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Arsenite Oxidation by a Facultative Chemolithotrophic Bacterium SDB1 Isolated from Mine Tailing

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An arsenite (As[III])-oxidizing bacterium, SDB1, was isolated from mine tailing collected from the Sangdong mine area in Korea and showed chemolithotrophic growth on As[III] and CO_2 as the respective electron and carbon sources. SDB1 is Gram-negative, rod-shaped, and belongs to the *Sinorhizobium-Ensifer* branch of α-Proteobacteria. Growth and As[III] oxidation was enhanced significantly by the presence of yeast extract (0.005%) in minimal salt medium containing 5 mM As[III]; decreasing the doubling time from 9.8 to 2.1 h and increasing the As[III] oxidation rate from 0.014 to 0.349 pmol As[III] oxidized $cell^{-1}$ h^{-1}. As[III] oxidation nearly stopped at pH around 4 and should be performed at pH 7~8 to be most effective. SDB1 was immobilized in calcium-alginate beads and the oxidation capacity was investigated. Specific As[III] oxidation rates obtained with SDB1 (10.1~33.7 mM As[III] oxidized g^{-1} dry cell h^{-1}) were 10~16-times higher than those reported previously with a heterotrophic bacterial strain (Simeonova *et al.*, 2005). The stability and reusability of immobilized SDB1 strongly suggested that the immobilized SDB1 cell system can make the As[III] oxidation process technically and economically feasible in practical applications.

***Keywords*:** arsenite oxidation, *Sinorhizobium-Ensifer*, chemolithotroph, mine tailing

Arsenic (As) is the 20th most abundant element in the Earth's crust and it is distributed widely in nature as a result of weathering, fire, volcanic activity, and anthropogenic input (Cullen and Reimer, 1989; Bumbla and Keefer, 1994). Arsenic is a toxic and carcinogenic element, representing the major toxic element in abandoned mine areas (Roussel *et al.*, 2000). The contamination of groundwater by arsenic has become a crucial water quality problem in many parts of the world, particularly in Bangladesh, West Bengal, India, and Vietnam (Berg *et al.*, 2001; Harvey *et al.*, 2002; Bhattacharjee *et al.*, 2005). Recent surveys in Bangladesh have revealed that around 60~70 million people are at risk of arsenic poisoning. This catastrophe has been described as the largest mass poisoning in history; the majority of the population is already affected and yet the disaster is just beginning to emerge (Halim *et al.*, 2009).

Arsenic is naturally present in the environment, primarily in its soluble inorganic forms as arsenate (As[V]) or arsenite (As[III]), with the latter form being considered 100-fold more toxic and having greater hydrologic mobility than the former (Cullen and Reimer, 1989; Neff, 1997). Thus, it is highly desirable to oxidize As[III] species to enhance the immobilization of arsenic (Niggemyer *et al.*, 2001; Lee *et al.*, 2003). Additionally, most arsenic removal technologies, such as coagulation, filtration, adsorption, and reverse osmosis target As[V] and require a preoxidation step, of As[III] to As[V], because As[III] is thermodynamically stable and non-ionic at the pH conditions in most natural environments (H_3AsO_3, pK_a=9.22) (Bissen and Frimmel, 2003; Mondal *et al.*, 2006). The oxidation of As[III] to As[V] can be achieved chemically using potent oxidants, such as ozone, chlorine, and H_2O_2; however, the major disadvantages of such chemical oxidation include the generation of additional pollutants and high cost (Mondal *et al.*, 2006). Recently, biological oxidation has been identified as an alternative method for removing arsenic from contaminated water in a cost-effective and eco-friendly way (Katsoyiannis and Zouboulis, 2004; Mondal *et al.*, 2006). As[III] oxidation is a thermodynamically exergonic reaction ($\Delta G^{o\prime}$=-256 kJ/reaction) and can provide sufficient energy for As[III]-oxidizing chemolithotrophs (Santini *et al.*, 2000):

$$2H_3AsO_3 + O_2 \rightarrow HAsO_4^{2-} + H_2AsO_4^{-} + 3H^+$$

Nevertheless most remediation studies focused on using heterotrophs which requires organic substrate for their growth (Simeonova *et al.*, 2005). Recently several chemolithotrophic oxidizers that are able to fix CO_2 coupled to the oxidation of As[III] to As[V] were reported mainly on their ecology, phylogenetic relationship, and physiological characteristics (Rhine *et al.*, 2005; Rhine *et al.*, 2007; Garcia-Dominguez *et al.*, 2008).

