WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 982-1000 Research Article ISSN 2278 – 4357

ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF ARSENIC RESISTANT BACTERIA (ARB) FROM RIVER GANGA AND GROUND WATER FROM NORTH BIHAR

Prangya Paramita Acharya[1], Dr. Surajit Das*[2] and Ajay Kumar Sahu[3]

[1]Dept. of Biotechnology, Sambalpur University, Odisha.

[2]Dept. of Life Science, National Institute of Technology, Rourkela, Odisha.

[3]Dept. of Microbiology, Bangalore University, Bangalore, Karnataka.

Article Received on
26 Jan. 2019,

Revised on 15 Fab. 2019,
Accepted on 08 Mar. 2019,
DOI: 10.20959/wjpps20194-13434

***Corresponding Author**
Dr. Surajit Das
2Dept. of Life Science, National Institute of Technology, Rourkela, Odisha.

ABSTRACT

12 arsenic resistant strains were isolated from water and sediment samples of river Ganga and ground water from Barauni, North Bihar. The strains BW041, MS133, MS122 showed high resistance towards arsenic and can be used for the bioremediation of arsenic. Out of the 12 isolates 8 were bio film forming. Among them 6 isolates have *arcs'* gene which encodes for arsenic reductase enzyme. Arsenate reductase activity was found to be conferred by this arsC gene. The isolates can tolerate wide range of pH and salinity. All the selected isolates gave positive catalyse test and negative for Voger Prosker test. Thus, such metabolically well-equipped bacterial strains with highest As^{3+} oxidation activities may be used for bioremediation of as contaminated water and effluents in the near future.

KEYWORDS: Arsenic, Bacteria, Bio film, Arsenic reductase.

INTRODUCTION

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1994). With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Duff, 2002). In recently years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their

use in several industrial, agricultural, domestic and technological applications. Reported sources of heavy metals in the environment include gynogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (Gyor, 2001). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations. Arsenic is a toxic metalloid or a semi-metallic element which is basically found in inorganic form as (III) which is more soluble in comparison to As (V). As (III) is widely distributed in nature with a crustal abundance of 0.001% (Nriagu, 2002). Arsenic is a known human carcinogen (Hughes, 2002). Arsenic is ubiquitous metalloids and widely distributed in terrestrial and surface ecosystem mainly because of geochemical and anthropogenic activity (Fig. 1). Arsenic is primarily released into environment through volcanic emission. They are more toxic in nature when they are present in water.

The accumulation of arsenic in ground water is a serious problem in many part of world especially in India, Bangladesh where arsenic contaminated ground water is used for drinking by over 40 million of people that represents various bacterial generations. Bangladesh agricultural field and water are very much contaminated with arsenic.

Elevated levels of arsenic have been reported in soils and groundwater worldwide. The maximum concentration limit (MCL) recommended for drinking water by the World Health Organization (WHO) is 10 mg/L. Elevated levels of arsenic in drinking water can seriously impact human health and have been implicated in human diseases and mortality. Around 6 million people in West Bengal and more than 46 million people in Bangladesh are estimated to be at risk from drinking water with arsenic above 50 mg/L.

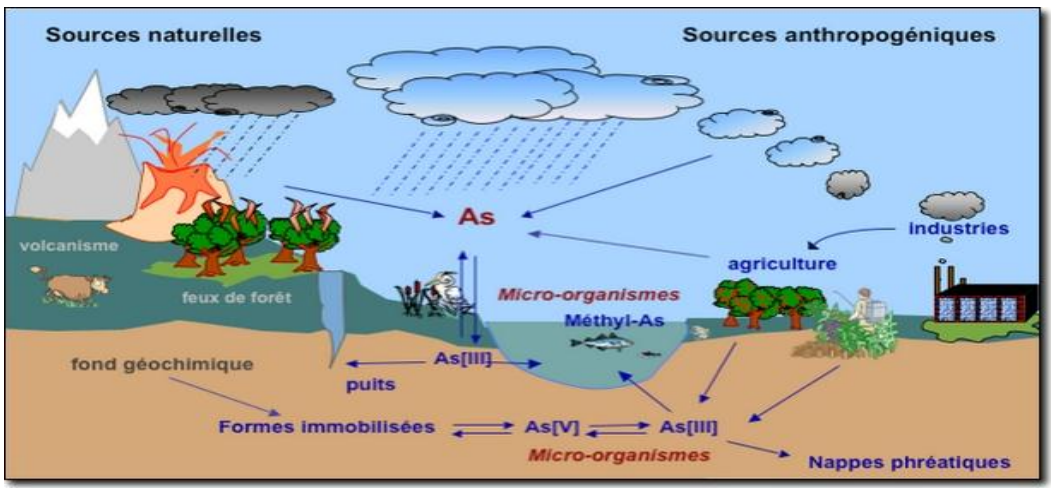


Figure: 1 Geological cycle of arsenic.

Remediation of arsenic contaminated soils and groundwater is necessary for providing safe drinking water. Bioremediation of arsenic contaminated soils and groundwater shows a great potential for future developments due to its environmental compatibility and possible cost effectiveness. It relies on microbial activity to reduce, mobilize, or immobilize arsenic through sorption.

Arsenic toxicity

Arsenic is a carcinogen, and is associated with animal and human skin, lung and bladder cancers. The WHO agrees and says that over the next decade these cancers are likely to become the principle human health concern due to As. Both As (III) and As (V) are toxic; as such inorganic arsenic is regarded as a major environmental pollutant based on USEPA's evaluation (Johnson and Derosa, 1995). However, they interrupt biological functions in different manner. Inorganic arsenic arsenates binds to proteins with sulfhydryl groups, interfering with their functions. It inhibits respiration by binding to vicinal thiols in pyruvate dehydrogenize and 2-oxo-glutarate dehydrogenize. Arsenate does not act directly as a mutagen but induces intra chromosomal homologous recombination (Helleday et al., 2000) and generates reactive oxygen species. As (V), on the other hand,

Conventional methods for treatment of water

A variety of conventional treatment technologies, based on the principle of precipitation, ion exchange, electrolysis, solvent extraction, reverse osmosis, membrane and biosorption process have been proposed and is tested for removal efficiency of different pollutants from potable water as well as industrial effluent (Baciocchi et al., 2005; McNeill and Edwards.

