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ISOLATION AND CHARACTERIZATION OF ARSENIC RESISTANT *PSEUDOMONAS STUTZERI* ASP3 FOR ITS POTENTIAL IN ARSENIC RESISTANCE AND REMOVAL

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ABSTRACT
Inorganic arsenic both arsenite As (III) and arsenate As (V) constitute the highest toxicological risk associated with arsenic in drinking water. Novel methods are in demand for its removal from water, especially in rural areas. For this purpose, the potential of different microbes in arsenic resistance and removal from water has gained interests worldwide. This study investigates the arsenic resistance and removal capacity of a bacterial strain isolated from arsenic enriched water of Rautahat district in Nepal. The concentration of arsenic was by hydride generation atomic absorption spectrophotometer. The isolated bacterium showed high resistance to sodium arsenate up to 4,680.15 mg/l and sodium arsenite up to 649.55 mg/l. The bacterium also conferred multiple heavy metal resistance to zinc chloride (136.28 mg/l), copper sulphate (249.68 mg/l), mercuric chloride (5.43 mg/l) and silver nitrate (3.39 mg/l). The growth rate calculated in the presence of 129.01 mg/l of sodium arsenite was 0.35 h^{-1} with doubling time of 1.96 h. The strain showed growth in the range of 25–45 °C (optimum 30-35 °C), pH 6 – 9 (optimum 7.5-8.5) and tolerated up to 10% of NaCl. The PCR-based 16S rDNA sequence analysis revealed that the isolated As resistant bacterium is a close relative to *P. stutzeri* (99%) with 30 hits. The bacterium removed 82.97 % of As (V) and 49.4% of As (III) from culture medium amended with 200 mg/l sodium arsenate and 74.92 mg/l of sodium arsenite respectively.

Keywords: Arsenate, arsenite, *Pseudomonas stutzeri* ASP3, arsenic removal

INTRODUCTION
Arsenic occurs in the environment in several forms but in natural waters, it is mostly found as trivalent arsenite [As (III)] or pentavalent arsenate [As (V)]. Arsenate generally is the dominant form in aerobic waters. In contrast, arsenite dominates in sulfidic and methanic waters including most geothermal water. Both forms are toxic; comparatively arsenite is the most toxic form [1]. While arsenic is a well known poison, a number of taxonomically diverse microorganisms have evolved biochemical mechanisms that either prevent arsenic from entering cells or rapidly extrude it back to the environment if it does enter [1]. These detoxification reactions are mostly centered on redox changes between the As (III) and As (V) oxidation states, and can alter the speciation of arsenic found in the surrounding aqueous medium. Other, defense mechanisms of microorganisms include a variety of methylation reactions that produce methylated oxyanions of both As (III) and As (V), or form highly toxic methylated arsine gases where the arsenic end product is in its most chemically reduced form [1]. Many bacterial strains contain genetic determinants that confer resistance to arsenic. In bacteria, these determinants are often found on plasmid, which has facilitated their study to the molecular level for example in *Staphylococcus aureus* and *Escherichia coli* [2, 3]. Bacteria can develop genetically determined resistance to arsenic [2, 4]. The mechanism of resistance in these bacteria involves intracellular reduction of arsenate to arsenite followed by efflux of the arsenite [5]. Some of the resistant organisms have the capacity to oxidize reduced form to arsenate and others to reduce oxidized forms [6]. *Bacillus arsenicoselenatis*

and *Bacillus selenitireducens* from Mono Lake, California, United states, were able to reduce arsenate [7]. Saltikov isolated *Shewanella sp.* Strain ANA-3 from arsenic treated wooden pier located in Eel Pond, Massachusetts that anaerobically respire arsenate as terminal electron acceptor. Besides anaerobic reduction it is also able to reduce arsenate aerobically to arsenite [6]. Takai *et al.* described *Sulfurihydrogenibium subterraneum* strain HGMKI from hot aquifer water of Hishikari gold mine, Japan [8]. Likewise, *Pseudomonas fluorescens* has been reported as active reducer under aerobic conditions [9]. Patel *et al.* reported reduction of arsenate to arsenite by *Pseudomanas sps* [10]. The oil-degrading bacterial isolates OSBH2 (similar to *P. stutzeri*) and OSBH5 (similar to *B. cereus*) showed efficient reduction of As (V) to As (III) [11]. Microorganisms and microbial products have been reported to efficiently remove soluble and particulate forms of metals, especially from dilute solutions, through bioaccumulation and therefore microbe-based technologies provide an alternative to the conventional techniques of metal removal/recovery [12]. This paper evaluates characteristics of *Pseudomonas stutzeri* ASP3 isolated from arsenic enriched water sources of Rautahat district, Nepal for its arsenic resistance and removal potential to be utilized in bioremediation.

