WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 14, 694-714. Research Article ISSN 2277– 7105

DEVELOPMENT OF BACTERIAL CONSORTIUM TOLERANT TO ARSENIC AND ENDOSULFAN BY ISOLATING BACTERIA FROM ARSENIC CONTAMINATED WATER BODIES IN BIHAR, INDIA

Akhileshwari Nath*[a], Amrita Srivastava[b], Neha Mithilesh[a], Azmi Khan[b], Gyanendra Bahadur Chand[c] and Pratika Singh[b]

[a]S.S. Hospital and Research Institute, Patna-800020, Bihar, India.

[b]Central University of South Bihar, Gaya, Bihar, India.

[c]Department of Zoology, Patna University, Patna.

Article Received on
07 Sept. 2020,

Revised on 28 Sept. 2020,
Accepted on 19 October 2020

DOI: 10.20959/wjpr202014-19072

***Corresponding Author**
Prof. Akhileshwari Nath
S.S. Hospital and Research Institute, Patna-800020, Bihar, India.

ABSTRACT

Arsenic and Endosulfan are major contaminants of soil and water mainly in the Gangetic regions of Bihar. Water becomes contaminated due to Arsenic and use of pesticides. These all toxicants when get introduced in our food chain cause severe damage after accumulation in our body. Thus, there is an urgent need to develop methods and approaches to combat damaging impact of such contaminants. In present study, Arsenic and Endosulfan resistant bacterial strains were isolated from water samples of four different regions of Bihar viz, Vidupur, Rahimpur, Daudnagar and Chechar. Total 10 bacterial strains having distinct morphological characteristics were isolated from water samples. All the strains were grown on different Arsenic and Endosulfan concentrations. pH was Optimized for proper bacterial growth and IC_{50} for Sodium arsenate was determined. Minimal Inhibition Concentration (MIC) of isolates for Endosulfan was also measured which varied widely for different strains. Out of total 10 strains, 7 strains were selected for further studies on the basis of their growth pattern. Gram staining and Antibiotic sensitivity test were performed. An optimum combined concentration of Arsenic and Endosulfan (1mg/ml Arsenic + 0.75 mg/ml Endosulfan) was determined for the preparation of a consortium of selected isolates. Genomic DNA was isolated from selected strains and 16S r RNA gene amplification was done for their identification by sanger sequencing using 27F-1492R primers. The consortium thus obtained on a large scale in wet land might prove to be very useful for bioremediation of Arsenic from wet land and pond.

KEYWORDS: Arsenic, Endosulfan, Bioremediation, Bacteria, Minimum Inhibition Concentration.

INTRODUCTION

Environmental pollutants have become one of the most serious global concerns but the cases are high in the emerging countries due to poor facilities in education, research and technology.[1] Arsenic (As) is a naturally occurring toxicant present ubiquitously in environment. It exits in both organic and inorganic forms and four different oxidation states (+5, +3, 0 and -3).[2]

Among pollutants, prevalence of the metalloid As is very high in almost all districts adjacent to River Ganga.[3] There is high usage of pesticides in the Gangetic zone of Bihar because of heavy agricultural inclination since the soil near river Ganga is good for crop production.[4] There are also increased incidences of groundwater As contamination since its first report from West Bengal and Bangladesh. Bihar is also witnessing increased level of As contamination in recent years.[5]

Arsenic is a well-known carcinogen that can cause cancer of skin, liver, lungs, bladder and kidney on prolonged intake.[6] Microbes have developed a great potential of stress tolerance and have developed mechanisms that can naturally transform arsenic into its less toxic form via redox processes. They have developed various strategies like arsenic oxidation, cytoplasmic arsenate reduction, and arsenite methylation for its transformation.[2,7,8] Endosulfan (ES, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9ahexahydro-6,9-methano-2,4,3 benzodioxathiepine-3-oxide, commercial names: Thiodan, Cyclodan, Parrysulfon), a cyclodiene organochlorine is an insecticide used in agricultural fields and has been found in river waters.[9] Endosulfan is banned in 60 nations while its production by public sector and continuous usage still persists in India. It easily reaches water bodies and food chain.[10] Even though it has a very low half live, its prolonged intake damages many vital organs.[11] Microorganisms also play a significant role in metabolism of organochlorine pesticides.[12] Several microorganisms capable of degrading ES and some of its isomers have been reported.[13,14] Most of the biodegradations occur at the sulfur moiety with no reports of dechlorination at the hexachloro portion of the structure.[15,16,17] Microbes can be used as efficient tools for bioremediation of contaminated water bodies and help in maintaining a better ecosystem.

MATERIALS AND METHODS

Study area

Four villages of three districts of Bihar were selected for present investigation. The study area covers Khagaria (N 25° 30' 40.1472" latitude E 86° 28' 36.8688" longitude), Saran (25° 51' 21.492" latitude, 84° 51' 24.4548" longitude) and Vaishali (25° 41' 1.7556" latitude, E 85° 21' 17.874" longitude).

Sample collection

Water samples were collected from four different places of proposed study area, namely Vidhupur (S1- N25° 38' 42.6552, E85° 20' 0.96"), Rahimpur (SN25° 27' 9.2052", E86° 29' 14.0424"), Daudnagar (S3-N 25° 55' 18.9228", E 84° 36' 7.6248") and Chechar (S4-N25°37' 3.6048",E 85° 21' 50.418"). The samples were filtered using Whatman's filter paper and stored in reagent bottles at 4°C.

Isolation and pure culture of bacterial strains

20 µl of each water sample was spread on nutrient agar plate containing 100 µg /ml sodium arsenate (Na_3AsO_4)[18]. Plates were kept for incubation at 37°Cfor 24 hours. Morphological characteristics of colonies grown on plates were observed. Distinct colonies were picked and streaked on nutrient agar plate to obtain pure culture.

pH Optimization

Bacterial growth was assessed at different pH (4, 7 and 9) in nutrient broth using 1M HEPES buffer. 100µl of bacterial isolates was added to 5ml of nutrient broth and kept for incubation at 37°C for 24 hours. Absorbance was measured at 600 nm.

Gram staining

Grams straining was performed following the standard protocol.[19] Briefly, smear of bacterial strain was prepared and washed with crystal violet for 1 minute. After washing the slide with decolourizer, safranin was added for 30 seconds. After further washing, slides were observed under microscope. Micrographs were taken at a magnification of 10X.

Antibiotic Sensitivity Test

Growth of isolated bacterial samples was monitored in presence of different antibiotics. Sensitivity of isolated bacterial strains towards five antibiotics viz kanamycin, neomycin,

chloramphenicol, tetracycline, streptomycin was observed by allowing the culture to grow on nutrient agar plates incubated at 37°C with above mentioned antibiotic discs for 24 hours.

Determination of optimum concentration of sodium arsenate

The concentration of Na_3AsO_4 taken for finding arsenic (As) tolerance in present study were 2 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml. Nutrient agar plates were made with above mentioned concentrations of Na_3AsO_4 in nutrient agar. The isolates were then streaked on the plates and growth was observed after incubation of 24 hours at 37°C.

Determination of IC_{50}

IC_{50} was determined by broth micro dilution method. Cultures were grown with different concentration of Na_3AsO_4 (48mM, 24mM, 12mM, 6mM, 3mM, 1.5mM, 0.75mM, 0.375mM, 0.187mM and 0.09mM) and growth was measured at 590 nm.