Bioremediation of Arsenic

Bioremediation is defined as degradation of environmental pollutant to less toxic form using living organisms like bacteria, fungi or plant. Microbial Bioremediation of As Currently bioremediation of heavy metals using microorganisms has received immense interest for its potential, cost effectiveness, and environmental friendly way for heavy-metal removal (Valls and Lorenzo 2002, Rakshit and Ghosh 2009). Microorganisms control the environmental fate of as through various mechanisms resulting changes.

Bioaccumulation

Microorganisms exhibit a strong ability to accumulate (bioaccumulation) As from a substrate containing very low concentrations of this element. Bioaccumulation is activated by two

processes, namely biosorption of as by microbial biomass and its byproducts and physiological uptake of as by microorganisms through metabolically active and passive processes. Factors such as soil pH, moisture and aeration, temperature, concentration and speciation of As, soil amendments, and rhizosphere are known to influence the process of bioaccumulation of As in microbial cells (Mahimairaja et al., 2005).

Microbial Redox Reaction

Microbial mediated redox reactions mainly act upon As (III) and As (V) species. Researchers have successfully isolated and characterized As resistant bacteria from different environmental samples and concluded that these bacteria have the capability to grow chemolithotrophically with oxygen as an electron acceptor and As (III) as an electron donor (Santini et al., 2000; Duquesne et al., 2008). Furthermore, Ilyaletdinov and Abdrashitova (1981) concluded that bacteria derive metabolic energy from As (III) oxidation. Strains of *Bacillus* and *Pseudomonas* spp. (Frankenberger and Losi, 1995) and Alcaligenes faecalis (Phillips and Taylor, 1976) and Alcaligenes spp. (Osborne and Ehrlich, 1976) were found capable of oxidizing As (III) to As(V).

MATERIALS AND METHODS

Sample collection

The water and sediment samples were collected alongside river Ganga and wells of Barauni, North Bihar on 27th January 2015. Samples were kept at 4ºC for longer storage.

Screening of arsenic resistance bacteria (ARB)

R2A agar was used in the screening of arsenic resistance bacteria from collected water and sediment samples. R2A agar was enriched with 50 ppm sodium arsenate. Collected samples were used to screen arsenic resistant bacteria. After streaking with water and sediment (after serial dilution) samples on R2A agar plates supplemented with 50 ppm $NaAsO_4$, incubated overnight at 37ºC. After 24 hour colonies were observed which are further sub cultured?

Bio film screening

Bio film formation in borosilicate glass tubes (10x75 mm) was assessed by the methods of O'Toole and Kolter (1998), with slight modifications. A colony was inoculated in LB broth and incubated at 37°C overnight. O/N culture was diluted to 1:100 in LB broth. From this, 1 ml was transferred to glass tubes and incubated under static condition at 37°C for 48 h. After

48 h, tubes were washed twice with water, stained with 0.2% crystal violet and distained with 95% ethanol. The tubes with attached visible cells were considered as bio film positive.

Characterization of bacterial isolates

Grams staining-: The grams staining (named after Christian gram, Danish scientist) divide the bacteria in to two group gram +ve and gram-ve. In gram staining the crystal violet dye is use as a primary stain with the help of this crystal violet dye we can identify the bacteria whether it is gram +ve or +ve bacteria.

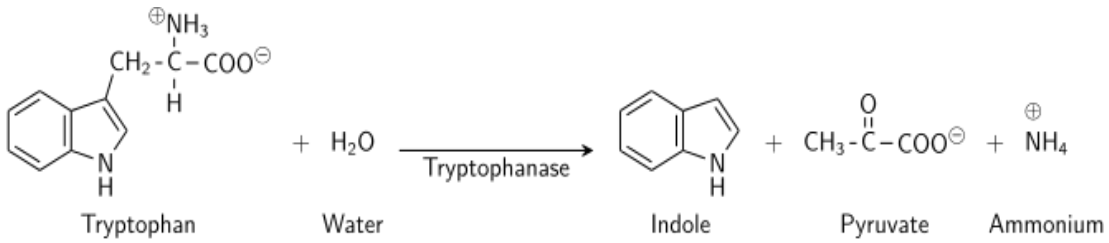
Biochemical characterization

Bacteria accomplish their growth and multiplication using raw materials and nutrients obtained from the environment. The biochemical transformation activities happened both inside and outside of the bacteria are governed by biocatalyst called enzyme. Single isolated colony was picked up and inoculated in 10ml of R2A broth and incubated at 37ºC for 24 hours.

IMViC TEST (Indole Methyl red Vogous-Proskauer Citrate utilization Test).

Principle

The conversion of tryptophan use into metabolic product is mediated by the enzyme tryptophanase the ability to hydrolyse tryptophan with the production of iodole is not a characteristic of all microorganism and therefore serves as biochemical marker.



Indole test used to detect the ability of an organism to split amino acid tryphan to form tryptophan to form the compound indole. Tryptophan is hydrolysed by tryptophanase to produce possible end product like indole and that indole cn be detective by the help of KOVAC'S reagent.

This reagent consists of para dimethyl benzaldehyde which is responsible for the red colour.

