Journal of Applied & Environmental Microbiology, 2015, Vol. 3, No. 4, 101-105
Available online at http://pubs.sciepub.com/jaem/3/4/2

DOI:10.12691/jaem-3-4-2

16S rDNA Identification of Arsenite-Oxidizing Bacillus sp. Isolated from Arsenic Contaminated Surface Water Uttar Pradesh, India

Bilal Ahmad Tantry[1,2,*], Divya Shrivastava[2], Ibrahim Taher[1], Mudasar Nabi Tantry[3]

[1]Department of Microbiology, college of medicine, Aljouf University, Sakaka,Saudi Arabia
[2]Department of life science, Jaipur National University, Jaipur India
[3]Department of Clinical Biochemistry, Kashmir University, Srinagar, India
*Corresponding author: batantry@ju.edu.sa

Abstract Three novel Arsenite oxidizing bacteria have been isolated from arsenic contaminated rivers from India. Samples were collected from the three districts of Uttar Pradesh (UP) viz., Ghaziabad, Moradabad and Gorakhpur Uttar Pradesh, India. All the three stains were observed as gram positive, aerobic, spore forming and rod shaped. The phylogentic analysis using 16S rDNA identification sequence showed that three isolates were belonged to the family of Bacillaceae and were closely related to Rummeliibacillus sp. (98%), Brevibacillus agri strain (88%) and Bacillus thuringiensis (99%). All the three strains are clearly distinguished from the related Bacillus species by its biochemical characteristics as well as the phylogentic relationship, therefore the three strains represent the novel sp. of the genus bacillus for which we proposed the names viz., Rummeliibacillus sp. AOBG-1, Brevibacillus sp. AOBMI-1 and Bacillus sp. AOB-GI-1 respectively. The GenBank accession numbers for the 16S rRNA gene sequence of the strains are KP295449, KP314033 and KP 308382.

Keywords: *Arsenite oxidizing bacteria, Firmicutes, bacillus sp., surface water and arsenic*

Cite This Article: Bilal Ahmad Tantry, Divya Shrivastava, Ibrahim Taher, and MudasarNabi Tantry, "16S rDNA Identification of Arsenite-Oxidizing Bacillus sp. Isolated from Arsenic Contaminated Surface Water Uttar Pradesh, India." *Journal of Applied & Environmental Microbiology*, vol. 3, no. 4 (2015): 101-105. doi: 10.12691/jaem-3-4-2.

1. Introduction

Arsenic (As) contamination in surface and ground water in the environment has been reported world wide [1,2,3]. As exists in four oxidation states viz,. -3, 0,+3 and +5. Arsenite As (III) is predominant in sub surface waters (anoxic reducing environments), it is more harmful, soluble, mobile and toxic than Arsenate As (V) [4,5]. The primary source of As in surface waters is release of As from arsenic rich sediments, geochemical factors, use of insecticides, phosphate fertilizers and mining, industrial processes etc [6]. The permissible limit of As in drinking is 10 ppb (0.01mg/L) as per provisional guidelines of WHO [5]. As level surface water in the districts of Uttar Pradesh is far exceeding the permissible limit of WHO [7,8]. large number of states of in India rely on As contaminated surface water for drinking and irrigation purpose [9]. In recent years, prokaryotes display the potential to remove the toxic form of As from aqueous medium [10]. Diversity of As resistant prokaryotes and their genes coding for As resistance have been found in various Arsenic contaminated environments [11]. The presence of ars operon on plasmids and chromosomes of As resistant bacteria explains the mechanisms of microbial arsenic oxidation [12]. Arsenite oxidation has been found in various prokaryotes viz., Agrobacterium, Pseudomonas and Alcaligenes [13,14,15]. Some prokaryotes use Arsenite as a sole electron donor as in lithotrophs, while as heterotrophic As-oxidizing bacteria use arsenite oxidation as a detoxification mechanism. As (III) oxidiation is catalyzed by arsenite oxidase enzyme [16,17]. Prokaryotic arsenite oxidation influence environmental As cycles better understanding of As resistant prokaryotic diversity will lead to the improvement of bioremediation process of As [18]. Microbial oxidation is very fast as compared to the chemical oxidation process of As. The As resistant communities in surface water of UP remain largely un studied. In our study we carried out the isolation of arsenite-oxidizing bacteria present in As contaminated surface waters (lakes and rivers) located in various districts of Uttar Pradesh India. The results revealed that all the three arsenite-oxidizing bacteria isolated were different to those previously identified.

