Isolation and Identification of Unique Arsenotolerant *Exiguobacterium indicum* DSAM62 from Arsenic Rich Environment

Debanjana Sengupta^{1,*}, Siddhartha Chakraborty¹, Sudeshna Shyam Choudhury¹, Sayak Ganguli², Arup Kumar Mitra¹

¹Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India

²Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata, India

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Abstract Arsenic infiltration via bio-geological and anthropogenic mean's has been a global concern in the developing countries due to the severe toxic effects of the compound which leaches to the ecological niche. Herein, we report one such arsenotolerant bacteria isolated from a contaminated soil collected from a district in West Bengal (India), where an arsenic concentration of the soil sample was found to be 0.51 mg/kg. In the present study, several bacteria were isolated from the soil sample, out of which LD₅₀ value of arsenic for the novel tolerant bacteria was found to be 500 mg/l which was also determined by INT assay. The efficacy of arsenate uptake by this bacterium was 52.20% from its respective LD₅₀ concentration. After 16S rDNA sequencing, the bacteria showed 99% similarity with *Exiguobacterium indicum* based on nucleotide homology and meta-phylogenetic analysis. LCMS FAME revealed that the unique fatty acids were monosaturated C:10:03 OH (0.53%), C12:1 (0.16%), C11:03 OH (0.38%), terminally branched Anteiso C14:0 (0.74%), monosaturatedC15:1w5C, monosaturatedC16:0 N Alcohol, Anteiso 16:0 (0.36%), Iso- C16:0 3OH (0.33%), Iso- C18:1 H (0.25%), C18:3w6C (6,9,12) (0.53%), C18:1 2OH (0.52%), which lead us to conclude that the bacteria was a new and unique bacterial strain. So, on the basis of FAME analysis, the unique bacteria were named as *Exiguobacterium indicum* Strain DSAM62, which can be used as a potential bio accumulator in agricultural fields for bioremediation of arsenic. The fully annotated sequence of the genome and the plasmid of the novel strain revealing key regulatory genes were also investigated and results can be correlated with this tolerant behavior. The annotated sequence was submitted to NCBI genome database which currently having an accession number of PRJNA555453.

Keywords *Exiguobacterium indicum* Strain DSAM 62,

Arsenic, Tolerance, Uptake

1. Introduction

Arsenic is a toxic element of the environment and it is a member of group V of the periodic table. So, it is classified as a heavy metal (Wackett, 2004). It mainly exists in the nature in two forms, the predominant pentavalent arsenic As [V] and trivalent arsenic As [III] (Cullen and Reimer, 1989) because arsenate As [V] is immobilized in the solid phase and arsenite As [III] primarily exists as uncharged H₃As⁰₃ with the pKa value of 9.2. Less toxic methylated form of arsenic dimethylarsinic (DMA) and monomethylarsonic (MMA) are generated by microorganisms under oxidizing conditions (Wang *et al.*, 2007). Groundwater contamination with bioavailable arsenic has been found in West-Bengal, India and Bangladesh (Smith *et al.*, 2002) as a direct result of irrigation in crop fields by contaminated water. Rice grains are also affected by bioaccumulation of arsenic (Meharg and Rahman, 2003) which exhibits the most common route of arsenic poisoning through food chain (Chowdhury, 2004) and causes skin cancer, liver and lung cancers (Wang *et al.*, 2001). The most common way for heavy metal removal techniques from industrial effluents are chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery (Ahluwalia and Goyal, 2007). These methods are expensive and have some drawbacks in the higher concentration of heavy metal removal (Nourbakhsh *et al.*, 1994). Therefore, innovative, low-cost and eco-friendly

microbes involving bioremediation techniques for heavy metal mitigation from the industrial sludge are developed. Bacteria can absorb, adsorb, chelate and biochemically modify arsenic species into much less toxic forms of reduced arsenic with the help of proteins helping in metal binding and modification, these activities are regulated by the inducible gene expression upon arsenic stress. The present study involves the isolation, characterization, arsenic tolerance studies, uptake ability and identification of a novel bacteria isolated from the arsenic contaminated soil from a rural area of West- Bengal.

2. Materials and Methods

2.1. Arsenic Content Analysis of Soil Sample by ICP-OES

Arsenic concentration of the collected soil sample from Shyamnagar was measured by ICP-OES after preparing the soil sample by wet digestion method (USEPA, 1996). 2 gm. of soil sample was mixed with 5 ml. of 69% HNO_3 in 100 ml Borosil glass conical flask and it was incubated for overnight. After the incubation the sample was kept in block tubes and the block tubes were kept in the Block Digester (AIM 600 Digestion System, Australia). Arsenic content of the soil sample was analyzed by Thermo Scientific, Model No. ICAP 6000 at 193.759 nm wavelength in UV region.

2.2. Characterization of the Collected Soil and Water Samples

The soil sample was collected from paddy rhizospheric soil from Shyamnagar. Then, the deionized water was added into the soil in 1:10 ratio. Then the mixtures were filtered and pH, EC content and specific gravity of all the collected samples were measured.

2.3. Bacterial Isolation by Serial Dilution Method

The colloidal soil sample was measured up to 1 ml and dissolved in 9 ml of sterile, deionized water to prepare a dilution of 10^{-1} . From this, 10^{-2} and 10^{-4} dilutions were prepared in sterile, de-ionized water. The 10^{-2} and 10^{-4} dilutions were used for pour-plating with fresh, sterile Nutrient Agar (HiMedia) media to obtain isolated bacterial colonies. For this, 0.2 ml of sample aliquots were placed in sterile petri-plates and to it, the nutrient agar (HiMedia) was added and swirled gently. After solidification, the plates were incubated for about 24 hours, at 37°C . Numerous colonies were obtained in each plate. Few colonies were selected on the basis of their frequency at random for further studies, i.e., morphological and biochemical characterization.

2.4. Biochemical Characterization of the Isolated Bacteria

Since bacterial organisms are similar in morphological and cultural characteristics, four biochemical tests are used to differentiate between these bacteria. InVic and Sugar Utilization test were performed for the identification of the bacteria at preliminary stage.

For indole test, the bacterial strains were inoculated in tryptophan broth and they were incubated at 37°C for 24 hours. After 24 hours of incubation, 0.5ml of Kovac's reagent was added to it, without shaking. Development of reddish ring was taken as a positive result.

For methyl red test, 48 hours old bacterial cultures in dextrose phosphate broth were taken and about 0.5 ml methyl red indicator was added to it, followed by shaking. Development of red coloration was taken as a positive result.

The bacterial strains were inoculated in dextrose phosphate broth for Voges-Proskauer test and they were incubated at 37°C in BOD Incubator Shaker for 48 hours old cultures in dextrose phosphate broth were taken and to it about 0.6ml Barritt's reagent was added with vigorous shaking. Development of copperish red color was considered as a positive result.

For citrate utilization test, the bacterial strains were inoculated in Simmon's Citrate Agar (HiMedia) media and they were incubated at 37°C for 24 hours. After 24 hours of incubation, the change in color of media from green to blue was taken as a positive result.

For chrome agar test, 24 hrs old cultures in Hi chrome agar (HiMedia) media were taken and the characteristic color of the respective cultures was observed.

For sugar utilization Test, each tube containing TSI agar (HiMedia) was taken. Small amount of the experimental bacteria from fresh culture was inoculated by using of the sterile technique into the tubes by means of slant and stab inoculation method with an inoculating loop. Then, the tubes were then incubated at 37°C for 24-48 hours in BOD Incubator. After 24-48 hours the color of both the stab and slant of agar slant cultures were observed.

2.5. Arsenic Tolerance Study

The bacterial cultures were inoculated into sterilized 30 ml. of nutrient broth supplemented with increasing concentrations of arsenic $[\text{As}(\text{v})]$ like 10 mg./l, 30 mg./l, 50 mg./l, 70 mg./l, 80 mg./l, 100 mg./l, 200 mg./l, 300 mg./l, 500 mg./l. 800 mg./l and 1000 mg./l and they were incubated at 37°C for 24 hours and 48 hours. After the incubation, OD_{580} values were taken for each bacterial sample to find the LD_{50} value and the maximum arsenic tolerance level of each bacterial strain.

2.6. Arsenic Analysis by ICP-OES

The bacterial cultures were centrifuged (Remi Cold

Centrifuge Machine, Model No.C-24) and the supernatants were taken for acid digestion. Then the samples were prepared with 69% w/v HNO_3 , using de-ionized water. Acidification to pH less than 2 was done 16 hours before the ICP-OES analysis. Necessary dilutions were made if the arsenic content exceeded the calibration standards. The nebulizer flow and plasma power were optimized (i.e., the plasma power adjusted between 1300-1400 W) using a 5 mg./l arsenic solution. Next, the nebulizer flow was incrementally adjusted between 0.90 and 0.60 L/min for radial and axial plasmas, in that order. After observing change in intensity, the best flow was established and used for the analyses. The most sensitive lines for arsenic lie in the UV region (193.759 nm.) and an appropriate spectrophotometer (Thermo Scientific, Model No. ICAP 6000 Duo) were used. For correlating the results, the arsenic standard is diluted with 1% ultrapure HNO_3 and analyzed on the instrument. After the run, the peaks and baselines are reset, the blanks are identified as global blanks, any interfering elements are identified for subsequent subtraction.

2.7. Cell viability Test Using INT Assay

The bacteria was grown in tryptic soy broth (HiMedia) with 500 mg./l, 300 mg./l arsenic As (V) and without arsenic As (V) at 37°C for 24 hours at BOD Incubator Shaker. After the incubation, 100µl. of the inoculums were inoculated in 3 ml. of 30% TSB containing 0.5 mg./ml of 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) (SRL). Then it was incubated for 30 minutes at 37°C in BOD Incubator Shaker at 40 rpm shaking speed. After the incubation the supernatant was separated and removed by centrifugation and 1ml. of Dimethyl Sulfoxide (DMSO) (Merck) was added to each tube. The cell viability was quantified by measuring the absorbance at 490 nm wavelength in UV-Visible Spectrophotometer (Optizen) using DMSO as a blank (Steifel, *et al.*, 2016).