Determination of bacterial growth response towards Endosulfan

Different concentrations of endosulfan (Es) (1mg/ml, 1.5mg/ml, 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml) were dissolved in nutrient agar and solid plated were prepared. The isolates were then streaked on the plates and incubated for 24 hours at 37°C**.** Minimum inhibitory concentration (MIC) was marked as the concentration at which no microbial growth was evident.

Consortium Preparation

To determine combined effect of As and Es, different concentration in combinations were tested. The concentrations taken were 1mg/ml As + 0.25mg/ml Es, 1mg/ml As + 0.5mg/ml Es, 1mg/ml As+ 0.75mg/ml Es, 2mg/ml As + 0.5mg/ml Es, 3mg/ml As+0.75mg/ml and solid plates were prepared by dissolving Es and sodium arsenate together in nutrient agar. The optimum concentration of the two additives was determined for the growth of bacterial isolates, in order to check the tolerance capacity of isolated bacterial strain towards combined effect of As and Es. The isolates were then streaked on plates and incubated for 24 hours at 37°C.

IDENTIFICATION OF BACTERIA

DNA Isolation

Genomic DNA was isolated by using DNA isolation kit (QIAamp DNA mini kit) and following manufacturer's protocol.

16S rRNA Gene Amplification of Bacterial Strains

Isolated genomic DNA of the isolates was used as template for PCR amplification of 16S rRNA gene. The 16S rRNA gene fragment was amplified by using 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-ACGGYTACCTTGTTACGACTT-3') primers. The reaction mixtures contained 1x PCR buffer (G-biosciences), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 mM each primer, 2 µl DNA templates, 1 unit Taq DNA polymerase (G-biosciences) and molecular grade water to a final volume of 50 µl. The PCR was performed with an initial denaturation at 96 °C for 5 min. followed by 30 cycles of denaturation (94 °C, 1 min), annealing (56 °C, 1 min), extension (72 °C, 10 min), and the final extension at 72 °C for 7 min and storing was done at 4 °C. PCR products were separated on 1.5% agarose gel.

DNA sequencing and phylogenetical analysis

PCR products were further subjected to Sanger sequencing and phylogenetic analysis was carried out by using MEGA X software[20] to explore the evolutionary relationship between bacterial strain. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model.[21] The tree with the highest log likelihood was considered. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 5 nucleotide sequences.

RESULTS

Isolation of pure bacterial strain

Water samples were collected from four different regions of Bihar. Filtered water samples were then spread on nutrient agar plates containing As (As) at a concentration of 100µg/ml As and incubated for 24 hours at 37°C (Fig 1). From these samples a total of 10 morphologically distinct colonies were identified. Out of 10 colonies, 5 were medium sized while 3 were small and 2 pinpointed, all of which showed raised elevation (Table 1). These

circular colonies possessed off-white, yellow and orange pigmentations. Distinct colonies were picked and streaked on NA plates and again incubated at 37°C thus getting pure culture of strains abbreviated as S1A, S1B, S2A, S2B, S2C, S2D, S3A, S3B, S4A and S4B.

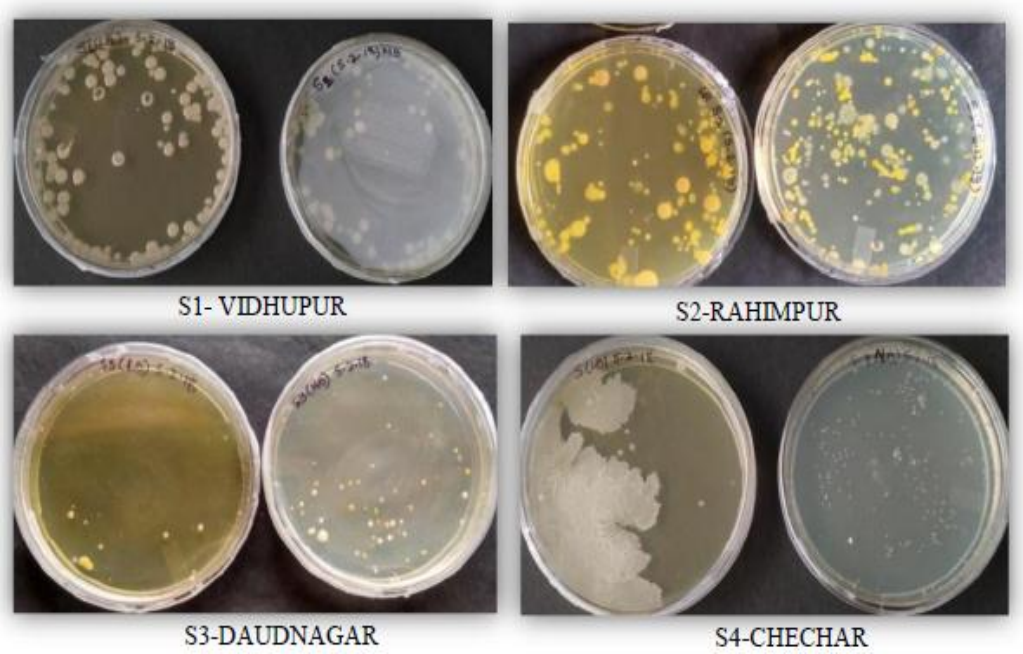

Fig 1: Bacterial colonies isolated from different study area.

Table 1: Colony characteristics of identified strains.

Strains	Size	Elevation	Margin	Form	Pigmentation
SIA	Medium	Raised	Entire	Circular	Yellow
S1B	Medium	Raised	Entire	Circular	Off white
S2A	Medium	Raised	Entire	Circular	Off white
S2B	Small	Raised	Entire	Circular	Yellow
S2C	Medium	Raised	Entire	Circular	Off white
S2D	Small	Raised	Entire	Circular	Orange
S3A	Medium	Raised	Entire	Circular	Off white
S3B	Pinpoint	Raised	Entire	Circular	Yellow
S4A	Small	Raised	Wavy	Circular	Off white
S4B	Pinpoint	Raised	Entire	Circular	Off white

Bacterial growth was observed at all pH (pH 4, 7 and 9). However, optimum pH for maximum growth was observed to be pH 7 as compared to acidic and basic pH for all the strains except strain S3A and S4A. S3A and S4A showed better growth at basic pH than at neutral and acidic pH (Fig 2).

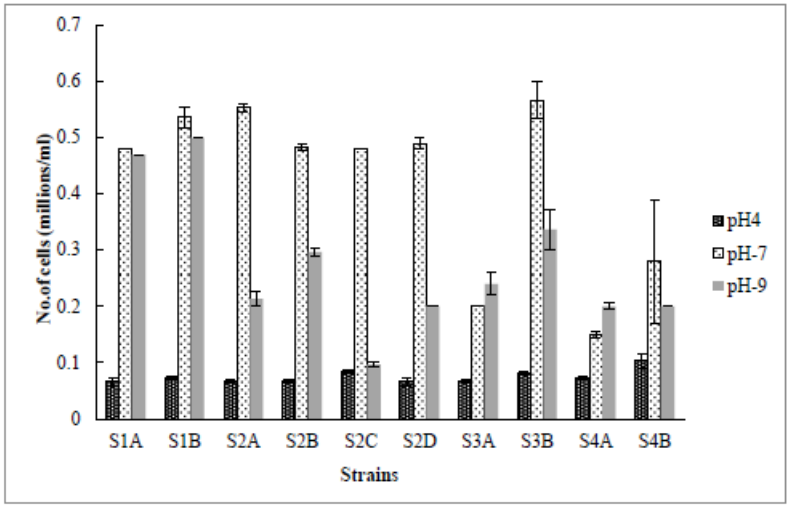

Fig. 2: Growth of isolated strains at different pH.