2. Material and Method

2.1. Study Sites and Sampling

The study was conducted in three districts viz., Gorakhpur (26° 45′ 31.68″,83° 22′ 10.92), Moradabad (28° 49′ 48″, 78° 46′ 48″) and Ghazibad (28° 40′ 12″, 77° 25′ 12″) of Uttar Pradesh. Surface water (lake) sample

collection was from grorakhpur, other water samples were collected from Ghaziabad (Hindon river) and Moradabad (Ram Ganga River). These rivers are highly polluted because of man made activities in the vicinity. To our knowledge, till now no detailed study has been designed to investigate the As level in these rivers. The samples of lake and river water were collected in polypropylene sterile bottle (250ml) and in each sample sodium arsenite (sigma Aldrich) was added and then the sample bottles were sealed, dated and transported to the laboratory in ice box. The samples were incubated for 7 days in dark at 25°C [19].

2.2. Culture Media

An aliquot of 100 µl of water sample was spread on plates containing chemically defined medium (CDM) the compostion of the media (g l^{-1}) viz., 8.12mM $MgSO_4$, (Sigma-Aldrich), 0.0574mM K_2HPO_4 (Sigma-Aldrich), 9.5mM $NaHCO_3$ (Sigma-Aldrich USA), 18.7mM NH_4Cl, 0.012mM Fe_2SO_4 (Sigma-Aldrich USA), 44.6 mM Na-Lactate (Sigma-Aldrich USA), 7 mM Na_2SO_4 (Sigma-Aldrich USA), 0.457mM $CaCl_2$ (Sigma-Aldrich USA), 2mM $NaAsO_2$ (Sigma-Aldrich USA) and 15g Agar. The final pH of CDM was 7.2 and media was sterilized by autoclaving at 120°C for 15min. the growth of bacterial colonies was observed after 48hr of incubation at 35°C, isolation was carried out in triplicates.

2.3. Isolation of As (III) Resistant Bacteria

The Triplicate sample cultures were sequentially streaked on As (III) supplemented CDM to get the pure colonies of arsenite oxidizing bacteria. The resulting enriched cultures were plated on the solidified R2A agar media (0.5/L glucose, 1g/L tryptone, 0.3g/L yeast extract, 0,3g KH2PO4, 0.05g MgSO4 and 1% agarose. cultures were incubated at 35°C for 48 h. The pure cultures were preserved in R2A agar medium.

2.4. Phenotypic Characterization

Isolated bacterial strains were cultured on R2A agar and were examined for morphological characteristics. Gram staining was done, and observed under light microscope (Leica DMD 108 digital microscope). Biochemical characterization was performed in terms of carbohydrate utilization (starch, dextrose, sucrose, maltose, rhamnose, fructose, lactose, citrate. Oxidase and catalase, IMVIC (Indole, methylred, vogues proausker and citrate) and hydrolysis of gelatin, casein, starch and esculin were done [20].

3. Results

3.1. Production of Biomass Selected Arsenic Resistant Bacteria in Different Medium

The three strains were inoculated in (50ml) of R2A broth and R2A broth containing 0.2% sodium arsenite at optimum pH 7.0 and temperature (35°C). The cultures were incubated and growth was observed after 48 hrs and then biomass was weighted (Figure 1) [23]. The biomass in R2A broth was found highest in the strain (AOB-2) AOB-MBI (392.4 mg/50ml) followed by the (AOB-4) AOB-G-I (387.6 mg/50ml). (AOB-5) AOB-GI-I (188 mg/50ml). while as the biomass production in arsenite supplemented media was found highest in the AOBG-I (167.2 mg/50ml), AOBMI-I (167 mg/50ml), AOB-GI-I (159.3 mg/50ml).

