e-ISSN: 2455-667X

Annals of Natural Sciences
(Peer Reviewed and UGC Approved International Journal)
Vol. 3(4), December 2017: 130-137
Journal's URL: http://www.crsdindia.com/ans.html
Email: crsdindia@gmail.com

Annals of Natural Sciences

ORIGINAL ARTICLE

Molecular Characterization and Expression of Arsenic Resistant *E. coli* Strain in Yamuna and Hindon River

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ABSTRACT

The present study proposed the solitude of arsenic resistant bacteria from weakened water Delhi NCR region two The Yamuna river (Delhi NCR region) Hindon river (District Ghaziabad UP). Only three bacterial isolates (E. coliA E. coli A2 and E. coliA3) were suitable to grow in high immersion of arsenic. The tiniest inhibitory immersion of arsenic against, E. coli A1 E. coli A2 and E. coli A3 were 300 mg/ L, 330 mg/ L and 370 mg/L single-handedly. The isolated strains showed maximum growth at 37 °C and at 7.0 pH in Control but in Arsenite stress Luria Bertani broth the bacterial growth is lower than control. All strains were arsenite oxidizing. All strains were biochemically characterized and ribotyping (16S rRNA) was done for the purpose of identification which supported That E. coli A was homologous to E. coli sp. while E. coli A2 and E. coli A3 showed their maximum homology with E. coli strain. The protein profiling of these strains showed in arsenic stressed and non stressed conditions, so no bands of gained proteins appeared in stressed-out conditions. The bacterial isolates can be exploited for bioremediation of arsenic containing wastes, since they feel to have the possibility to oxidize the arsenite (more poisoned) into arsenate (less poisoned) form.

Key words: *Arsenite Oxidizing Bacteria, Bioremediation. E. coli sp., Klebsiella pneumoniae*

Received: 9th Oct. 2017, Revised: 16th Nov. 2017, Accepted: 23rd Nov. 2017

How to cite this article:
Kumar D. (2017): Molecular Characterization and Expression of Arsenic Resistant *E. coli* Strain in Yamuna and Hindon River. Annals of Natural Sciences, Vol. 3[4]: December, 2017: 130-137.

INTRODUCTION

Non-m*et al* inorganic basics having the characteristics of material are called m*et al*loids. Arsenic is also included in the genus of m*et al*loid that's present in trace quanta in water, soil, gems, air and all living matter at the attention of 2 mg/ kg on average (Patel *et al*., 2007). Arsenic occupies the position in group 15/ V (A) of the periodic table having parcels that form cocktails with different material like sulphur, carbon, hydrogen and oxygen. Arsenic can breathe in 4 different valency countries; − 3 (arsine), 0 (introductory arsenic), 3 (arsenite), 5 (arsenate). Generally arsenic is present in the forms of arsenite (III) and arsenate (V). Arsenic itself isn't resolvable in water but when it combines with different grammar either it has wide solubility in water (Aitio and Becking, 2001). As (III) species are more poisonous than As (V) species. Arsenate species are predominant at moderate and high redox potentialities, while arsenite species do under fresh reducing conditions. Makeup arsenites are much more resolvable than the corresponding makeup arsenates, and arsenites are adsorbed less by solid phases.

Now-a-days, the impurity of drinking water with arsenic refers a big public health problem in the whole world (Smith *et al*., 2002). The long term exposure of arsenic causes habitual toxin. Exposure of arsenic through drinking water for a long time leads to murderous conditions like cancer of skin, digestive system, bladder and lungs. Enthrallment of arsenic in drinking water fresh than 50 µg/ L raises the pitfalls of lung

and bladder cancer and yea if the enthrallment is minor than 50 µg/ L, it may increases the pitfalls of skin cancer (Aksu *et al*., 2010). Gastrointestinal symptoms due to ingestion of large quantum of arsenic include severe vomiting, abdominal pain, fatigue, abnormal heart measure, abnormal bruising and destruction of nervous system. It also causes flaws in blood curl and sometime leading to death. When not deadly, it breaks up red blood cells in blood curl, causes less product of blood cells, darkening of skin, telephoto of liver, damage of brain, legs and needle like sensation in hands and nadirs and loss of competence of sensation in the branches. Non-cancer personal effects may also invoke by arsenic like diabetes, nervous and bulletproof system problems, reproductive problems and cardiovascular problems including heart fever, stroke and high blood pressure (Tseng, 2004).