In this study, a chemolithotrophic As[III]-oxidizing bacterium, SDB1, was isolated from mine tailing collected near the Sangdong mine area in Korea and its As[III] oxidation activity was characterized. As[III] oxidation by SDB1 immobilized in Ca-alginate beads was also investigated to improve the potential use of SDB1 as a remediation tool for As[III]-

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contaminated waste. These results will lead to improved arsenic risk management by presenting an alternative cost-effective and ecofriendly approach to conventional As[III] oxidation techniques.

Materials and Methods

Enrichment, isolation, and growth characteristics of As[III]-oxidizing bacteria

One gram of tailing sample from the Sangdong mine area in Korea was inoculated into 100 ml of minimal salt medium ($Na_2SO_4 \cdot 10H_2O$, 0.07; KH_2PO_4, 0.17; KCl, 0.05; $MgCl_2 \cdot 6H_2O$, 0.04; $CaCl_2 \cdot 2H_2O$, 0.05; KNO_3, 0.15; $(NH_4)_2SO_4$, 0.1; $NaHCO_3$, 0.5 in g/L; plus trace elements and vitamins) as described by Santini *et al.* (2000). The concentration of As[III] in the form of $NaAsO_2$ was 5 mM and the initial pH of the medium was 8. All incubations were carried out at 28℃ with shaking at 150 rpm. After several rounds of subculturing, the enriched culture was serially diluted and spread onto the same minimal salt agar medium containing 5 mM As[III] to isolate As[III]-oxidizing bacteria (AOB).

Growth curves were obtained based on direct counts of the collected culture medium. Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min and counted under an epifluorescence microscope (Olympus BX51 with UPlan Apo 100×/1.35 oil objective). The fluorescence was observed using ViewfinderLite® (v. 1.0.125), coupled with StudioLite® (v.01.0.124) programs for viewing and data storage, respectively.

To determine the substrate utilization capabilities of SDB1, 150 μl of cell suspension (OD_{600}=0.1) in minimal salt medium was inoculated to a 96-well microplate (Biolog® GN2 microplate), the wells of which contained different carbon substrates. After incubation at 28℃ for 48 h, substrate utilization was assessed by measuring light absorbance at 595 nm with a microplate reader (model *V*max; Molecular Devices Corp., USA).

Sequencing and phylogenetic analysis

Genomic DNA of strain SDB1 was extracted using a genomic DNA Prep kit (Solgent, Korea), following the manufacturer's protocol. PCR was carried out in a 50 μl reaction mixture containing the extracted DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM primer 27F; 5'-AGAG TTTGATCMTGGCTCAG-3', 0.2 μM primer 1492R; 5'-G GTTACCTTGTTACGACTT-3', 5% DMSO (Sigma, USA), 0.1% BSA (BMS, USA) and 1 U of *Taq* polymerase (TaKaRa, Japan). The reactions were incubated in a thermocycler (Biometra, Netherlands) with an initial denaturation at 95℃ for 10 min, followed by 25 cycles of 95℃ for 1 min, 55℃ for 1 min and 72℃ for 2 min, followed by a final extension period at 72℃ for 3 min. The PCR products were purified (AccuPrep PCR Purification kit; Bioneer, Korea) and then used as templates for sequencing reactions (BI PRISM Big Dye Terminator v3.1 Cycle Sequencing kit; Applied Biosystems). The products were purified (Wizard MagneSil sequencing reaction clean-up system; Promega, Germany) and the sequences were determined using an ABI Prism 3100 Genetic Analyzer. The 16S rDNA sequences were edited with Chromas, version 2.0 (Technelysium, Australia). BLAST sequence similarity searches were performed (NCBI, USA). The closest relatives were initially identified from 16S rDNA sequences aligned with known sequences in the GenBank database, using the gapped BLAST tool and the Ribosomal Database Project databases. Sequences were assembled, aligned, and analyzed with the PHYDIT program (ver. 3.2). Then, a phylogenetic tree was constructed using the neighbor-joining method, based on Jukes & Cantor distance. The sequences determined in this study have been deposited in the GenBank database under accession number FJ774943.