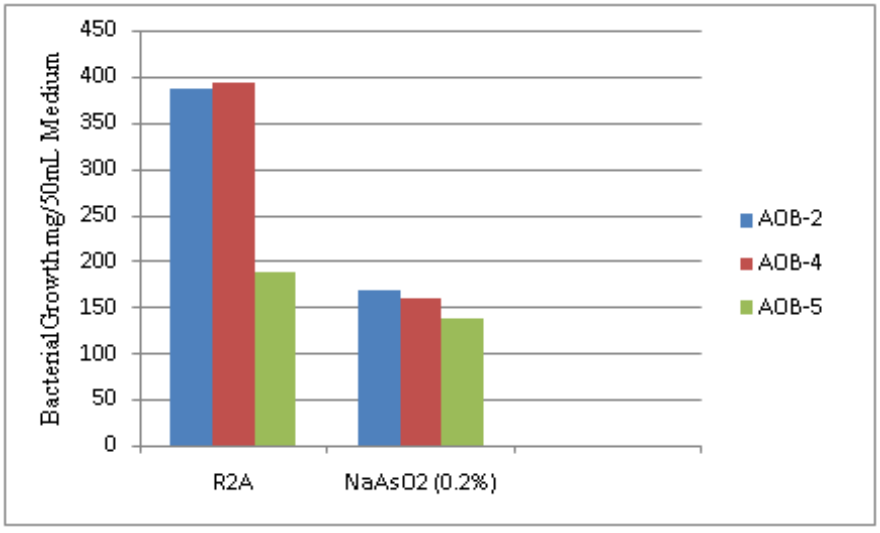

Figure 1. Production of biomass selected arsenic resistant bacteria in different medium

3.2. DNA Isolation, DNA Sequencing and Phylogenetic Analysis

Total DNA was isolated from overnight broth using the Invitrogen kit USA, according to instruction mentioned in protocol. The 16S rDNA of the bacteria was amplified by PCR using universal primers 8f 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 926r 5′-CCG TCA ATT CCT TTR AGT TT-3′ [24]. PCR amplification was performed in ABI 9700 Thermal cycler (USA) in a 50 µL volume containing 1 µL DNA, 1 µL of each primer, 2 µL of dNTPs, 1.5 µL of $MgCl_2$, 2.5µL of PCR buffer (trisHCl 100Mm), 1 µL of DNA polymerase, and adding of nuclease free water to a final concentration of 50 µL. After the amplification of the 16S rDNA, the samples were electrophoresed on 1% Agarose gel using buffer 1X

TAE. ladders were loaded along the sides of the samples. For analysis 5µl of PCR product was taken and 10µl of 6x Gel loading dye was added and loaded to the Agarose gel. For purification of the PCR products an inhouse Gel elution kit has been used. The required band is excised from the gel and re suspended in Buffer which contains components for dissolution of the gel keeping the DNA intact. After dissolution of the gel the solution loaded onto a purification column. Wash buffer is used to get rid of all the salts and other contaminants. Pure DNA is eluted from the column in 10mM Tris buffer or Nuclease free water. DNA sequencing analysis was performed by using ABI 3730XLS DNA analyzer and ABI Big dye Terminator kit. The resulting 16SrRNA sequences of the As-resistant bacteria were analyzed by BLASTn. The phylogenetic trees was constructed by the neighbor- joining distance method,using program phylogenetic.fr (Figure 2) [25,26].