Methyl Red (MR)

Test determines whether the microbe performs mixed acids fermentation when supplied glucose. Types and proportion of fermentation products produced by anaerobic fermentation of glucose is one of the key taxonomic characteristics which help to differentiate various genera of enteric bacteria. The pH indicator present in methyl red detect the pH change to the acid range as a result of acidic end product vary depending on enzyme pathways present in bacteria. In case of bacterial growth the grown bacteria that cause the acid production. The pH production by the methyl red indicator and that may indicates the colour change. That is positive result and if yellow colour than negative result occurred.

Vogous-Proskauer test

This test used to determine the capability of some microorganism to produce non acidic neutral product, such as acetyl methyl carbinol, from the organic acids that result from glucose metabolism. The reagent used in this test is Barrett's reagent, consists of a mixture of alcoholic a-naphthol and 40% potassium hydroxide solution. Detection of acetyl methyl carbinol requires this end product to be oxidized to a diacety compound. This reaction will occur in the presence of the a-naphthol catalyst and a guanidine group that is present in the peptone of the MR-VP medium. As a result, a pink complex is formed, imparting a rose colour to the medium. Development of a deep rose colour in the culture 15 minutes following the addition of Barrett's reagent is indicative of the presence of acetyl methyl carbinol and represents a positive result. The absence of rose colour is a negative result.

Barrett's reagent= alcoholic napthol + 40% hydroxide solution.

Citrate utilization test

Microorganisms are capable of utilizing citrate as their sole carbon source in absence of the fermentable glucose and lactose because the citrate was 1st intermediate of krebs cycle formed from the condensation of active acidic oxaloacitic acid.

Triple sugar iron test (TSI test)

TSI agar test usually use to identification of enteric bacteria. TSI agar slants contain 1% lactose and sucrose and 0.1% glucose. The phenol red act as pH indicator in the media used to detect the production of acid. The TSI slants are inoculated with isolated bacteria to check the carbohydrate fermentation.

Catalase test Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme.

Urease test

Urease is a hydrolytic enzyme that attacks the nitrogen and carbon bond in amide compound such as urea and forms alkaline end product ammonia. The presence of urea's in detectable when organism are grown in urea broth. The medium containing pH indicators, phenol red. Urea splits in to product the presence ammonia and as a result phenol red to in to deep pink this cause the positive results.

Antibiotic sensitivity test

New antibiotics are continuously being developed and discovered thus there is increasing demand on clinical laboratory to determine antibiotic susceptibility of resistant of various pathogenic bacteria and metal resistant bacteria. The method use to check the arsenic resistant bacteria is in the method of disc method named as KIRBY-BAURE method (named after W. Kirby and A.W. Bauer 1996). This method used to measure the sensitivity of any microorganism and susceptibility of any microorganism to the variety of antimicrobial agent. In case of this experiment we test the antibiotics susceptibility of resistance of various arsenic resistant bacteria and saw the result of which strain can resist which antibiotic.

Procedure

a) Well autoclaved plates were poured with R2A agar.
b) After solidification the plates were streaked with the help of cotton swab which were immersed in bacterial culture on the surface of agar rotating 60o after each streaking covering each edge of the plates.
c) After keeping 5-10 min in room temperature, the antibiotics were dispensed in to the plates with the help of dispenser gently pressing the antibiotics.
d) Kept in incubator for 16-18 hour.
e) After that zone of inhibition was occurred. Results were reported.

Minimum inhibitory concentration

MIC means the lowest concentration of metal that completely prevented bacterium growth (De and Ramaiah, 2007; Gupta et al., 2005). MIC test was done by Broth serial dilution

method and the antibacterial activity method. The bacterial cultures supplemented with the metals and with serial dilution process with different concentration. The micro titter plates used in this process.

Procedure

a) 300 µl of (1500 ppm of As) was added in 1st column
b) Remaining wells were filled with 150 µl of sterilised R2A broth.
c) Serial dilution was performed. 150 µl form 1st well is taken and transferred to next well and subsequently till 10th well.
d) 150 µl of content was discarded from10th well.
e) 20 µl of bacterial culture was added to the entire well except 12th column wells which acts as a negative control.
f) After all this the plate was incubated at 37°C for 24 h.
g) Finally, after 24 h absorbance was taken at 595 nm in the ELISA plate reader.
h) According to the negative control the MIC was determined after carefully inspection of each well at 595nm.

Physicochemical parameters

The physicochemical character of the microorganism can be studied by the pH test and salinity tests.

Estimation of pH tolerance of bacterial isolates

The pH tolerance test was conducted to study the cardinal pH of the arsenic resistant bacteria (ARB) (Buchanan and Gibbons, 1977; Holt et al., 1994). The procedure for estimation of pH tolerance is as follows.

a) 3 ml of the medium was taken in different test tubes and the pH was adjusted: from 3-10 respectively with the help of 1N HCl, 1N NaOH.
b) 30 µl of the overnight culture was dispensed into the test tubes and incubated at 37ºC for 24 hours.
c) Growth can be seen after the incubation and result can be recorded.

Estimation of salinity

The procedure for study the effect of salinity on bacterial growth is as follows.

- 5ml of the medium was taken in different test tubes and the salinity was adjusted as 1% to 9% respectively with the help of 1N NaCl.

- 200µl of the overnight culture (R2A) was dispensed into the test
- Tubes and incubated at 37ºC for 24 hours.
- After incubation the growth can be seen in different salinity and result can be noted.

Amplification of *arsc* gene in resistant isolates

Preparation of bacterial lysate

Before going for the gene amplification we need to prepare the bacterial lysate. the following steps are included for making of bacterial lysate.

a) 200 microliter of overnight grown culture was taken.
b) Centrifuged at 6000 rpm for 5 min.
c) Supernatant were discarded.
d) 200 microliter miliQ water dissolved properly.
e) The mixture was boiled for 10-15 min at 100oC.
f) Immediately it was kept on ice for 15min.
g) After 15min it was centrifuged again for 10min at 6000rpm for 4Oc.
h) The supernatant was collected in fresh eppendorf tube and kept on ice.

