91	70	406	225	263	255	43	64	10	0	0	41	47	80	167	64	146	70	33	5619	
		<i>Caldimicrobium rimae</i>	98.42	0	0	1705	467	2168	594	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	177
<i>Caldimicrobium rimae</i>		95.26	0	0	0	0	0	0	0	237	418	42	0	0	0	0	0	0	0	0	51	0	25	0	0	0	0	0	0	28	
<i>Caldimicrobium thiodismutans</i>		100.00	0	47	33	65	0	206	344	8840	15137	4032	90	286	12928	3244	36	3361	89	196	361	1012	20	273	399	215	135	2806	1945		
<i>Caldimicrobium thiodismutans</i>		99.60	0	0	0	0	0	0	0	401	157	0	0	0	0	1939	638	0	0	0	0	0	0	0	0	0	0	0	0	103	
<i>Caldimicrobium thiodismutans</i>		99.60	0	0	0	0	0	0	0	404	215	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	
<i>Thermodesulfobacterium geofontis</i>		98.80	0	337	0	1790	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	76	
<i>Thermodesulfobacterium geofontis</i>		98.02	0	0	166	0	0	0	0	0	0	348	0	0	0	0	0	0	0	0	0	128	0	47	0	0	0	0	0	25	