Conventional Ways for removing spirit from raw streamlets are chemical rush, chemical oxidation or reduction, natural process, filtration, electrochemical treatment, forward osmosis, membrane technologies and evaporation recovery (Ahluwalia and Goyal, 2007). These processes could also be ineffective or extremely valuable especially when the spirits in result are within the range of 1=100 mg/L. So, it's important to develop an innovative, low cost andeco-friendly way for scrapping of venomous heavy spirit ions from the water and wastewater (Lou and Chin, 2008). A wide variety of microorganisms are suitable of growth in the presence of heavy spirit ions and tolerates high immersion (Gaballa and Helmann, 2003; Rehman *et al*., 2007). Anderson and Cook (2004) reported strains of Aeromonas, Exiguobacterium, Acinetobacter, Bacillus and Pseudomonas, that can tolerate high immersion of arsenic species (up to 100 mM arsenate or up to 20 mM arsenite). Since heavy spirits are far and wide present in our surroundings, microorganisms have developed mechanisms to withstand the venomous things of those heavy spirits. Several bacteria belonging to the types Acidithiobacillus, Bacillus, Deinococcus, Desulfitobacterium and Pseudomonas have been reported to be resistant to arsenic (Oremland *et al*., 2004; Suresh *et al*., 2004a).

Resistance to arsenic species in both Gram-positive and Gram-negative organisms results from energy-dependent efflux of either arsenate or arsenite from the cell intermediated via the ars operon (Tseng, 2007). In *E. coli*, an ArsA-ArsB complex functions as a primary arsenite pump (Yoon, 2005). InS. aureus, ArsB alone is sufficient to act as a chemiosmotic secondary transport system for arsenite resistance without the presence of an ArsA ATPase (Wolfe-Simon *et al*., 2011). A farther gene, arsC, has been shown to jumble for an arsenate reductase that mediates reduction of arsenate foregoing to arsenite efflux (Liao *et al*., 2011).

The present study deals with the secludedness and characterization of arsenic resistant bacteria from a dilute contexture, the capacity of the bacteria to oxidize arsenite, and optimization of temperature and pH for maximum arsenite oxidation.

MATERIAL AND METHODS

SAMPLE COLLECTION AND BACTERIAL ISOLATION

For the privacy of arsenic resistant bacteria, wastewater samples were collected from Hindon River near Hindon vihar metro station Ghaziabad Uttar Pradesh India and Yamuna racecourse near Okhala New Delhi, in screw confined fixed bottles. Some Physio-chemical parameters like pH, temperature, dissolved oxygen and Arsenic (µg/ mL) were measured. About 50 µL water samples were spread on Luria Bertani agar plates that contain10.01 µg/ L of Arsenite for the privacy of arsenic resistant bacteria (Shakoori *et al*., 2010). After 24 hr of incubation at 37.5 °C the expansion of bacteria was observed. Individual colonies were picked and banded on Luria Bertani agar medium containing 20 mg/ L of arsenite. The Luria Bertani agar was prepared by mixing 5 g of sodium chloride, 5.01 g of impulse passage, 10 g of trypton and 19 to 20 gm of agar per 1000 mL of water, pH of medium was conditioned between7.0 and7.2.

After mixing 100 ml medium was taken in a pail and covered with cotton draw. The medium was autoclaved at 15 lb per inch square pressure121.5 °C for 15 min. After 24 hr the expansion of bacterial colonies was observed at37.1 °C of incubation. The goods of arsenite on bacterial strains growth was checked in acetate minimum medium which contained g/ L0.51 gm impulse extract, g magnesium sulphate ($MgSO_4$), gm sodium acetate, 1.0 gm ferric sulphate ($FeSO_4$), g calcium chloride ($CaCl_2$), gm potassium phosphate (KH_2PO_4) and1.0 g ammonium chloride (NH_4Cl) in 1000 mL of distilled water. After mixing exhaustively 5 mL medium was taken in each test tube and autoclaved at 121 °C, 15 lb per inch square pressure for 15–18 min. After bacterial inoculation in acetate minimum medium test tubes were kept in shaker incubator at 37 °C for twenty-24 hr

