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Arsenite Oxidation by *Bacillus* sp. Strain SeaH-As22w Isolated from Coastal Seawater in Yeosu Bay

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Abstract

This study was conducted to evaluated seawater bacteria and their seasonal characteristics in the arsenic contaminated coastal seawater of Yeosu Bay, the Republic of Korea. Arsenite-oxidizing bacteria play an important role in the seawater of the arsenic contaminated bay, with a variety of arsenic resistance system (*ars*) genotypes being present during summer. Specifically, *Bacillus* sp. strain SeaH-As22w (FJ607342), isolated from the bay, were found to contain the *ars*B, *arr*A and *aox*R type operons, which are involved in arsenic resistance. The isolated bacteria showed relatively high tolerance to sodium arsenite (III; $NaAsO_2$) at concentrations as high as 50 mM. Additionally, batch seawater experiments showed that *Bacillus* sp. strain SeaH-As22w completely oxidized 1 mM of As (III) to As (V) within 10 days. Ecologically, the arsenic-oxidizing potential plays an important role in arsenic toxicity and mobility in As-contaminated coastal seawater of Yeosu Bay during all seasons because it facilitates the activity of *Bacillus* sp. groups.

Keywords : *ars* genotype, Arsenic-oxidizing bacteria, Arsenic resistance system (*ars*), *Bacillus* sp

1. Introduction

Arsenic compounds are ubiquitous in nature and commonly found in environmental samples involved in many different biological-chemical interactions, including arsenite-oxidation[1-3]. Arsenic compounds naturally occur in marine systems and are concentrated in a variety of ores[2, 4]. Arsenic in marine systems is predominantly in inorganic forms, such as arsenite (As (III)) and arsenate (As (V)), and organic forms, such as methylarsenate (MA), dimethylarsinate (DMA), trimethylarsine oxide (TMAO), teramethylarsonim ion (TMA), arsenocholine (AC) and arsenobetaine (AB). A study of arsenic in an aquatic system was conducted by Atkins and Wilson in 1927, and their results were similar to these reported for generally inconsiderable higher than those in seawater[4]. To date, seawater samples have provided the greatest number of marine arsenic compounds, although recent studies have revealed that terrestrial samples also contain many of these compounds. A significant percentage of the arsenic in surface water from marine environments is oxidized to arsenate, and is then methylated[2]. Previous studies have shown that the arsenic-resistant bacteria isolated from marine environments, are able to accumulate arsenic[6, 7]. Furthermore, arsenic cycling has been shown to respond to biological redox in seawater as well as in the demethylation assay.

It is well known that arsenic redox transformations by microorganisms are mediated by specific enzymes or respiratory chains[8]. The results of these studies suggest that arsenic species change in response to bacteria with the *ars* genotype; therefore, evaluation of the ecological role of such bacteria may lead to a better understanding of the molecular geomicrobiology. In addition, arsenic-resistant bacteria play an important role in controlling the speciation and cycling of arsenic in marine systems[3, 7] via a variety of *ars* mechanisms that affect the transformation between soluble and insoluble, as well as toxic and non-toxic forms of arsenic[9]. In addition, bioremediation has been achieved through the use of arsenic-resistant bacteria, with several of the mechanisms involved in the detoxification of arsenic having been identified using the *ars* operons of *Pseudomonas* sp[9]. The arsenite oxidase operons are generally found on metal-resistant *β-Proteobacterium*, such as strain ULPAs1. Rapid arsenite oxidation generally occurs via the bacterial chromosome of bacteria with the *ars* genotype, such as *Alcaligenes faecalis* and *Pseudomonas putida* OS-5 (AY952321)[10,