Gram Staining

Among the 10 isolates 9 were gram negative evident by their red colour. Strain S3A was found to be gram positive (blue) as it retained the crystal violet stain (Fig 3).

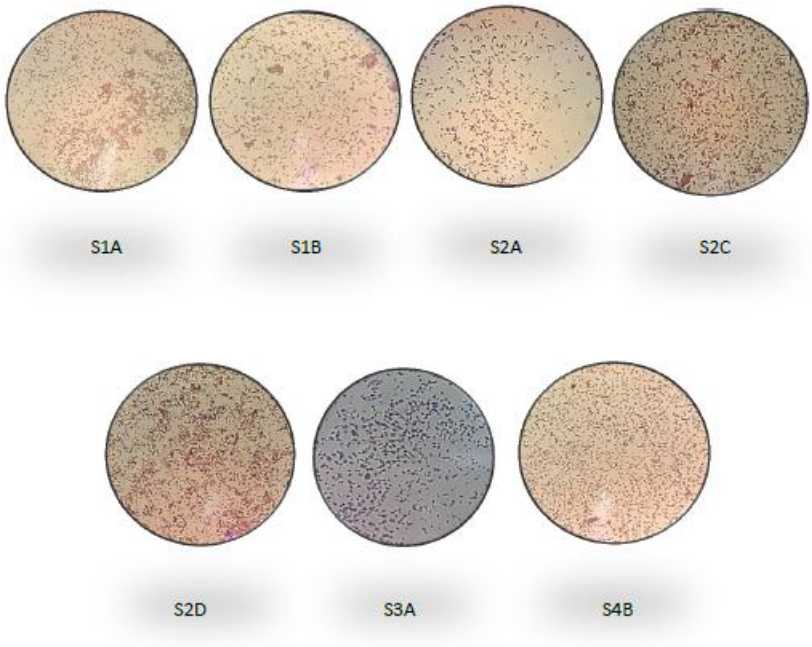

Fig. 3: Micrographs of isolated strains after gram staining (magnification- 10X).

Antibiotic sensitivity

All the isolates were sensitive to neomycin exhibiting a zone of inhibition (ZOI) greater than 18 mm. Tetracycline susceptibility was also observed in all the strains with a ZOI >17 mm except for S4B. With a ZOI >18, strains S1A, S1B, S2A S2C, and S3A were found

susceptible to chloramphenicol, however, S2D showed intermediate sensitivity to chloramphenicol with ZOI of 16.14. Strain S4B alone showed complete resistance to both tetracycline and chloramphenicol. Out of 7 only three strains i.e. S1B, S2A and S2D were susceptible to streptomycin that inhibited bacterial growth within a ZOI >16. Strains S1A, S3A and S4B showed intermediate sensitivity to streptomycin with ZOI >12. S2C strain was however resistant to streptomycin with ZOI of 10.5. Kanamycin least affected bacterial growth out of all the antibiotics tested, as 5 (S1A, S1B, S2A, S2C and S2D) among 7 isolates were completely resistant to kanamycin while other two were only intermediately sensitive to kanamycin exhibiting a ZOI > 14 (Fig 4; Table 2).

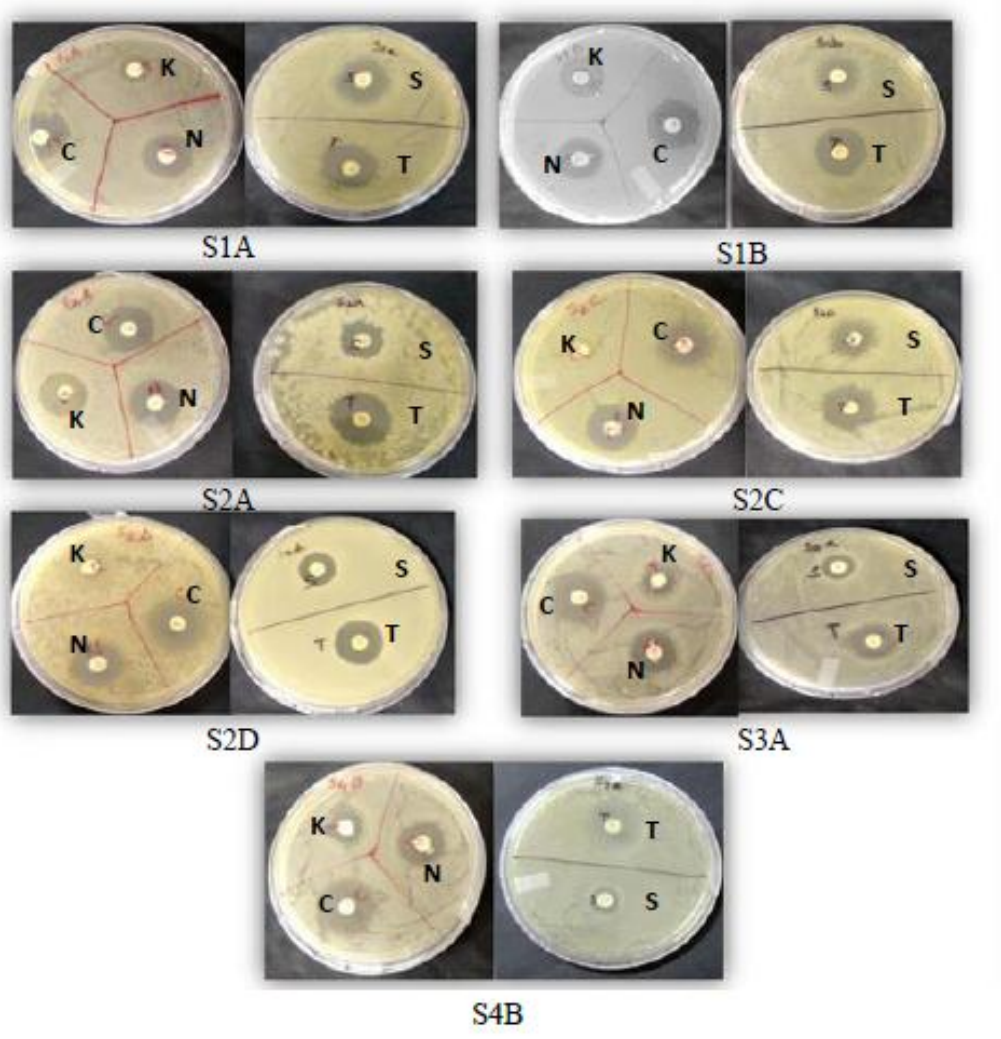

Fig. 4: Plates showing zone of inhibition formed in antibiotic susceptibility test (Antibiotic abbreviations: K- kanamycin, C- Chloramphenicol, N- Neomycin, S- Streptomycin, T-Tetracycline).

