Distribution of arsenite-oxidizing bacteria and its correlation with environmental factors in geothermal areas of Tengchong, Yunnan, China

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Abstract. Arsenic (As) is an ubiquitous constituent in geothermal water. Arsenite (As_{III}) is oxidized via microbial processes as the waters equilibrate with oxygen in the geothermal effluent. The distribution of arsenite oxdizing bacteria and its correlation with environment factors were studied in Tengchong geothermal areas of Yunnan, China. A total of 230 *aio*A clone sequences were obtained and these sequences were affiliated with four phyla: *Betaproteobacteria*, *Alphaproteobacteria*, *Deinococcus-Thermus* and *Aquificae*. Temperature was negatively correlated with *aio*A diversity and was the only environment factor that had correlation with diversity index. *Betaproteobacteria* was mainly distributed in low temperature (T = 28 to 43 ºC) and circumneutral or light alkaline (pH = 7 to 9) springs; *Alphaproteobacteria* was mainly predominant in low pH (pH = 3.3 to 3.6) springs; *Deinococcus-Thermus* and *Aquificae* mainly inhabited in high temperature (T=55 to 78 ºC) springs with a wide range of pH. Usually, *Deinococcus-Thermus* was dominant when springs had a pH within 4.0 to 8.0. *Aquificae* was dominated in springs with pH > 8.0 or pH < 4.0.

1 Diversity of *aio*A gene

A total of 230 *aio*A gene clone sequences from 10 sample sites were subjected to sequence similarity analysis. The coverage values of the clone libraries were greater than 78 % for all except one sample ZZQ2 for which the coverage was 42% (Table 1). The quantity of operational taxonomic units (OTUs) in different samples were mostly under 6, and Chaol diversity indices were not higher than 5.0 except for samples ZZQ2 and SRBZ (20.3 and 9.0, respectively.

The correlation between environmental factors and the microbial diversities indicated by the quantity of OTUs, and the Shannon and Chaol was analyzed using the statistical analysis software SPSS (Table 2). The only statistically significant correlation is for that between temperature and the Shannon index ($P \leq 0.05$, correlation coefficient -0.654).

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Table 1. Diversity of *aio*A genes of 10 samples collected from Tengchong（97% OTU level）

Sample	Quantity of clone	Coverage	Quantity of OTU	Shannon	Chaol
LXS	23	85%	5	1.31	5.0
XXS	39	95%	2	0.33	2.0
SPS	19	95%	1	0.00	1.0
ZZQ1	19	95%	1	0.00	1.0
ZZQ2	19	42%	11	2.03	20.3
LGG1	15	80%	3	0.85	3.0
LGG2	12	83%	2	0.45	2.0
SRBZ	28	79%	6	1.25	9.0
HYDW	37	95%	2	0.52	2.0
QLDW	19	79%	4	1.06	4.0

The translated amino acid sequences of the *aio*A genes were generally classified into five putative groups and affiliated with four phyla **(Fig. 1)**. Group I and Group II belong to *Betaproteobacteria*. Group III, Group IV and group V belong to *Deinococcus-Thermus*, *Alphaproteobacteria* and *Aquificae*, respectively.

Table 2. Statistical correlations between environmental factors and *aio*A gene diversities.

Environmental factors	Diversity		
	OTU	Shannon	Chaol
T	-0.624	**-0.654***	-0.62
pH	0.558	0.505	0.538
S^{2-}(mg/L)	-0.08	-0.058	-0.068
Na^{+}(mg/L)	0.009	-0.013	0.047
Cl^{-}(mg/L)	0.571	0.493	0.601
SO_4^{2-}(mg/L)	-0.192	-0.129	-0.176
HCO_3^{-}(mg/L)	-0.226	-0.183	-0.217
Total Fe(mg/L)	-0.251	-0.2	-0.234
Total As(μg /L)	0.151	0.022	0.185

+ and −, positive and negative relationship, respectively; * P-value < 0.05;

2 Correlation with environmental factors

Correlations between the *aio*A gene diversity distributions and environmental factors were examined **(**Table 3**).** According to the data, Group II is significantly negatively correlated with temperature (R=-0.651，P≤0.05), but Group IV showed positive correlation with temperature (R=0.722，P≤0.05). The Cl concentration was significantly positively correlated with Group I and Group II. Group III was significantly correlated with the concentrations of SO_4^{2-}, Fe^{2+}, and total Fe. Other environmental factors, including arsenic

species and HCO_3^-, did not show significantly effects on community structure of the *aio*A gene.