2.8. SEM-EDX

To study the effect of arsenic on cellular morphology of S6C2 bacteria, 1% inoculum was added into LB broth containing LD_{50} concentration of arsenic [500 mg./l of As(V)] and without arsenic (control) and incubated at 37°C in BOD Incubator Shaker incubator (100 rpm). Samples were harvested at mid- log phase and the samples were prepared for SEM and EDX according to De *et al.* (2008) and Daware and Gade, (2015). Following incubation, both control and arsenic stressed bacterial cells were harvested by centrifugation at 6,000g for 10 min, 4°C. The pelleted cells were washed twice with 0.1M potassium phosphate buffer solution (PBS; pH 7.2) and fixed overnight in 2.5 % glutaraldehyde in 0.1M PBS at 8°C. The cells were washed twice again with PBS. The cells were dehydrated through a

series of different concentrations (from 30% to absolute) 1 ml. of ethanol at 10,000 rpm for 2 minutes. Finally, the pellet was dissolved in 200 µl of 100% ethanol concentration for half an hour. Finally, 2 µl of each sample was placed on 1mm clean and grease free mini round glass cover slips. The glass cover slips were coated with platinum and examined under SEM with the acceleration voltage of 20 kV (Zeiss Evo 18 Special Edition). Energy dispersive x-ray spectroscopy (EDS) (Zeiss Evo 18 Special Edition) was performed to detect the presence of arsenic over cell surface.

2.8.1. Identification of Bacteria by 16 S rDNA Sequencing

According to Paul *et al.*, 2015, DNA was isolated from S6C2 bacteria. Its quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by 27F and 1492R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved using Agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program MEGA 7 followed by construction of the distance matrix and construction of the phylogenetic tree.

2.8.2. Identification of Strain Specific Bacteria by Fatty Acid Methyl Ester (FAME) Analysis

According to Kunitsky *et al.*, 2005, the fatty acids were extracted from the growing bacterial culture by a procedure which consists of saponification in dilute sodium hydroxide/methanol solution (15g sodium hydroxide, 50ml methanol, and 50ml distilled water) followed by derivatization with dilute hydrochloric acid/methanol solution (40.62ml certified 6.0N hydrochloric acid and 34.37ml methyl alcohol pH value below 1.5) to give the respective methyl esters (FAMES). The FAMES were then extracted from the aqueous phase by the use of an organic solvent (50ml hexane and 50ml methyl tert-butyl ether) and the resulting extract was analyzed by GC using Agilent HP-ULTRA 2 column which was of 25m length, 0.200mm diameter, 0.33µm film and its temperature limit is 60°C to 325°C. In that gas chromatography, Hydrogen was used as mobile phase, Flame ionization detector as detector and its flow rate was 1.3ml/min. The software automated all analytical operations and used a sophisticated pattern recognition algorithm to match the unknown FAME profile to the stored library entries for identification by RTBA6 method.

2.8.3. Whole Genome and Plasmid Sequencing

The DNA extraction from the bacteria S6C2 was performed using standard kit based protocols (Oyedara *et al.*, 2018). The DNA sample quality were checked using Nanodrop and quantitated using Qubit ds DNA BR assay (Thermo Scientific, USA). The genomic DNA was fragmented using Covaris to generate 300-400bp fragments. 100 ng of fragmented DNA was used to generate sequencing library using NEBNext Ultra II DNA Library Prep Kit for Illumina (#E7645S). In brief, the fragmented DNA was subjected to end repair followed by A-tailing and adapter ligation. Ampure bead based size selection was performed to obtain the library of desired size. The size selected DNA were enriched by PCR amplification using Illumina index adapter primers. The amplified product was purified using ampure beads to remove unused primers. The library was quantitated using Qubit DNA High Sensitivity quantitation assay and library quality was checked on 2100 using Agilent 7500 DNA Kit. The library was quantitated using Qubit DNA HS quantitation assay (Thermo Scientific) which specifically quantitates dsDNA assay. For sequencing, the libraries were diluted to 3Nm Using RSB and 5 uLs of the diluted library was mixed with an equal volume of freshly prepared 70.1 N NaOH to allow the library to get denatured. The pH of the reaction was neutralized by adding 5 uL of freshly prepared 200Mm Tris-HCl and were taken on to Cbot for cluster generation on a HiSeqX high output flow cell. Cluster generation and sequencing was performed on the Illumina 2000 system. The following pipelines were used FastQC – To run and provide a QC report to check the quality of the sequences Galaxy (Unicycler + MOB) – To assemble the draft genome and to identify plasmid presence. RAST – To annotate the nuclear genome & Plasmid genome CONTIGuator – To finish the Genome

assembly RNAmmer – To identify the 5s/8s, 16s/18s, and 23s/28s ribosomal RNA in full genome sequences. Genomic DNA. The circular plasmid map of the bacteria was created by CGVIEW Server. After the whole genome annotation, the existence of unique ClpB protein was identified and the bacterial whole genome sequences were submitted at NCBI under the Accession Number PRJNA555453.

2.8.4. Isolation of Cellular Protein and SDS-PAGE Analysis

The sample preparation was done according to Mathabe, 2014. The bacteria (S6C2) was grown in control (without arsenic) and 500 mg./l of As(V) treated condition. After the incubation the bacterial cells were centrifuged 15,000rpm for 15 minutes (Remi C-24). The pellet was obtained and the cellular proteins were extracted with 100 µl of 0.4% Triton-X. Then, the samples were centrifuged at 15,000 rpm for 15 minutes. The resulting pellet was resuspended in 4X loading buffer (consisting of 10Mm Tris-HCl pH 6, 277 mM SDS and 40% Glycerol) and it was sonicated (Hielscher UP 200S, Hielscher Ultrasonics GmbH, Germany) to recover the cellular proteins. After that, it was centrifuged and the supernatant was retained. SDS-PAGE analysis (Biorad, Richmond, CA) of the crude extract of the cellular proteins of the control and 500 mg./l As(V) treated samples were done.

3. Results

3.1. Arsenic Concentration of Soil Sample

Arsenic concentration of the collected soil sample from Shyamnagar was 0.51 mg./l (Table-1).

Table 1. Characterization of Soil Sample

Sample Name	Texture of the Soil Sample	Location	pH	Temperature (°C)	EC value (ds/m) of the Soil Sample	Specific Gravity	Arsenic Content	Inference
S-6	Clay	Paddy rhizospheric soil from Shyamnagar	7.34	25	0.00247	0.997	0.51±0.001	Neutral, non saline in nature

3.2. Physical Characterization of Soil Sample

Some physical characterization of the soil sample was done. The temperature of the soil sample was 25°C. It was clay type because its pH value was 7.34. EC value and Specific Gravity value were 0.00247 dS/m and 0.997 respectively (Table 1).

3.3. Isolation of Bacteria

The bacterial colonies were isolated from 10^{-2} and 10^{-4} dilution plates. Numerous colonies were obtained in each plate. The four colonies were selected at random for further studies, i.e., morphological and biochemical characterization [Table 2 and Figure. 1.(a), (b)].

Table 2. Morphology of the Bacterial Strains

Bacteria	Colony morphology	Gram's nature
S6C1 Bacteria	Moderate in size, yellowish white, slimy, irregular, lobate margin, flat	Gram (+), short rods
S6C2 Bacteria	Small in size, whitish, slimy, circular, entire margin, flat	Gram (+), short rods
S6C3 Bacteria	Small in size, slightly yellowish, slimy, lobate margin, flat	Gram (+), short rods
S6C4 Bacteria	Large in size, whitish, slimy, irregular, undulate margin, slightly raised	Gram (-), short rods

Figure 1. (a), (b), (c) and (d). Gram staining of S6C1, S6C2, S6C3 and S6C4 Bacteria under 40 X Magnification of Compound Light Microscope

3.4. Biochemical Characterization of the Isolated Bacteria

The results are tabulated in Table 3 and Table 4.

Table 3. Biochemical Characterization of the Bacteria

Bacteria	Indole test	MR test	VP test	Citrate test	Chrome agar test
S6C1	-	-	-	+	Dark Blue Colony
S6C2	-	+	+	-	Brownish Orange Colony
S6C3	-	-	-	+	Dark Blue Colony
S6C4	-	-	-	+	Brownish Orange Colony

Table 4. Sugar Utilization Test

Bacteria	Result	Interpretation
S6C1	Red slant/Yellow Stab	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products.
S6C2	Red slant/Yellow Stab	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products.
S6C3	Red slant/Yellow Stab	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products.
S6C4	Red slant/Yellow Stab	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products.

3.5. Arsenic Tolerance Study

The isolated bacterial strains were inoculated in the nutrient broth (HiMedia) supplemented with increasing concentration of arsenic [As (V)] from 5 mg./l to 1000 mg./l because the bacterial strains have arsenate reduction ability. The LD₅₀ value of S6C1, S6C2, S6C3 and S6C4 were 30 mg./l, 500 mg./l, 300 mg./l and 50 mg./l respectively [Figure. 2.(a),(b),(c),(d),(e),(f),(g) and (h)].

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

Figure 2. (a), (b), (c), (d), (e), (f), (g) and (h). Arsenic Tolerance Study of S6C1, S6C2, S6C3 and S6C4 Bacteria at 37°C in BOD Incubator Shaker after 24 Hours and 48 Hours of Incubation respectively

3.6. Uptake of Arsenic by the Bacteria

The percentage uptake of arsenic [As (V)] by S6C1, S6C2, S6C3 and S6C4 bacteria were 11.46%, 52.20%, 11.56% and 10.84% respectively (Table 5).

Table 5. Arsenic Uptake by Bacteria

Bacteria	Concentration of Arsenic (mg/l)	Residual Arsenic in the Medium (mg/l)	Uptake of Arsenic by the Bacteria (mg/l)	% Uptake of Arsenic by the Bacteria
S6C1	30	26.56 ± 0.1	3.44	11.46%
S6C2	500	239 ± 0.1	261	52.2%
S6C3	300	265.3 ± 0.1	34.7	11.56%
S6C4	50	44.58 ± 0.1	5.42	10.84%

3.7. SEM-EDX

The scanning electron micrographs of S6C2 bacterial cells without arsenic (control) and with exposure to arsenic [500 mg./l of As(V)] were studied in mid-log phase. Cellular morphology of S6C2 bacteria in the presence of As(V) and was changed in mid-log phase and the cells became elongated [Fig. 3.(a) and (b)]. The energy dispersive X-ray spectroscopy (EDX) analysis showed a distinct EDX signal corresponding to arsenic peak was observed in presence of As(V) loaded cells [Fig. 3.(d)] however, no such peak was observed in control [Fig. 3.(c)].