MINIMUM INHIBITORY CONCENTRATION OF BACTERIAL STRAINS

For the determination of slightest inhibitory enthrallment of the bacterial strains5.001 mL of acetate slightest medium was added in each test tube and different enthrallments of arsenite were added from30.001 to 370 mg/ L. They were steeped and incubated at 37 °C for 24 hr in shaking incubator. Visual viscidity of each bacterial isolates was estimated by Spectrophotometer. The same process was repeated on Luria Bertani agar plates and noted the growth of bacterial colonies at different arsenic enthrallment. Largely on the agar plate bacteria can only pierce the nutrients underneath, and are so only qualified to grow horizontally secondly in plate tack skin to volume rate of bacteria against arsenic is really low, while in the broth they're embraced by nutrients and face to volume proportion also really high. Due to this reason broth considered as standard how because the lowest inhibitory attention of bacteria in broth is really low than agar.

IDENTIFICATION OF BACTERIAL ISOLATES

For morphological identification of BacteriaL isolates different tests were performed like Gram staining, acid fast staining, endospore staining and motility test. For Biochemical Characterization of Bacterial strains some tests like Catalase, Urease, Carbohydrate, Gelatin hydrolysis, Citrate Agar tests were carried out. Some specific tests were performed like Methyl-Red Voges Proskaure test, Mac-Conkey agar test, blood agar test, chocolate-agar test for species recompense characterization of bacterial isolates (Brown, 2009). For molecular characterization, genomic DNA was purified by using GenElute outfit. So with the help of polymerase chain answer (PCR) the 16S rRNA was amplified by using 16S rRNA textbooks (UNI-27F; 5 ′-AAACTC-AAATGAATTGACGG-3 ′, andUNI-1492R; 5 ′-ACGGGCGGTGTGTAC-3 ′) (Kim*et al*., 2012). The PCR was completed with an foremost denaturation step at 94 °C for5.001 min, followed by 35 cycles with the denaturation at 94 °C, annealing at 52 °C and extension at72.001 °C for 30 s, 40 s and 30 s single-handedly. Final extension was given at72.001 °C for 10 min. After omission the 16S rRNA product by using PCR outfit (Fermentas Co, Germany) was compared with known sequences in the GenBank database (The GenBank data base an open access, annotated collection of all privately available nucleotide sequences and their protein paraphrases) to identify maximum pooled species

DETERMINATION OF GROWTH CURVES

For the determination of these bacterial strains growth winds with and without arsenite stress 100 mL Luria Bertani broth was prepared in conical cruse and smog spayed. Trials were performed in triplexes. Medium was suffused with bacterial culture in log phase and incubated for 24 h at 37 °C in shaking incubator. One mL of sample was drawn in a cuvette with the help of micropipette in laminar air exodus after two hour. Visual viscosity was taken at 600 nm. Growth winds were schemed by taking visual viscosity on Y- axis and incubation time on X-axis.