MATERIALS AND METHODS

Sample collection and analysis

The water samples were collected randomly from different water sources located at Dumaria (sample STJ1 and STNJ4), Bagahi village (sample SB7) and Mardhar village (sample SRC10) in Rautahat District of Nepal (Fig. 1). Water samples (100 ml each) were collected aseptically in sterile sampling bottles from each site and brought to laboratory at Kathmandu University. Within eight hours the samples were processed for heterotrophic plate count as well as isolation of arsenic resistant bacteria. Some physiochemical parameters namely: temperature, pH, dissolved oxygen (DO), Total organic carbon (TOC), nitrate, iron (Fe), manganese (Mn) and arsenic (As) concentration were measured according to the standard methods for the examination of water and wastewater (APHA) [13].

Figure1 Sample collection site indicated by white star (☆)

Isolation of arsenic resistant bacteria

For isolation of arsenic resistant bacteria, samples were randomly collected from river SRC10, toribari jungle well STJ1, toribari non-jungle well STNJ4 and Bagahi well SB7 containing varied arsenic concentrations of 6µg/l, 19µg/l, 42µg/l and 25µg/l, respectively. Samples were serially diluted in sterile distilled water to 0, 10^{-1} and 10^{-2} dilutions then plated on plate count agar supplemented with different concentrations of sodium arsenate, $Na_2HAsO_4 \cdot 7H_2O$ (0, 0.5, 10, 40, 80, 160 mg/l) by standard pour plate method [14]. Three

replicate plates for each dilution and arsenate concentration were incubated for 24 h (hour) at 30°C. Viable counts of bacteria capable of resisting each concentration of arsenate were determined by counting visible colonies as colony forming unit per ml (cfu/ml). One of the highly abundant and resistant isolate was screened for detail study.

Identification and characterization of the bacterial isolate

Arsenic resistant bacterium obtained was initially characterized in terms of colony morphology (color, shape, size, elevation, margin, consistency, opacity) and basic microscopic observations (gram stain, spore stain, size). The isolate was analyzed for different biochemical tests with the help of Hi media test kits. These tests were used to identify the isolates referring to the Bergey's manual of systematic bacteriology [15], determinative bacteriology and probabilistic identification matrix. The Willcox probability (P) matrix was used to assign and test the isolates with P scores of 0.9 indicated a positive identification then further confirmed by 16S rDNA sequence analysis.

16S rDNA sequence determination

A colony PCR method was used for amplification of 16S rDNA. A single colony of bacterial isolate was suspended into 50µl sterile distilled water. Boiled for 0.25 h at 95˚ C and centrifuged at maximum speed in microcentrifuge for 0.17 h to pellet cell debris. 5 µl of the supernatant was used as DNA templates for PCR. Bacterial 16S rDNA was amplified from the extracted genomic DNA by using the universal bacterial 16S rDNA primers Bact8F forward primer (5'-AGA GTT TGA TCC TGG CTCAG-3') and Bact1492R reverse primer (5'-GGT TACC TTG TTACGA CTT-3'). PCR was performed with a 25 µl reaction mixture containing 5 µl of DNA extract as the template, 0.2 mM of primers Bact 8F and Bact1492R, 0.2 mM of dNTPs and 1 U of Taq polymerase with its supplied 1X buffer (Fermentas, Hanover, Germany). The thermal cycling was performed in MJ minicycler (MJ research, PTC 100,US) and consists of an initial 95°C denaturation for 0.05 h followed by 30 cycles of 95°C for 3 sec, 55°C for 1 min, 72°C for 2 min, followed by a final extension at 72°C for 6 min. PCR product of 0.5 kb was analysed by electrophoresis in 1.5% (w/v) agarose gel in 1x TAE buffer with ethidiumbromide (0.5 µg/ml) before being subjected to further analysis. The amplification products were purified with spin column (Centricon 100 columns, Amicon, USA) by following the specifications of the manufacturer, followed by ethanol precipitation. The amplified 16S rDNA fragments were used as templates for DNA sequencing. The DNA sequencing was carried out in Gene way Research, USA.