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11]. The potential detoxification processes of arsenic by microoganisms may be related to the *ars* gene, arsenic respiratory reduction genes (*arr*), or arsenite oxidation genes (a*ox* etc.)[8, 9, 11]. It has been reported that arsenite-oxidizing bacteria, such as *Pseudomonas putida* OS-3 (AY952322), which has *ars*B genes, and *Agrobacterium tumefaciens*, which has a signal transduction system composed of *aox*S-*aox*R-*aox*A-*aox*B-cytc2, have been isolated from arsenic-contaminated water and soil[8, 9, 10]. Also, arsenic resistance systems, such as ars ATPase, have ArsA/ArsB efflux/influx pumps to the inner membrane as well as change the As species and mobility within environment (soil/water/sediment), which play a significantly ecological role in allowing arsenic oxidizing/reducing bacteria to control biogeochemical cycles within As-contaminated environments. The above studies have suggested that arsenic-oxidases plays critical roles in arsenic detoxification processes via the *aox*A-*aox*B-*aox*C-*aox*D and *aox*S-*aox*R or *aox*AB systems, which explains the regulatory control of the *ars*R-*ars*D reader gene.

In this study, arsenic-resistant bacterial strains were isolated from the eastern coastal seawater of Yeosu Bay during spring, summer, autumn and winter, and then identified based on their *ars* genotype characteristics. In addition, arsenic resistant bacteria were used to confirm that As oxidization was occurring at Yeosu Bay. Arsenic resistance bacteria are able to influence As speciation in marine environments by detoxification and *ars* mechanisms in biogeochemical systems. The characteristics of the *ars* genotype and the arsenic detoxification by this indigenous bacterium are described in relation to biogeochemical environments.

2. Experimental Methods

2.1. Isolation and Growth of Arsenic Oxidizing Bacteria

Seawater samples were collected for isolating pure bacteria from the Yeosu Bay, Republic of Korea. To isolate arsenic-resistant microorganisms from the samples collected during spring, summer, autumn and winter (Fig. 1), 1 mL of seawater was added to MSB medium[8, 9] containing sodium arsenite ($NaAsO_2$) (Sigma, USA). After several transfers, isolated colonies were assessed for the amounts of As (III) present, with a single isolate selected for further study. The isolates (7th pass) were then cultures on media containing sodium arsenite at concentrations ranging from 0 to 26 mM (As (III): $NaAsO_2$; 0, 5, 10, 15, 20 and 26 mM). The isolated arsenic resistant bacteria were tested for their ability to oxidize As (III). The arsenite-oxidizing microorganisms collected during spring, summer, autumn and winter were cultured at 22°C in MSB (Stranier`Basal Medium, pH 7), with 1 mM D(+)-glucose as the carbon source. This medium was then autoclaved at 121°C for 25 minutes before use. Prior to inoculation, the bacteria were harvested (14 kg, 20 min). After incubation, colonies were removed using a sterile syringe, with anoxic conditions maintained by continuous flushing of the culture tube with 22°C O_2-headspace, with H_2 (10% in N_2) as the electron donor in lieu of As (III). The isolated colonies were again assessed for their levels of sodium arsenite ($NaAsO_2$), after which a single isolate was selected. Next, arsenic resistance experiments were carried out using the same arsenic concentrations used in the aerobic tests. For anoxic liquid cultures, MSB (Stranier`Basal Medium, pH 7) was first preincubated in the glove box of a chamber for at least 3 days. The arsenic resistant bacterium grew in MSB plus (1mM) D(+)-glucose.

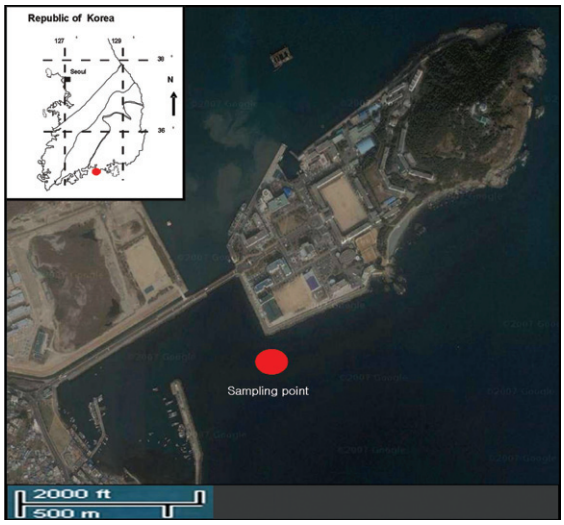

Fig. 1. Location of sample collection points in Yeosu Bay, Republic of Korea, during spring, summer, autumn and winter.