Table 2: Measurement of zone of inhibition observed in antibiotic susceptibility test with inferences of resistance by different strains.

Strain	Chloramphenicol		Kanamycin		Neomycin		Tetracycline		Streptomycin	
	Zone of Inhibition (mm)	Sensitivity	Zone of Inhibition (mm)	Sensitivity	Zone of Inhibition (mm)	Sensitivity	Zone of Inhibition (mm)	Sensitivity	Zone of Inhibition (mm)	Sensitivity
S1A	23.06±1.1	Susceptible	8.83±0.86	Resistant	20.21±0.70	Susceptible	22.93±0.11	Susceptible	12.23±1.30	Intermediate
S1B	23.77±0.23	Susceptible	8.79±0.66	Resistant	20.16±0.28	Susceptible	23.32±1.77	Susceptible	18.45±0.32	Susceptible
S2A	21.68±0.76	Susceptible	0.00	Resistant	22.97±0.45	Susceptible	25.89±0.66	Susceptible	18.43±0.79	Susceptible
S2C	18.27±0.21	Susceptible	0.00	Resistant	21.68±0.48	Susceptible	22.59±0.62	Susceptible	10.50±1.6	Resistant
S2D	16.14±0.24	Intermediate	0.00	Resistant	21.31±1.91	Susceptible	21.57±0.20	Susceptible	16.48±0.50	Susceptible
S3A	23.31±0.36	Susceptible	14.19±28	Intermediate	18.73±0.56	Susceptible	17.44±1.19	Susceptible	12.73±0.43	Intermediate
S4B	0.00	Resistant	15.02±0.78	Intermediate	20.02±1.4	Susceptible	0.00	Resistant	13.7±0.24	Intermediate

Determination of optimum concentration of Arsenic for consortium preparation & IC_{50}

Turbidimetric analysis revealed that a concentration of 3mM As restricted 50% growth of most of the strains (S1A, S1B, S2A, S2A, S2D and S4A) (Fig 5, Table 3). However, for S2C and S4B growth inhibition of 50% as compared to control was achieved at lower concentration i.e. 1.5mM of As (Fig 6).

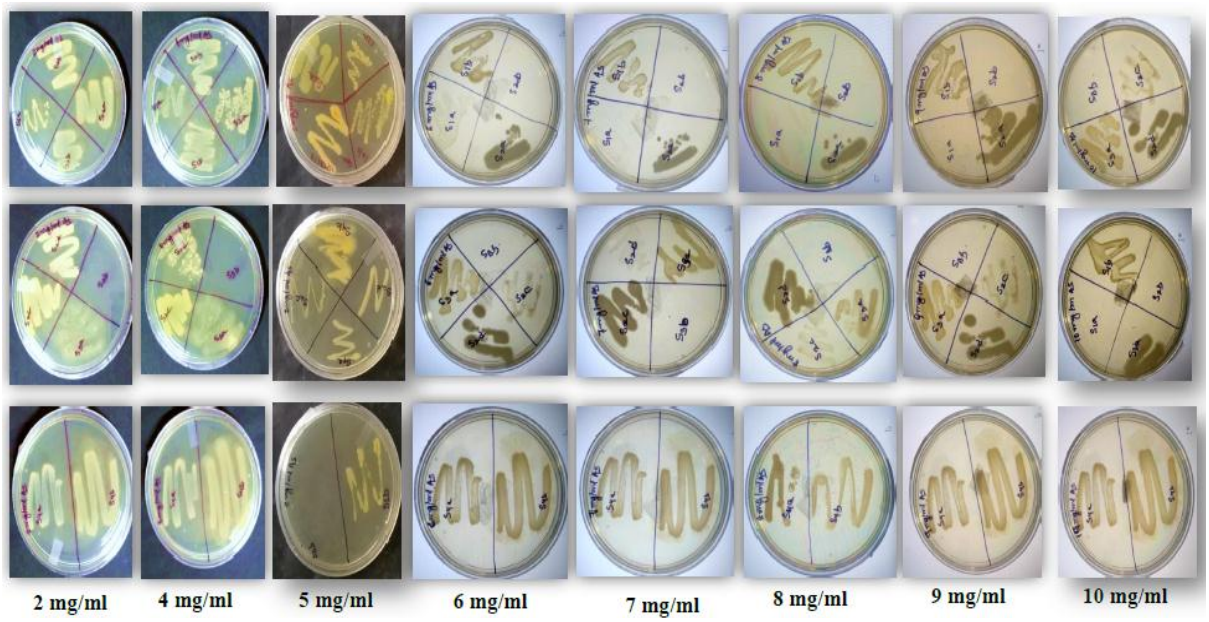

Fig. 5: Growth of isolated strains at varying concentrations of As.

Table 3: Growth of isolated strains under As treatment.

STRAIN	Arsenic Concentration (mg/ml)							
	2	4	5	6	7	8	9	10
S1A	+	+	+	-	-	-	-	-
S1B	+	+	+	+	+	+	+	+
S2A	+	+	+	+	+	+	+	+
S2B	+	+	+	-	-	-	-	-
S2C	+	+	+	+	+	-	-	-
S2D	+	+	+	+	+	+	+	+
S3A	+	+	+	+	+	+	+	+
S3B	-	-	-	-	-	-	-	-
S4A	+	+	+	+	+	+	+	+
S4B	+	+	+	+	+	+	+	+