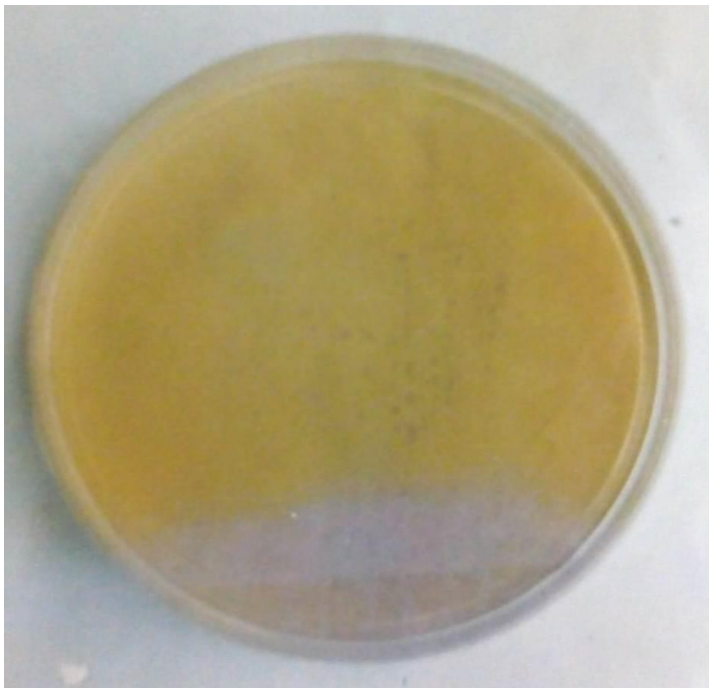
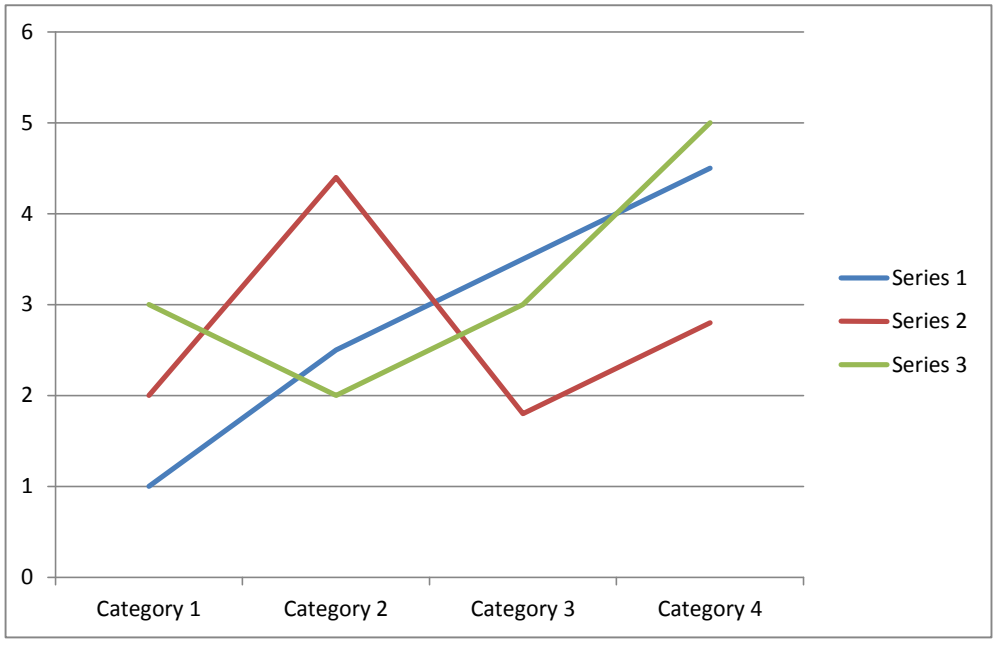