Nucleotide sequencing, alignment, and phylogeny

Sequences were matched with previously published bacterial 16S rDNA sequences in the National Center for Biotechnology Information (NCBI) databases using Basic local alignment search tool using nucleotide query (BLASTN) (http://www.ncbi.nlm.nih.gov/). Based on the scoring index the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA by using Clustal X version 2.1 to identify the most similar sequence alignment. The phylogenetic relationship was generated using bootstrap Neighbour- joining algorithm [16].

Determination of optimal growth conditions

For optimal growth conditions, three parameters pH, temperature and salinity were considered. The strain was grown in nutrient medium with varying pH values, i.e. 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 and incubated in water bath shaker at varying temperatures of 25°C, 30°C, 35°C, 45°C, 50°C and 60°C respectively for 48 h. The effect of salinity was studied in

nutrient broth amended with different concentrations of NaCl as 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% respectively, and incubated at 30°C for 48 h. The optical density of the growing cultures in the above mentioned conditions were observed at 600nm using an ultraviolet visible spectrophotometer (Electra, USA) and optimal growth was determined as a function of biomass by measuring the absorbance at 600nm against blank media [17].

Growth kinetics of *Pseudomonas stutzeri ASP3*

Culture slants were made and kept at 4 °C. The bacterium was grown at 30 °C in nutrient broth medium with continuous shaking at 150 rpm in the orbital shaker in the presence and absence of 129.90mg/l sodium arsenite and 624.02 mg/l sodium arsenate. Optical density was measured after different time intervals at 600 nm using UV-visible spectrophotometer. The growth rate constant (k) for the log phase of growth was determined by plotting the $\log_{10}$ of the optical density against time. Experiments were performed in triplicate [3].

Determination of Maximum Tolerance Concentration (MTC)

Maximum resistance of the selected isolate against increasing concentrations of As on nutrient broth/agar plate was evaluated until the strain was unable to give growth on the respective media. The growing colonies at a given concentration were subsequently transferred to the next higher concentration. Nutrient medium supplemented with the respective arsenic salt concentration i.e. sodium arsenite ($NaAsO_2$) 0, 0.5,10, 40, 80, 129.91, 259.82, 389.73, 519.64, 649.55, 779.46 and 909.37 mg/l; sodium arsenate ($Na_2HAsO_4.7H_2O$) 0, 0.5,10, 40, 80, 160, 200, 300, 400, 500, 600, 700, 800, 900,1000, 1562.5, 3120.1, 3746, 4680.15 and 6250 mg/l were inoculated with cells from fresh overnight cultures and incubated for 48 hrs at 30˚C [3].

Screening for As (III) Oxidation and As (V) reduction Activity

The isolate was tested for the abilities to oxidize As (III) or reduce As (V) using a qualitative $KMnO_4$ screening method [18]. The arsenic resistant bacterium was cultured to nutrient broth medium containing either 312.01 mg/l sodium arsenate or 129.91 mg/l sodium arsenite. After forty eight hours of incubation 1 ml of the culture was pipette out in sterile eppendorf in triplicate. The cell pellet was separated by centrifugation at 5000 rpm for 0.25 h. The pellet was washed twice in distilled water and suspended in 20 µl of sterile distilled water and 80 µl of sterile Tris HCl buffer pH 7.4. 0.1 µl of either 312.01 mg/l sodium arsenate or 129.91 mg/l sodium arsenite was added to bring the final concentration of 1 mM and incubated at 30 °C for 48 h. Then, 20 µl of 0.01 M $KMnO_4$ was added to the culture. A pink colour of the mixture indicate positive arsenite oxidation reaction [formation of As (V)], and a yellow colour indicate positive arsenate reduction reaction [formation of As (III)] [18].

Resistance to heavy metal ions

The cross heavy metal resistance of bacterial isolate was determined by using stock solutions of different metal salts (zinc chloride, copper sulphate, mercuric chloride, silver nitrate). Nutrient agar plates and nutrient broth flasks containing 50 ml medium amended with metal ions were inoculated with overnight bacterial culture and incubated at 30°C for 24 h. Growth was confirmed as presence of colonies in nutrient agar plate and by measuring optical density at 600nm [17].