(a)

(b)

Figure 3. (a). Scanning Electron Micrographs of S6C2 Bacteria in Control, (b). Scanning Electron Micrographs of S6C2 Bacteria in Arsenic Treatment [As(V)], (c). Energy Dispersive X Ray Spectrum of S6C2 Bacteria in control and (d). Energy Dispersive X Ray Spectrum of S6C2 Bacteria in Arsenic Treatment [As(V)] condition

3.8. Bacterial Viability Test by INT Assay

Based on the higher arsenic As (V) tolerant ability, S6C2 and S6C3 bacterial cells were tested for viability assay with the help of INT. The decreasing amount of cell viability of S6C2 and S6C3 bacteria were 41.34% and 43.35% respectively [Supplementary Table 1, Figure. 1.(a),(b) and 2.(a) and (b)].

3.8.1. Bacterial Identification by 16S rDNA Sequence Analysis

S6C2 bacteria showed high similarity with *Exiguobacterium indicum* based on nucleotide homology and phylogenetic analysis (Figure. 4 and Table 6).

Figure 4. Molecular Phylogenetic analysis by Maximum Likelihood method on the Kimura 2-parameter model. Strains in the tree are: *Exiguobacterium indicum* strainBR18_1A (JN644531.1), *Exiguobacterium indicum* strainK1 (KY928088.1), *Exiguobacterium indicum* strain BPC2 (KF387706.1), *Exiguobacterium acetylicum* strainQD-3 (FJ970034.1), *Exiguobacterium indicum* strain HHS 31 (NR_042347.1), *Exiguobacterium acetylicum* strain DSM20416 (NR_043479.1), *Exiguobacterium indicum* strainS2KU058432.1, *Exiguobacterium indicum* strainV5 (KU058437.1), *Exiguobacterium acetylicum* strainS1 (KU058431.1), *Exiguobacterium indicum* strain IHBB10090 (KR233792.1).

S6C2		0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.002
JN644531.1	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
KY928088.1	0.002	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
KF387706.1	0.001	0.002	0.002		0.002	0.000	0.002	0.002	0.002	0.002	0.002
FJ970034.1	0.003	0.002	0.002	0.004		0.002	0.001	0.001	0.001	0.001	0.002
NR_042347.1	0.001	0.002	0.002	0.000	0.004		0.002	0.002	0.002	0.002	0.002
NR_043479.1	0.004	0.003	0.003	0.005	0.003	0.005		0.001	0.001	0.001	0.002
KU058432.1	0.002	0.001	0.001	0.003	0.001	0.003	0.002		0.000	0.000	0.002
KU058437.1	0.002	0.001	0.001	0.003	0.001	0.003	0.002	0.000		0.000	0.002
KU058431.1	0.002	0.001	0.001	0.003	0.001	0.003	0.002	0.000	0.000		0.002
KR233792.1	0.004	0.005	0.003	0.004	0.005	0.004	0.006	0.005	0.005	0.005	

3.8.2. Fatty Acid Methyl Ester (FAME) Analysis

(20.30%), Iso-C17:0 (15.07%), C16:0 (9.62%), Iso-C13:0 (7.36%), Anteiso C15:0 (11.37%), Anteiso C15:0 (7.98%) and Anteiso C17:0 (6.22%). However unique existence of C:10:03 OH (0.53%), C12:1 at 11-12 (0.16%), C11:03 OH (0.38%), Anteiso C14:0 (Terminally branched Myristic Acid) (0.74%), C15:1w5C (0.25%), C16:0 N Alcohol (0.45%), Anteiso 16:0 (Anteiso Palmitic Acid) (0.36%), Iso- C16:0 3OH (3 Hydroxy Hexanoic acid/ Palmitic acid) (0.33%), Iso- C18:1 H (Oleic Acid) (0.25%), C18:3w6C (6,9,12) (Alpha Linolenic acid) (0.53%), C18:1 2OH (2 Hydroxy Oleic acid (0.52%) fatty acids were observed in the fatty acid profile of the unique bacterial strain. So, the unique bacteria was named as *Exiguobacterium indicum* Strain DSAM62 (Figure.5 and Supplementary Table 2).

Figure 5. Chromatograph of Fatty Acids of *Exiguobacterium indicum* Strain DSAM 62

3.8.3. Genome Sequencing

Whole genome and plasmid sequencing showed that the total assembled genome size of *Exiguobacterium indicum* Strain DSAM 62 was 3.1 Mb with 19 scaffolds. Out of these, 18 scaffolds were present in 2.9 Mb genomic DNA and 1 scaffold was present in 263 Kb of plasmid DNA. The bacteria had the several genes which could be related to arsenic resistance behavior (Supplementary Table 3). The whole genome sequence is a complete structural and functional annotated sequence which will be an important data to correlate the experimental and computational studies.

3.8.4. Cellular Protein Isolation and SDS-PAGE Analysis

Two distinct bands were found in the As(V) treated sample at about 96 kDa and 76 kDa. Among these two bands only 96 kDa molecular weight band was found in case of 500 mg./l of As(V) treated sample, which may correspond to be ClpB protein as the gene for that protein is present in the genome assembly and previous reports (Tsuchiya *et al.*, 2019) do predict the role of the protein in response to arsenic stress (Figure. 6). This needs to be tested further to confirm the role of the protein under conditions of arsenic uptake in this bacterium.

Figure 6. SDS-PAGE Analysis of the Membrane Protein of S6C2 Bacteria, Lane 1, Prestained Protein Ladder, Lane 2 Control and Lane 3 As(V) (500 mg./l) Treated *Exiguobacterium indicum* Strain DSAM 62

4. Discussion

According to Castro-Severyn *et al.*, 2017 and Albarracin *et al.*, 2016, psychrophilic bacteria *Exiguobacterium* was normally found in the extreme environmental condition of Chilean Altiplano, Atacama desert, Antarctic ice (Carneiro *et al.*, 2012), Himalayan glaciers (Chaturvedi and Shivaji, 2006), soda lakes (Borsodi *et al.*, 2005), hyperthermophilic hot springs (Vishnivetskaya *et al.*, 2009), and deep-sea hydrothermal vents (Vishnivetskaya *et al.*, 2009 and Crapart, 2007). They had been reported to persist in effluent of paper mills and in rhizospheres of *Lemna minor* (Kashamaet *et al.*, 2009 and Tang *et al.*, 2013). The genetic differentiation of *Exiguobacterium* were found due to the ecology of diverse habitats (e.g., CuatroCiénagas: Rebollar *et al.*, 2012). According to Castro-Severyn *et al.*, 2017 *Exiguobacterium* sp. Strain SH31 was arsenic resistant and it was able to survive in the media supplemented with 100 mM of Arsenic; i.e. As(V). Pandey and Bhatt, 2015 described in their study that *Exiguobacterium* sp. (As-09) was tolerant to arsenic upto 700 mM, which was isolated from an arsenic contaminated soil from Rajnandgaon district, Chattisgarh, India. Sacheti *et al.*, 2013, isolated *Exiguobacterium* sp. PS from the arsenic contaminated site in India. According to the study of Belifore, *et al.*, 2013, that arsenic resistant *Exiguobacterium* sp. S17 was isolated from a high-altitude Andean lake stromatolite.

In the present study, the four bacteria were isolated from the soil sample of Shyamnagar, 24 Parganas (North). The LD₅₀ value of the isolated S6C1, S6C2, S6C3 and S6C4 bacteria were 30 mg./l, 500 mg./l, 300 mg./l and 50 mg./l of arsenic As (V) respectively. The uptake ability of S6C1, S6C2, S6C3 and S6C4 bacteria were 11.46%, 52.2%, 11.56% and 10.84% respectively. Depending on their higher arsenic tolerance ability the cell viability test through INT assay were done in case of two bacteria viz. S6C2 and S6C3 bacteria among these four bacteria. In this assay, the decreasing cell viability value of S6C2 and S6C3 bacteria were found to be 41.34% and 43.35% in their respective LD₅₀ value i.e 500 mg./l and 300 mg./l of arsenic As(V) respectively and the amount was the near about 50%. From these results, it was concluded the fact that the bacteria S6C2 isolated shows an extreme tolerance towards this heavy metal and this indicates that further molecular studies must be performed in order to understand the molecular basis of this tolerance in order to shed light on the fact that how they can be used in contaminated fields as a potential bioremediator of this heavy metal. Metal tolerant bacteria usually have the ability to internalize and metabolize heavy metals to more non toxic gaseous forms (Chen *et al.* 2014) such that a part of the arsenic in the biomass can be encompassed within the atmosphere essentially reducing the gross quantitated level of arsenic from the soil.

On the basis of the higher arsenic tolerance and uptake

efficacy, S6C2 bacteria was identified as *Exiguobacterium indicum* by showing high similarity based on nucleotide homology and phylogenetic analysis. After fatty acid methyl ester (FAME) analysis, the major fatty acid (>5%) S6C2 Bacteria were terminally branched Iso-C15:0 (20.30%), Iso-C17:0 (15.07%), straight chain saturated C16:0 (9.62%), terminally branched Iso-C13:0 (7.36%), Anteiso C13:0 (11.37%), terminally branched Anteiso C15:0 (7.98%) and terminally branched Anteiso C17:0 (6.22%). However unique presence monosaturated of C:10:03 OH (0.53%), C12:1 at 11-12 (0.16%), C11:03 OH (0.38%), Anteiso C14:0 (0.74%), monosaturated C15:1w5C, C16:0 N Alcohol, Anteiso 16:0 (0.36%), Iso-C16:0 3OH (0.33%), Iso- C18:1 H (0.25%), C18:3w6C (6,9,12) (0.53%), C18:1 2OH (0.52%) fatty acids were found in the fatty acid profile of the unique bacterial strain. Upon identifying this unique strain, our goal was to identify broadly the genes which were present in the species as a whole in order to get hold of an idea of the gross genomic blueprint which would enable to draw better conclusions on this tolerance. So, the further molecular level of studies was initiated. Whole genome and plasmid sequencing revealed that the bacteria indeed harbors several genes which could be linked to this unique resistance behavior. The whole genome sequence is a complete structural and functional annotated sequence which will be an excellent tool to substantiate experimental and computational studies. Further biochemical activity based experiments would reveal probable molecular mechanisms of the metal resistance of the novel extremophile. The unique bacteria was identified and named as *Exiguobacterium indicum* Strain DSAM62.