Amplification of conserved regions of arsC

Following primer was used for the amplification of *arsC* gene: F1merA-5'. The sequences of the primers pair was as follows: *arsC* F (5-TCG CGT AAT ACGCTG GAG AT-3) and *arsC* R: (5-TCA CGC AAT ACC CTTGAA ATG ATC/50-ACC TTT TCA CCG TCC TCT TTCGT). The PCR conditions were as follows: initial denaturation step at 94°C for 5 min, 30 cycles of 94°C for 30 s, 54°C/59°C for 30 s, 72°C for 30 s with a final extension of 72°C for 10 min (Fig. 2) (Angana et al, 2013.). The total volume was 25µl for the amplification reaction; the reaction was performed by using thermal cycler (BioRad). To perform this experiment we prepared a PCR mixture were of; 11.4 µl of Milli Q, 2.5 µl of 1X enzyme buffer, 1.5 µl of MgCl2, 0.6 µl of dNTPs, 2.0 µl of *arsC* F, 2.0 µl of *arsC* R, 11.4 µl of Taq Pol and 4.0µl of template.

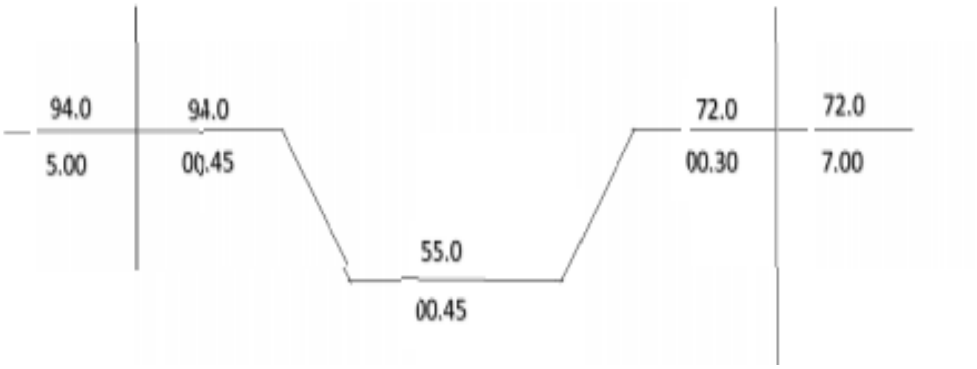


Fig 2: PCR programme.

The PCR products were examined using agarose gel electrophoresis (1.5% agar) and visualized under UV light in Gel Documentation System (Bio-Rad).

RESULTS

Sample collection and screening of ARB

The enrichment methods used in this study resulted in the selection of isolates which were able to tolerate significant level of As. As metals stress were provided during the screening and isolation to support the growth of potential marine bacterial isolates those can be used to study its bioremediation potential of heavy metal arsenic. 12 isolates with different morphological features were obtained (Fig. 3). The isolates were designated as (1) BW041, (2) MS122, (3) MS131, (4) MS231 (5) MS332, (6) BW051, (7) MW011, (8) MS331, (9) MS331, (10) MS121, (11) MS124 and (12) BW512 (Fig. 3).

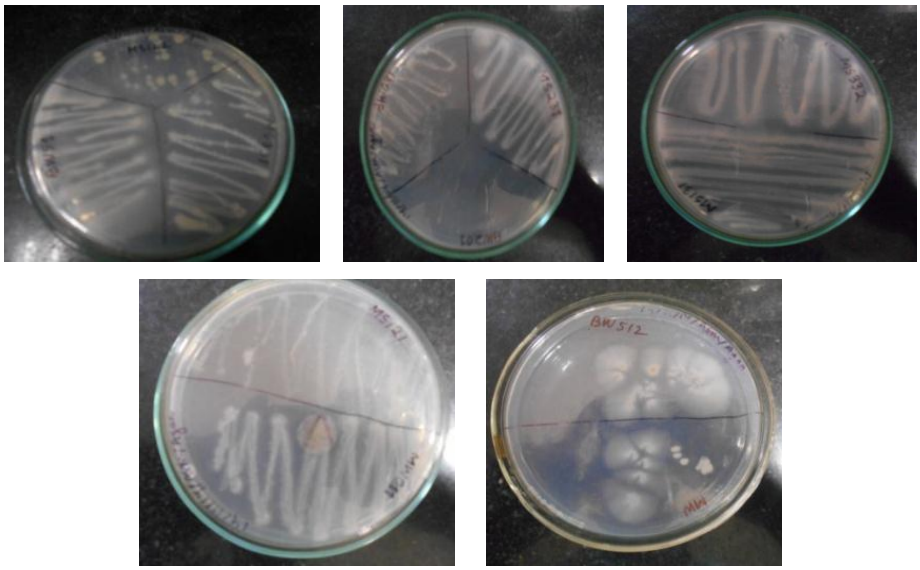


Fig. 3. Isolated colonies on R2A+ 50 ppm Na_2AsO_4 plates.

Bio film screening

Out of 12 isolates, 8 were found to be biofilm formers (Fig. 4). Biofilm is a surface associated growth, composed of cells and EPS, which is stained by crystal violet. Biofilm or surface attachment by bacteria can be visually seen after destining with 95% ethanol (Jain et al. 2013).