Removal of arsenate and arsenite by *P. stutzeri* ASP3

The bioaccumulation of arsenic was carried out using bacteria grown in 250 ml conical flasks containing 50 ml of nutrient broth medium supplemented with arsenic salt at the concentration of 129.91mg/l of sodium arsenite or 200 mg/l of sodium arsenate. The

incubation was done at 30° C for 96 h. At selected intervals of time, samples were harvested by centrifugation at 5000 rpm; supernatant was collected and stored at –20° C for arsenic analysis. The arsenic was analyzed by Hydride generation Atomic Absorption Spectrophotometer (HG-AAS), (Thermo electron corporation, UK). The amount of metals in samples was estimated by using known concentrations of metals in the medium as control [16].

RESULTS AND DISCUSSION

Physicochemical characteristics

Basic physicochemical characteristics of the groundwater and river water sources enriched with arsenic and arsenic free or low background with noted influence on microbial growth and arsenic were ascertained (Table 1). World Health Organization (WHO) guideline value for arsenic in drinking water is 10 µg/l and Nepal Standard is 50 µg/l. Iron (Fe) and manganese (Mn) are primarily nuisance chemicals with characteristic staining properties, although high levels can impart a bittersweet or metallic taste to drinking water. For these reasons, iron and manganese are regulated by secondary drinking water standards established by the U.S. Environmental Protection Agency for public water supplies. The drinking water standard for iron is 0.3 milligrams per liter (mg/l), and the standard for manganese is 0.05 mg/l. Manganese is an important water quality parameter because it can catalyze the oxidation of arsenic (III) to arsenic (V). Similarly, iron hydroxide is a strong adsorbent for arsenic [19]. Iron is one of the essential elements for microbial growth. Arsenic reported to be mobilized in groundwater as a result of As - oxyanion adsorbed onto Fe- and Mn- oxides as well as reductive dissolution of these surface reactive phases from the sediments [20].
Similarly, high organic carbon content influence the abundance of heterotrophs and arsenic resistant bacteria in rice soil [21].

Table 1 Physical and chemical parameters of collected water samples

Sample code	Depth (m)	pH	Temperature (°C)	(TOC) mg/l	Nitrate mg/l	DO mg/l	Arsenic (µg/l)	Iron mg/l	Mn (mg/l)
STJ1	16.77	7.5	24.2	58.04	9.43	7.5	19.0	1.25	3.93
STNJ4	16.77	7.3	24.4	151.6	0.93	7.6	42.0	2.80	0.48
SB7	8.53	7.3	24.1	69.77	0.93	7.8	25.0	10.15	0.67
SRC10	surface	8.4	22.0	61.78	0.44	8.5	6.0	0.57	0.28

Enumeration and isolation of arsenic resistant strain

Viable counts of the total heterotrophic and arsenate resistant bacteria counted as cell forming unit per milliliter of water (cfu/ml) in water sources from four selected sites showed that the water samples contained very high numbers of culturable arsenate resistant bacteria, despite the diverse arsenic level (Figure 1).

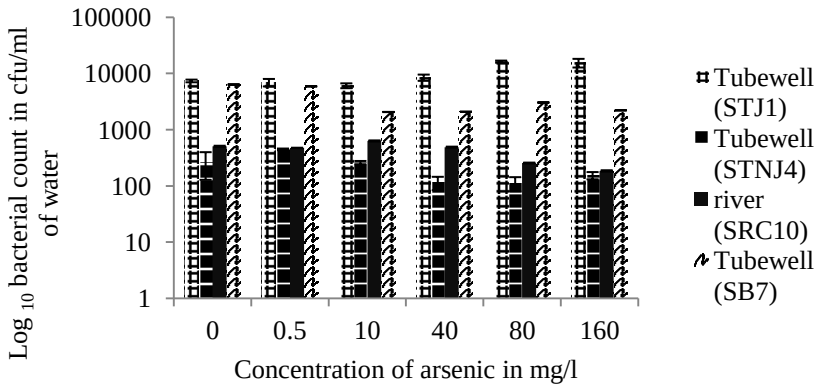

Figure 2 Enumeration of arsenic resistant bacteria from four selected water sources of Rautahat district, Nepal. Data are expressed as mean ± standard deviation of triplicate.