Quantitative determination of As[III] oxidation

Minimal salt medium containing As[III] (5 mM) was added to an overnight culture of strain SDB1. After growth at 28℃, 150 rpm for 48 h, the cells were harvested by centrifugation (10,000×g, 20 min, 4℃) and washed with saline, then centrifuged again. The resultant cell pellet was resuspended in minimal salt medium to an optical density (OD_{600}) of 0.1. Aliquots of this suspension (100 μl) were dispensed into 500 ml Erlenmeyer flasks and As[III] was added to a final concentration of 5 mM. Following the adjustment of pH or the concentration of medium additives, depending on the purpose of experiments, cells were incubated at 28℃ and 150 rpm. All experiments were conducted in triplicate. Identical flasks without cells served as negative controls and any changes in these controls were subtracted from the determinations in the active flasks. At various time intervals, subsamples were retrieved and arsenic species were separated by anion-exchange chromatography, followed by atomic absorption spectrometry. For the speciation of the inorganic arsenic (As[III] and As[V]), strong anion-exchange resin (AG1-X8, 100~200 mesh chloride form, BioRad) was slurry packed into a glass column (7×1 cm) and kept moist with deionized water. The pH of the sample was adjusted to around 4.6 before passing over the resin, because this is the optimum pH for efficient speciation of As[III] and As[V]. At this pH, As[III] exists in solution as a neutral species, while As[V] exists primarily as an anionic species. The As[III] in the pH-adjusted sample would flow through the anion-exchange resin and the negatively charged As[V] would be retained. Then, As[V] was neutralized and eluted from the resin by a more acidic solution (0.5 M HCl) (Ficklin, 1983; Kim, 2001). The collected arsenic solutions were digested with HCl (2%, v/v). Subsequently, the amount of arsenic was determined using a SpectrAA 220 FS (Varian) atomic absorption spectrophotometer (AAS), equipped with a deuterium lamp background corrector and arsenic hollow cathode lamp. The wavelength was set at 193.7 nm.

As[III] oxidation with immobilized SDB1

SDB1 cells immobilized on alginate beads were prepared to take advantage of these free-living cells in the treatment of arsenic-contaminated wastewater. A cell suspension of SDB1 in minimal medium was mixed with a solution of sodium alginate (3%, w/v) to yield a final cell concentration of 4.0±0.5 mg cell dry weight per g wet weight of alginate beads. The prepared cell-alginate solution was extruded dropwise through a 10 ml syringe with a 19-gauge needle into $CaCl_2$ solution (0.2 M) using a syringe pump (KD Scientific,

USA) at a flow rate of 1 ml/min. The beads were soaked in the $CaCl_2$ solution for 45 min to ensure hardness, washed with sterile distilled water, and immediately used as an inoculum. The cells entrapped in the beads were estimated by the viable counting method. Five beads with entrapped cells were dissolved in 10 ml of 2% (w/v) sodium citrate solution (pH 6) (Elibol and Moreira, 2003), spread on agar plates, and then incubated at 28°C for 48 h. The colonies formed on the plates were counted and the number of viable cells was estimated. Additionally, aliquots of liquid medium were taken and spread on agar plates to monitor the number of cells that were released from the beads into the liquid medium during the incubation.

A stirred tank reactor containing 1 L of minimal medium plus yeast extract (0.005%) was inoculated with 10 g of SDB1-immobilized beads. Then, the prepared reactors received As[III] at five different concentrations (1, 2, 3, 5, and 8 mM). During the experiment, the pH and temperature of each reactor was maintained at pH 7~8 and 28°C, respectively. The As[III] concentration was monitored to determine the specific oxidation rate of As[III] at given concentrations of As[III]. The specific oxidation rate is defined as the amount of As[III] oxidized per unit amount of dry cells per hour. A reactor containing beads with no SDB1 cells and 2% glutaraldehyde served as a background control for As[III] oxidation.