5. Conclusions

From the present study it is concluded that isolated unique *Exiguobacterium indicum* Strain DSAM62 is highly arsenic tolerant and its efficacy of arsenic uptake is 52.2% from its respective LD₅₀ dose of arsenic, which will be utilized in arsenic mitigation strategy of agricultural and industrial sludge treatment. 16S rDNA sequence of *Exiguobacterium indicum* Strain DSAM62 was deposited at NCBI GenBank under Accession Number MH819520. Whole genome sequence including plasmid sequence of *Exiguobacterium indicum* Strain DSAM62 was deposited at NCBI under Accession Number PRJNA555453.

Acknowledgements

We would like to acknowledge Department of Science & Technology, Government of West-Bengal for funding this research work.

Supplementary Tables and Figures

Table 1. Cell Viability Test by INT Assay

Name	Condition	Arsenic Concentration	OD Value at 490 nm. With SE	% of Increase or decrease
S6C2 Bacteria	Control	-	0.179 ± 0.001	-
S6C2 Bacteria	Arsenic Treatment	500mg/l	0.105 ± 0.002	41.34% of decrease
S6C3 Bacteria	Control	-	0.092 ± 0.003	-
S6C3 Bacteria	Arsenic Treatment	300 mg/l	0.039 ± 0.002	43.35 % of decrease

Figure 1. (a) and (b). Growth of S6C2 and S6C3 Bacteria in INT supplemented TSB in Control (Second and Third Tube) and Arsenic Treated Condition (Fourth and Fifth Tube) Respectively, (First tube was for Reagent Blank).

Figure 2. (a) and (b). S6C2 and S6C3 Bacterial Viability Test by INT Assay using DMSO in Control (Second and Third Tube) and Arsenic Condition (Fourth and Fifth Tube) Respectively, (First tube was for Reagent Blank).

Table 2. FAME Results of S6C2 Bacteria by RTBA6 Method

Name of the Organism	Serial Number	IUPAC Name of the Major Fatty Acids	Common Name of the Major Fatty Acid	IUPAC Name of the Unique Fatty Acids	Common Name of the Unique Fatty Acids	Retention time (Minutes)	ECL Value	Percentage
<i>Exiguobacterium indicum</i> Strain DSAM 62	1.	Iso-C 15:0	13-Methyltetradecanoic acid	-	-	2.3755	14.6307	20.30%
	2.	Iso-C17:0	Not available	-	-	3.0132	16.6342	15.07%
	3.	Anteiso C13:0	Not available	-	-	1.8123	12.7179	11.37%
	4.	C16:0	Palmitic acid	-	-	2.8097	15.9988	9.62%
	5.	Anteiso C15:0	Not available	-	-	2.4055	14.7275	7.98%
	6.	Iso-C13:0	Not available	-	-	1.7867	12.6215	7.36%
	7.	Anteiso C17:0	Not available	-	-	3.0431	16.7274	6.22%
	8.	-	-	Anteiso C14:0	Terminally branched Myristic Acid	2.1017	13.7324	0.74%
	9.	-	-	C10:03 OH	Not available	1.4964	11.4551	0.53%
	10.	-	-	C18:3w6c(6,9,12)	Alpha linolenic acid	3.3210	17.5957	0.53%
	11.	-	-	C18:1 2OH	2 Hydroxy Oleic acid	3.8081	19.1463	0.52%
	12.	-	-	C16:0N alcohol	Not available	2.6728	15.5713	0.45%
	13.	-	-	C11:03OH	Not available	1.7427	12.4555	0.38%
	14.	-	-	Anteiso C16:0	Anteiso Palmitic Acid	2.7214	15.7233	0.36%
	15.	-	-	C16:0 iso 3OH	3 Hydroxy Hexanedecanoic acid/ Palmitic acid	3.1862	17.1743	0.33%
	16.	-	-	C15:1w5c	Not available	2.4681	14.92.92	0.25%
	17.	-	-	C18:1 iso H	Oleic Acid	3.2854	17.4842	0.25%
	18.	-	-	C12:1 at 11-12	Not available	1.6042	11.9231	0.16%

Table 3. Details of Arsenic Metabolism Associated Genes in *Exiguobacterium indicum* Strain DSAM 62 Genome.

contig_id	type	location	start	stop	strand	function	evidence_codes	nucleotide_sequence	aa_sequence
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_156539_158269	156539	158269	+	Efflux ABC transporter for glutathione/L-cysteine, essential for assembly of bd-type respiratory oxidases => CydD subunit		atgaatccattaaaagggaatcgtccagattccacgcagttattctcattcaattcattattgagcttttctaattgggggttgcggtcgtcggtcaatcgtaggtatcggttgacggtcgtatcggtttctgaaagaacaacggttgaagccgtcattccattgctactcgtcgtctcgggatgctgtgctccgggtagcagtgacgtattggaacggctcgtcgtgacgacgttatcgccacacatgaagcagcgcttcgggaagcgtcgtcgtcgaatacacgggtcgttcggtcgagatgatctacaagggaagcgggacagaaggtcagtgcttctcgacgctgtagacgagatggacagctactacagtcaatactgcgaaaagtcgtccagacgacgacgttcccttgatgctcctgattgcggcattcagctatgactggatcaccggtcgtcgtcatgatgacgacgacgcttccgttcttttatatcatcatcgccatcatgacgaaaagcgggcccgtatgctcaactcgagaagatgacggcattctcgggaacgttcttgatacgttgcagggaactgacgacctgaagttattggtcgcgcgaaacgacaactgacgtcatcgaaaagcagcttctgattccgagatgcgacgttaacgcttgaagctcgccttctgtcgtcgtgatgcttgcagttcatctcgtatgctcagatgggaatgattgcactgaagtcagtcggttgattctgttcaaaagcatcaccttcattccggcgttctctgatgctcgtgttggcaccagaatactatcttccttaaaagagatgggagctgcttccatacgggacgcggtagtgctcggcagcgaaacagatcgacccgaactaacggcagacagatgctcgttgccttgggcaatcagaactttcgattaacgtccaccagaatcgttttgatgaagttgattttacgtatcaagaagacgcttgccttacaagacgtgacacttgcgatcgaaccgtatc aaaagtggtgctgatcggtcgcagtggtgccgtaagtcgactgtcttcgattgattccggactcgcgatccaaaagaaggacggattctgcttgacggatcgagcgcagtcaggtggcggaagcagctggttccgtcagttgagctacatctcgcaacatccgtatctgtatccgggaacgtggcagaaaaattgcgatcggtgaactccgtcaagcgacccggaagagattttgagggcgcgaggcgaggaactcgagga gttgattcgacgttacctgaaggattgacacagtaattgagaaggcggacgtggtctatcagtggtcgagaacacgcgcttgcgttggcacgagcgttcttgaacgaccgaacgtcatcttcttgatgaaccgacgacgactgacgtcaaaacgga gcgactgctcaacaagcgatcgaaacgcttggacaacacgcgacagtcattactgtcgacaccgctgcataatcgagcgtcagatcaaatcgtcgtcctcgacg gcggacgaatcgttgatcgttggtacgcacgatgaactacttgacgtgattcgag tatccccggatcgctgtagctccagagaggggaggaacacgatga	MNPLKGVQIPRSILIQFIIAFLMGVAVVG QSYGIVLTVDRIFLKEQPF EAVIPLLLVVLG MLLLRVAVTYWNGRLGTTLSAHMKQALR EALVAKYTGRSVEMMLQQAGQKVS VLL DAVDEMDSYYSQYLPKVVQTTIVPLM VLI AAFSYDWITGLVMMITAPFIPLFYIIIGIMTQ KRADAQLEKMTAFSGTFLD TLQGLTTLKL FGRAKRQRDVIEKSSLD FRDATLTVLKLAF LSSLMLFISMLSMGMIALEVSLRLILFQSIT FIPAFMLMLVLAPEYYLALKEMGA AFHTGR GSVAAAKQIAAELTADDRPVAFGQSELSIN RPPRIVLDEVDFTYQEGRFALQDVTLAIEP YQKVALIGRSGAGKSTVLQLIAGLADPK EG RILLDGIERSQVAEASWFRQLSYISQHPYLY AGTLAENIAIGELRQATPEEIFEAAQAAGLE ELIATLPEGIDTVIGEGGRGLSGGEKQRVAL ARAFKRPNNVVFDEPTTGLDVKTERLLQQ AIEQLGQTATVITVAHRLHTIERSDQIVVLD GGRIVDRGTHDELLGRDSEYARMRSVQRG EETR