Isolation and identification of arsenic resistant strain

Numbers of indigenous arsenic tolerant bacteria were isolated from the water sources. *A* bacterial strain showing growth up to 160 mg/l was selected as one of the potent strains for study. The strain formed non-fluorescent yellowish wrinkled colonies in nutrient agar (Fig. 3). Cells are aerobic, Gram-negative, rod shaped, motile and non-spore formers, measuring approximately 1x0.5µm (Fig.4).

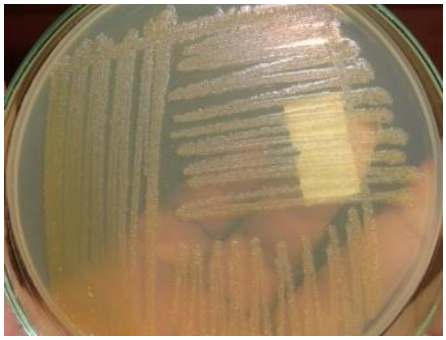

Figure 3 Pure culture of *P. stutzeri* ASP3 in nutrient agar

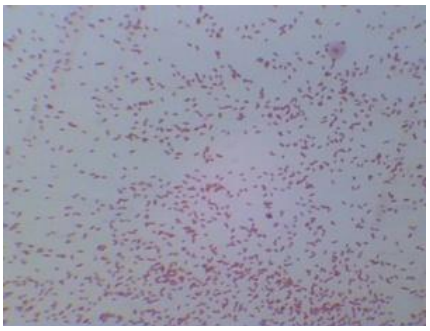

Figure 4 Microscopic image of the of *P. stutzeri* ASP3 isolated from arsenic contaminated water sources of Rautahat district, Nepal, obtained after performing Gram stain

Table 2 Phenotypic characteristics of *P. stutzeri* ASP3 isolated from arsenic contaminated water sources of Rautahat district, Nepal

Tests	Result		Result
Catalase	+	Trybutyrin Test	-
Oxidase	+	Motility Test	+
Oxidative/fermentative	O/F	Starch hydrolysis Test	-
Indole	-	Gelatin liquefaction	-
Methyl red	-	Casein hydrolysis	-
Voges Proskauer's	+	Malonate utilization	+
Citrate utilization	+	Alkaline phosphatase	-
Triple sugar Iron agar	R/R	Arginine utilization	+
Nitrate reduction	+	Lysine utilization	+
Urease	-	Ornithine utilization	+
H_2S production	-	ONPG	-

Note: R/R = Alkaline/ Alkaline, O/F =Oxidative and fermentative

Table 3 Phenotypic characteristics of *P. stutzeri* ASP3 isolated from arsenic contaminated water sources of Rautahat district, Nepal

Tests	Results	Tests	Results
Phenylalanine Deamination	-	Inositol	-
Arabinose	-	Inulin	-
Adonitol	-	Lactose	-
Cellobiose	-	Maltose	-
Dextrose	-	Mannitol	-
Dulcitol	-	Melezitose	-
Fructose	+	Melibiose	-
Glucosamine	+	Mannose	-
Glucose	+	α-Methyl-D glucoside	-
Glycerol	-	α-Methyl-D mannoside	-
Galactose	-	Raffinose	-
Ribose	-	Sorbitol	-
Rhamnose	-	sorbose	-
Saccharose	+	Trehalose	-
Sodium gluconate	-	Xylitol	-
Sucrose	-	Xylose	-
Salicin	-		

Note: positive test = +; negative test = –

Based on the morphological, physiological, biochemical characteristics (Table 2, Table 3) showed that the strain is close to the members of genus *Pseudomonas*. The partially amplifed 1121 bp fragment of 16S rDNA sequence was submitted to NCBI database search using Blastn to confirm the species of the bacterium. The highest sequence similarity revealed that it is closely related to *P. stutzeri* (99%) with 30 hits, *Pseudomonas putida* isolate SR21 (99%)

accession number, DQ288952, Bacterium enrichment culture clone CL-9 (99%) accession number, HM459846 and 99% similarity with uncultured bacterium with 38 hits. The multiple sequence alignment and the phylogenetic relationship confirmed the highest sequence similarity with *P. stutzeri* (HQ680964.1). The calculation and classification of phylogenetic relationship was determined by bootstrap neighbor - joining algorithm using ClastalX (2.0) (Fig. 5).