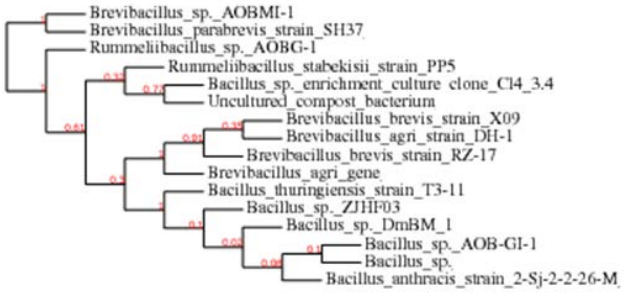

Figure 2. Phylogenetic tree of the three most arsenic resistant bacteria based on 16s rRNA sequences

3.3. Nucleotide Sequence Accession Numbers

The sequences determined in this study for strains Rummeliibacillus sp. AOBG-1, Brevibacillus sp. AOBMI-1 and Bacillus sp. AOB-GI-1 have been deposited in Gen- Bank under the accession numbers KP295449, KP314033 and KP 308382 respectively.

The three new arsenite oxidizing bacteria AOBG-1, AOBMI-1 and AOB-GI-1 were isolated from As contaminated surface water. The characterization of the isolates was done on the basis of morphology and gram staining characteristics. All the three starins were gram positive, oxidase negative, catalase positive, rod shaped, aerobic. The optimum growth temperature and pH was 35°C and 7.0 respectively. Biochemical characterization was performed in terms of H_2S formation, Hydrolysis of (esculin, casein, starch and gelatin), carbohydrate utilization (starch, maltose, lactose, glucose, galactose, N-acetylglucosamine, arabinose, rahamnose, sorbitol, xylose and trehalose. (Table 1) shows the biochemical test of all the three strains.

Table 1. Phenotypic and Biochemical Characterization of the 03 Arsenite oxidizing bacterial isolates

Biochemical test	AOBG-I	AOBMI-1	AOB-GI-1
Gram stain	+ve	+ve	+ve
0.8% NaCl	+	+	+
Growth at pH(7.0)	+	+	+
Growth at (35°C)	+	+	+
Cell shape	Rod	Rod	Rod
Spore formation	+	+	+
Oxidase	-	-	-
Catalase	+	+	+
Motility	+	+	+
Methyl red	+	+	-
Voges-proskauer	-	-	+
Indole	+	-	-
Urease	-	-	-
H_2S formation	-	-	-
Citrate utilization	-	-	-
Hydrolysis of			
Esculin	-	ND	ND
Casein	-	+	+
Starch	+	+	+
Gelatin	ND	+	+
Acid production			
Sucrose	-	+	+
Maltose	-	+	+
Lactose	+	+	+
Galactose	-	-	-
N – acetylglucosamine	-	+	+
Arabinose	-	-	-
Rahamnose	-	-	-
Sorbitol	-	-	-
Xylose	-	-	-
Trehalose	-	+	+

+(Positive), - (Negative), ND (Not Done).

The ability of the isolates to oxidize arsenite were tested using qualitative potassium permanganate ($KMnO_4$) method [21]. Each isolate resistant to arsenite was inoculated in R2A broth with final concentration of 900 µM sodium arsenite and then shaken on rotatory shaker at 180 rpm for 7 days at 30°C. 1 ml of cultured isolate was added to a 2 ml of glass tube containing 0.01 M $KMnO_4$ and change in color is monitored. A pink color indicates arsenite oxidation (As V) [22].

Table 2. Arsenite oxidizing bacterial strains and their 16S rDNA Identification

Sample code	Isolate Accession Number	Strain name	Base pair	Closest Neighbor to known strain (Similarity %)
AOBG-1	KP295449	Rummeliibacillus sp. AOBG-1	1536	Rummeliibacillus (98%)
AOBMI-1	KP314033	Brevibacillus sp. AOBMI-1	1402	Brevibacillus agri (88%)
AOB-GI-1	KP308382	Bacillus sp. AOB-GI-1	1397	Bacillus thuringiensis (99%)