Table 3 continuous

PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_158266_159975	158266	159975	+	Efflux ABC transporter for glutathione/L-cysteine, essential for assembly of bd-type respiratory oxidases => CydC subunit	<p>atgaaggaaactactaggagctcttctgttgatggctgcatcaaaacgcgatatcgcc tggctgatcttcttgggttcctcgctggagtgacggcggttgattcgagca agtggttatctgatttcaaaagcagccttattgccaccgattcagacgcttgcctctt gattcgctacaaaaaatctcgaagtctgacgcgcgcctcagtcgatatgcagaa cggttatcttcgacccggcgacgtttacgacccctcagtgatatccggacatcgctt accggagactcgagccgctcgccaggaaatttcggcaataaccggagtgggg atttacttcccggatcgtcggggacgtcgaaggtctcaaaatagcttctacgtgt tctatccaccgacgtcttctgttcttctctgtacgacgttttctgttagttcttc tcgtggacgattcgccgggattctacttctgttctgattcgaccgcttctgattcc gggttgggttcggtaccgtgaacaacgttccgacgttcggtccgtgcccgcgtg gtgatctgtcagacaagtgacagagttgttccaaggctatcggtattaaagattt accagcaactgatcaaaaagaacaggacttgaatccgtcgcaaacggtatgt cgaggaagaaaaacggaatgggttacatgctgtttcgaaacctggcctgaatacga tggtagcgtgatcatctcttggctcgttctgtgctgggtcctatctcgtcgcgagt ggacagctcgaagggttctctcgttctactcgtacatcgatcgacggttttg aaaaacgcgcacggatggcgattttaccgggcttttcgaagatagccgcatgct gcgaacgactgatgaagtcgtgaccgagcaagaagaaccaacttatgacacgt ttgttctcgtacaggccacctcttgaacgaacgcgttgacgttcttccctgat gcaccgcgtccggtcttactgacgtcaccgtctcgttctcgcgcaagaaga cggcgattgtcggggcgagtggttccgggaatccacgttacttcaattgatgttac ggatgtatccgacagcggattaacgattgctggtcaagagtcagacggttgat ccggaaggactgtggcaacacgcaaatgtcgtcgaacagaatcattctttat ggaacgcttcgcgacaatctattactcgcgaacgaacgacgaacgagcg atgctgcaagcactcgtgatgttgggttaacgacgttcgaattaaacgacgtgat ttgaaaaaggcgagaacttcttgaggagaaaaacaacgcttagcgttgcctg tgtccttttgcgggaagcgtcgttcttactgcttgacgaaccgacgttccgttgatg ctttgacggaacagatgattctcaatcgactgttcgagcagagggacgcgaca cttgttctcgtcagccaccgggttagcggtcttgaaagcgtggtgatcaatcatcgtc atggatcaaggtcacgtcatcgaagtcggaacatataccgaattgatggcgcaac aggagcggttctatgagttaaaacaagtcgaacgtctgttctcgtccgattggct aa</p>	<p>MKELLGVFRLMVHQKRDIAWSIFFGVLAG VTAVGLFAASGYLISKAALLPPIQTLAVLIA LQKISSLTRAVSRYAERYSHRATFTILSDI RTSFYRRLEPLAPGIFGKYRSGDLLARIVGD VESLQNTFLRVLYPPIVLLLVFFCTIFFVSFF SWTIAGILLGLILTGFLIPGWFAFREQRF RSVRVRRGDLSTEVELFQGYRDLKIYQQL DQKEQDLNAVAKRYVEEKRNLHVASN LALNTMVTLLHISWLVLGVGAYLVASGQLE GVFLALLVMTSLTVFENAAPMAILPGFFED SRHAARKLDEVVTEQEEPTYDTFVLDQAP SFETNALTFTFPDAPRPVLRDVTVPISAGKK TAIVGASGSGKSTLLQLMLRMYPDTGLTIA GQESQTVDPDEGLWQHANVVLQNNHYFYG TLRDNLLLANEATDEAMRQALDDVGLTT FELNDRVFEEKGENLSGGEKQRLAIARVLLR EASLYLLDEPTSSVDALTEQMILNRLFMR EDATLVLVSHRLAGLEAMDQIIVMDQGHV IEVGYTYELMAQQGAFYELKQVEQSVFAP I</p>
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_165288_165917	165288	165917	+	Transcriptional regulator, AcrR family	<p>atgtcatcttctcaaccagaaaaatcgataaacgtcatctcgccacgggtcgagc acgcgagaaactgttgagtcggcgacgtgacgtcttcttagaacaagggtttcaaa cggcaacgatttcacaaagtgaattaaacaagcggcgctgcgctacggaacagccta tgtccatttcgaagggaagatgagctactcgtcgttttgatggaagatgtcatgag ccgttccacgcgatcgcggaacgaacattcgaaccgacgacgacccgctcgagg caaaggaaacgcatcgtgacgcaagcgacggtattcttactcgtcgcggaagaag aacgagcgatattacggattgtcgaagcaatcggcgtgtcttcatcgtccgt gcgaatggaaagcgttcgggaacggttcatgacggtcagtcaggtatcc agtatgcgcaagaagcaggtctgcgcggcaggtatgctgactgaaactcgtcg cccgtggttcttcttgaatgagatgtattattcgaagtcgccggaagagc gacagcgacgatcgaagagattgcgcacgtcttagcgacgatttatcgaagga cttaccggatcgaggattga</p>	<p>MSSSQPEKIDKRHLRTRVTRKLLSAARDV FLEQGFQTATISQVIKQAGVGYGTAYVHFE GKDELLVVLMDVMSRFHAIERTFEPTTP LEAKERIVTQATDFLRLAEERAILRIVEQA IGVSYIVRAKWKAIRERFIDRISQDIQYAO TGRLARQDVDSKLVARGWFFANEMYLFV VREEATATIEIAHVLAITYTQGLYRIED</p>

Table 3 continuous

PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_370367_370816	370367	370816	+	Universal stress protein family	atggagagccagatcatcacgatttttagtggcagtcgatggatcaaaagcggcag atcttgctttgaaaagcagtgaaatcgctaaactcgatcatgccaaactcgcat cgctcatgtcattgatattcagacatttctaagtacgtgccataagcgtttcattga tcaaaagtcacggaatgacacaaggactgctcgatcggtacgagaagcgggca cgagaagaaggcgtcgagacaattgaactggatcgcgaaatggctctccaaaa cattgattccgaaggtcatcgaccacgtgtcggtgctgggtgatcgtctcgggg gcggctgggatgaatgcgatggaacgcacatcatgcggagtgctctgaaaaa tctcccgttatgcgaaatgcgatatctatctcgtccgccgaaaccttgaagtga	MESQYHTILVAVDGSKTADLAFEKAVKFA KLDHAKLVIAHVIDIQTFANVRANKRFIDQ NVTEYAQGLLDREYKREAREEGVETIELVIE NGSPKTLIPKVIAPRVGAGLIVCGAVGMNA MERIMIGSVSENISRYAKCDIYIVRPKTLK
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_378282_379592	378282	379592	+	ABC transporter, substrate-binding protein (cluster 1, maltose/g3p/polyamine/iron)	atgaaattcaaaaaaacgaaactggtgctgcagcatctgtcctgacattatccgat tccttgctgcttggtggagaagatgagcaacaacgaaaacgaaagcggtaa gacgatcgtcacatggtgggctgggcgccacaacctgaagcggggaagcag tcgttgatcggttcaacgaatcgcaagacaatacgtcgttcaattcaaacgctaca gtgaagactatgaaaaacaattacaagtcgcgatgtgtcaggtgaagggtccagac atcatcggtctaaaaagaccgatgatccacagtagaaggatcgtgctgaccgt tgattcgtacatggataaagcagccggcaaaaggctggaagataaagttcgtcgag cttggaattgaacagcagacatcgacggttaacaatacgcggtcccaatcggtt cacaggtcaagcgtacttctgtgtaacaacagcagatgctcgacaatatgctgtg acaccaccgaaactacaagaacagtcagcgaatcaagaagattaaagac tcaggcgataaagtcatccactcatgctggagcaaaagatcggtggatcgatat cgacgtctacaacgtcctcagtcacgaagtgccgaggttacatccaaaagttc tttcgggtgaagcgaagtgagacagacgacagatggtcaaaacagcacaatct ggcaagacctctttaagataaagttccaagaaggtgcacttggtctagcgacat acaatgacgggatgaaccaatttctgataaaaaagcagcgtgtgggtcatcggt tcatgggaagcgcactcgatgacgacgaagagaaaaaagaaaaatggaaaatc gatgatgagctcggttgcgttccactcccgaacttagcaggcggaacagagcaac ccgtcatcgatcgatcgatggcacttggtgtaacaagaagtcacaaaca agaaggtgcagcggatgctcgttcatgagccaaggaaaaggacaagaagt ctacatgggtgagtgagatggcacctggcatcaaggacgtcaaaagtcgattca gatgctgcttcacatcggaagtgaaaaagagtcgtacaagatgttgacgaca cattagcaaaagcgggtgctagccgtggtatccgggatacgaattaaacgatatc ctcggtgaaggaactcaaaacatcgacagagacaagatccgaagaagcattac aacgcgttcaagacgcagcgatcaatcagcgaataa	MKFKKTKLVAAASVLTLSAFLAACGGEDE QQTKTKDGKTIIVTWGWAPQPEAGKAVV DAFNESQDKYVVQFKRYSEDEYKQLQVA MLSGKGPDIIGLKPEMIQYKDRVVPVDSY MDKAAGKGWKDFVELGIEQTTLDGKQY AVPIGFTQAYLMYNTMLDKYGVTPPKN YKETVSAIKKIKDSGDKVIPLMLGAKDAWI DIDVYNVLSHQVAPGYIQKVLSEAKWTD DEMVKTAQIWQDLFDKVFQEGALGLAT YNDGMNQFFDKAAMWVIGSWEAHSMT TKEKKEKWKIDDELGFVPLPNLAGGTEQP VIASIDMALGVNKEKQQEGAAEFIAFMSQ GKGQEVYMGFEFEMAPIKDVKVDSDAAF TSEGEKESYKMLNDTLAKAVASRGIRDTK LNDILGKELQNIATGQDPKEALQVRQDAA DQSAK
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_413828_413229	413828	413229	-	Transcriptional regulator, ArsR family	atggaacaaaaagcattcacatattagagagctatgaacagttaaaagtgatcag tgatccgttacgaacaaaaacttattgttctcgtcgaaaaaccacacaggtacat cagttagccacttgctgacgttatctcgcgcgaaggtcctctatcacgtacgggaa ctgaaaaagcagtgacttgattcgtctcgtccggagcgaagaacagggcggaatct attgaataatcatcaagcgattgctcgcaggatcattcctgccgatcatctactgaac ttcgtcgagacgaatgaagcgaccggcagtcgtatctcgaagtattgacgggg caaagacacgcgttcttacggctccgacagctacttttctccttcatctccgaact cgaggactggatgagcttatcacttcaaaaggaaactgacgatgtctgaagccgact ttcaacgcttctacaaaatctgtgcttattggacgagttcacacgggtccttca ctagacaaaaagataaaaaacacttctatatcatgacgactgccttcgcaattgat gaaccactgtttgagaacgacgaggtctaa	MEQKAIHILESIEQLKVISDPLRTKILMFLV EKPTHVHQLAHLTLSSRAKVLVYHVRELEK HDLIRLVRTEEQGGNLLKYQAIARGYIPA DHLNLFVETNEATRSYLEVLDRAKTRVL TAPTATFSPSPNVNEDWMSLSLQKELTMSE ADFQRFATKYRALLDEFTRSPSLDKKDKK HFYIMTTAFAIDEPLFENDEV