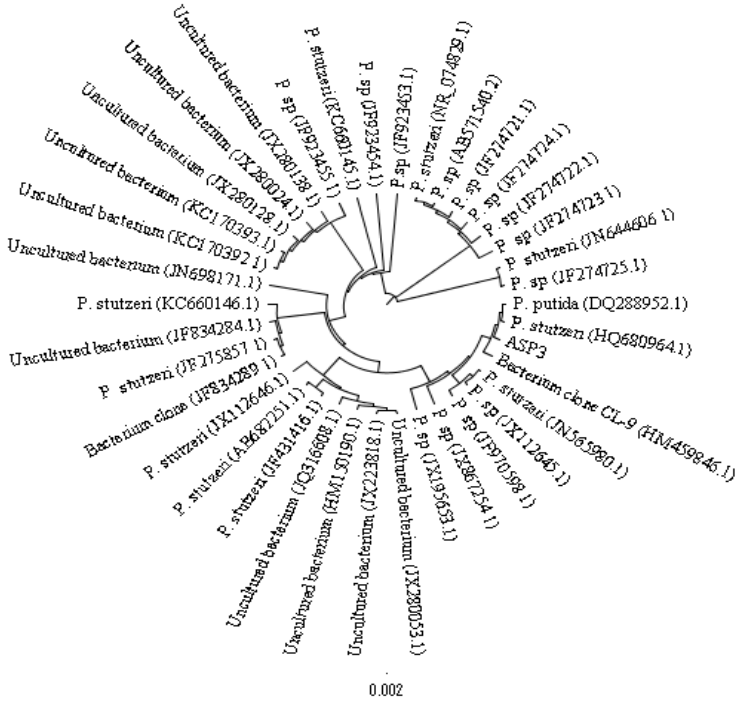

Figure 5 Phylogenetic relationship based on 16S rDNA sequence comparison showing the position of ASP3 and representative species. The GeneBank accession numbers for the 16S rDNA sequences are given after the strain in parenthesis.

Determination of optimal growth conditions

The strain showed growth in the range of 25–45 °C (optimum 30-35 °C), pH 6 – 9 (optimum 7.5-8.5) and tolerated up to 10% of sodium chloride (NaCl). The cellular growth of the bacterium was found to be better in presence of 74.92 mg/l sodium arsenite compared to149.84 mg/l sodium arsenate and in absence of arsenic in nutrient broth (Fig. 6). The growth rate calculated in the presence of arsenite was 0.35 h^{-1} with doubling time of 1.96 h. The pH of the extracellular medium was found to increase gradually from 7.8 to 8.97 from the lag to the stationary phase in the medium with arsenate (Fig.7) while the pH increased from 7.69 to 7.83 in presence of arsenite (Fig.8). Growth pattern of *R. eutropha*, *P. putida* and *B. indicus* in absence as well as in presence of arsenic were reported to be similar due to prior acclimatization of the bacteria in the arsenic containing media [22]. It may be true in our investigation as well since the bacteria were subcultured from arsenite containing medium (74.92 mg/l).

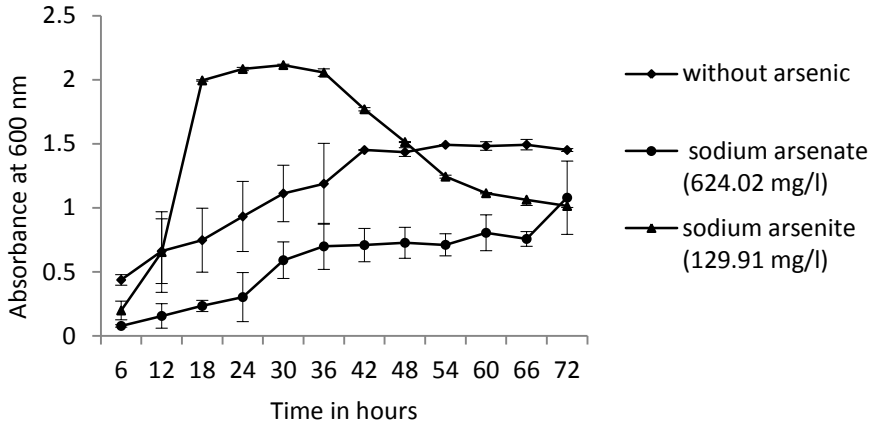

Figure 6 Growth of P. *stutzeri ASP3* in the presence of arsenate, arsenite and in absence of arsenic. Data are expressed as mean ± standard deviation.