The temperatures and pH values were measured using an Orion model 290A portable meter, employing an Orion model 9170 electrode. To determine the total concentrations of arsenic, 25 mL of distilled water were added to each sample. The samples were then shaken, after which the water phase was filtered through a membrane PTFE filter (Whatman 0.45-μm pore size, 13 mm), with 1 mL of HNO_3 and 3 mL of HCl added to 0.25 g of the residue. The samples were again filtered through a membrane PTFE filter (Whatman 0.2-μm pore size, 13 mm), after which the arsenic concentrations were measured using a hydride-generation atomic absorption spectrophotometer (HG-AAS, Perkin Elmer 5100, Waltham, MA, USA), with a detection limit of 1 μmol/L[12]. All seawater of samples were collected from depth of 1 ~ 5 m. Analyses of heavy metals and arsenic were conducted according to standard procedures[13, 14].

2.2. PCR Amplification of the 16S rDNA and ars Genes

Bacterial genomic DNA was prepared from the bacterial cultures using standard methods[9, 15]. after which they were placed in a 1 mL microcentrifuge tube, with an individual colony of bacteria, to a final concentration of 10^8 CFU/mL. Each culture was then incubated overnight at 22°C, with intermittent shaking, and then placed in a 1.5 mL microcentrifuge tube, with 1 mL of TES (10 mL Tris-HCl, 50 mM EDTA, 10% sodium dodecyl sulfate) and 10 μL of proteinase K (50 mg l^{-1}), which was subsequently reacted in a 55°C tremulous cistern for 10-12 hours to digest the protein. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the reaction mixture. This solution was then manually mixed for 3 minutes and centrifuged at 14,240 g for 15 minutes, after which the supernatant was removed. This process was repeated 3 times, after which the supernatants were combined and mixed with 0.1 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100 % ethanol. The tubes were then shaken slowly, with the genomic DNA gently removed and then washed with 75 % ethanol, dried, and dissolved in TE (10 mM Tris pH 8.0; 1 mM EDTA pH 7.2). The purity of the DNA was then determined based on the ratio of the absorbance at 260 and 280 nm, with only DNA with a ratio of 1.7 to 2.0 used for subsequent experiments.

Table 1. Oligonucleotide primers used for amplification of the arsenic-resistant system genes Update sources: Chang et al.[8, 9, 11]