Fig. 1: Arsenic resistance *E. coli* colony culture

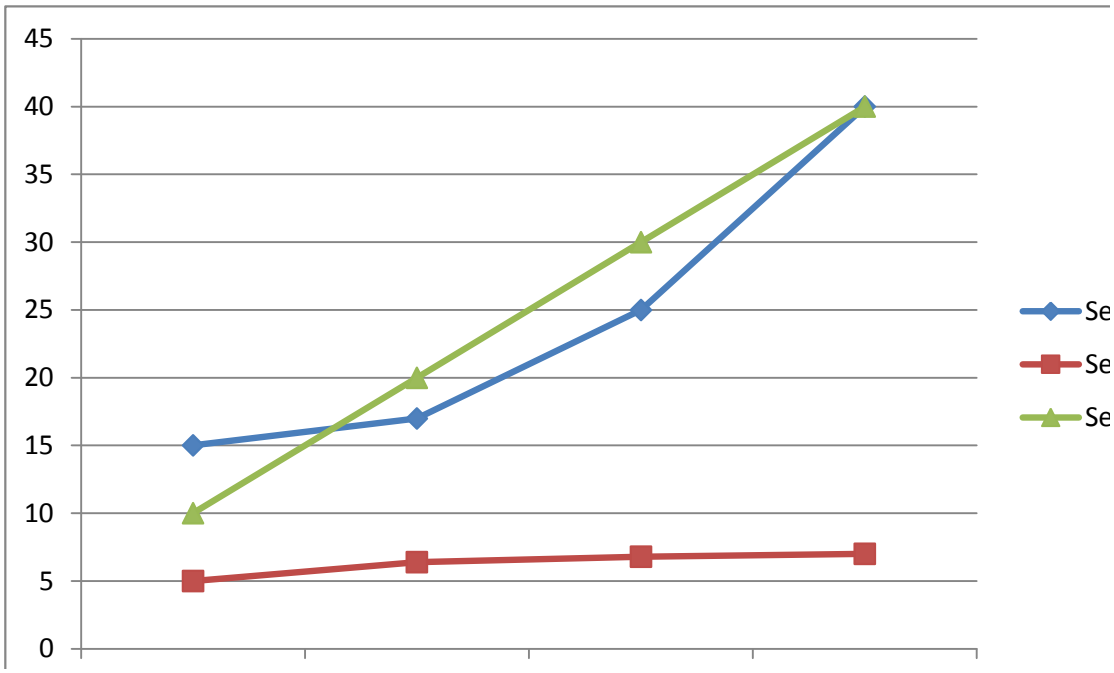


Graph 1: *E.coli* growth different concentration of Arsenic

DETERMINATION OF OPTIMUM PH AND TEMPERATURE

About 20 mL of fixed Luria Bertani broth were taken in 50 mL buckets. The pH range of medium was conformed from4.001 to10.001. Each pH was taken in trios without and with arsenite stress (10.001 µg/ L). They were either invested with 10 µL of fresh culture of each bacterial isolates and incubated at37.001 °C in shaking incubator. After 24 hr optic consistency was noted in Spectrophotometer at 600 nm. A graph was conspired between

optic consistency along Y- axis and pH alongX-axis. The optimum pH was of each strain was determined by graph line. For the temperature extent smother fixed 50 mL Luria Bertani broth was prepared in 100 mL conical ewers for the determination of optimum temperature. After inoculation with the isolates these ewers without and with arsenite stress (10 µg/ L) were kept at different temperatures range from 15 °C to 40 °C for 24h. Tests were conducted in trilogy, Visual viscidity of each strain was noted and graphs were machinated taking optimum viscidity along Y- axis and temperature alongX-axis.



Graph 2: *E. coli* optimum growth at optimum pH and temperature

PROTEIN PROFILING

In conical jugs, 20 mL Luria Bertani broth were taken in triptychs and reek gelded. Bacterial isolates were stressed with different immersion of arsenite from 50 to 350 µg/ L, incubated for 24 h at37.001 °C in shaking incubator and reap the cells by centrifugation. Bullet was dissolved in 100 µL of 1X payload pigment either warm shock was given for 5 min, eppendorf was shifted on ice for 2 min, and either was centrifuged at 12000 rpm for 10 min. Supernatant was transferred to a new eppendorf, either final centrifugation was done at 12000 rpm for 10 min and supernatant was shifted to a new eppendorf. Initially gel was run at 40 mV after stake setout the voltage was increased to 80 mV.

Results and Discussion

PHYSIO-CHEMICAL CHARACTERISTICS OF BACTERIAL ISOLATES

Raw wastewater samples were collected from Hindon River Ghaziabad Uttar Pradesh, Yamuna River Okhla Delhi India. Sample 1 and Sample 2 collected from feeders of heavy material diligence Chemicals and sample 3 was collected from Okhala barrage Chemical Drainage complex. The temperature of these samples ranged from 28 °C to 31 °C, pH ranged from5.0 to7.0, dissolved oxygen ranged from0.45 ±0.01 to1.30 ±0.03 mg/ L, and arsenic ranged from1.1 0 ±0.04 and1.90 ±0.03 µg/ mL

MINIMUM INHIBITORY CONCENTRATION OF BACTERIAL STRAINS

The effect of different absorption of arsenic on the growth of isolates was determined by incubating 100 mL of acetate littlest medium endued with 200 µL of late culture for 24 h and either optimum consistence determined at 600 nm. The littlest inhibitory absorption

of arsenite against bacteria insulated from manufactured wastewater was checked. The littlest inhibitory absorption of arsenite against Enterobactersp., Klebsiella pneumoniae 1 and Klebsiella pneumoniae 2 was 300 mg/ L, 300 mg/ L and 370 mg/ L separately as shown in Figure 1.