Table 3 continuous

PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_482465_485041	482465	485041	+	ClpB protein	isu;Proteolysis_in_bacteria_ATP-dependen nt isu;USS-D B-7 icw(1);Pro tein_chaperones isu;Type_VI_secretion_systems	atggatttgaacgtttaacggatcgggcacacgagggaaatcgtctctcccaagcgatcgcg aaacaacgtcgtcatagtaaatcacagaatggcatttgttacttgccttacttgcctcaagaaga agggattgcacgagtcgtcttgaaaagtgaatcaacggattgagacattgaatgagcgatt gacgagcgcatcggtaagcttctccttgccttcccaaggcagcagcctcgatcggggct cgttgctgaacgtgtgacggaagcagagacggaagcgcgtctcatgcaggatgactatgtc tccgtcgaacactattgttagcattgatcaacaatcaagtcacgagcgtcagctacctacgtcg tcaaggcatcacggaagagacactccgggaagcgatcgatgtagcaggcaatcggaa ggtagcagcgaataatcctgaagagacgtttgatgtgctgaaaaatatggacgtgatctcgt agcggatttccggcgggaaaaagatccgggtcatcgccgtgatgatgagattcgacgtg tcatccggattttagtcggaagacaaaaacaatcccgcttggattggagaacccggtgtcg ggaaaacggcaatcgttgaaggactcgcgcaacggatctccgaggggatgtaccggaga gtctgaaaaacaagcagttgtttagacatgagtcgctcgtcgtggtgcgaagtacc ggggtgagttcgaagaacccctgcaagcagtccttgaatgaagtcgaagaacccgaaggaca gattctcttattcatcgatgagctgcatacgatcgttgggtccggaagacgagcgcgcaat ggatcccggaacatgctcaagccgatgcttcacgaggagaattgcactgtatcgggtcgga cgacgtgtagtgacgggaagtatatcgaaaaggaccggcgctcgaaacgtcgcttcca acaagtcctcgtcgcggaaccgatgtcgaggatcagatttccattctcgttgattaaaaga acgattcgagattcaccacgggtgtcggattcatgataatgcgtcgtcgcggcgatgtt atccgatcggatatcacggatcgattcatccggataagcgatcgatcgtcgtgatgaagc atgtcgatgattcgaccgatatggagtcagtccagctgaactcgattcactcgttcgtcgg gtcatgcagctgaaatcgaggaaacggcattaaagaaagacgagatgaagcattccgg aaacgacttgcctcttacgacaagaactcagctctgtccgggaagcagagatgcgcgtcgc gacacgatgggaacgggaaaaagagaggttcgcaaaagtctcaacagtacgcgcgcatctt gagaaggcaaaactggcattacaagaagcgggaaggcgctatgacttgaaccgagcatcg gaaatcaagtacggtcaaatccggatcttgaggcacgactgaaagtcgcggaagatcatct gaacacgtctcacacgaactcgttcgagaagcgggtgacgggaagaagatattccgacatcg tttcgaaatggactggcattccgggtgacgcgcttgcgcaggggcgaacgcgaaaattactt atcttgaagatacgttcatagcgcgtcttcgggtcaagacgaagcgggttcgtctcgcgg acgctgtcattcgagcgcgcgggcatcaaggatccgaaccgccgattggctcgttctgt tcctcggtcgcgaggggtcgggaagacagaactgccaaagccttagcggcgcgatgtt cgatagtgaagatcatatcgtccgcatcgatatgtcggagtatatgaaaaacacatgtctcc cgtctcgtcgggtcgcctccgggatatgtcggttatgaagaagcgggtcaattgacggaagc gtcgcgacggagcccatttcagtgctgtgtttgatgagatcgaanaagcgcacggagacgt ctttaatatcttgcctcagttgtctcgatgatgtgcactgacggatgcgcaagacgtgtcgtcg acttcaagaacacgacgtcgtcatcatgacgtcaaacatcggggcaccaatcctgttgaagcag caaaagatggtgtcatcgatgcagcgggaagaagaagccgttcgccaagaactgaagcgcca cttcgaccggaggttctgaaccggatcgatgatacattttatccactccaccggacg gaaatcgaacgaatcatcgacaaagcggtcgaaaaatgagttcacgtttatctggacgtggc attacgattgatgttacggatgctgcgaagacgttgatttgcgaagaagccttgcaaccacaata cggcgacgtccaatcactatatacgaacgaacgattgagacgaacttcccgtgcctt gatcagtgggcgatacaagatggtgcccgattgcgttgacgtagaagacggcgaaatcg gtcgtccacggatga
								MAKDYYQTLGVTKDASNQDIKRAYRKLAKQYHPDVNKEASADQRFKDIQEAFDVLGDEQKRAQYDRYGSNFDQMAGAGAGGAGDYEDLFRQFGGGGRTRPGQSFGEFDMFGSFSQEEVSTDEELETVPVLGHLSTNEKVTVRLATGSIQLTLPKDVYDGKKVRLRGKSSMRNRQGVAGDVYVTHLKDQDQFRRVDQDVISTVRVHPTTLVLGGEVVADTLEGKRIKLKVKPGTKPGARLRIPNRGLSQANGHRGALLVQLEVKLPEMDRAFYEWEWRLAVKE

Table 3 continuous

PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_7 82930_783 418	782930	783418	+	Thioredoxin	idu(2);CBSS- 315749.4.peg .3658 idu(2);Glycin e_reductase, sarcosine_red uctase_and_b etaine_reduct ase	gtgtcaagaagtcgaaacgtaaaacacgaaaaaccgcaatccaaggcgaattggattacag cgggagtcacgccttgatcgtattgggtggaggattactctcgtcttcacggatcgagaagattc tgcacggaagaacggcaacctaacggctacacaaagtcgcagataaaagtaaaatccggatgaa ttctatacgtatttctaccaaacgggttgggttcactgtgaaaaagtaaaaccatatctcgtccgctt gggtgaaaaacaagacattccgttcgaacagatcgatcttgcctgctgcagcagtcggcttgggatac gtttggcatcgagggtacaccaactgtcgttactttaagacggtaaaagtcggacgcgtag cagggtgaacaaacagaagacagctataaagagttcttcgctgaaaagacgtaaaagactcaaa agaagaatcatctggtgatctcaatgattaa	MDFERLTDRAHEGIVSAQAIKQRRHSEITEWHL LLALLAQEEGIARVVFELNQRITLNGAIDEAIG KLPALSQATTPRIGGSLNLVTEAEARLMOQDD YVSVHELLLLALIKQSSPAAQYLRRQGITEETLREAI VSMRGNRKVTTKNPEETFDVLKKGRLVADFR SGKTDPVIGRDDEIRRVRILSRKTKNNPVLIGEPG VGKTAIVEGLAQIRVRGDPVPSLKNKQLFSLDMS ALVAGAKYRGEFEERLQAVLNEVKEAEGQILLFI DELHTIVGAGKTDGAMDAGNMLKPM LARGELH CIGATTLDEYRKYIEKDPALERRFQQVLVAEPDVE DTISILRGLKERFEIHGHVRIHDNALVAAAMLSDR YITDRFMPDKAIDLVEACAMIRDTMESMPAELD SLVRRVMQLEIEEAALKKETDEASRKRLAVLQOE LSSVREDESALRTRWEREKESQSVQQLRADLEK AKLALQEAEGRYDLNRASEIKYGGIPDLEARLKV AEESSEHVSHELVREAVTEEEISDIVSWTGIPVTR LAQGEREKLLYLEDTLHERVFGQDEAVRLVADA VIRARAGIKDPNRPISFLFLGPTGVGKTELAKAL AAAMFDESDHIVRIDMSEYMEKHNVSRVLGAPPG YVGYEEGGQLTEAVRRSPYSVLLFDEIEKAHGDV FNILLQLDDGRLTDAQGRVVDKNTIVIMTSNIG APILLEAAKDGVIDAEEEEAVRQELKRHRPEFLN RIDDITLHFPLHRTIEIRIIDKAVEKMSSRLSGRGIT IDVTDAAKTLIFEEAFEPQYGARPINRYMQRTIET KLARALISGAIQDGARIALDVEDGELVVHG
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_7 98111_799 256	798111	799256	+	Hypothetical radical SAM family enzyme in heat shock gene cluster, similarity with CPO of BS HemN-type		atgggaatcgcgcgcgatgtccacatcccttttgtgaacatatgtttactgtgatttcaata aagtctttttaagaatcagcccgatcgaatacctcgatgcgctcgaacgcgaatcgaactga cgttgaacaatacccgacggatcatctcgagacgatcttcatcgggtggcgaacacacacccgct tgaatgaaccacagatgcaacgggtgatggaatcatccagaagcacttgttgcactgacagg cgatgatttagatgacacggctcgaagtcgaacccggacggtgatcgaagagaaactcgatatca tgaagcgggcggtgtcaaccgcgtcagtttgggtccaatcattgatgacgggttgcctgaac gcacggctcggacgcaccgggaagcgaagtcgcgcagacgctcgacgcagcagcaaaacg gtttgataacatctccgctgacttgatgttcgggttaccgaatcagacgctcgatcaggtccgctac gatgtcacgcgggacttcaattgccgatcgcacatatctcttattcattgattttagaacgcgat acggcttcttcgattcaagagcgaaggaagaaagttacctttaccaacgcgaagatctcgaggctga gatgtatcaagtgatgattgatacgcgaagcagggtgatatgcgcagatgagatcgcgaact cgcgcaaacaggaagagagtcgtcataaccgcgtctactgggaaaacgatgagtactacgg attcgggtcaggatcacacagctatatcaatcagacgcgtcgtgcgaacatagcaccaattccgc actatatcaaggcgaagggttaccggttcgaaaagagacgcgcttgacgaacgtagagcggat ggaagaagagatgttctcggacttcaatgaaggtggcgtatcactcgaacgtttccggacga aatatgagtagcttttgaagatgtgttgggtgatgcatgaaacgtttattgccaacgggtcgtcgc aacagacggagacacatccggttaacgccagccgggttccattagcaaacgaagcttctgcg gaattcatcggtgaagcgcagggtcaatga	MSKKS KRKN TKKPQSKANWITAGVIALIVLVGGL LLVFTDREDSASKNGNLATQVADKVKSGDEFYIT YFYQTGCVHCEKVKPYLVPLGEKQDIPFEQIDLA VEQSAWDTFGIEGTPTVVHFKDQKEVGRVAGEQ TEDSYKEFFAGKDVKDSKESSGDLND

PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_965059_966243	965059	966243	+	SAM-dependent methyltransferase	gtggaaactacatgaattaaaagcagctcttgccctcgcaaaattacgaaagaaacatgatccatgcacgataagtaatccgcgtcaaaaatcaaatgatctccgacgaatcaaggtaaaaccgattctattaagagatacggatgatgcaggtagagtttcaatttgaaacgcgttatgaagcatgaaaaatttttagtggacgatttcgtcggcgactcgaatgactgggtccaggaattccgccaattcttattccgttttgaagaagaggaatggcagttccagctctcgaaaaagctgaaaagtcgcgcctcaagacatcca aaaaagagccgatgaagcctctcttctgcatgaccgcaaaaaaacctatctattagaagacgggtgtaccggttcgttttcgattcgccttaggcgtcatgacggaagcgggtcgaagtcacaaaacagaagtaacgataagtttaacacaaatcaatcgtttcttgaaattcgtcgaggatagcatcgctgcttgccaaaaggaaaaaaactccggatcttggacttcggatgcgggaagtcgtatttgacgtttgctctctatcattatctaaaagtcgtaaaaggattcgaacctgaagtgacaggcttgatttaaagaagaa gtcatcgaagaatgtacacaaattgcgcgtgatctggaattataccgatctgacattccgtgtcggagacgttcatgattaca cagggggagacgggaagtcgacctgatgggtgacacttcacgtcttgatgtcgcgaaccgacgtcgcaactcgcaacgacgtg ttgactggaaatgatcgtgatccttgagcgtaccgtgctgtcgtcaaaaggaaatgaaccgtcaactggactgttcgccactcg atgtcatgctccagcagcgactgatcaagaaaaattcgcgtcactggcgacagactcgattcgtgccgagttgtcgacg cttctcgatcgaagcgcagttgctcgaattcatcgatctgcgacacacccgaagaacatcttgattcgcgcgtatataa acaaaacaagcgcaccaacggaagaaaaatcgaccgtacatcgcgttccgtgattgttgatgccccgaccatttttag aaactgaattaaagcggcagacttggacagatttcaccgaaactcgtaacataa	MGIRAAVYHIPFCEHICYCDFNKVFLKNQPVDEYLDALEREIELTLKQYPTDHFLETFIFGGGTP TALNEPQMQRLEMEIQKHLPLTGDDLEYTVESNPDGVSEKLDIMKAGGVNRVSFGVQSF DDGLLERIGRTHREAKVAQPLDAAAARKFDNISVDLMFGLPNQTLQDQVRYDVTRALQLPITHIS SYSLILEPHTVFAIQERKKGKLPQTQDLEAM YQVMIDTIEAGGYAQYEISNFAQTGKESRHN RVYWENDEYYGFGAGSHSYNQTRRANIAPHYIKAEGLPVRKETPLTNVERMEEMLFGL RMKDGVSLEFRFTKYGVAFEDVFGDVMKRL LPNGLVEQTETHIRLTPAGVPLANEVFAEFIG EAQVQ
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_1217221_1217535	1217221	1217535	+	Arsenical resistance operon repressor	ttgatcgagacagcaaaacgggtgaagagatgagtcagttcttgaagctactgagcgaatgaacacgtcttgaattttg cgaagactacacgttcgggagcattgtgtctgtgatttcgtcgagatgtttgataatttcgaaccggcagtcagtcgat ctaaaattattgctgcaatcccgctcatcttgaacgacgggaacgacaaatggatttatttcgaatcaatccagatcacc cacaacacagattgttattgacgattttgaagaagtgacatgccggacatcccgatagaagttgctaa	MELHELKASLASQITQGTLIHATISNPRQKSN DLRRIKVKPILLRDTVMMQVEFQFERVMKHE NILVDDFVGRLLDDWFTEFRQFLRFEEEEWQ FQLSKKLKVALKTSKKEPMKALLSHDRKKT YLLEDGVPVPFLIRLGVMTEAGQVKKQKYD KFKQINRFLEFVEDSIAALPKGKKLRILDFGC GKSYLTFALYHYLKVVKGFDELEVTLGLDKK EVIECTQIARDLDYTDLTFRVGDVHDYTGTE TEVDLMVTLHACDVATDVALARAQWNAS VILSVPCCKQLKLNRLQDCSPLDVMLQHLIKE KFASLATDSIRAELLTLVGYEALQLEFIDLEH TPKNILIRAYQKNKRPTTEEKIDRYIAFRDLLH ARPFLETLSGRLQGISPKLVQ
PseudoContig_NC_010556.1	peg	PseudoContig_NC_010556.1_1217665_1218969	1217665	1218969	+	Arsenic efflux pump protein	gtgaacgcattgaccggtacacatttactagcgtcggaaattttctgatcaccttattcttaatcattaaacaaccgaaagg actcggcatcggttactccgcgatgggaatggcgtcgttgctcgctattgacggagtcgaacctcgaggatgtctcga cggctcggggcatcatcatctggaatgccacctttatctatcgtctgatcatcatctcattgattcttgatgagattgggtcttc gaatggcgccgcatcccatatggccagcatcgacgagtggtgtgtcaactattcgtgtacatgacattactcgtgtc tgtctgtgtcttatttcggaacgatggggcagccttgattctgacaccaatcgctcgcgatgggtcgtcacttgaac ttccgattatgtcgtgttagcttacgtcttcgcttcggattcattgccgatacagccagtttgcggttcgtcgtcgaacct cgtcaacatcgtctcagcagactctttaacatcgggtttacggagatgcgtcacggatgggtgttcgtaactctctttctg atcgcgagtcgtgatcgtctgtatctatttccgaagagatttccaaagaattacgatgtcgcgcgaactcgtcgcacccg cgacagcgaatcaagatcaacggatgtttaagctttcagttgctcctcattttgctgtcgttggaatttaccagtgacg tcactggcaatcccgctcagttctgtcgtggaatcgcgtttgttcttctattgatggcagcgcgttcacccgagcgtaccga cgaaaaaggctatcgggtgtgcaccgtggcgatcgtctcttttcttacttggcatgtacatcgtcgtctacggactgcaaa atgccgggttgacacaagtactcgtgacatcattgatggcttgcacgaatttagcagccgcaacatttggcatgggct tcttggctccatcttctgtcgtcatgaataatcgccgaccgtcatgatcgatcccttgcgatcgacttgagccagga aacgtgaacgattcgagaagcgtcatctatgcgaacgtatcggatctgatcttgaccgaagatcacgccaatcggtga gtctagcgacattacttggttacagctgcttcggacgaaaggcgtgaagattgggttggggcaatactttaagattggaa cgattctgacgatcccgacacttgcctacatttgccttgatatacagcttgccttattga	MIETAKRVEEMSQFLKLLSDGTRLEILRRLHV REHCVCDFVEMFDISQPAVSRHLKLLRQSQLI LERRERQWIYFRINPDHPQHRLLLTILEEMDM PDIPDRSC

Table 3 continuous

PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_1 238155_12 39072	1238155	1239072	+	Efflux ABC transporter, ATP-binding protein	atgattcatgtcgaacttgacgaagcgtacgatggaagaatgtcgtcaaggcatctcgttcgat gtcgaggcaggagaatcttcttcttaggaccgaagcgagcaggaaaatcaacgacctccaga tgctgacgacgttgattccgattccgggtgtcggcgacgattcaagaatttgatgtcgaacagg aaaaacagggttcgtctgcaaatcgagtgcttgcagataccggcatcgtatgaggatataaccggg ctgaattactcgtcctacaaggaaaattattcggattaaagtcagacggaagcaaaatacgggcaga ggaattactacgactcgtcggactcgtacgaagcgaagcgtcgtcaggcacttactccggcgg gatgaaacgacggcttgatcgtacgtgtcgtcgtccatcaaccgacctgttcttctcgaacacc aacgaccggactgacccggcgtcgtcgtcgtcaaatctggaacgaggtccggcgtcgaacgaag agttcgaacgacgactcttttgacgacacagtacttggaaagggccgacgtacgtacggaccgat cgcgatcatcaatgaaggcaactgattccggaaggacggcagctgagctgaaggcagcaaacg gagaagagcggatcgaattgacacttgcggaagagagcgaacaaatcgttttagagagctttgc cggctcgtcaggaagaccgtcgtcgtcattccttcgcggggaacgagacactgcgcgcgtcgt ccgttttctcgtatgaacggaagatggttgcgacgtcactggtcgtcaaacaccgtcacttgatgatgt cttcattcgtttgaccggacaagcttataaggaggaatcgtga	MNALTGTHLLAIGIFLITLFLIIKQPKGLGIGYS AMGMALVALLTGVVNLEDVSTVWGIIWNAT FTFIALIIISILDEIGFFEWAAALHMARIAGGG VKLFVYMTLLGAVVAALFANDGAALITPIV LAMVRHLKLPDYVVLAYVFASGFIADTASLP FVVSNLVNIVSADFFNIGFTEYASRMVFVNLF SVIGSLIVLYL YFRKSPKNDVAQLVAPATAI KDQRMFKLSWFWLILLVVG YFTSDVIGIPVSL VAGIILFFLLMARRSPSVPTKKVIGGAPWAI VFFSLGMYIVVYGLQNAGLTQVLAIDIIDGFA TNLAAATFGMGFLAAILSSIMNNMPTVMIDA LAIDL SQATGTIREALIYANVIGSDLGPKITPIG SLATLLWLHLRRTKGVKIGWGQYFKIGTILTI PTLAITLLGLYISLVLY
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_1 697908_16 97198	1697908	1697198	-	SAM-dependent methyltransferase	atgacagaacacagacgattgatacgtaggagatatttaaatgctcgcacgacgattgcgtga accagaaccgttttggacaagtattatgaagatcggcgaaccggctccgtttttaagaacattcct gacgagaacttagttcgtctacgtcgaacagggtcgtcgtcgtcgtgacgacattagatcgcgaac aggaccgggtcgaatgcgtatctatcgcgaacacgaggtttctcgtgacggagctggtatcag caaccgcgttagactggcgaacggaacagcgcagcagactggggcacatgttgcgttcgaaagc aagattttctagtgacgaccgaacagaatacatatgattcattttagcattccgctgctttcatcatatgg cgccacatcagcgtgtgacttatgtgacgcgactgaagagatgttgcacaggaggatatttcgg attgacgtgttttggcggggcggcgtcgttggcggggcgcgactgacggaactgggaggtctatcaag aaaatagctccaaagggtgactcgatacacgaaagagcgaattactcctcattttagtcattcgacg tcacgaactaacggaatgaacccgtcgacaatccggaggcattatttggctgctgctgactgtgg actggtcgttttgattaaaggattga	MIHVENLTKTYDGKDVVKGISFDVEAGEIFA FLGPNAGAKSTTVQMLTTLIPISGRATIQEF DVVKQEKGQVRLQIGVALQDGTIDE DLTGLEL LVLQGLFLGSLQTEAKIRAEELLRLVGLDQD GKRRSGTYSGGMKRRDLALTLVHQPTVFL DEPTTGLDPASRLQIWEVRRLEEF GTTIFL TTQYLEEADQLADRIAHNEGQLIAEGTAEL KAANGEERIELTAEKEDEQIVLET FAGRQEK DRLVIPSRGTETLRAVVRFLDERKMOVATSLV VKQPSLDDVFIRLTGQAYKEES
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_1 882552_18 81563	1882552	1881563	-	Thioredoxin reductase (EC 1.8.1.9)	atgtagattgtatcgtaatcgggtcaggaccgtgtgggttatcgcagcgatcgaatgcaagatcg gggactggacgtcgaagtgctcgaaggggaatatcgtcgaacgcatctatcgttatccgacgcat caaacgttctttcaagtagtgaagttagaattgggtgtgacctgtcatcaataaagaattaaaaacc gcgacgtctcgcgcgtcgtctattatcgagaagtcgtcaaacgaaacaattgacgattcgtccgtt tgagacggctcgcgcggattgaacgtcaagcggatcatttatcatcactcggagcgttccggggaa aaaagaagccgagaagctcgtccgtcgtgcttgcgacagggttattggtttaccaaacggatgga tgttctggagaggaaattacctatgtctcgcactactttacggaaggacatccgtattttgatcaagatg ttgtcgttatcgggtgaaaaaattcgacgttgatgcagcgaattgaactgaaaaagcaggagcacgt gtgaccgtctttaccgtggaccggagtatagtcacatcaaacatgggtgttgcgaacttcgaa tctctcgttcgttcgaaaaaggtcaagatgatttttggggcgtcaatcgaagaattacggagcaagag gtcgtttatcaaatagatgagataaacagaccgtcgtgctgattttgtttgcgatgacagggttacac acccgatgtcggattgtcgtgacacgggcattatgattgatcgtaaaacaggattatccaaagtcacga cgaagagacgatggaatcgaatgtccgggcatctatcgcaggtgtcgtcgcagcaggatgatgac gccaataaaatattcattgaaaacggacgttttcacgtgtcgaagcattgccaacctcgttgaacgg ctacgtgggtgcatcgatacaggcctaa	MKKWFVILMLIPVTLSSGCLFPDSQKPSNRVP YPQQLRTVQDAVDAYQKDTGVLPKTM EAD TPLMERYQIELRLIPRYMSDPPSNSFE GGT FIYLLVDVDTKPTVKLMDLLVAEELQKLQVR IDAYRKKNEKYPFKGSFGKNQFTLDYKKLFI KKEPTVSPSPYDEKLSIYVDGQGRLFINYLPE LKKAVQTAKKKPKVGQDIRYLLYEDQPFPVA YSQSYTINEKGEPVFSEESIEESEN