Amplified region[a]	Primer sequence	Tm[b] (°C)
SEL0904 Ars sense (universal)	5` - ATC ATG GCT CAG ATT GAA CGC - 3`	55
SEL1226 Ars Anti-sense (universal)	5` - T ACC TTG TTA CGA CTT CTA CCT - 3`	
AGL0609 ArsR sense	5` - ATC CAG CTC TTC AAA ACC - 3`	53
AGL0310 ArsR Anti-sense	5` - GTT TTT CAG CTT CAT AC - 3`	
AGL0725 ArsD sense	5` - ATG TGC TGC AGT ACC GCC GT - 3`	60
AGL0726 ArsD Anti-sense	5` - TAT TAC CAC CAC AGC AAC - 3`	
AGL0923 ArsA sense	5` - ACC CAC GCT TAG CAA TAT CAT CGA - 3`	55
AGL1230 ArsA Anti-sense	5` - TGA AAG TCT TCA TAT AGG TCT TCC - 3`	
AGL0929 ArsB sense	5` - GTG GAA TAT CGT CTG GAA TGC GAC - 3`	57
AGL0226 ArsB Anti-sense	5` - GGT AAT TTT CGG CCC CAA ATC G - `3	
AGL1117 ArsC sense	5` - TGC GGC ACT TCG TGA AAC AC - 3`	57
AGL0822 ArsC Anti-sense	5` - AAG TAT ATC CAG AAC CAC TT - `3	
AGL0507 ArsH sense	5` - ATG GAC CAG TTC CCA GAC - 3`	55
AGL1103 ArsH Anti-sense	5` - CTG ATT GGG GAT GGT GAA CA - `3	
AGL-*arr*A sense	5` - CTT TGA AGA AAT TCA AAC GTA CG - 3`	59
AGL-*arr*A Anti-sense	5` - TCA AAG TTT CGC TGT AAA ACT CA -3`	
AGL-*arr*B sense	5` - AAC ACG AAC GAC GGT ATT CAC TGG -3`	59
AGL-*arr*B Anti-sense	5` - ATA CCT TGC TCT GTG GAT CAT CTA -3`	
AGL-*aro*A sense	5` - GT ATG TCA CGT TGT CAA AAC - 3`	59
AGL-*aro*A Anti-sense	5` - TTA TAG AAC GTT GGA CAG AC -3`	
AGL-*aro*B sense	5` - ACT CTT CAC CTA TAT CGC CGA -3`	59
AGL-*aro*B Anti-sense	5` - TTC TCG TAA CCG AAC ATG ACA -3`	
AGL-*aox*A 0415 sense	5` - ATA AAG TAA GTC GTC GCA ATT - 3`	55
AGL-*aox*A 1106 Anti-sense	5` - GAG CTG AAT CTG AGG CAG ATT - 3`	
AGL-*aox*B 0125 sense	5` - TGC GGC TAC CAC GCC TAC ACC -3`	59
AGL-*aox*B 1022 Anti-sense	5` - TGC CCC AGG TGT TTT CGT AAC -3`	
AGL-*aox*C 0710 sense	5` - TGG CAT CGG GAG GAG GAT -3`	57
AGL-*aox*C 1118 Anti-sense	5` - TGA CCT GGG AAG TAT GGC -3`	
AGL-*aox*D 1025 sense	5` - ATA TGC CAT TGC TAT TTG -3`	57
AGL-*aox*D 1124 Anti-sense	5` - TAC TTG GCT CCA GCC AAT -3`	
AGL-*aox*S 0507 sense	5` - TTC ATC ATC TCC GAA CGC GTA TTG -3`	59
AGL-*aox*S 0705 Anti-sense	5` - TTG CGT TTA GCA CGA GGT TCA AAA -3`	
AGL-*aox*R 0725 sense	5` - AAT CGC TCA TCC AGC GAC TTT CGC -3`	57
AGL-*aox*R 1103 Anti-sense	5` - TTG CGT CCT CGC CAA GCG TAC TGA -3`	

[a] GenBank sequence similarity was evaluated using the BLAST program. (BLAST; National Center for Biotechnology Information [http:www.ncbi.nlm.nih.gov].

[b] Tm, annealing temperature used in PCR cycle.

Bacterial genomic DNA was isolated from the cultures using standard methods[9, 15], and then placed in a 1 mL microcentrifuge tube with a single appropriate colony. The 16S rRNA gene was then amplified using oligonucleotide primers designed based on the published sequence. The sequences of each of the primers used to amplify the arsenic resistance gene are shown in Table 1. The PCR amplification was conducted by subjecting the reaction mixture, in a final volume of 50 μL, which contained 0.5 μg genomic DNA and 10 pmol of each of the primers, to 35 cycles of denaturation for 5min at 94°C, annealing at 55°C for 1minute, and extension at 72°C for 2 minutes, followed by an additional extension for 7 minutes at 72°C. Ars primers were designed to obtain a T_m of approximately 55 to 60°C, with specificity obtained by aligning the sequences using the BLAST Search Tool (BLAST; National Center for Biotechnology Information [http:www.ncbi.nlm.nih.gov]). The PCR was conducted in a Mastercycler Gradient (Eppendorf, Germany), with the PCR products analyzed in 0.7 to 1.5 % agarose gels (Sigma, USA). The ars primers were designed for the *ars*R, *ars*D, *ars*A, *ars*B, *ars*C, *ars*H, *arr*A, *arr*B, *aro*A, *aro*B, *aox*A, *aox*B, *aox*C, *aox*D, *aox*S, and *aox*M genes of the ars operon. The primer sequences included targeted regions for the specific genes (Table 1).