The 16S rRNA sequence analysis and phylogenetic relationship showed that the three arsenite-oxidizing bacteria were identified including Rummeliibacillus sp. AOBG-1 (Firmicutes), Brevibacillus sp. AOBMI-1 (Firmicutes), and Bacillus sp. AOB-GI-1 (Firmicutes). All the three isolates were found novel and the gene sequences were submitted to GenBank NCBI, Acession numbers were assigned to all the three strains (Table 2) shows the percentage similarity and accession numbers. The16S rDNA identities of these strains were analysed and compared to the following related arsenite-oxidizing bacteria and it was found that the Rummeliibacillus sp. AOBG-1 shared 98% 16S rDNA identity to Rummeliibacillus stabekisii. Brevibacillus sp. AOBMI-1 showed 88% 16S rDNA identities to Brevibacillusagri strain and 16S rDNA identity of Bacillus spp. AOB-GI-1

was 99% to Bacillus thuringiensis The three newly isolated arsentie oxidizing bacteria are the members of the firmicutes (Table 2).

4. Discussion

The microbial community that oxidize arsenite are mainly composed of gamma proteo bacteria, and delta proteobacteria, with alpha proteobacteria and beta Proteobacteria [27]. The microbial reduction of arsenic has been reported in numerous genres including Alcaligenes, Escherichia [28], Pseudomonas, Bacillus, Desulfovibrio [29], Shewanella, Enterobacter, [30]. In our study all the arsenite oxidizing bacteria that we found belong to phyla firmicutes all the strains are gram positive, rod shaped, oxidase negative and catalase positive. The three isolates (AOBG-1, AOB-MI-1 and AOB-GI-1) are new arsenite oxidizing bacteria and we named them as Rummeliibacillus sp. AOBG-1 (Accession No. KP295449, 1536bp), Brevibacillus sp. AOBMI-1 (Accession No. KP314033, 1465bp) and Bacillus sp. AOB-GI-1 (Accession No. KP 308382. 1397bp). The isolate AOBG-1 shows the similarity with Rummeliibacillus sp. (98%, accession no. HE586361), Bacillus sp (98%, accession no AB243841) and baclillus sp. (97% accession no AB243839). Similarly the isolate AOB-MI-1 shows the similarity with Brevibacillus sp (98%, accession no KM403208), Brevibacillus agri (98%, accession no HE993879), Brevibacillus agri (98%, accession no GQ927168), Streptomyces sp. (98%, accession no AB731746), Bacillu sp. (98%, accession no AB533759) and the isolate AOB-GI-1 shows the similarity with Bacillus sp (95%, accession no JN975197), Bacillus thuringiensis (95%, accession no CP010577), Bacillus thuringiensis (95% accession no KP006649), Bacillus thuringiensis (95%, accession no CP003687), Bacillus sp.(95%, accession no JX402416), bacillus sp. (95%, accession no JQ030915).Microorganisms belonging to the class of gamma proteo bacteria, such as Stenotrophomonas strain MM7 and, Pseudomonas strain TS44, which were recently reported to oxidize As(III) in various environments and were found to tolerate high arsenic concentration levels [31,32] and Alkali limnicola ehrlichii MLHE-1 a denitrifying halophilic microorganism isolated from a salt lake with naturally high As concentration levels [33]. There are numerous bacterial species capable of oxidizing arsenic and using this compound as an electron donor for growth chemolithotroph, although very few organisms can grow through this mechanism among these is arsenitoxidans Pseudomonas strain NT-26 arsenopyrite samples isolated from a gold mine. Microbial species that bio transform arsenic between oxidation states with differing environmental behaviors control the release of adsorbed arsenic by sediments into groundwater and may potentially be utilized for bioremediation. To date, most investigations of bacterial communities only focused on analyzing population shifts in incubation experiments using environmental samples from contaminated sediments in Southeast Asia. Although previous reports supported the biogeochemical basis for arsenic mobilization, such incubation experiments failed to identify the responsible in situ microbial populations [34].

References

[1] Sohel, N., Persson,L.A.,Rahman,M., Streat field, P.K.,Yunus,M., Ekstrom,E.C., Vahter, M., 2009.Arsenic in drinking water and adult mortality a population- based cohort study in rural Bangladesh. Epidemiology20, 824-830.