Table 3 continuous

PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_2 235507_22 35196	2235507	2235196	-	Thioredoxin	idu(2);CBSS-315749.4.peg.3658 idu(2);Glycine_reductase_sarcosine_reductase_and_betaetaine_reductase	atggcaatcgtacacgcaactagccaatcatttaaagaagaacacacaagaagactcgtccttgtgattttgggcaacatggtgtggaccatgctgtagctcgcacactgttcttgaagaactcgtcgtgacatgcaagatgtataaatcgtcaaatgcgacgtagatgcaaacccagaagtagctggagcattccaagtccaagtatccgacactcgttctctcaagacggacacacctgtcaaaaaacaatggcttcacgccgaagacgcacttaagaattcgttgaacatctaactaa	MAKFTIVDKDTCIACGACGAAAPDIYDYDDEGLAYVILDDNNGTAEIPEALFDDMIDAFEGCPTDSIKVADESFDGALKFE
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_2 389480_23 90478	2389480	2390478	+	Thioredoxin reductase (EC 1.8.1.9)	idu(3);pyrimidine_conversions idu(3);Thioredoxin-disulfide_reductase idu(3);Glycine_reductase_sarcosine_reductase_and_betaetaine_reductase	atgcaccaaccatgatgtcacgatcgcggcggtccggtcggactgttcaccgcgtttttatcaggatgcgccagatgaagacgaaggatcatcgagagctctacctcaattagtggtcaactcgtcagctttatcctgaaaaatatatacatattgcccgttttccgaaagttaaaagcacaggaactggtcgtcgttgcctgaacaaatgaattcgtacacatacgtcgtcggtgagacagtcacgtttacgaacggatgatgacggaatcatccgtctcgtcacgaacaaagccgagcattatacgaagaccgtcatcttgacggcaggaacggctcgttcgccgcgcgtccactaggagtgccgagtcgaagcgttcgaagaacaaacttacactttctgcaacgacatggaacgtttcaaggatcgcaagtctcttactgggtggcggtgactccgagttgattgctcattgatgcttgagccgattgcgaatccgtcacgctcgtccaccgtcgtgataagttccgtgccatgaacattcagtcgaactgttacacgattcgtccgcaacgtcatgacgccgtatagcgttgaatcgggtcacggtgagacgcacatcgagacgctgacgttcaaacatgctgaaagtggtgaagtgcgtcgtcgtccgacgatgctctgtaactcggcttctcgtcgtcactcgaccgtcaaaagaatgggaagtcgaattcgaacgcaattcgattcgtgtaattcgaagatggagactcgattccggcgctctcgttctgtgataatcgcaacttacgaaggacgcgtcaaacatgatcgcaacgggttcgggaagcacaatccgcttaacaaacgaaacttctcgttgatccatctgctcgtcaccgcaacactcgacgagctctgttgcgaaagtaacgaatcattcttga	MAIVHATSQSFKETQEGLVLVDFWATWCGPCRMLAPVLEELDADMQDVKIVKVDVDANPEVAGAFQVQSIPTLVLFKDGQPVNKTMGFMPKDALKEFVETSN
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_2 417640_24 17002	2417640	2417002	-	SAM-dependent methytransferase YrrT	icw(2);Methionine_Biosynthesis	atgacagtacgttttggatgttttcacagaatgggcatcgacgtatgacgagacagtaccggacacgattccggagtataaagagggttttctgctgatacgtgagattttgatacagtagcggataaagcgcagtcgcgggtcattgaattcggagcagggcacagggaacctgacacacgtctcttggcacgtcagcagccgtactgcctcgaaccaagtccggagatcgggcaattctgacggaaaagattcgtcactgatgtacaagacggctcacttctgtcgttcgaagcgaaggagcagcaggttttgggtgacgtatcggtttcatcatctaagatgagagaaaaggagaagcaatcgaactgatgcatcaccattaccgagcaggaataatcgtctacgcgatagatgttctcgaagagcagcgttgcaaacgattcgtgaagcagctgtcgaaggttcgacggttagcggagatcgaacgcgaggttttaccattgattccagttatggaagaattgttacagcagcgggattcactgttaaatttatacatacaatcacttctcgtcgtcgtagaagccgagaaaaatggggataa	MHQPVDVTIIGGGPVGLFTAFYSGMRQMKTKVIESLPQLGGQLATLYPEKYIYDIAGFPKVKAEQELVDRLLLEQANEFDPYVVLGETVIAYERMDDGIIRLVTNKGEHYTKTVILTAGNSFAARPLGVADADERFEETNLHYFVNDMERFKDRQVVLGGGDSADVWSLMLEPIAKSVTLVHRRDKFRAHEHSVELLHDSSVNVMTPTYLTESVTGETHIEITLTFKHAESGEVVVVAADDVVCNFGFVSSLGPLKEWEVEFERNSIRVNSKMETAIIPGVFACGDIATYEGRVKLIA TGFGEAPIAVNQAKLLVDPSARHPQHSTSLFEKVTNHS

Table 3 continuous

PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_2 418462_24 18181	2418462	2418181	-	Thioredoxin	idu(2);CBSS-315749.4.peg.3658 idu(2);Glycine_reductase_sarcosine_reductase_and_beta_reductase	atgcaagaggtcacgaccgtcccgaaaacgggctattgtatgtctatgcaccgatgtgcgggacatgcgccatcgcggagcgtatgcttgatctgcgaagcaacggaatcaggatcaatcgattcagaagcgaacgggaatttcattccggaattcttagaagaagcccgctgctcatgagtgcttctgcgttggtgcgaattgaagaaaatcaagtcgtagagcgactttatgcttccagtcggttcaaacgtctttgctttctgaagaa cggtaa	MTVRFMDVFTEWASTYDETVTGHDPEYKEVFRR YDEILDTVADKAQSPVIEFGAGTGNTLTQRLRLARH DAVLAVEPSPEMRAILTEKIPSLDVQDGHFLSFEA KEARSFVSTYAFHHLTDEEKGEAIELMASHLPAD GKIVYADTMFVSEEARLQTIREARAQGF DALAED LEREFYPLIPVMEELFTARGFTVKFIQYNHFVWLVEAEKMGE
PseudoCo ntig_NC_0 10556.1	peg	PseudoCo ntig_NC_0 10556.1_2 425524_24 26171	2425524	2426171	+	SAM-dependent methyltransferase		atgagtc aaacgagcatgtatggaatcaaaagccgctgactggcgatcgcgctgacgatatgtggacgaacggcagtcggaaaacggtttgcccgttcttacgaaaacgatcaccggtggctgcttgcttgatctcgggttgcggtgatgggtgcagcctgtcgttactgacaccggactttcagatagtcggacttgatgtttcagacgagatgatccggatcgcccgtagaagaatcaccgagcagacgttcacgttggaacgggtgaagcattgccgttcgccgatcgcgtttgatgtcgtcctcgccgtcaactcactcgaatgggtcgacacgtcccggtcaagtcgtcgaagaaatcgaaacgctggtcccgctgtgctcatcagcttactcgtcgcacgagcaccacggcaacttgcttaccgtcgtctctatggtgaggaagtcgcgatgggaaatac gatgatgccgtgggaactgttcagctagcaaaagaacaggcgtggacgttactcgatgaagcgtcatccaaaaagaaggtgccgtcatgacggggcatcggttactggatcaagcccatgccttctcatgtttgttgcttttcagacgacagctgtgagggaagataa	MQEVTTVPENGLLYVYAPMCGTCAIAERMLDIVE ATESGQSIQKANGNFIPDFLEEARVMSVPALLRIEENQVVERLYAFQSVQNVFAFCQER
								MNTGKVKWFNAEKGFGFIEVEGGEDVVFVHFSAIT GEGFKTLDEGQVEFEITEGARGAQAANVVKL	

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