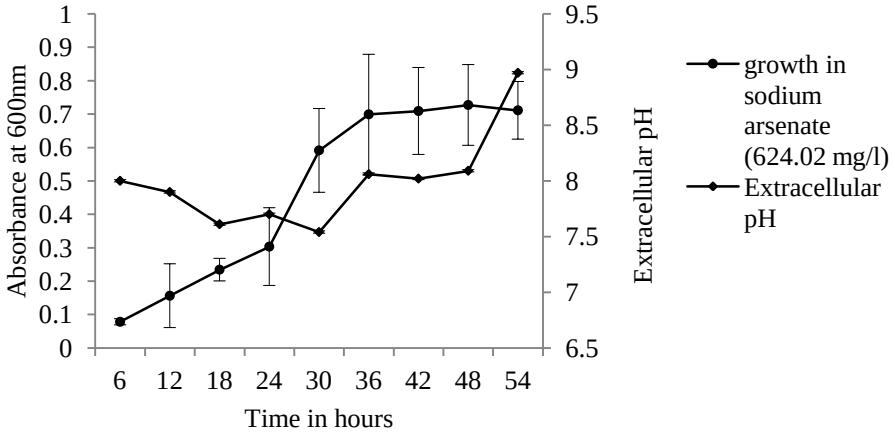

Figure 7 Growth *of P. stutzeri* ASP3 in the presence of sodium arsenate versus extracellular pH of the medium. Data are expressed as mean ± standard deviation.

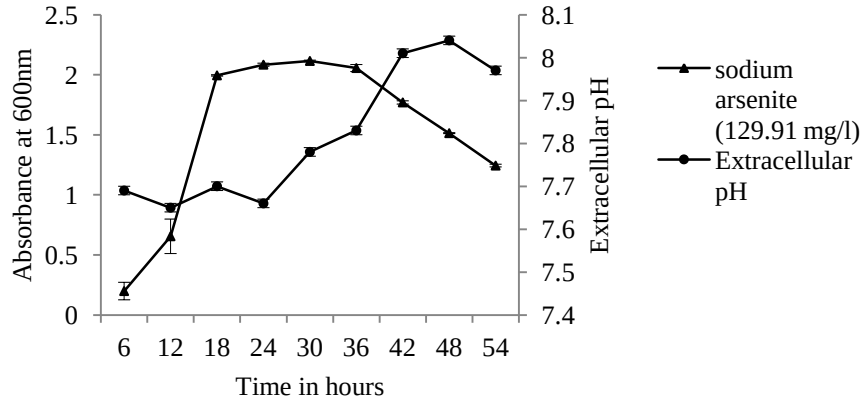

Figure 8 Growth *of P. stutzeri* ASP3 in the presence of sodium arsenite versus extracellular pH of the medium. Data are expressed as mean ± standard deviation.

Multiple metal resistance and As (V) reduction Activity

The maximum tolerance concentration was found to be 4680.15 mg/l for As (V) as sodium arsenate and 649.55 mg/l for As (III) as sodium arsenite. One of the most arsenic resistant

bacteria isolated so far is *Corynebacterium glutamicum*. It can grow at up to ~15,589.92 mg/l arsenite, compared to ~649.55 for *E. coli* and > 1, 24,804 mg/l arsenate [23]. The *Staphylococcus sp* strain NBRIEAG-8 isolated from arsenic contaminated site of West Bengal was able to grow at higher concentration of As (V) level up to 30,000 mg /1 and As (III) level up to 1,500 mg/1[3]. The *P. stutzeri* ASP3 also showed resistant to all the other tested heavy metals; zinc chloride, copper sulphate, mercuric chloride and silver nitrate at concentrations of 136.28 mg/l, 249.68 mg/l, 5.432 mg/l and 3.3974 mg/l respectively. Likewise, the bacterium was screened positive for arsenate reduction test while could not oxidize arsenite.