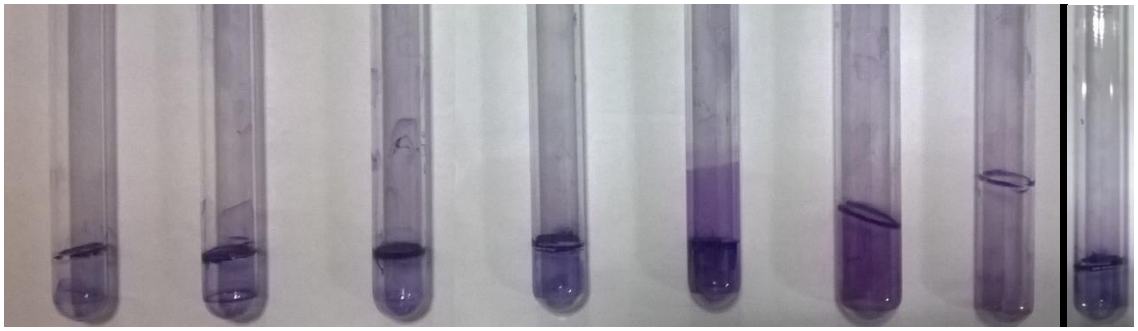


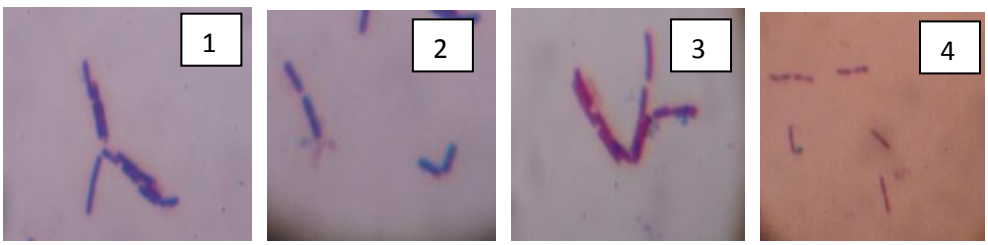
Fig. 4: Biofilm formation over borosilicate glass tube surface. Biofilm visible as attached cell mass after crystal violet staining. (1) BW041, (2) MS122, (3) MS131, (4) MS231 (5) MS332, (6) BW051, (7) MW011, (8) MS331.

Characterization of strains

Total eight strains were selected for further study. Cell morphology of strains was studied by gram staining and observing under oil immersion microscope, the result of which has been given in the Table 1 and Fig. 5.3.

Table 1: Gram character of the Isolated Strains.

Isolate	Colour	Gram Character and shape
BW041	Violet	Gram positive rods
MS122	Violet	Gram positive rods
MS131	Pink	Gram negative rods
MS231	Pink	Gram negative rods
MS332	Pink	Gram negative rods
BW051	Violet	Gram positive rods
MW011	Pink	Gram negative rods
MS331	Pink	Gram negative rods



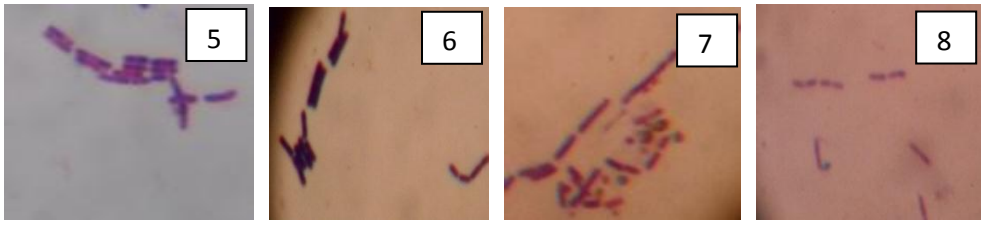


Fig 5: Gram Staining results of the isolates [(1) BW041, (2) MS122, (3) MS131, (4) MS231 (5) MS332, (6) BW051, (7) MW011 and (8) MS331.

Biochemical Characterization of the isolates

Different biochemical tests were performed to characterize the arsenic resistant bacterial strains and observations are collectively given in Table 2 and Fig.6.

Table 2: Biochemical Test Results.

TEST	MS131	MS231	MS122	MS331	MS332	MW011	BW041	BW051
INDOLE	-	+	-	+	+	+	+	+
METHYL RED	+	-	-	-	-	+	+	+
V P	-	-	-	-	-	-	-	-
CITRATE	+	-	+	+	+	+	+	-
TSI	+	+	+	+	+	+	+	+
CATALASE	+	+	+	+	+	+	+	+
UREASE	-	-	-	-	-	-	+	-

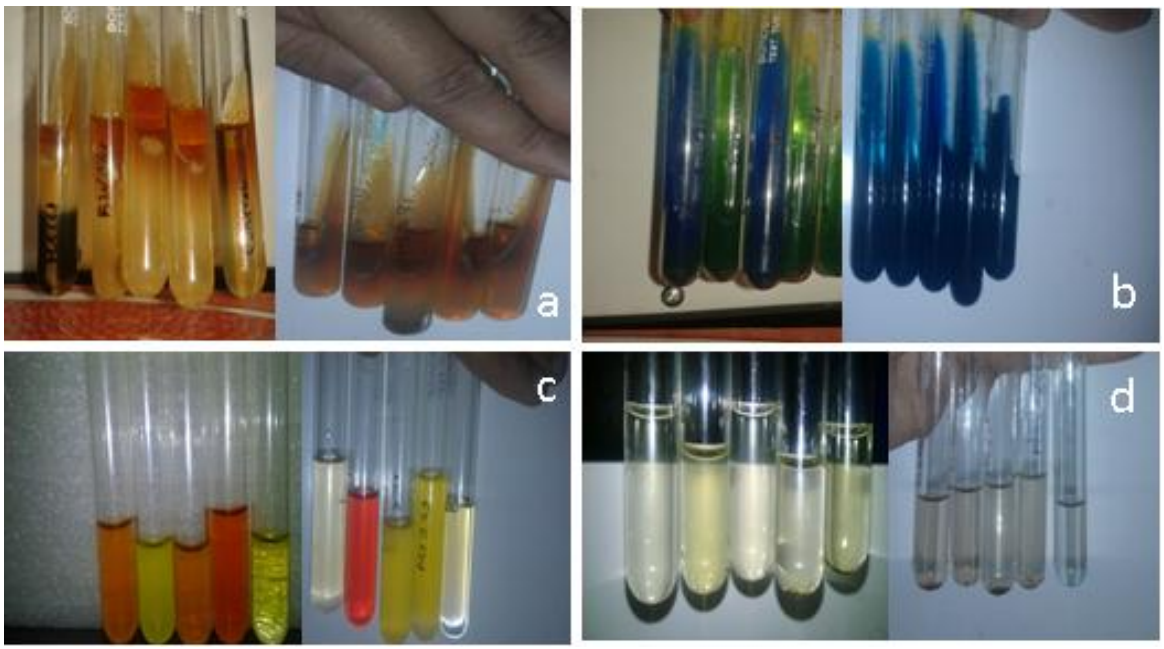


Fig 6: IMVIC test (a) Indole test (b) Citrate utilization test (c) Methyl red test (d) Vogues Proskaur test.