[2] Mandal, B.K., Roy Chowdhury,T., Samanta,G., Basu,G.K., Chowdhury,P.P., Chanda, C.R., Lodh,D., 1996. Arsenic in ground water in seven districts of West Bengal, India-the biggest arsenic calamity in the world. Curr.Sci. 70, 976-985.

[3] Welch, A.H., Lico, M.S., Hughes, J.L., 1988. Arsenic in ground water of the western United States. Ground Water 26, 333-347.

[4] Argos, M., Kalra,T., Rathouz,P.J., Chen,Y., Pierce,B., Parvez, F., Islam, T., Ahmed, A., Rakibuz - Zaman, M., Hasan,R., Sarwar, G., Slavkovich, V., van Geen, A., Graziano, J., Ahsan, H., 2010 . Arsenic exposure from drinking water,andall-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. Lancet 376, 252-258.

[5] WHO, Arsenic in drinking-water,World Health Organization, 2011.And Bureau of Indian Standards 10500: 1991, Amendment II, 2003.

[6] Bundschuh, Bhattacharya, P., Sracek, O., Mellano, M.F., Ramírez, A.E., Storniolo, A.R., Martín, R.A., Cortés, J., Litter, M.I., Jean, J.S., 2011. Arsenic removal from ground water of the Chaco-Pampeanplain (Argentina) using natural geological materials asadsorbents. J. Environ.Sci. Health 46, 1297-1310.

[7] Ahamed, S., Sengupta, M. K., Mukherjee, A., Amir Hossain, M., Das, B., Nayak, B., et al. (2006). Arsenic groundwater contamination and its health effects in the state of Uttar Pradesh (UP) in upper and middle Ganga plain. India: a severe danger. Science of the Total Environment, 370, 310-332.

[8] Saumya Srivastava ,Yogesh Kumar Sharma. (2013) Arsenic occurrence and accumulation in soil and water of eastern districts of Uttar Pradesh, India. Environ Monit Assess 185:4995-5002.

[9] Abedin, M.J., Cresser, M.S., Meharg, A.A., Feldmann,J., Cotter-Howells, J., 2002a. Arsenic accumulation and metabolism in rice (Oryzasativa L.). Environ. Sci. Technol. 36, 962-968.

[10] Shivaji,S., Suresh,K., Chaturvedi,P., Dube,S., Sengupta, S., 2005. Bacillus arsenicus sp. nov., an arsenic-resistant bacterium isolated from asiderite concretionin West Bengal, India. Int. J.Syst. Evol. Microbiol. 55, 1123-1127.

[11] Sri Lakshmi Sunita, M., Prashant, S., Bramha Chari, P.V., NageswaraRao, S., Balaravi, P., KaviKishor, P.B., 2012. Molecular identification of arsenic-resistant estuarine Bacteria and characterization of their ars genotype. Ecotoxicology 21, 202-212.

[12] Tsai, S.L, Singh, S., Chen, W., 2009. Arsenic metabolism by microbes in nature and the impact on arsenic remediation. Curr. Opin. Biotechnol. 20, 659-667.

[13] Turner AW: Bacterial oxidation of arsenite. I. Description of bacteria isolated from arsenical cattle-dipping fluids. Aust J BiolSci 1954, 7(4):452-478.

[14] Osborne FH, Enrlich HL: Oxidation of arsenite by a soil isolate of Alcaligenes. J ApplBacteriol 1976, 41(2):295-305. 8. Bruneel O, Personne JC, Casiot C, Leblanc M, Elbaz-Poulichet F, Mahler BJ, Le Fleche A, Grimont PA: Mediation of arsenic oxidation by Thiomonas sp. in acid-mine drainage (Carnoules, France). J ApplMicrobiol 2003, 95(3):492-499.

[15] Salmassi TM, Venkateswaren K, Satomi M, Nealson KH, Newman DK, Hering JG: Oxidation of arsenite by Agrobacterium albertimagni, AOL15, spnov., isolated from Hot Creek, California. Geomicrobiol J 2002, 19(1):53-66.

[16] Mukhopadhyay R, Rosen BP, Phung LT, Silver S: Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev 2002, 26(3):311-325.