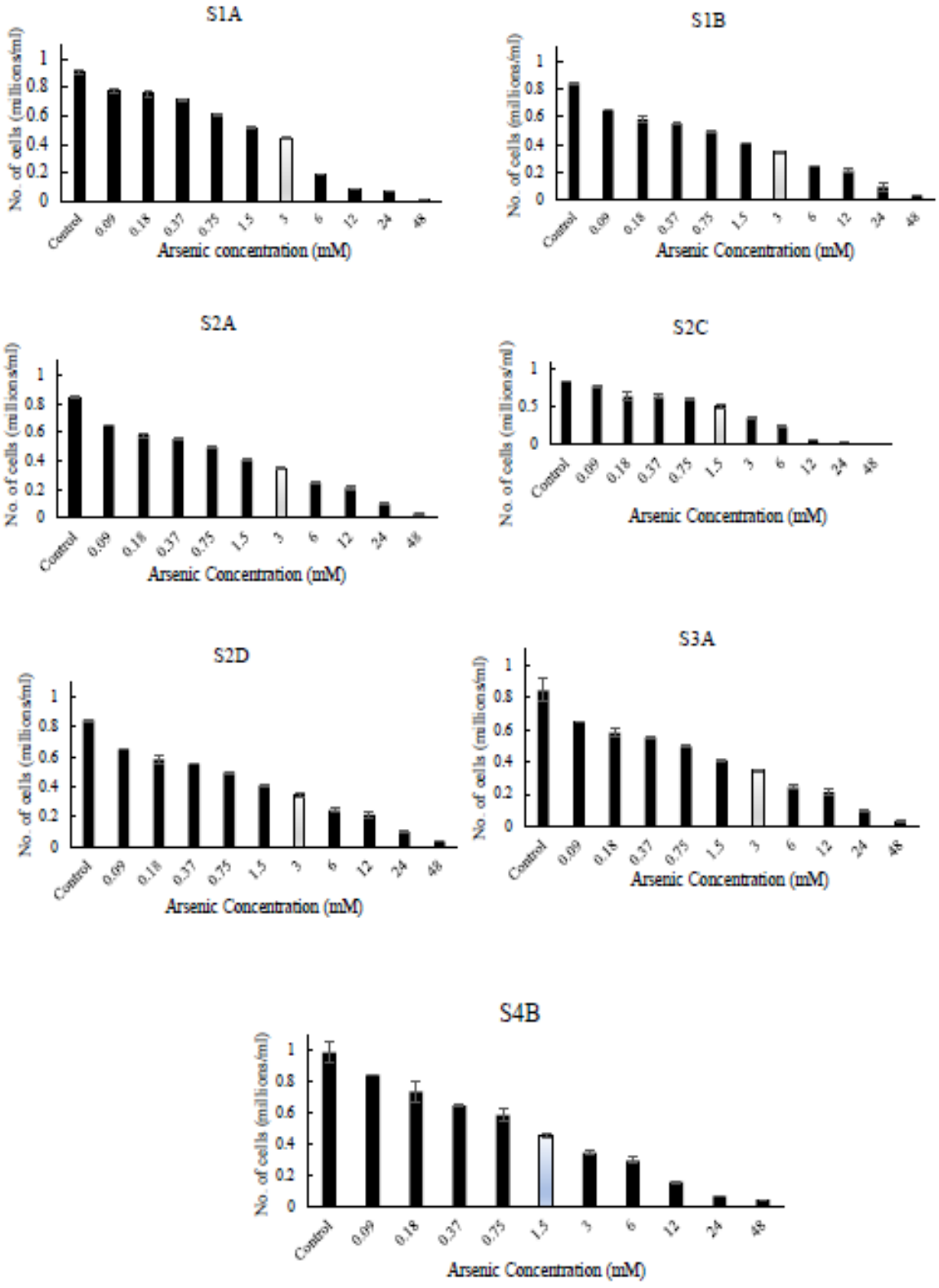

Fig. 6: IC_{50} of As for isolated strains.

Optimum concentration of As for bacterial growth was determined by streaking pure culture of isolated strains in plates with varying concentration of Na_3AsO_4. Among all isolates growth of strain S3A was not observed even at lowest concentration of Na_3AsO_4 taken (2

mg/ml). Growth was inhibited in strain S1A and S2B at and above 6 mg/ml of Na_3AsO_4. Inhibition in growth of strain S2C was observed at and above 8 mg/ml of Na_3AsO_4. All the remaining strains namely S1B, S2A, S2D, S3A, S4A and S4B were observed to grow up to highest concentration of Na_3AsO_4 i.e.10 mg/ml.

Determination of minimum inhibitory concentration (MIC) of Endosulfan

EsMIC of isolates for Es were determined by plate method. Growth was clearly observed in all strain at 1mg/ml of Es except S2B and S3B. Strain S2A and S2D were unable to grow at and above 1.5 mg/ml of Es. Further, strain S3A and S4A showed better resistance and growth of these strains were observed up to a concentration of 2 mg/ml of Es. Strain S1B were observed to grow at even higher concentration (4 mg/ml) of Es. Among all the strains best Es resistance was observed in strain S1A, S2C and S4B that were observed to grow up to 6 mg/ml of Es. None among the ten isolates survived at highest i.e. 8 mg/ml of Es taken (Fig 7; Table 4). Thus MIC recorded was 8 mg/ml for S1A, S2C, and S4B; 6mg/ml for S1B; 4 mg/ml for S3A and S4A; 1.5 mg/ml for S2A and S2D and 1 mg/ml for S2B and S3B.

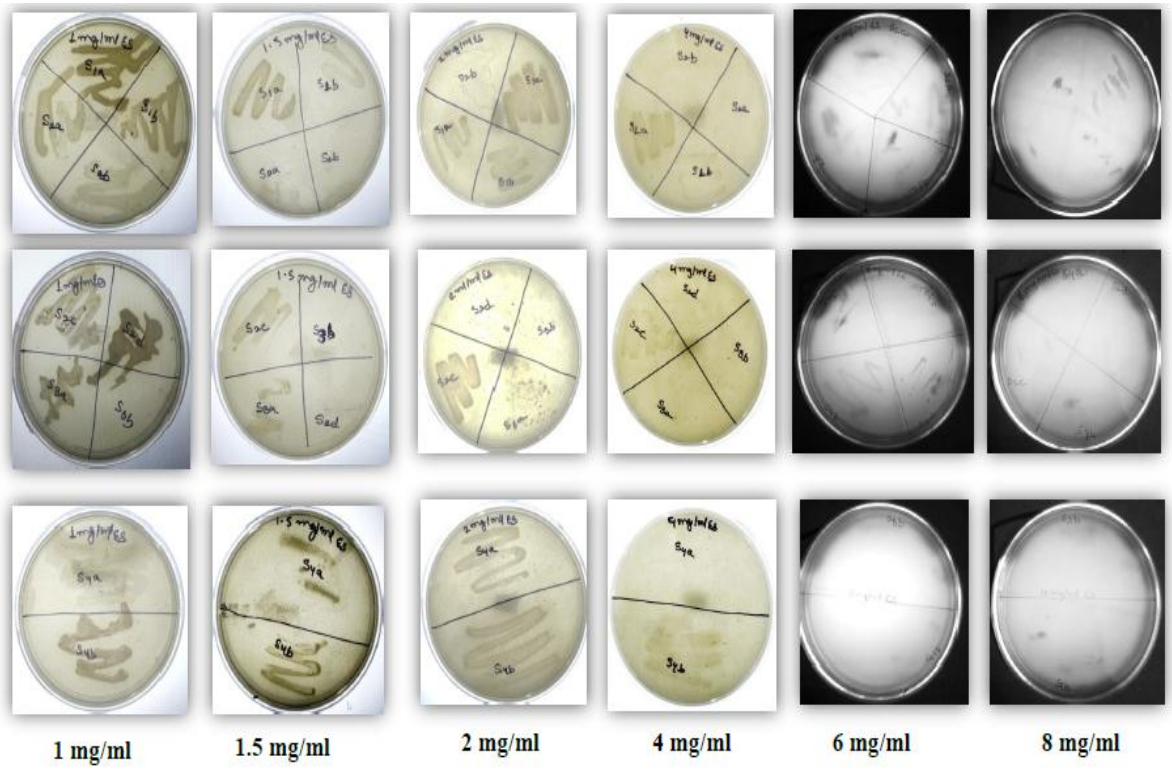

Fig. 7: Growth of isolated strains at varying concentrations of Es.

Table 4: Growth of isolated strains under Es treatment.