Triple Sugar Iron Test

After glucose after utilization oxidative deamination of peptone occurs and thus NH_3 is produced giving pink colour alkaline slant (i.e. aerobic condition).

MS131, MS231, MS331, MS332 alkaline slant and acidic butt. M122, MWO11, BW051-acid slant and acid butt and only BW041 have acidic slant and acidic butt (Fig. 7).

Fig 7: Triple Sugar Iron Test.

Catalase production test

The lack of catalase is evident by a lack of or weak bubble production with the addition of hydrogen peroxide. Catalase-positive bacteria include strict aerobes as well as facultative anaerobes. They all have the ability to respire using oxygen as a terminal electron acceptor they results the strong bubble production in this experiment all strains were catalase positive (Fig. 8).

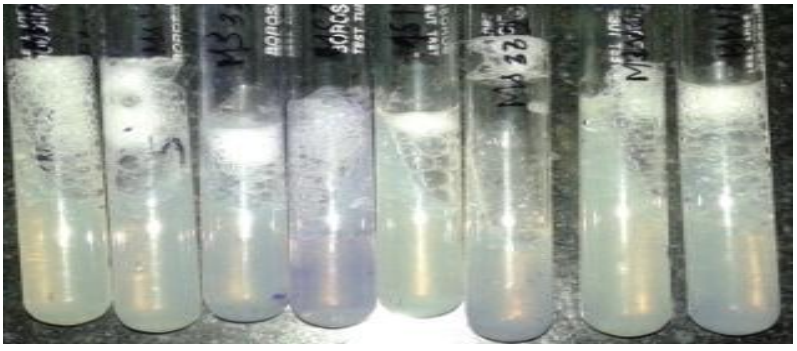


Fig 8: Catalase activity test.

Urease activity test

The presence of urease could be detected with the help of pH indicator present in urea broth. Urea splits in to product the presence ammonia and as a result phenol reds to in to deep pink

this cause the positive result. In this experiment the strain **BW041** only showed the positive result (Fig. 9).

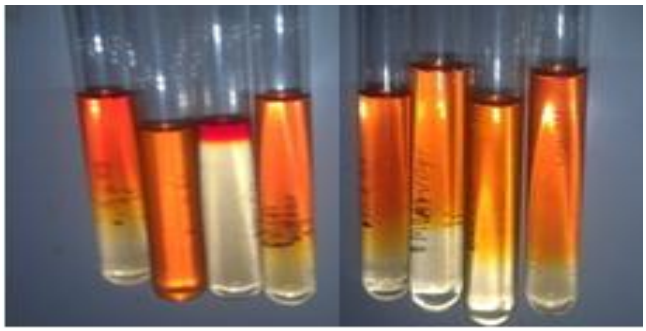


Fig. 9 Urease activity test.

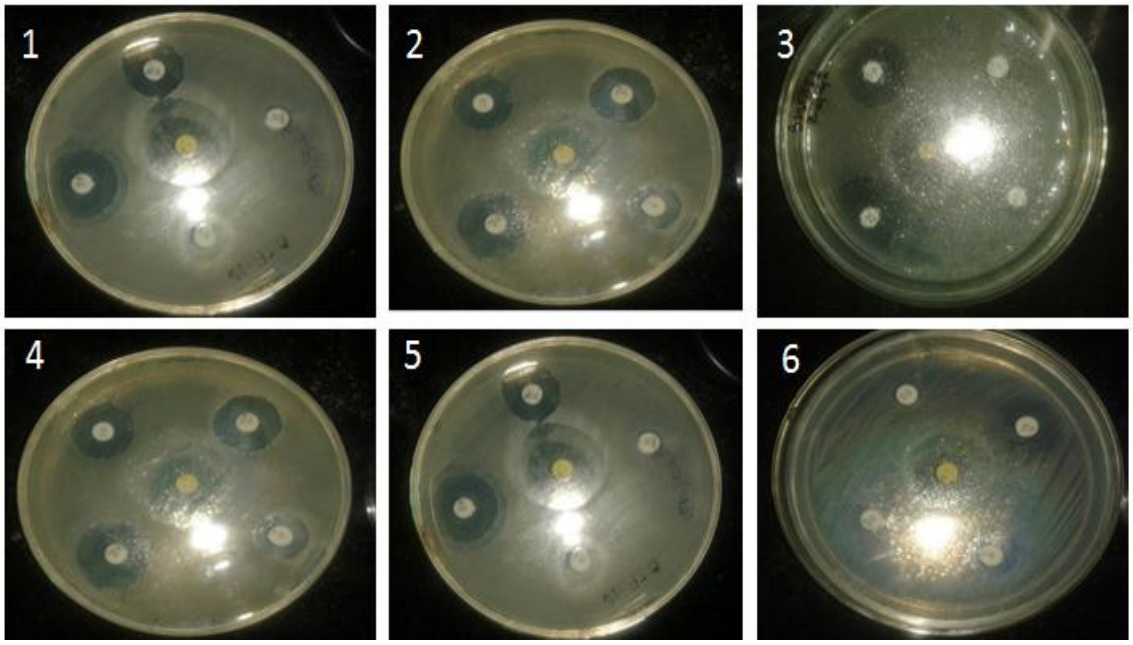
Antibiotic Susceptibility Test

Antibiotic susceptibility test of the bacterial strains is shown in Table 3 and Fig. 10. Most of the strains were resistant towards ampicillin.