The repeated use of alginate beads was also tested in a batch reactor containing 1 g of beads (4.0 mg dry cells) in 100 ml liquid medium containing 5 mM As[III] and 0.005% yeast extract. The inoculated reactors were incubated at 28°C in a rotary shaker (150 rpm) without pH control as opposed to the above experiment. At various time intervals, an adequate amount of the medium and beads were collected for the determination of As[III, V] and the cell numbers in the medium and on the beads. Once the pH of the medium dropped to around pH 4.5~3.5, the beads were collected by decantation and transferred into fresh medium containing 5 mM As[III] and the cycle was restarted.

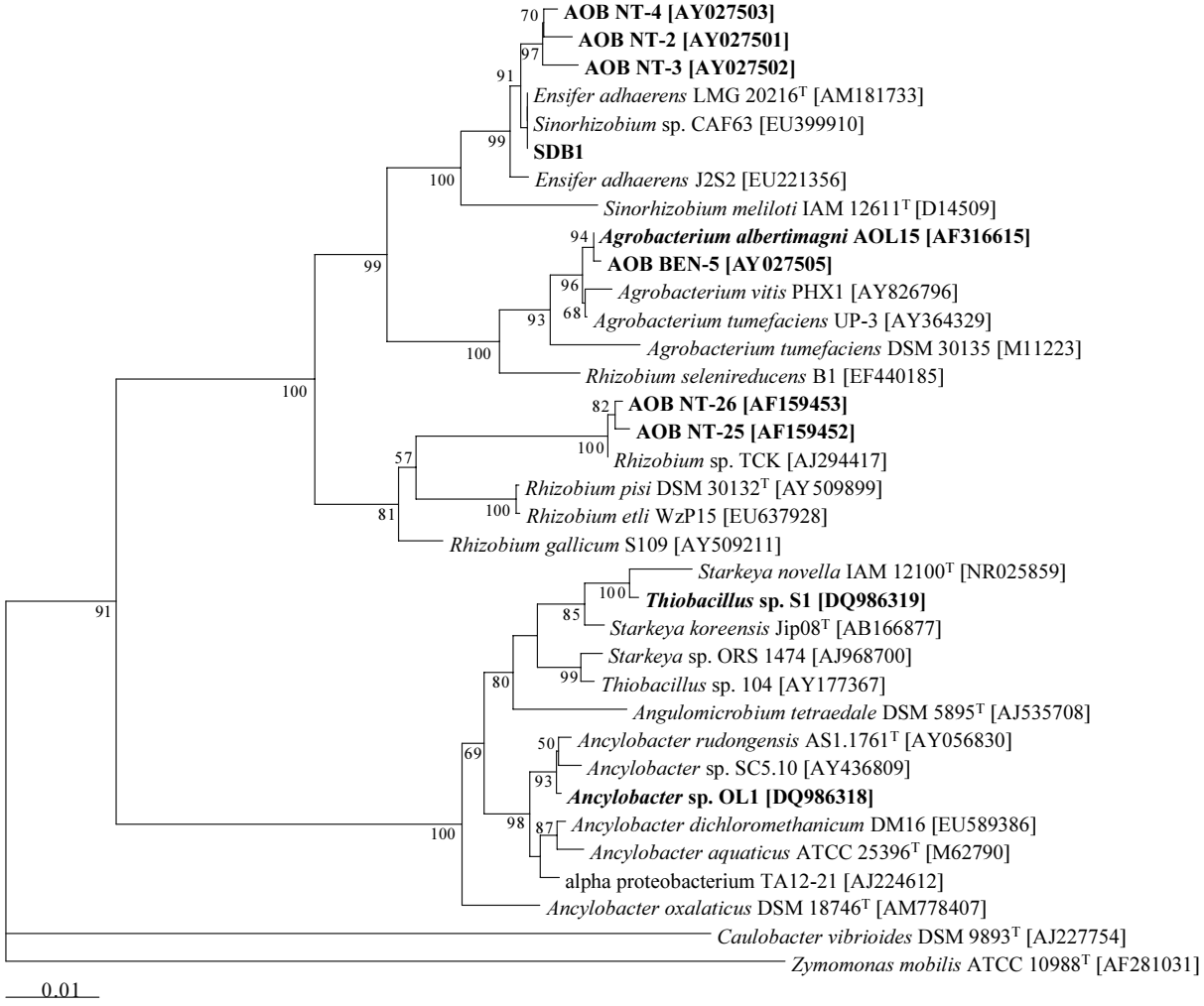

Fig. 1. Rooted neighbor-joining tree based on 16S rRNA gene sequences showing the relationship between chemolithotrophic As[III]-oxidizing bacterium SDB1 and closely related sequences retrieved from GenBank. Previously reported As[III]-oxidizing microorganisms are presented in bold typeface. Percentage numbers on each node are levels of bootstrap support (>50%) from 1,000 resampled datasets. *Caulobacter vibrioides* DSM 9893^T and *Zymomonas mobilis* ATCC 10988^T were used as outgroup. Bar, 1 substitution per 100 bases.

The control was tested to estimate abiotic loss in which As[III] was absorbed onto the beads.

Results and Discussion