2.3. 16S rDNA Sequencing and Phylogenetic Analysis

The nucleotide sequences were determined using the dideoxy-chain termination method, with a PRISM Ready Reaction Dye terminator/primer cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CT, USA). The oligonucleotide primers included in this investigation were specific for the 16S rDNA gene. Sequencing was conducted in a reaction mixture with a final volume of 6 μL, which contained 15 to 50 ng of the PCR product and 1 pmol of each primer. The samples were analyzed using an automated DNA sequencer (Model 3100; ABI PRISM Genetic Analyzer System Profile, USA), with the 16S rDNA sequencing results compared

with sequences from the NCBI database. The BLAST algorithm, integrated with the Vector NTI Suite v5.5.1 (InforMax, USA), was used to determine the sequence homologies. Database sequences with fewer than 1,500 nucleotides were excluded from the phylogenetic analysis. The full 16S rDNA gene sequences were compiled using the Vector NTI Suite v5.5.1 (InforMax, USA). Phylogenetic analysis was conducted using the neighbor-joining method[16].

2.4. Arsenic Oxidation and Reduction Assays

To test the ability of the strains to oxidize arsenite and/or reduce arsenate, the isolates were inoculated in 250-mL glass flasks containing 60 mL of seasalts (Sigma, USA) and 1 mM sodium arsenite. For the batch tests, MSB medium, supplemented with 1 mM sodium arsenite, was inoculated with each strain (10^7/CFU), with the concentration of As (III) in the culture then determined. All experiments were conducted in triplicate, using 60-mL Erlenmeyer flasks; the bacteria were incubated aerobically at 22°C for 10 d, with shaking (170 rpm). For controls, sterile medium (i.e., un-inoculated), with 1mM sodium arsenite, was incubated under the same conditions. Periodically, 2 mL samples were taken, and the cell density measured and the arsenic speciation determined. These samples were centrifuged at 14,240 g for 10 min, decanted, and then stored at 4°C prior to arsenic analysis[12]. The oxidation of As (III) to As (V) throughout the incubation period was monitored by determining the concentration of As (III) based on the concentration of As (V), using a silica-based strong anion cartridge (LC-SAX SPE, Supelco)[17]. The As (III) and As (V) concentrations were then measured using a hydride generation atomic absorption spectrophotometer (HG-AAS, Perkin Elmer 5100). All analytical measurements were performed in duplicate.

3. Results and Discussion

3.1. Isolation and Characterization of Arsenic Oxidation by Seawater Microbial Strains

Arsenic was not found in the coastal seawater from Yeosu bay in every season; spring (AsIII; 15.4 μg/L$\pm$0.1, AsV;4.9 μg/L$\pm$0.1), summer (AsIII; 9.9 μg/L$\pm$0.1, AsV;12.9 μg/L$\pm$0.1), autumn (not detected) and winter (not detected) (Fig. 1). The concentration of arsenic required to influence biological-chemical interactions is known to range from 0.5 to 3 μg/L, with a mean of about 1.7 μg/L.[1] Additionally, arsenic is a suspected carcinogen and As-related cancer of the skin may occur following environmental exposure, such as the harvest of fish products from water containing arsenic at 0.0175 μg/L (at the 10^{-6} cancer risk level[18]. Arsenic contamination has a complex seawater biogeochemistry, with important implications of its toxicity towards organisms, including humans[19]. The arsenic concentrations observed during spring and summer in this study were high.