[17] Santini JM, Hoven RN vanden: Molybdenum-containing arsenite oxidase of the chemolithoautotrophicarsenite oxidizer NT- 26. J Bacteriol 2004, 186(6):1614-1619.

[18] Oremland RS, Stolz JF, Hollibaugh JT: The microbial arsenic cycle in Mono Lake, California. FEMS MicrobiolEcol 2004, 48(1):15-27.

[19] Davolos, D., Pietrangeli, B., 2011. Phylogenetic analysis on the arsenic resistant bacteria isolated from three different freshwater environments. Chem. Ecol. 27, 1-9.

[20] Krieg, N.R. Bergey's Manual of Systematic Bacteriology, Vol 1. Baltimore, MD: Williams and Wilkins; 2010.

[21] Salmassi, T.M., Venkateswaren, K., Satomi, M., Nealson, K.H., Newman, D.K. and Hering, J.G. (2002) Oxidation of arsenite by

Agrobacterium albertimagni, AOL15, sp. nov., isolated from Hot Creek, California. Geomicrobiol J 19, 53-66.

[22] Simeonova,D. ; Lievremon,t D.; Lagarde, F.; Muller,D.; Groudeva, V.; Lett, M.C (2004). Micro plate screening assay for detection of arsenite oxidizing and arsenate-reducing bacteria. FEMS Microbiol. Lett., 237, 249-253.

[23] Dhar, R.K.; Zheng,Y.; Rubenstone, J.; van Geen,A. A rapid colorimetric method of measuring arsenic concentrations in groundwater. Anal. Chim. Acta 2004, 526, 203-209.

[24] Wilson KH, Blitchington RB, Greene RC: Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J Clin Microbiol 1990, 28(9):1942-1946.

[25] Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0.Syst Biol. May;59(3):307-21. (PubMed)

[26] Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O. Phylogeny.fr (2008): robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. Jul 1; 36(Web Server issue):W465-9. Epub 2008 Apr 19.

[27] Muller, D., C. Medigue, S. Koechler, V. Barbe, M. Barakat, E. Talla, V. Bonnefoy, E. Krin, F. Arsene-Ploetze, C. Carapito, et al. 2007. A tale of two oxidation states: bacterial colonization of arsenic-rich environments. PLoS Genet. 3: e53.

[28] Pommier, J., V. Mejean, G. Giordano, and C. Iobbi-Nivol. 1998. TorD, a cytoplasmic chaperone that interacts with the unfolded trimethylamine Noxide reductase enzyme (TorA) in Escherichia coli. J. Biol. Chem. 273: 16615-16620.

[29] Widdel, F., and N. Pfennig. 1981. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of Desulfobacter postgatei gen. nov., sp. nov. Arch. Microbiol. 129:395-400.

[30] Saltikov, C. W., R. A. Wildman, Jr., and D. K. Newman. 2005. Expression dynamics of arsenic respiration and detoxification in Shewanella sp. strain ANA-3. J. Bacteriol. 187:7390-7396.

[31] L.Cai, G.H.Liu, C.Rensing, G.J.Wang (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils, BMC Microbiology 94.

[32] M.M.Bahar, M. Megharaj, R. Naidu, Arsenic bioremediation potential of a new arsenite-oxidizing bacterium Stenotrophomonas sp. MM-7 isolated from soil, Biodegradation 23(2012) 803-812.

[33] R.S. Oremland, S.E. Hoeft, J.A. Santini, N.Bano, R.A. Hollibaugh, J.T. Hol-libaugh, Anaerobic oxidation 0f arsenite in Mono Lake water and by facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1, Appliedand Environmen- tal Microbiology 68(2002)4795-4802.

[34] Rowland, H.A., Boothman, C., Pancost, R., Gault, A.G., Polya, D.A., Lloyd, J.R., 2009. The role of indigenous microorganisms in the biodegradation of naturally occurring petroleum, the reduction of iron, and the mobilization of arsenite from west bengal aquifer sediments. J. Environ. Qual. 38, 1598-1607.