Isolation and characterization of SDB1

With the given minimal salt medium and enrichment conditions, As[III]-oxidizing bacterial cultures were obtained from a Sangdong mine tailing sample. Among the pure cultures obtained as As[III]-oxidizers, strain SDB1 was selected, based on its high oxidizing activity. The strain is Gram-negative, non-motile, and short rod-shaped, typically 0.5×1.5 μm in dimension. The phylogenetic tree shows the position of SDB1 among other related bacteria, including previously identified As[III]-oxidizers (Fig. 1). SDB1 belongs to α-*Proteobacteria* and sequence similarity indicated 100% match (1,310 bp) with *Ensifer adhaerens* LMG 20216^T (AM181733) and *Sinorhizobium* sp. CAF63 (EU399910) which had not been considered as As[III]-oxidizers. Among the previously described chemolithotrophic As[III]-oxidizers, strains NT-2, NT-3, and NT-4 are the closest to SDB1 (99.3~99.5% similarity); all of these strains belong to the *Sinorhizobium-Ensifer* lineage (Santini *et al.*, 2002a). Other As[III]-oxidizers, such as NT-25 and NT-26, fall in *Agrobacterium/Rhizobium* lineage (Santini *et al.*, 2002a) and are less related to SDB1 (94.3~94.4% similarity). Most chemolithotrophic As[III]-oxidizers isolated to date, including strain SDB1, are members of the α-*Proteobacteria*, while the heterotrophic oxidizers are members of the β-*Proteobacteria*.