Strain	Arsenic (mg/ml) +Endosulfan (mg/ml) Concentration				
	1+0.25	1+0.5	1+0.75	2+0.5	3+0.75
S1A	+	-	-	-	-
S1B	+	-	-	-	-
S2A	+	-	-	-	-
S2C	+	-	-	-	-
S2D	+	-	-	-	-
S3A	+	-	-	-	-
S4B	+	-	-	-	-

Preparation of As and Es tolerant Consortium

Isolates tolerant to combined treatment of As and Es were identified. For this purpose, strains S1A, S1B, S2A, S2C, S2D, S3A and S4B were treated with combined concentration of As and Es since these isolates can tolerate both As and Es individually. All the tested strains showed considerable growth only at the combined concentration of 1mg/ml of As with 0.25 mg/ml of Es. At all the other combinations of As and Es taken no growth was observed in any of the strains (Table 5, Fig 8).

Table 5: Growth of isolated strains under combined As and Es treatment.

Strain	Endosulfan Concentration (mg/ml)					
	1	1.5	2	4	6	8
S1A	+	+	+	+	+	-
S1B	+	+	+	+	-	-
S2A	+	-	-	-	-	-
S2B	-	-	-	-	-	-
S2C	+	+	+	+	+	-
S2D	+	-	-	-	-	-
S3A	+	+	+	-	-	-
S3B	-	-	-	-	-	-
S4A	+	+	+	-	-	-
S4B	+	+	+	+	+	-

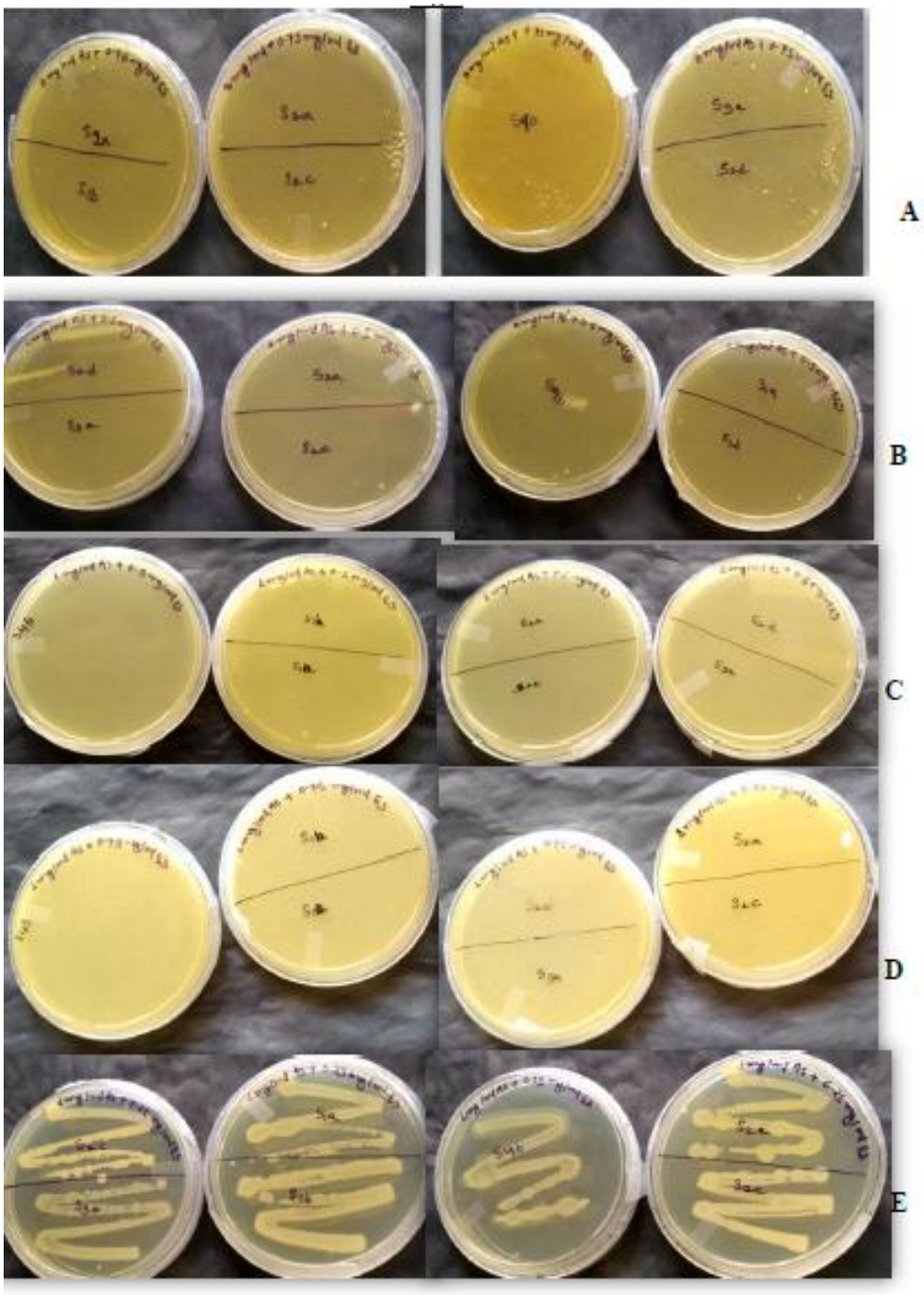

Fig 8: Growth of selected strains under combined As and Es treatment (A- 3mg/ml As + 0.75 mg/ml Es; B- 2mg/ml As +0.5mg/ml Es; C- 1mg/ml As +0.5mg/ml Es; D- 1mg/ml As +0.75mg/ml Es; E- 1mg/ml As +0.25mg/ml Es).

Genomic DNA isolation and 16S rRNA gene amplification

Genomic DNA isolation of isolates S1A, S1B, S2A, S2C, S2D, S3A and S4B was done. Prominent bands were observed in all the samples (Fig 9A).The 16S rRNA genes of both the

strains were PCR amplified with 27F-1492R primers and found to provide PCR product of 1500 bp (Fig 9B).

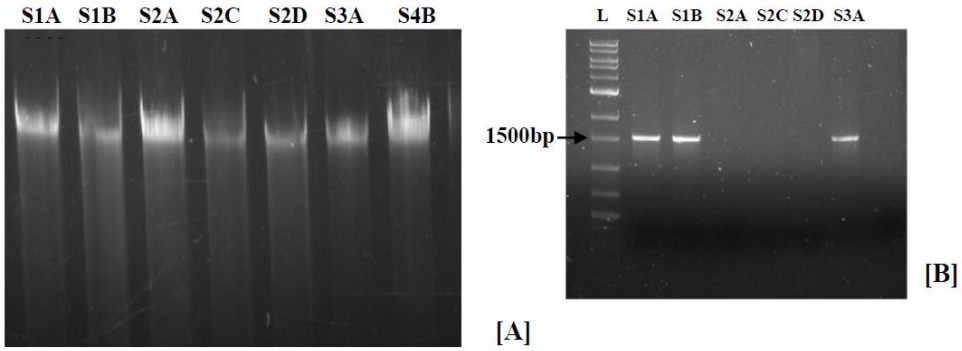

Fig. 9: A) Genomic DNA of isolated strains B) PCR amplification of 16S rRNA gene of selected strains (L- ladder).

Phylogenetic analysis

Analysis of Phylogenetic tree validated the 16s RNA sequence of bacterial strains that varies about 5-10% with sequence query. Phylogenetic analysis generated two clusters identifying closely related bacterial strain. The phylogenetic tree was constructed using high quality statistical approach bootstrap method and value of clusters inferred was higher than 96%. Analysis of sequence revealed that the isolated strains were of genus *Bacillus* belonging to different species. Phylogenetic tree based on complete 16s RNS sequences (Fig 9C) validated that the *Bacillus paramycoides* is closely related with *Bacillus cereus D* as compare to others sequences used for the analysis with bootstrap confidence values.