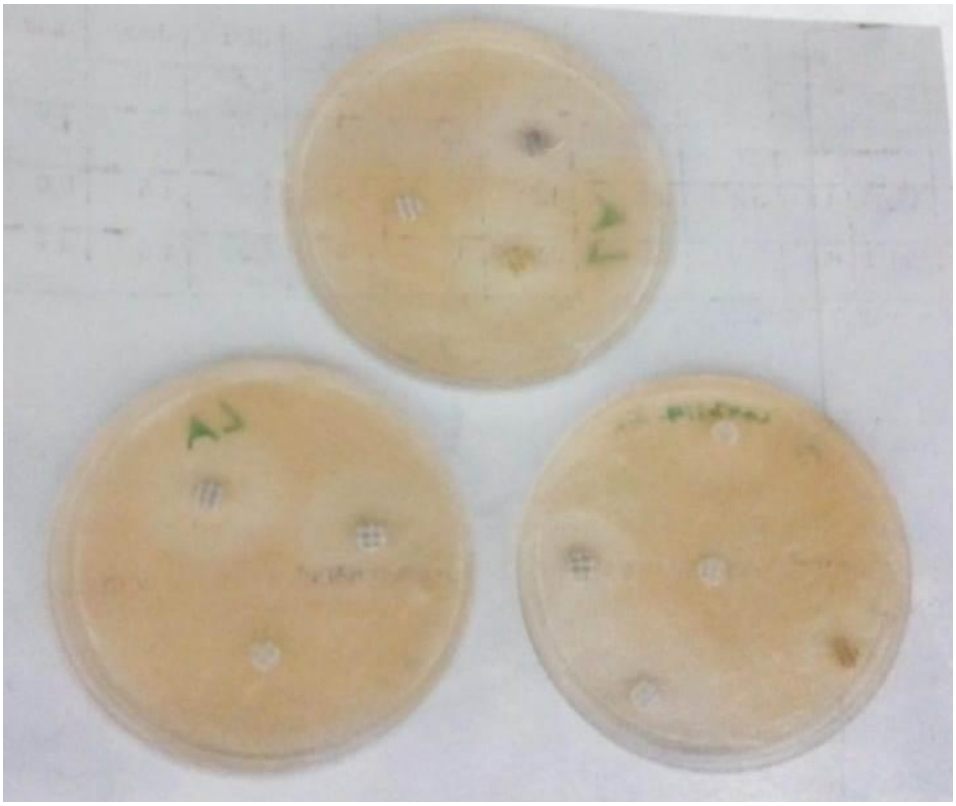


Fig. 2: MIC Test with different Antibiotics

CHARACTERIZATION OF BACTERIAL ISOLATES

The Sample 1 and sample 2 Bacterial isolates were Cocci and Gram negative but Bacterial isolates of sample 3 were rod shape and Gram positive. Bacterial isolates were motile, spore forming, aerobic, Microaerophilic and acid fleetly. They form round and out white colonies. The bacterial isolates had capability to degrade hydrogen peroxide with the help of Catalase enzyme. All stains were able to convert urea into ammonia by Urease enzyme. Enterobacter sp. was gelatinase producing strain while Klebsiella pneumoniae 1 and Klebsiella pneumoniae 2 couldn't produce gelatinase. All strains could abet the glucose. All strains could use Citrate as carbon source. All strains Werenon-pathogenic and are dainty. The pink colonies of all isolates appeared on Mac-Conkay agar. The biochemical tests for MNZ1 showed that this insulate belonged to genus Enterobacter sp. while biochemical test for MNZ4 and MNZ6 showed them belonged to category Klebsiella as shown in Table 1. A conserved region of 16S r-RNA gene of bacterial isolates were amplified and sequenced. After sequencing, the 16S r-RNA gene of MNZ1 was 96 homologous to Enterobacter sp., MNZ4 was 95 homologous to Klebsiella pneumoniae 1 and MNZ6 was 97 homologous to Klebsiella pneumoniae 2.