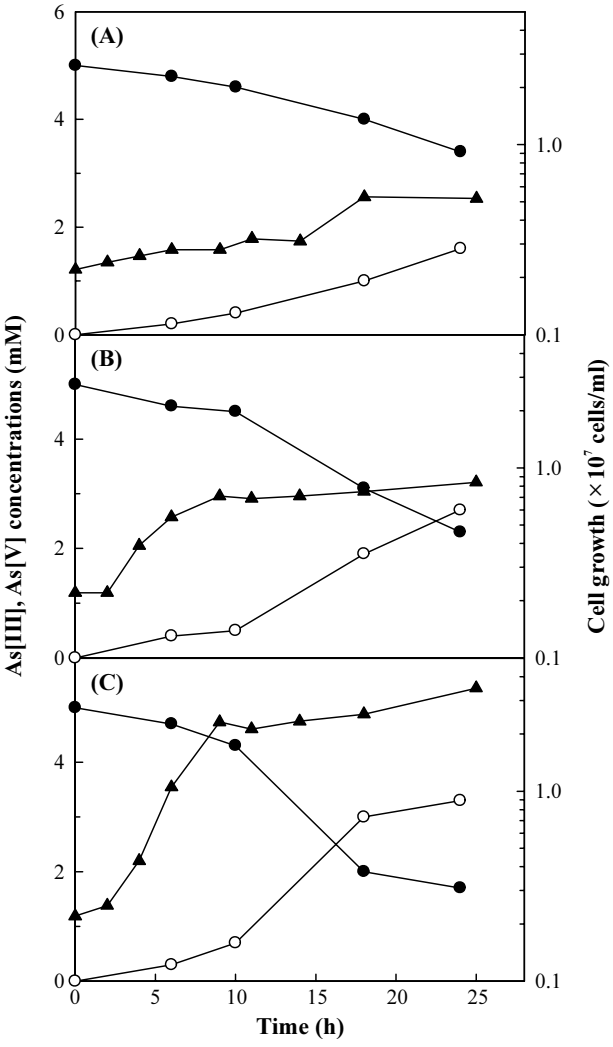

Fig. 2. Effects of yeast extract additions [(A), no addition; (B), 0.001%; (C), 0.005%] on As[III] oxidation (●, As[III]; ○, As[V]) and cell growth (▲) of SDB1. Chemical oxidation of As[III] was found to be marginal during the incubation.

Arsenite oxidation by SDB1

Since the first As[III]-oxidizing bacterium, *Bacillus arsenoxydans*, was described in 1918 (Green, 1918), numerous As[III]-oxidizing strains have been reported (Phillips and Taylor, 1976; vanden Hoven and Santini, 2004). In many cases, the bacteria isolated were heterotrophs, which generally oxidize As[III] for detoxification (Anderson *et al.*, 1992; Martin and Pedersen, 2004). Only a limited number of organisms have been reported as chemolithotrophic As[III]-oxidizers, using As[III] as an electron donor and CO_2 as a sole carbon source. Except for a few anaerobic As[III]-oxidizing strains including MLHE-1 (Oremland *et al.*, 2002) and a thermophilic As[III]-oxidizing chemolithotroph, AO5 (D'Imperio *et al.*, 2007), six As[III]-oxidizers isolated from gold mines (Santini *et al.*, 2002a) and four recently isolated As[III]-oxidizers (Rhine *et al.*, 2007) are documented as aerobic, mesophilic As[III]-oxidizing chemolithotrophs.

As shown in Fig. 2, SDB1 grew chemolithotrophically in minimal medium with As[III] and CO_2 as the electron donor and carbon source, respectively. However, the addition of yeast extract (YE) significantly stimulated As[III] oxidation, as well as the growth of SDB1. Based on direct cell counts, the doubling time (t_d) was clearly shortened in the presence of YE, from 9.8 h (0% YE), to 3.4 h (0.001% YE), and 2.1 h (0.005% YE). Specific As[III] oxidation rates were 0.014, 0.165, and 0.349 pmol As[III] oxidized cell^{-1} h^{-1} at 0, 0.001, and 0.005% YE, respectively. The enhancement of specific As[III] oxidation rates in the presence of YE indicated that SDB1 gained energy from the YE as well as As[III].

The stimulatory effects of YE on growth and As[III] oxidation by SDB1 prompted us to test its carbon utilization capabilities. Among the 95 carbon substrates on the Biolog® GN2 Microplate, SDB1 exhibited utilization of various carbohydrates (glucose, fructose, galactose, sucrose, and rhamnose) and amino acids (alanine, glutamic acid, histidine, and proline); however, it displayed little growth on organic acids (acetic acid, citric acid, D-gluconic acid, succinic acid, and α-ketoglutaric acid). These results indicated that SDB1 can be characterized as a facultative chemolithotroph in the sense that it can grow chemolithotrophically or chemoorganotrophically, similar to the previously identified As[III]-oxidizers, NT-2, NT-3, NT-25, NT-26 (Santini *et al.*, 2002a), OL-1, S-1, CL-3 (Garcia-Dominguez *et al.*, 2008), and MLHE-1 (Oremland *et al.*, 2002). In this study, As[III] oxidation seemed to occur roughly at the end of the exponential phase and the beginning of the stationary phase (Fig. 2). Similar results were reported by Ehrlich (1978) sugges-