Removal of arsenate and arsenite by *P. stutzeri* ASP3

P. stutzeri ASP3 removed 82.97% of 200 mg/l of As (V) and 49.4% of 129.91 mg/l of As (III) in 48 hr and 24 hr respectively from the culture media Fig. 9.

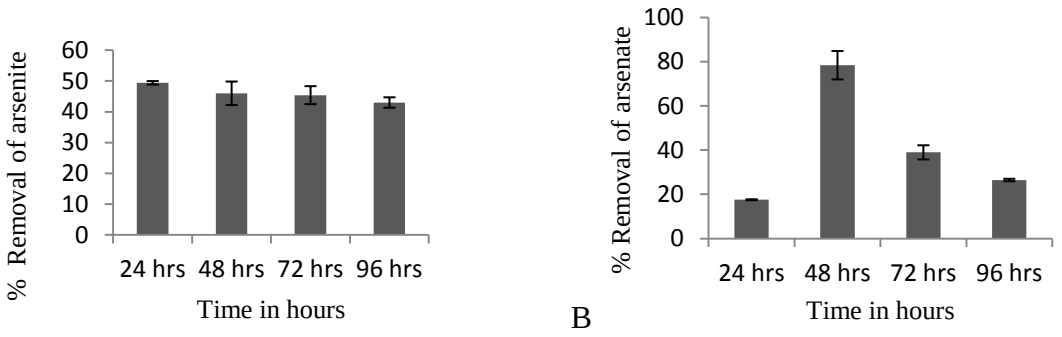

Figure 9 Removal of arsenite (A) and arsenate (B) by *P. stutzeri* ASP3. Data are expressed as mean ± standard deviation.

The arsenic resistant bacterium isolated in this study is *P. stutzeri* ASP3 based on the 16S rDNA sequence analysis. The genus *Pseudomonas* composed of ubiquitious bacteria endowed with a remarkable adaptability to diverse environments. Many species of *Pseudomonas* isolated from waste water, soil are reported to contain heavy metal resistant as well as arsenic resistant capability [11]. *Pseudomonas* and *Bacillus* are broadly reported among arsenic resistant bacterial strains isolated from arsenic contaminated sites [23]. Some of the bacteria having arsenic removal capability are *Alcaligenes faecalis, Agrobacterium tumefaciens,* bacteria *NT26, Bacillus indicus, Bacillus subtilis, Corynebacterium glutamicum, Desulfovibrio desulfuricans, Galleonella ferruginea, Leptothrix ocracia, Pseudomonas putida, Pseudomonas arsenitoxidans, Ralstonia picketti, Thiomonas ynys1, Acidithiobacillus ferrooxidans, Ralstonia eutropha etc*. Bacteria from genus *Pseudomonas, Bacillus, Psychrobacter, Vibrio, Citrobacter, Enterobacter* and *Bosea* are also reported as arsenic resistant a well as arsenic transforming genera [24, 25]. Likewise, arsenic removal capability has been reported in *Ralstonia eutropha* MTCC 2487, *Bacillus indicus* MTCC 4374 and *Pseudomonas putida* MTCC 1194 [25]. Microbial transformation of arsenic has great implications on its geochemical cycling in the environment because different forms of arsenic vary in solubility, mobility, bioavailability, and toxicity [26]. Bacteria that convert As (V) to As (III) are of environmental significance due to the formation of toxic As (III), which has higher environmental mobility than As (V). Mobility and toxicity are the most important issues pertaining to As contamination on both the regional and global scales. In aerobic environments, microbial reduction of As (V) to As (III) is primarily a detoxification mechanism whereby As is excreted by the cell via a As (III) specific transporter. Recently,

the oil-degrading bacterial isolates OSBH2 (similar *to P. stutzeri*) and OSBH5 (similar *to B. Cereus*) have been described to efficiently reduce As (V) to As(III) with significant role in the mobilization of As in the more toxic As(III) form that affects biotic life [11]. Therefore, the present study isolated and characterized the indigenous arsenate as well as arsenite resistant *P. stutzeri* ASP3 from groundwater sources of Rautahat, Nepal that also showed positive screening test for arsenate reduction.

CONCLUSION

The efficient arsenic removing capabilities, arsenic transforming ability, multiple heavy metals resistance and its stable growth in presence of highly toxic As (III) under aerobic conditions encourage for future in-depth study to explore its role in arsenic mobilization and bioremediation.

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