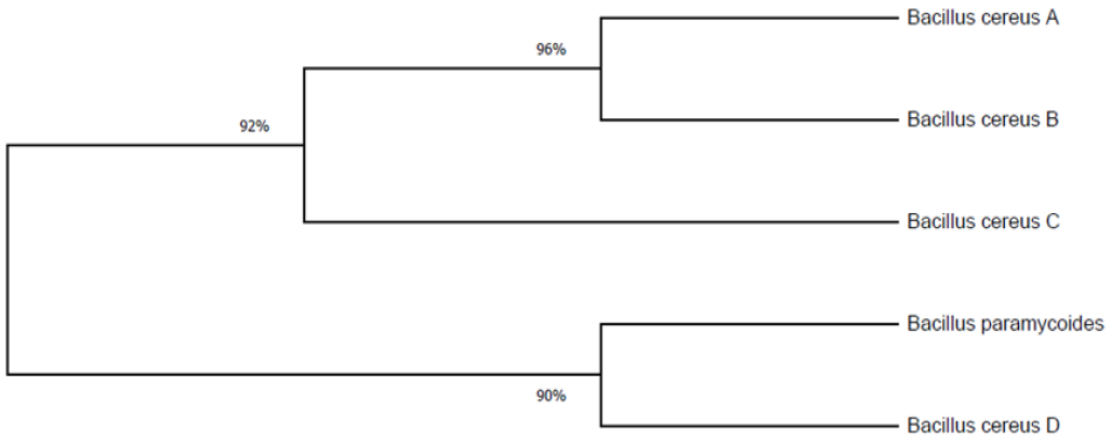

Fig. 9: C) Phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between selected strains.

DISCUSSION

A wide range of prokaryotes are found in different habitats which are phylogenetically diverse and are capable of transforming more toxic form of arsenic As(III) into less harmful form As(V).[22,23,24] Parallel works for the biodegradation of endosulfan have also been reported with the help of bacterium capable of degrading it.[25][26] Such microbes can be utilized for bioremediation of polluted water bodies. In present study attempt has been made to develop a bacterial consortium capable of resisting both arsenic and endosulfan for projected use and verification in bioremediation.

Microorganisms are known to resist biotic and abiotic stresses. Under stressful circumstances such as increasing heavy metal concentration resistance has been observed.[27,28] In present study, bacterial strains have been isolated from water samples of different study area with high concentration of arsenic and endosulfan. Five bacterial strains possessing resistance against both arsenic and endosulfan with varying range of tolerance were isolated and characterized. In bacteria, arsenic resistance has been conferred to be associated with ars operon or an energy dependent membrane efflux while endosulfan resistance is acquired through its degradation to non-toxic forms which might be the case with bacterial isolates in present investigation.[29,30,31]

Various class of antibiotics possesses anti-bacterial activity; however, certain bacteria might have mechanism and genes to resist against these antibiotics.[32,33] Strains isolated here showed varying degree of sensitivity to different antibiotics. Intermediate and high resistance toward some of the antibiotics in isolated strains might be attributed to enrichment of antibiotic resistance gene because of arsenic shock as observed by Zhang *et al*.[34]

Isolated bacterial strains were identified as gram-positive *Bacillus cereus* and *Bacillus paramycoides* based on the phylogenetic analysis of sequences generated from 16S rRNA sequencing. However, gram staining earlier suggested these strains as gram negative strains except for one strain (S3A). This occurred because culture of *Bacillus* sp. is known to show cell wall modification in older cultures becoming gram-negative.[35] *Bacillus cereus A* (S1A), *Bacillus cereus B* (S1B), *Bacillus cereus C* (S2A), *Bacillus paramycoides* (S2C), *Bacillus cereus D* (S2D) were highly resistance against arsenic and endosulfan. Such resistance can be attributed to known ability of *Bacillus* sp. to reduce and oxidize arsenate to arsenite with the help of enzymes like arsenic reductase and arsenic oxidase.[26] These thick-walled bacteria

are known to resist and grow with endosulfan by degrading it to less toxic endosulfan diol, lactone or ether, thus, confirming our analysis.[31]

For bacterial culture, pH is an important condition to maintain proper growth. Growth of isolated strains at neutral pH was in accordance with similar work by Satyapal *et al.*[2] Low growth of isolated strains at pH 4 might be due to alteration in metabolic redox processes and respiration kinetics.[36]

Inhibition of bacterial growth is known to be dose dependent for arsenic. Taran *et al.*[37] observed that the maximum tolerance for arsenic by bacterial species ranges between 90 -225 µg/mL. The IC_{50} of isolates in present study was also achieved at low arsenic concentration. However, growth pattern of isolates changed with increasing arsenic concentration. Resistance up to certain concentration of arsenic can be attributed to presence of aox operon in these isolates which causes detoxification of arsenic.[38] It has been reported that bacteria can tolerate up to 800 µg/ml of endosulfan.[39] But, in present study, some isolates show tolerance range up to as high as 6 mg/ml. Such tolerance can be attributed to the presence of detoxifying mechanism similar to *Mycobacterium* (ESD) strain and *Pseudomonas* species KS-29. The strain of *Mycobacterium* (ESD) degrades the endosulfan in order to access the sulphur as a nutrient source.[40] *Mycobacterium* strain possess *esd* gene encoding a monooxygenase enzyme capable of degrading both α- and β-ES with specificity towards β-isomer which might be the case here.[41] Also, *Bacillus* can sometimes utilize endosulfan as sulphur source for growth.[42] However, further analysis is required to confirm the presence of arsenic or endosulfan resistance genes and enzymes in isolated strains.

Microbial consortium having potential of degrading specific pollutant can be used in bioremediation.[43] In present investigation, different strains presented varying degree of tolerance towards tested compounds. Thus, a combination of As and Es was tested for consortium preparation using strains that showed tolerance at individual levels. Co-tolerance against arsenic and endosulfan suggests co-activation of individual mechanisms of arsenic and endosulfan resistance. This consortium could be used for decontamination of water bodies, polluted with arsenic and endosulfan after some necessary field trials.

ACKNOWLEDGEMENT

Authors want to thank ICMR, New Delhi for financial support. Author Azmi khan is thankful to University grant Commission, India for providing financial support as fellowship.