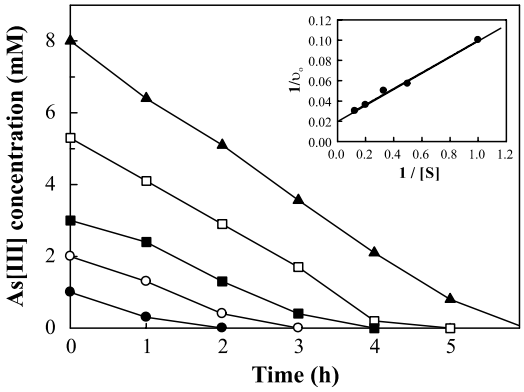

Fig. 3. Time course profile of As[III] oxidation by alginate-immobilized SDB1 at various concentrations of As[III] (●, 1 mM; ○, 2 mM; ■, 3 mM; □, 5 mM; ▲, 8 mM). Specific oxidation rates vs. As[III] concentrations followed saturation kinetics, and V_{max} and K_m was determined as 48.5 mM As[III] oxidized g^{-1} dry cell h^{-1} and 3.83 mM, respectively, based on the reciprocal plot of the saturation curve (Fig. inset, r^2=0.993).

ting that microorganisms used some of the energy available from As[III] oxidation for cell maintenance in the resting phase during mixotrophic growth.

Among the chemolithotrophic As[III]-oxidizing strains isolated by many researchers (Santini *et al.*, 2002a; Garcia-Dominguez *et al.*, 2008), NT-26 was the most efficient As[III]-oxidizer ever reported. NT-26 has a doubling time of 7.6 h, which is a little faster than that of SDB1 (9.8 h). However, NT-26 was so sensitive to pH that growth slowed significantly at pH below 7.4 and the doubling time increased to 15 h at pH 7.4 (Santini *et al.*, 2000). In contrast with NT-26, SDB1 is not so sensitive to low pH and its growth was not inhibited until the pH reached near 5 (data not shown). When NT-26 was grown in the presence of YE (0.004%), the doubling time was reduced from 7.6 h to 3.2 h (Santini *et al.*, 2002b), which is comparable to that of SDB1 (3.4 h with 0.001% YE and 2.5 h with 0.005% YE).

As[III] oxidation with immobilized cells

It is known that immobilized cell systems greatly improve the efficiency of bioreactor operation, compared with suspended cell systems, due to, for example, the maintenance of stable and active biocatalysts, high volumetric productivity, improved process control, tolerance to shock loadings, and reusability of the immobilized biocatalyst (Scott, 1987; Chen, 2007). In particular, the operation of immobilized-cell bioreactors is essential for maintaining high activities of slow-growing microbes, such as chemolithotrophs, to prevent the total washout of cells during long-term continuous operation (Chen *et al.*, 2005).

To test whether the alginate-immobilized SDB1 cells could perform efficient oxidation, a time-course profile of As[III] oxidation was monitored in a stirred tank reactor containing 1 L of minimal medium with various concentrations of As[III]. As shown in Fig. 3, As[III] was oxidized without any apparent lag period. The specific oxidation rates of As[III] (v_o) when plotted against As[III] concentration (S) followed saturation kinetics, indicating an arsenite oxidase activity. From a reciprocal plot of the saturation curve, parameters V_{max} and K_m were determined as 48.5 mM As[III] g^{-1} dry cell h^{-1} and 3.83 mM, respectively (Fig. 3; inset). During the experiment, the pH of the medium was maintained at pH 7~8, and the number of cells entrapped in the beads remained in the range of $4.4\sim9.2\times10^{12}$ cells/reactor; although some cells (<5%) were released into the liquid medium. Assuming that protein constitutes approximately 50% of dry cell mass, V_{max} and K_m values can be recalculated as 0.097 mM As[III] mg^{-1} protein h^{-1} and 0.047 mM, respectively. These values are comparable to the values (V_{max}; 0.042~0.077 mM mg^{-1} protein h^{-1}, K_m; 0.1423~0.2700 mM) obtained with recently isolated chemoautotrophic As[III]-oxidizers (Garcia-Dominguez *et al.*, 2008).