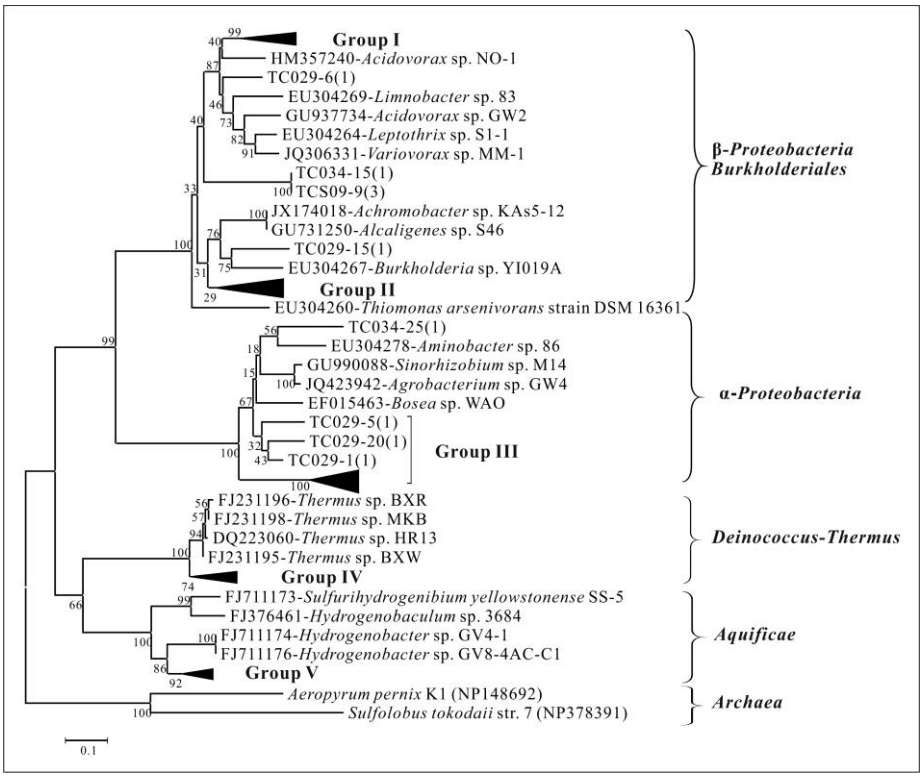

Fig. 1. Phylogeny of *aio*A sequences deduced from clone sequences detected in hot spring in Yunnan. *Lower-case letters* show the variety of OTU.

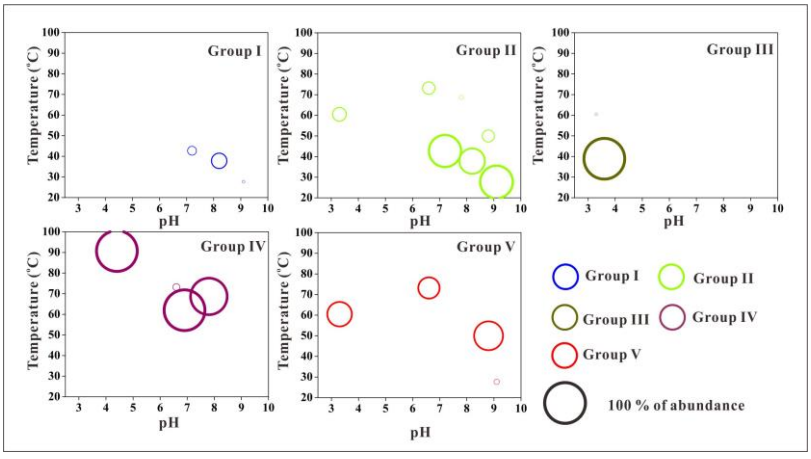

Fig. 2. Relationship between *aio*A gene group and temperature and pH, wheres the size of each circle represents the proportion of different group in the library.

The correlations between the *aio*A gene in our samples and pH and temperature were analyzed (Figure 2**)**. The data indicated that the Group distribution had a particular relationships with the hot spring temperature and pH. Group I and Group II were mainly distributed in low-temperature and high-pH environments, and Group II had the main advantage in these environments. The temperature distribution range of Group II was wider than the other groups. Group III only appeared in low-pH hot springs. Due to the small number of low-pH sample locations and the small temperature range covered, the correlation between Group III and temperature cannot be determined. Both Group IV and Group V were concentrated in hot springs with high temperatures and had a wide range of pH adaptability. However, in the range of pH 4.0-8.0, Group IV was usually dominant in hot springs, while Group V existed in more extreme environments with pH < 4.0 or pH > 8.0.

Table 3. Statistical correlations between environmental factors and *aio*A gene groups.

Environmental factors	community structure of *aio*A gene				
	Group I	Group II	Group III	Group IV	Group V
T(°C)	-0.492	-.779**	0.073	.724*	-0.157
pH	0.371	0.544	-0.579	-0.096	0.023
NH^{4+}(mg/L)	-0.137	-0.334	-0.142	0.518	-0.24
S^{2-}(mg/L)	-0.064	-0.213	.943**	-0.366	-0.184
Ca^{2+}(mg/L)	0.4	0.226	0.327	-0.377	-0.182
Mg^{2+}(mg/L)	0.399	0.481	-0.079	-0.226	-0.257
Na^{+}(mg/L)	0.59	0.611	-0.336	-0.083	-0.429
Cl^{-}(mg/L)	.875**	.790**	-0.289	-0.328	-0.413
SO_4^{2-}(mg/L)	-0.245	-0.25	.676*	-0.292	0.108
HCO_3^{-}(mg/L)	0.401	0.435	-0.246	-0.057	-0.293
Fe^{2+} (mg/L)	-0.122	-0.319	.986**	-0.273	-0.235
Total Fe (mg/L)	-0.174	-0.315	.961**	-0.312	-0.129
As^{3+}(μg/L)	0.221	0.139	-0.076	0.164	-0.423
Total As(μg/L)	0.036	-0.01	-0.373	0.482	-0.362

+ and −, positive and negative relationship, respectively; * P-value < 0.05; **P-value < 0.01.

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