REFERENCES

1. Briggs D. Environmental pollution and the global burden of disease. Br Med Bull, 2003; 68(1): 1–24.
2. Satyapal G, Srivastava A, Kumar N. Possible bioremediation of arsenic toxicity by isolating indigenous bacteria from the middle Gangetic plain of Bihar, India. Biotechnol Rep., 2018; 17: 117-125
3. Chakraborti D, Mukherjee SC, Pati S, Sengupta MK, Rahman MM, Chowdhury UK et al. Arsenic groundwater contamination in middle ganga plain, Bihar, India: a future danger? Environ Health Perspect, 2003; 111(9): 1194.
4. Mutiyar PK, Mittal AK. Status of organochlorine pesticides in Ganga river basin: anthropogenic or glacial? Drink. Water Eng Sci Discuss, 2012; 5: 1–30.
5. Das D, Samanta G, Mandal BK et al. Arsenic in groundwater in six districts of West Bengal, India. Environ Geochem Health, 1996; 18: 5–15.
6. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environ Health Perspect, 1992; 97: 259-67.
7. Dhuldhaj U, Sharma NK, Singh S. Microbial removal of arsenic: An overview. In: Bioremediation of Pollutants, IK International Publishing House, New Delhi, 2012; 112-127.
8. Satyapal GK, Rani S, Kumar M, Kumar N. Potential role of arsenic resistant bacteria in - bioremediation: current status and future prospects. J Microb Biochem Technol, 2016; 8(3): 256–258.
9. Agnihotri NP, Gajbhiye VT, Kumar M et al. Organochlorine insecticide residues in Ganga river water near Farrukhabad, India. Environ Monit Assess, 1994; 30**:** 105–112
10. Shivaramaiah H, Sánchez-Bayo F, Al-rifai J, Kennedy IR. The fate of endosulfan in water. J Environ Sci Health B, 2005; 40: 711-20.
11. Alva S, Damodar D, D'Souza A, D'Souza UJ. Endosulfan induced early pathological changes in vital organs of rat: a biochemical approach. Indian J Pharmacol, 2012; 44(4): 512-515.
12. Barragán-Huerta BE, Costa-Pérez C, Peralta-Cruz J, Barrera-Cortés J, Esparza-García F, Rodríguez-Vázquez R. Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. *Int Biodeter Biodegradation*, 2017; 239-244.

13. R Martens, Degradation of [8,9,-14C] endosulfan by soil microorganisms. Appl Environ Microbiol, 1976; 31(6): 853-858
14. Awasthi N, Singh AK, Jain RK, Khangarot BS, Kumar A, Degradation and detoxification of endosulfan isomers by a defined co-culture of two *Bacillus* strains. Appl Microbiol Biotechnol, 2003; 62: 279–283.
15. Bhagobaty RK, Joshi SR, Malik A. Microbial Degradation of Organophosphorous Pesticide: Chlorpyrifos (Mini-Review). Internet J Microbiol, 2007; 4(1): 1-12.
16. Jesitha K, Nimisha KM, Manjusha CM et al. Biodegradation of Endosulfan by *Pseudomonas fluorescens*. Environ Process, 2015; 2**:** 225–240.
17. Sutherland TD, Horne I, Lacey MJ, Harcourt RL, Russel RJ, Oakeshott JG. Enrichment of an endosulfan-degrading mixed bacterial culture. Appl Environ Microbiol, 2000; 66: 2822–2828.
18. Nath A, Behera S, Singh K. Isolation of bacteria with arsenite mobilization capacity from culture and water sample from arsenic area of Bihar, India. ISOR J Environ Sci Toxicol Food Technol, 2017; 11(12): 1-8.
19. Bartholomew JW, Mittwer T. The Gram stain. Bacteriol Rev., 1952; 16(1): 1–29.
20. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol, 2008; 35: 1547-1549.
21. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol, 1993; 10: 512-526.
22. Rhine ED, Phelps CD, Young LY. Anaerobic arsenite oxidation by novel denitrifying isolates. Environ Microbiol, 2006; 8(5): 899–908.
23. Handley KM, Héry M, Lloyd JR. Redox cycling of arsenic by the hydrothermal marine bacterium *Marinobacter santoriniensis*. Environ Microbiol, 2009; 11(6): 1601–1611.
24. Osborne TH, Jamieson HE, Hudson-Edwards KA et al. Microbial oxidation of arsenite in a subarctic environment: diversity of arsenite oxidase genes and identification of a psychrotolerant arsenite oxidizer. BMC Microbiol, 2010; 10: 205.
25. Shivaramaiah HM, Kennedy IR. Biodegradation of endosulfan by a soil bacterium. J Environ Sci Health B, 2006; 41(6): 895–905.
26. Kumar SM, Raju NS. Biotransformation of chlorpyrifos and endosulfan by bacteria and fungi. Appl Microbiol Biotechnol, 2017; 101(15): 5961-5971.
27. Boyd ES, Barkay T. The mercury resistance operon: from an origin in a geothermal environment to an efficient detoxification machine. Front microbiol, 2012; 3: 349.

28. Khan A, Gupta A, Singh P, Mishra AK, Ranjan RK, Srivastava A. Siderophore-assisted cadmium hyperaccumulation in Bacillus subtilis. Int Microbiol, 2019; 23(2): 277-286.
29. Silver S, Keach D. Energy-dependent arsenate efflux: the mechanism of plasmid-mediated resistance. Proc Nat Acad Sci USA, 1982; 79(20): 6114-6118.
30. Carlin A, Shi W, Dey S, Rosen BP. The ars operon of *Escherichia coli* confers arsenical and antimonial resistance. J Bacteriol, 1995; 177(4): 981-986.
31. Kafilzadeh F, Ebrahimnezhad M, Tahery Y. Isolation and identification of endosulfan-degrading bacteria and evaluation of their bioremediation in kor river, Iran. Osong public health res perspect, 2015; 6(1): 39–46.
32. Girgis SH, Alison KH, Tavazoie S. Genetic Architecture of Intrinsic Antibiotic Susceptibility. Plos ONE, 2009; 4(5): e5629.
33. Sabir S, Anjum AA, Ijaz T, Ali A, Khan MURM, Nawaz M. Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. Pak J med sci., 2014; 30(2): 389–392.
34. Zhang M, Wan K, Zeng J, Lin W, Ye C, Yu X. Co-selection and stability of bacterial antibiotic resistance by arsenic pollution accidents in source water. Environ Int, 2020; 135: 105351.
35. Dey U, Chatterjee S, Mondal NK, Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation, Biotechnol Rep., 2016; 10: 1-7.
36. Jin Q, Kirk MF. pH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective. Front Environ Sci., 2018; 6: 21.
37. Taran M, Fateh R, Rezaei S, Gholi MK. Isolation of arsenic accumulating bacteria from garbage leachates for possible application in bioremediation. Iran J microbiol, 2019; 11(1): 60-66.
38. Turnbull PCB. Bacillus. In: Baron S, eds. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston, 1996.
39. Tripathi D, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis JMF. Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotechnol, 2007; 25(4): 158-165.
40. Shafiani S, Malik A. Tolerance of pesticides and antibiotic resistance in bacteria isolated from waste water irrigated soil. World J Microbiol Biotechnol, 2003; 19: 897–901.
41. Sutherland TD, Horne I, Russell RJ, Oakeshott JG. Gene cloning and molecular characterization of a two-enzyme system catalyzing the oxidative detoxification of β-endosulfan. Appl Environ Microbiol, 2002; 68: 6237–6245.

42. Kumar A, Bhoot N, Soni I, John PJ. Isolation and characterization of a *Bacillus subtilis* strain that degrades endosulfan and endosulfan sulfate. 3 Biotech, 2014; 4: 467-475.
43. Sutherland TD, Weir KM, Lacey MJ, Horne I, Russell RJ, Oakeshott JG. Enrichment of a microbial culture capable of degrading endosulfate, the toxic metabolite of endosulfan. J Appl Microbiol, 2002; 92: 541–548.