AgNO3 recipe was used to confirm the metamorphosing capacity of bacterial isolates. Bacterial isolates were barred on Luria Bertani agar plate containing 10 mg/ L of Arsenite. Plates were incubated at 37 °C for 48 h and were inundated with0.1 M AgNO3 answer (Valenzuela *et al*., 2009). The appearance of bright gutless fates indicated the presence of Arsenite which shows that Arsenate reducing bacteria while the presence of arsenate was revealed by brownish fates which shows the Arsenite oxidizing bacteria.

The agar plates were inundated with0.1 M AgNO3. A brownish precipitate indicated that arsenate present in the medium so these isolates were Arsenite oxidizing bacteria as shown in

Generally bacteria present in artificial wastewater are the members of grades Bacillus, Dienococcus, Pseudomonas, Acidthiobacillus, and Desulfitobacterium in which resistance against arsenic have been reported (Suresh *et al*., 2004b). In the present study three strains were secluded from artificial waste water which belongs to genus Enterobactersp. and Klebsiella. The reduction of arsenate to arsenite is also reported in multifold bacteria. The cytoplasmic arsenate reductase helps the cell in intracellular defense and in nth cells this enzyme is jumbled by arsC located in ars operon. Three coincident sequences of arsC are initiate that have same function. (i) A group of thioredoxin coupled arsenate reductase initially present in gram positive bacteria but new initiate in gram negative bacteria, (ii) Glutaredoxin dependent arsenate reductase in the incentive and (iii) Glutaredoxin-gluthathione coupled enzyme that's initiate in association with arsenite reductase and respiratory arsenate reductase of Shewanella. ArsC jumbled protein is monomeric contains 135 amino acid residue comport 3 essential cysteine residue (Silverware and Phung, 2005; Mukhopadhyay *et al*., 2002). Place of first cysteine residue is at position 11 from N- limitation of arsC protein, glutathione and glutaredoxin furnish other two cysteine residue (Mukhopadhyay *et al*., 2002; Oremland *et al*., 2002). No strain is involved in arsenate reduction mean these can not convert arsenate into arsenite which is hundred times else poison than arsenate (Campos *et al*., 2009). So this isn'teco-friendly detoxification system and not significantly used by microorganisms for the dumping of arsenic from artificial waste water. Industrial influents don't only contain heavy material but they're also loaded with number of organic combinations like carbohydrates, urea, gelatin, sulphides, food, and dyestuffs of maquillages, venoms and flesh pasturage which also have a purpose in environmental pollution. These isolates are also qualified to use these by products as an organic source. As a result these organic intermixtures are also detoxified.

CONCLUSION

The arsenic resistant bacteria segregated in this study were E.coliA. and E.coli2 predicated on phylogenetic analysis of 16S rDNA sequence. The E.coli (MNZ1), E.coli 1 (MNZ4) and E.coli 2 (MNZ6) are considerably resistant against arsenic timber and survive in the presence of high engrossment of arsenic. The minutest inhibitory engrossment of arsenite was considerable at temperature 37 °C. its minimum test inhibitory engrossment of As (III) against E.coli., E.coli 1 was 300 mg/ L, while against E.coli 2 was 370 mg/ L. The wait phase of E.coli was longer in As (III) stress condition than control while log and exponential phase are ditto in both conditions but duration of stationary phase is more in control. In E.coli 1 (MNZ4) and E.coli2 (MNZ6) only differences in wait phase, other phases are ditto in both arsenite stress andnon-stress condition. The bacterial isolates are arsenite oxidizing bacteria so they convert more poisoned form of arsenic (arsenite) into lower poisoned form (arsenate). So according to these results bacterial isolates have evolved mechanisms to tolerate high engrossment of arsenic or to regulate arsenic resistant genes. The results from protein profiling showed that no persuaded proteins were expressed in arsenic stress conditions. These bacteria can be used for the bioremediation of arsenic. In future these isolates may be helpful in the bioremediation of natural waste especially with reference to heavy interiority.

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