Four indigenous arsenite oxidizing *Bacillus* sp. strains (spring; SeaH-As11w (FJ607341), summer; SeaH-As22w (FJ607342), autumn; SeaH-As33w (FJ607343) and winter; SeaH-As44w (FJ607344)) isolated from the bay were capable of oxidizing arsenite (Table 2). Two of these isolates, strain SeaH-As11w and SeaH-As22w, oxidized greater levels of As (III) to As (V) than the other 2 strains. As shown in Table 2, during the culture of strains SeaH-As11w and SeaH-As22w in arsenite-containing artificial seawater medium, a lag in growth occurred during the first 3 days, after which both isolates entered a typical exponential growth phase (doubling times of 48 and 24 hs, respectively). The concentration of sodium arsenite (III) also slowly decreased during the initial 3-4 days, and then rapidly decreased until the end of cultivation. During the growth of SeaH-As11 and SeaH-As22w in MSB medium containing 1000 μM of As (III), almost 100% of the initial arsenite was decomposed within 9 -10 days (Fig. 4). Takeuchi et al.[7] evaluated arsenic resistance and removal in nine bacterial strains of marine and non-marine organs. They found that up to 2290 μg As g^{-1} accumulated in cells incubated in artificial seawater medium containing 5 mg As 1-1 of As (V). The results of studies conducted by Oremland et al.[3] and Sanders[2] suggested that arsenite-oxidizing systems may be an inducible form of As-detoxification in marine systems.

Table 2. Bacterial strains, their identification, *ars* genotypes, and tolerance to arsenic under facultative anaerobic conditions. Each strain was analyzed for the presence of the *ars*R, *ars*D, *ars*A, *ars*B, *ars*AB, *ars*C, *ars*H, *arr*A, *arr*B, *aox*A, *aox*B, *aox*C, *aox*D, *aox*S, *aox*M, *aro*A and *aro*B genes within its genomic DNA using a PCR, as described in Materials and Methods

Strain or iIsolat	isolate accession no.	16S rDNA Similarly(%)to known bacteria	As (III) Tolerance (mM);day	*ars* Genotype[b]	Doubling time (h)	Arsenic measurements in sample points ($\pm$ standard diviation)		
Season[c]						As (III)	As (V)by difference	As total
spring SeaH-As11w	FJ607341	99 / *Bacillus* sp.	40 ; 20	*nd*	48	15.4 μg/L$\pm$0.1	4.9 μg/L$\pm$0.1	20.3 μg/L$\pm$0.1
summer SeaH-As22w	FJ607342	99 / *Bacillus* sp.	50 ; 10	*arsB*$^+$ *arrA*$^+$ *aoxR*$^+$	24	9.9 μg/L$\pm$0.1	12.9 μg/L$\pm$0.1	22.8 μg/L$\pm$ 0.2
autumn SeaH-As33w	FJ607343	99 / *Bacillus* sp.	25 ; 12	*aroB*$^+$	28	ND	ND	ND
winter SeaH-As44w	FJ607344	99 / *Bacillus* sp.	30 ; 10	*aoxC*$^+$	30	ND	ND	ND

[a] Arsenic concertration in mM test/survival (days).
[b] *ars* resistance relationship analyzed for ars genotype: +, positive PCR product generated.
[c] Test strains: Candidate YeoSu sea for SRAHERO Test-bed construction of Korea.
Nd; Not detected.