Table 1. Performance of a batch reactor containing alginate-immobilized cells of SDB1 during several cycles of oxidation-decantation period

Cycles	Duration[a]	As[III] oxidized[b] (%)	Cells retained in beads[c] (cells/g bead)
1st	40 h	73	1.02×10^{11}
2nd	40 h	83	1.50×10^{11}
3rd	26 h	72	4.05×10^{10}
4th	12 h	65	9.28×10^{10}
5th	18 h	81	1.14×10^{11}
6th	18 h	76	1.38×10^{11}
7th	20 h	78	1.06×10^{11}

[a] Time spent for the liquid pH dropped to near 4.0
[b] Initial conc. of As[III] was 5.0 mM
[c] Cells were estimated after the entrapped cells were dissolved into sodium citrate solution.

Prior to this study, the only described As[III] oxidation by immobilized cells was As[III] oxidation in 8-ml batch reactors with an alginate-immobilized ULPAs1 strain, which is a heterotrophic As[III]-oxidizer (Simeonova *et al.*, 2005). As[III] was oxidized more efficiently by the alginate-immobilized ULPAs1 than by the suspended cells. However, ULPAs1 required organic substances as carbon and energy sources for the oxidation; moreover, the As[III]-oxidizing activity was inhibited at As[III] concentrations as low as 1.33 mM. Unlike ULPAs1, the immobilized SDB1 cells were able to obtain energy from As[III] oxidation during chemolithotrophic growth and did not exhibit any inhibition at As[III] concentrations up to 8 mM (Fig. 3). When the efficiencies of As[III] oxidation were compared, the specific As[III] oxidation rates obtained with SDB1 (10.1~33.7 mM As[III] oxidized g^{-1} dry cell h^{-1}) were 10~16-times higher than those reported by Simeonova *et al.* (2005) with the ULPAs1 strain (1.0~2.1 mM As[III] g^{-1} dry cell h^{-1}). The difference between the two strains is probably related to the hypothesis that most of the heterotrophs oxidize As[III] to the less toxic As[V] merely as a means of detoxification (Ehrlich, 2002). However, because chemolithotrophic microorganisms, such as SDB1, use As[III] as their sole source of energy, those microorganisms should therefore have sufficient tolerance against As[III], and extensive oxidizing capabilities as well.

The immobilized SDB1 could be reused for several cycles

without significant loss of retained cells or As[III] oxidation capability (Table 1). In this experiment, the initial pH of the medium was adjusted to 7.0 and the pH was not controlled thereafter. Nevertheless, the % oxidation was 75.4%, on average, which seemed to be more complete when the medium pH was maintained near neutral. The SDB1-immobilized beads were transferred into fresh minimal medium containing 5 mM As[III] whenever the pH dropped to around pH 3.5∼4.5. During each oxidation period, a relatively small portion of the cells (0.5∼7.8%) in the beads was released into the liquid medium and 0.5∼1.6% of the total As[III] in the reactor was found absorbed on the beads. At similar cell density (40 mg dry cell weight L^{-1}), % As[III] oxidation with suspended cells was also estimated as 74.7% on average (n=7, data not shown) close to the value obtained with the immobilized cells. Therefore, the convenience of cell transfer and reusability of the beads seemed to be the merits of using immobilized cells over suspended cells for the As[III] oxidation.

Conclusions

In conclusion, SDB1 is one of the very few chemolithotrophic As[III]-oxidizing bacteria and grows on As[III] and CO_2 as rapidly as strain NT-26, which was the most rapidly growing chemolithotrophic As[III]-oxidizer reported to date. The As[III] oxidation capacity was enhanced substantially when the cells were immobilized in alginate beads and the pH of the medium was maintained in the range of 7∼8. The successful demonstration of repeated batch experiments supported the mechanical strength, as well as reusability of the immobilized cells, thereby making their use in the As[III]-contaminated wastewater treatment process technically and economically feasible for practical applications.

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