

Arsenic transformation and possible mobilization by indigenous microbes in hot spring environment

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ABSTRACT: Indigenous bacteria play an important role for arsenic (As) mobilization in aqueous environment. In present study, investigated reduction characteristics of arsenate by different indigenous bacterial isolates KTL (GU329910), CL (GU329907) and BL (GU329908), which was isolated from Kuan-Tzu-Ling (KTL), Chung-Lun (CL) and Bao-Lai (BL) hot springs (Taiwan) respectively. Morphological and 16S-rRNA analysis exhibits that the pure culture of isolate KTL (GU329910), CL (GU329907) and BL (GU329908) are similar (99% similarity) to *Bacillus pocheonensis*, *Desulfovibrio psychrotolerans* (sulfate reducing deltaproteobacterium), and *Clostridium sulfidigenes* (mesophilic, proteolytic, thiosulfate- and sulfur-reducing bacterium), respectively. *D. psychrotolerans* (GU329907) and *C. sulfidigenes* (GU329908) reduced SO_4^{2-} to S^{2-} and As(V) to As(III), efficiently. The growth rate and arsenic reduction was exhibited higher in presence of *D. psychrotolerans* (GU329907) compared to *C. sulfidigenes* (GU329908). Thus, the sulfate reducing bacteria contributes in arsenic mobilization process and forms more toxic As(III) species, which affects the biotic life.

1 INTRODUCTION

Arsenic have been found in geothermal systems of different geographical locations (e.g. USA, Turkey, Taiwan etc.), throughout the world. There are more than 100 different types of hot springs, cold spring, and mud spring, are located in Taiwan (Maity *et al.*, 2017).

In general, arsenic mobilization occurs through food chain or due to the oxidation and reduction process, through chemically and microbiologically in environment. Often, the reductive mobilization process plays an important role in arsenic transportation and mobilization at anaerobic environment.

Considering this background, the study was to investigate the arsenic and sulfate reduction characteristics of indigenous bacteria in hot springs in Taiwan, by which arsenic can mobilize to environment.

2 MATERIALS AND METHODS

2.1 Study area and sampling

Hot spring water were collected from Kuan-Tzu-Ling (KTL), Chung-Lun (CL) and Bao-Lai (BL) hot spring in Taiwan; and stored under anaerobic condition in laboratory at -20°C , after flushing with N_2 . Environmental parameters were estimated in field.

2.2 Isolation and identification of indigenous bacteria

Indigenous bacteria were isolated and identified (16S-rRNA with $\geq 99\%$ similarity) (CL, BL and KTL) using arsenic rich sulfate reducing media (serum bottle and dilution plate) (Hi-Media, India), in anaerobic environment. By the forward and reverse primer, such as 16S 27F (forward): $5' - \text{AGAGTTTGAT CCTGGCTCAG} - 3'$ and 16S 1492R (reverse): $5' - \text{GTTTACCTTGTTACGACTT} - 3'$ respectively.

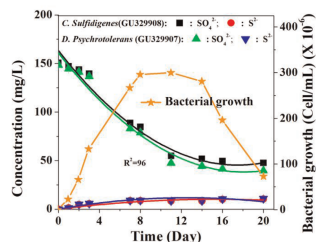


Figure 1. Sulfate reduction by indigenous bacteria.

2.3 Analysis of arsenic and sulfate reduction

Arsenic transformation [(0–2000 $\mu\text{g/L}$) As(V) to As(III)] and sulfate reduction (0–150 mg/L SO_4^{2-}) was performed using two efficient strain,

D. psychrotolerans (GU329907) and *C. sulfidigenes* (GU329908), at 28°C up to 20 days. SO_4^{2-} and S concentrations determined by IC and Microprocessor-Controlled Photometer-PC MultiDirect. Arsenic was measured with the ICP-MS (Hewlett-Packard 4500, Japan) and HPLC-AFS. Accuracy was checked by a certified material (TMDW; Lot # 623609, HPS, USA), within $\pm 5\%$ of certified values (accuracy: $\pm 5\%$ and precision: $\pm 2\%$).