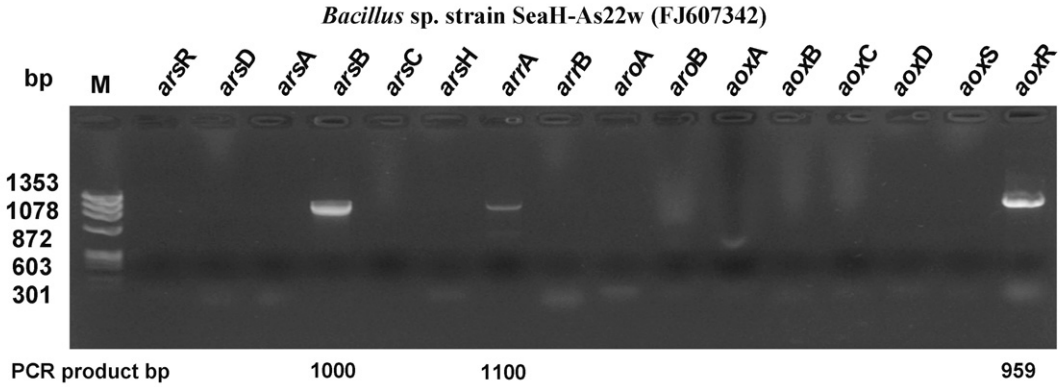

Fig. 2. Agarose gels (0.7 to 2.0%) showing PCR products amplified from the genomes of several arsenite-resistant bacterial strains. Lanes M: Lambda DNA/HindIII size mark (Promega, USA). The strain shown is SeaH-As22w (FJ607342).

Ecologically, arsenic-oxidizing potential plays an important role in As-detoxification/mobility in coastal seawater areas, as it facilitates marine-biogeochemical cycling activity of the *Bacillus* sp. group[19, 20]. However, further studies are required to understand the roles played by the arsenic resistant system in bacterial respiration within different Bays.

3.2. Sequencing of 16S rDNA and Phylogenetic Analysis

The phylogenetic relationships were analyzed based on the partial 16S rDNA sequences obtained from the 4 arsenic-resistant isolates, with Bacillales as an out-group, revealed all orders of the arsenic resistant Beta-proteobacteria. Four indigenous seawater bacteria strains were isolated; 2 As (III)-oxidizing bacteria and 2 arsenic resistant bacteria. According to our results, most isolates, except SeaH-As11w (FJ607341), SeaH-As22w (FJ607342), SeaH-As33w (FJ607343) and SeaH-As44w (FJ607344), were closely related to *Bacillus* sp. The G + C contents of the 16S rRNA were as follows: SeaH-As11w; 53 %, SeaH-As22w; 54 %, SeaH-As33w; 53 %, and SeaH-As44w and 55 mol% (Fig. 3, Table 2). Phylogenetic analysis of the 16S rRNA gene sequence (1,472 bp) of the SeaH-As22w strain, isolated during summer, revealed that its sequence was 99 % homologous with that of *Bacillus* sp. (Fig. 3, Table 2). The nearest known phyogenetic relatives to *Bacillus* sp. strain were Bacillus infemus strain TH-23 (U20385) and Bacillus infemus TH-22 (U20384) of Yeosu Bay *Bacillus* sp. was also previously shown to be comparable, with 90% similarities[19].

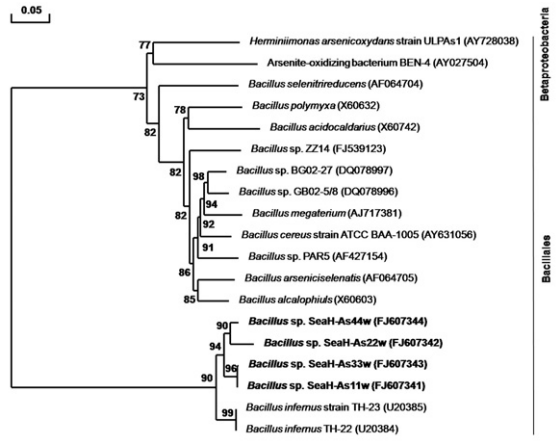

Fig. 3. Phylogenetic tree based on the 16S rDNA sequence, showing the positions of the arsenite-oxidizing bacterial isolates SeaH-As22w (FJ607342) and Bacillales. The tree was constructed from a matrix of pair-wise genetic distances using the neighbor-joining tree method. The phylogenetic data were obtained by aligning the different arsenic-resistant bacterial sequences in the Search Tool (BLAST; National Center for Biotechnology Information [http:www.ncbi.nlm.nih.gov]) using standard parameters. The scale bar represents 0.05 substitutions per 100 nucleotides within the16S rDNA sequence.