Table 3: Antibiotic Susceptibility Test Result.

Antibiotic/ Isolates	MS131	MS231	MS122	MS331	MS332	MW011	BW041	BW051
Ampicillin	R	S	S	R	R	S	S	R
Neomycin	S	I	I	I	S	S	S	R
Chloramphenicol	S	S	S	S	S	I	I	I
Gentamycin	S	S	S	S	S	S	S	S
Kanamycin	S	S	I	I	I	S	S	I

R- RESISTANT S- SUSCEPTIBLE I- INTERMEDIATE



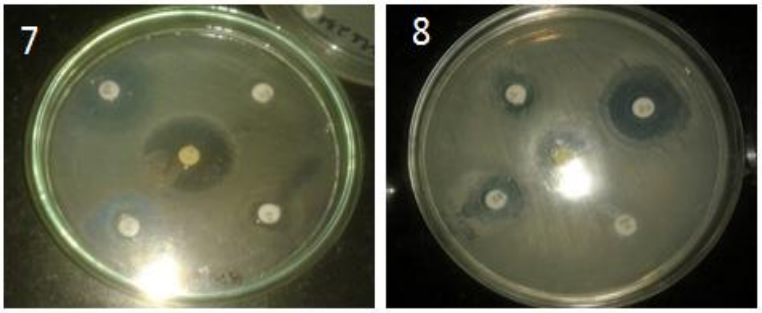


Fig. 10: Antibiotic Susceptibility Test Results (1) BW041, (2) MS122, (3) MS131, (4) MS231 (5) MS332, (6) BW051, (7) MW011, (8) MS331.

Minimum Inhibitory Concentration refers to the minimum concentration of arsenic at which bacterial growth can be inhibited. After the test it was concluded that all the 8isolates have their MIC value in the range of 250-750 ppm. The results of Minimum Inhibitory Concentration Test (MIC) of **Na_2AsO_4** for 8 strains have been shown in Table 3.

Table 3: MIC Results of Bacterial Isolates.

Bacterial Isolates	As(III) MIC in ppm
MS131	750
MS231	250
BWO41	500
BWO51	250
MW011	250
MS122	500
MS331	250
MS332	250

Estimation of pH and salinity tolerance of bacterial isolates

Physiological properties like growth at different pH and salinity ranges were studied using the 8 test bacterial isolates (Table 4.). Most of the isolates showed their ability to grow over abroad range of pH (pH 5.0-10.0), temperature range and salinity (1–9%).

Table 4: Estimation of pH and salinity tolerance of bacterial isolates.

TEST	MS131	MS231	MS122	MS331	MS332	MW011	BW041	BW051
pH	5-11	5-11	6-9	5-10	6-10	5-10	5-11	4-10
SALINITY (%)	1-3	1-3	1-8	3-9	3-8	1-7	1-3	1

Amplification of *arsC* gene in the resistant isolates

arsC coding for arsenic reductase genes were found in 6 isolates out of the eight selected isolates. A thick single band of 270 bp was observed in case of MS122, MS131, MS231, MS332, MW011, MS331 (Fig. 11).

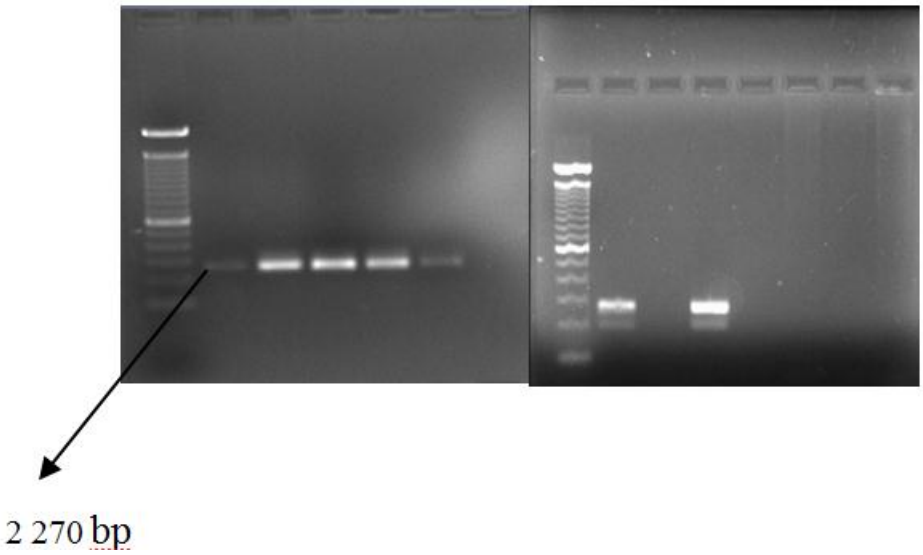


Fig 11: Gel photograph showing *arsC* amplification in the isolated strains.

CONCLUSION

Twelve arsenic resistant strains were isolated from river Ganga and ground water from Barauni, North Bihar. The strains BW041, MS133, MS122 showed high resistant of arsenic and can be used for the bioremediation of arsenic. All the isolates selected for study were bio film former. Among them 6 isolates have *arsC* gene encoding arsenic reductase. So it is suggested that arsenic elimination ability of these bacteria should be evaluated. More ever these isolates can be genetically engineered to reach better result in removal of arsenic. For further biotechnological approach for arsenic detoxification further investigation needs to be carried out in laboratory scale and in-situ metal reduction potential of the isolates has to be assessed.