3 RESULTS AND DISCUSSION

3.1 Environmental parameter

Temperature of the KTL, BL and CL hot springs was $82.2 \pm 1.2^\circ\text{C}$, $62.0 \pm 0.7^\circ\text{C}$ and $50.0 \pm 0.4^\circ\text{C}$, respectively. The pH of the KTL CL, and BL water noted 7.01 ± 0.02 , 7.41 ± 0.02 and 8.00 ± 0.02 respectively. The Eh of KTL (-396 mV) and CL (-360 mV) water were low compared with BL (-185 mV), suggesting reducing nature of spring water.

3.2 Identification of indigenous bacteria

The nucleotides similarities (%) in between KTL (GU329910) and *B. pocheonensis* (FJ009384); *B. drementensis* (FJ009411) and *B. soli strain G8* (FJ009379) were found 99.0, 98.0, and 98.0. Therefore, bacterial isolates from KTL hot spring was assigned to be closely related to *B. pocheonensis*. The nucleotides similarities (%) in between BL(GU329908) and *C. sulfidigenes* (EF199998); *C. thiosulforeducens* (AY024332) and *C. subterminale* (AF241844) were 99.0, 99.0 and 98.0, respectively. Therefore, isolates from BL hot spring can be assigned to *C. sulfidigenes*. The nucleotides similarities (%) between CL (GU329907) and *D. psychrotolerans* (AM418397), *D. acrylicus* (NR025978) and *D. desulfuricans* (FJ655909) were noted as 99.0, 98.0, and 98.0, respectively. Therefore, isolates from BL hot spring were assigned to *D. psychrotolerans*.

3.3 Sulfate reduction by indigenous bacteria in hot spring environment

Hot spring water was rich in different types of reducing bacteria. The indigenous strain *D. psychrotolerans* (GU329907) and *C. sulfidigenes* (GU329908) were transformed the 150 mg/L of sulfate to sulfide with increasing time. The transformation rate for both the strain noticed higher order upto 11days; and after, the rate was observed slower (Figure 1). Therefore, the redox sensitive elements may release to hot springs water by the action of reducing bacteria (e.g. SO_4^{2-} transformed into S^{2-} by sulfate-reducing bacteria as *C. sulfidigenes* and *D. psychrotolerans*) and exposed to the environment.

3.4 Arsenic reduction and possible mobilization by indigenous bacteria in hot spring environment

The biotransformation of arsenic (As(V) to As(III)) by *D. psychrotolerans* (GU329907) and *C. sulfidigenes* (GU329908) are shown in Figure 2, with different initial concentrations of As(V) (0–2000 $\mu\text{g/L}$). The transformation results reflecting the relationship between incubation time with the concentration of As(V) and As(III) revealed that *D. psychrotolerans* (GU329907) and *C. sulfidigenes* (GU329908) transformed 2000 $\mu\text{g/L}$ of As(V) to As(III) within 10 and 14 days, respectively. In contrast in between two strain, the *D. psychrotolerans* (GU329907) are more efficient than *C. sulfidigenes* (GU329908). So, the sulfate reducing bacteria transform the As(V) to As(III) in hot spring environment, which help to mobilized the arsenic in surface and subsurface environment.

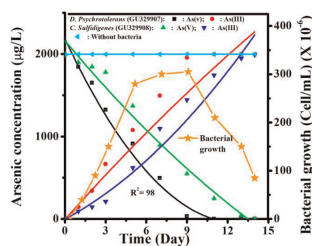


Figure 2. Arsenic reduction by indigenous bacteria.

4 CONCLUSIONS

Hot springs are rich with reducing bacteria. The indigenous sulfate reducing bacteria (*D. psychrotolerans* (GU329907) and *C. sulfidigenes* (GU329908)) transforms the As(V) to As(III) in hot spring environment, which help to mobilized the arsenic in surface and subsurface environment. However, different indigenous sulfate reducing bacteria exhibits the different rate of arsenic transformation from As(V) to As(III).

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