3.3. *ars* Genotype Profiling of Arsenite-Oxidizing Bacteria

The arsenic resistance system has been described based on an evaluation of the oxidation in the presence of bacteria containing the arsR, -D, -A, -AB, -B, -C, -H, *arr*A, *arr*B, *aro*A, *aro*B, *aox*A, *aox*B,

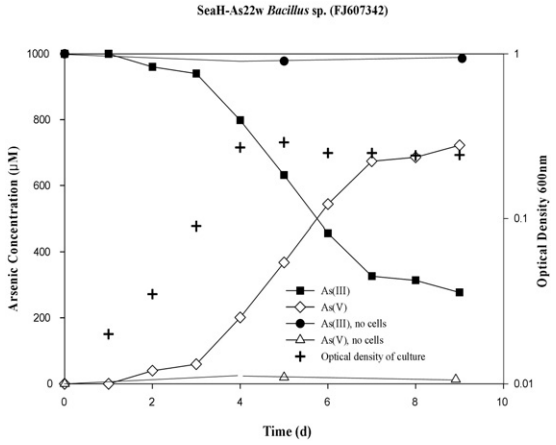

Fig. 4. Arsenite oxidation, arsenate reduction, and culture density under facultative anaerobic conditions for SeaH-As22w *Bacillus* sp. (FJ607342). The experiments were performed independently, in triplicate, in the batch modes, with a working volume of 60 mL and temperature of 22°C. Each data point represents the average value for the readings from triplicate experiments.

*aox*C, *aox*D, *aox*S and *aox*R genes[8, 9, 21, 22]. The resistance of the strains differed greatly depending on the genes they carried. For example, strain SeaH-As22w (FJ607342) encoded the *ars*B, *arr*A and *aox*R genes (Fig. 2, Table 2). Interestingly, the gene encoding the *aox*R was found in an isolate of *Bacillus* sp. strain SeaH-As22w (FJ607342). The results of the genotyping analysis are shown in Table 2 and Fig. 2. This oxidation reaction may be considered an arsenic detoxification process. The results of a batch test showed that SeaH-As22w *Bacillus* sp. (FJ607342) completely oxidized 1 mM of arsenite to arsenate within 9-10 days (Fig. 4). This is the first known report of a member of an arsenic-resistant Bacillus sp. detoxifying arsenic in a seawater ecosystem in the presence of an ars genotype profile. Studies conducted by Sanders[2] and Phillips and Taylor[23] revealed that the microbial demethylation and oxidation of methylated arsenic in seawater, and the oxidation of arsenite to arsenate by *Alcaligenes faecalis* were important mechanisms responsible for detoxification of marine systems. In addition, the results of a study conducted by Oremland et al.[3] were comparable to those of a previous investigation on the biological oxidation of arsenite in seawater. This arsenic-oxidizing bacterium is capable of changing the arsenic species, as well as As-toxicity and mobility in the coastal areas of the bay; therefore, it is a very important ecological *ars* genotype because it allows control of the biogeochemical cycle of seawater and the transformation of arsenic in marine environments[21, 24]. Furthermore, the isolates were found to be able to survive in growth medium containing excessively high arsenic concentrations, which indicates their arsenic resistant and suggests they would be useful for the bioremediation of As contaminated seawaters in the Republic of Korea.

4. Conclusions

Herein is described the first investigation of As-oxidizing bacteria and *ars* genotype profiling based on the presence of an arsenic-resistant system in bacteria from seawater. One of four isolates (SeaH-As22w (FJ607342)), which was isolated during summer, was determined to be *Bacillus* sp. This strain was found to have arsenic-oxidizing potential, which plays an important role in the marine-biogeochemical cycling activity. Therefore, this organism could be used to enhance the arsenic detoxification of coastal seawater in the bay during summer because it facilitates the biogeochemical cycling activity of *Bacillus* sp. groups in marine environments. However, further studies are required to understand the roles played by the *ars* genotype in bacterial respiration during different seasons.

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