REFERENCES

1. Alexander, D. R., & Ferguson, J. W. (1994). Low-temperature Rosseland opacities. The Astrophysical Journal, 437: 879-891.
2. Baldi, F. (1994). Microbial transformation of metals in relation to the biogeochemical cycle. In Chemistry of Aquatic Systems: Local and Global Perspectives (pp.121-152). Springer Netherlands.
3. Baldwin, D. R., & Marshall, W. J. (1999). Heavy metal poisoning and its laboratory investigation. Annals of Clinical Biochemistry, 36: 267-300.
4. Barkay, T., Miller, S. M., & Summers, A. O. (2003). Bacterial mercury resistance from atoms to ecosystems. FEMS microbiology reviews, 27: 355-384.

5. Bogdanova, E. S., Bass, I. A., Minakhin, L. S., Petrova, M. A., Mindlin, S. Z., Volodin, A. A. & Nikiforov, V. G. (1998). Horizontal spread of mer operons among Gram positive bacteria in natural environments. Microbiology, 144: 609-620.
6. Bradl, H. (Ed.). (2005). Heavy metals in the environment: origin, interaction and remediation: origin, interaction and remediation (Vol. 6). Academic Press.
7. Bridges, C. C., & Zalups, R. K. (2004). Homocysteine, system b 0, + and the renal epithelial transport and toxicity of inorganic mercury. The American journal of pathology, 165(4): 1385-1394.
8. Cervantes, C., Ramirez, J., & Silver, S. (1994). Resistance to arsenic compounds in microorganisms. FEMS microbiology reviews, 15: 355-367.
9. Craig, P. J. (1989). Biological and environmental methylation of metals. The Metal-Carbon Bond, 5: 438-463.
10. Dopp, E., Hartmann, L. M., Florea, A. M., Von Recklinghausen, U., Pieper, R., Shokouhi, B., & Obe, G. (2004). Uptake of inorganic and organic derivatives of arsenic associated with induced cytotoxic and genotoxic effects in Chinese hamster ovary (CHO) cells. Toxicology and applied pharmacology, 201: 156-165.
11. Duffus, J. H. (2001). "Heavy metals": a meaningless term? Chemistry International, 23.
12. Frankenberger Jr, W. T. (Ed.). (2001). Environmental chemistry of arsenic. CRC Press.
13. Frankenberger Jr, W. T., & Arshad, M. (2002). Volatilization of arsenic. Environmental chemistry of arsenic, 363-380.
14. Ghosh, S., & Sar, P. (2013). Identification and characterization of metabolic properties of bacterial populations recovered from arsenic contaminated ground water of North East India (Assam). Water research, 47: 6992-7005.
15. He, Z. L., Yang, X. E., & Stoffella, P. J. (2005). Trace elements in agro ecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, 19: 125-140.
16. Inskeep, W. P., McDermott, T. R., & Fendorf, S. (2001). Arsenic (V)/(III) cycling in soils and natural waters: Chemical and microbiological processes. Environmental chemistry of arsenic, 183.
17. Johnson, B. L., & DeRosa, C. T. (1995). Chemical mixtures released from hazardous waste sites: implications for health risk assessment. Toxicology, 105: 145-156.
18. Kinniburgh, D. G., & Smedley, P. (2001). Arsenic contamination of groundwater in Bangladesh.

19. Klaassen, C. D. (Ed.). (2013). Casarett and Doull's toxicology: the basic science of poisons (Vol. 1236). New York (NY): McGraw-Hill.
20. Knowles, F. C., & Benson, A. A. (1983). The biochemistry of arsenic. Trends in Biochemical Sciences, 8: 178-180.
21. Kumar, J. I., & Oommen, C. (2012). Removal of heavy metals by biosorption using freshwater alga Spirogyra hyalina.
22. Kumar, J. N., Oommen, C., & Kumar, R. N. (2009). Biosorption of heavy metals from aqueous solution by green marine macroalgae from Okha Port, Gulf of Kutch, India. American-Eurasian J. Agric. & Environ. Sci, 6: 317-323.
23. Lillis, T. O., & Bissonnette, G. K. (2001). Detection and characterization of filterable heterotrophic bacteria from rural groundwater supplies. Letters in applied microbiology, 32: 268-272.
24. Mac Faddin, J. F. (1976). Biochemical tests for identification of medical bacteria. Williams & Wilkins Co.
25. Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: a review. Talanta, 58: 201-235.
26. Nedkovska, M., & Atanassov, A. I. (1998). Metallothionein genes and expression for heavy metal resistance. Biotechnology & Biotechnological Equipment, 12: 11-16.
27. Ng, J. C., Wang, J., & Shraim, A. (2003). A global health problem caused by arsenic from natural sources. Chemosphere, 52: 1353-1359.
28. Ning, P., Cao, H., Liu, C., Li, Y., & Zhang, Y. (2009). Characterization and prevention of interfacial crud produced during the extraction of vanadium and chromium by primary amine. Hydrometallurgy, 97: 131-136.
29. Ning, R. Y. (2002). Arsenic removal by reverse osmosis. Desalination, 143: 237-241.
30. Santini, J. M., Sly, L. I., Schnagl, R. D., & Macy, J. M. (2000). A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. Applied and environmental microbiology, 66: 92-97.
31. Sarkar, A., Kazy, S. K., & Sar, P. (2013). Characterization of arsenic resistant bacteria from arsenic rich groundwater of West Bengal, India. Ecotoxicology, 22: 363-376.
32. Singh, N., Ma, L. Q., Srivastava, M., & Rathinasabapathi, B. (2006). Metabolic adaptations to arsenic-induced oxidative stress in *Pterisvittata L* and *Pterisensiformis L*. Plant Science, 170: 274-282.

33. Wang, S., & Mulligan, C. N. (2006). Occurrence of arsenic contamination in Canada: sources, behavior and distribution. Science of the Total Environment, 366: 701-721.