Arsenic- resistant plant growth-promoting bacterial strains in the contaminated soils of West Bengal, India

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ABSTRACT: *Soil arsenic contamination has become a serious concern worldwide. In our present study, arsenic (As^{V} , As^{III}) resistant plant growth promoting bacteria Burkholderia capacia (CSAR-1,accession no. MK747363), and Bacillus flexus (GSAR-6, accession no. MK747247) were isolated from the arsenic contaminated agricultural soils and evaluated for the plant growth promoting activities and arsenic bioremediation. Assessment of different plant growth promoting charecteristics, such as phosphate solubilization, nodulation, indole-3-acetic acid production,siderophore production revealed that the both strains Burkholderia capacia (CSAR-1), Bacillus flexus (GSAR-6), served as an efficient plant growth promoter under laboratory conditions.*

Key Words: *Arsenic resistant bacteria, Plant growth promoting bacteria, Bioremidiation.*

I. Introduction

Arsenic pollution is a serious environmental problem throughout the world. (Meharg, 2004; Pandey, 2006). Arsenic is a toxic metal or metalloid caused serious health hazards were described as "the greatest mass poisoning in human history" by WHO (World Health Organization, 2001) and it is recognized as "Class-1 human carcinogen" by the USEPA (United States Environmental Protection Agency) as a global concern (Ng *et al*., 2003). In periodic table arsenic (As) belongs to group 15, period 4, P block element which has an electronic configuration as (Ar) $3d^{10}$ $4s^{2}$ $4p^{3}$. Arsenic can occur in four oxidation states (-3, 0, +3 and +5) in nature. Arsenate (V) and Arsenite (III) are the most predominant form exits in environment .Arsenite (III) is more toxic and mobile than arsenate (III) (Rhine et al., 2006). Acute and chronic Arsenic, both poisoning to human has raised a great concerns, especially in highly contaminated zones in different parts of India especially in West Bengal (Majumder *et al*., 2013). The soaking of agricultural croplands by arsenic-contaminated water causes its accumulation in soil (Abedin *et al*., 2002) and thus leading to arsenic uptake by plants.

Use of arsenic contaminated groundwater continuously for crop irrigations significantly increased the arsenic level in the soil in some part of West Bengal (Sanyal and Dhillon, 2005).

Therefore, there is a potential risk of As contamination in different crops leading to food chain contamination resulted in the deleterious human health problem (Williams *et al*., 2009; Li *et al*., 2018). Due to excessive use of arsenical pesticides, application of fertilizers, dust from the burning of fossil fuels, medicines, disposal of industrial, metallurgy and animal wastes (Nordstrom, 2002) arsenic accumulation in the soil exceeds natural sources by 3:1. In other hand, elevated concentration of As in soil is absorbed by the roots and transported to different plant parts leading to impaired metabolism (Zhao *et al*., 2013), reduced growth and decreased crop production (John *et al*., 2009).

Heavy metals are basically non degradable and only change may occur in their nuclear structure . Although several detoxification methods such as stabilization, containment, and solidification etc. have been proposed for the remediation of onsite polluted soils, appropriate controls are needed by all of these methods (Chang *et al*., 2011).

Microbes copes with arsenic in different ways (act as precipitation, chelation, compartmentalization, extrusion and biochemical transformation) and play an important role in the geocycling of As in environment (Mukhopadhyay *et al*., 2002) as they can either oxidize As(III) to As(V) or reduce As(V) to

As(III). The more widespread chromosomal and plasmid Ars system takes up As(V) into the cell and reduces it to As(III) and exudes the As(III) through an ATP-dependent efflux pump.

The universal distribution of 16S ribosomal DNA (Weisburg et al., 1991) and its species-specific variable regions (Kataoka et al., 1997; McCabe et al., 1999) have been utilized for the identification of most frequent As resistant bacteria belonging to several genera like *Exiguobacterium, Aeromonas, Bacillus, Pseudomonas, Escherichia, Acinetobacter, Planococcus, Achromobacter, Alcaligene, Arthrobacter, Ochrobactrum* (Anderson and Cook, 2004; Jackson et al., 2005; Banerjee et al., 2011; Sarkar et al., 2012).

Some of the bacterial isolates obtained from such arsenic -contaminated soils were found to have additional advantage of plant-growth-promoting (PGP) traits such as indole-3-acetic acid production (IAA) production, P solubilization, nodulation (Burd *et al*., 1998; Sheng and Xia 2006; Zaidi *et al*., 2006; Jiang *et al*., 2011). Thus they can solve two purposes one is bioremediation of arsenic and other is plant growth promotion.

So the aim of our present study was to investigate Plant Growth Promoting bacteria (PGP microbes) in As-resistant bacterial strains isolated from the agricultural soil of the West Bengal .

II. Materials and methods

Experimental zone:

Chakdaha block of Nadia district, West Bengal, India was chosen for the present study which was previously reported for arsenic contamination (Sarkar *et al*., 2012). The Study area is situated at New Alluvial Zone of West Bengal at 23º5.3'N latitude and 83º5.3'E longitude.

soil sampling and Physico-chemical analysis:

Soil samples (0–10 cm depth) were collected from six different sites of arsenic contaminated agricultural lentil field of the Chakdaha in Nadia districts West Bengal. Where As concentrations in the ground water exceed WHO-defined permissible limits (Sarkar *et al*., 2012). Individual soil cores (2 cm diameter,10 cm depth) were taken with a sample probe from four different places within each As-enriched site. Each sample was divided into two subsamples (for soil physiochemical and microbial analyses).

The physiochemical properties of the soil such as pH (Jackson, 1967), total As (Majumder et al., 2013) and NaHCO3-extractable As (Johnson and Barnard, 1979) levels were determined using standard protocols.

Isolation and Enrichment of As-resistant bacteria

Soil from arsenic contaminated agricultural field were collected aseptically and suspensed with 1 ml of sterial distilled water. This 1 ml of suspension were resuspended in LB (Luria-Bertani) liquid medium supplemented with 1 mM As(III) and As(V) and incubated at 37 °C for 48 h (Kinegam *et al*., 2008). The cultures were enriched by transferring 2 mL of culture into the same medium. This process was repeated twice, and the final enriched culture was used for the isolation of arsenic tolarent bacteria. Approximately 0.1 mL of enriched culture was plated on LB Agar medium amended with As and ten distinct colonies were selected for isolation of bacteria.

Determination resistant to As(III) and As(V)

Minimum inhibitory concentration (MIC) has been defined as the lowest concentration of As(III) or As(V) added that completely inhibits bacterial growth (Daims *et al*., 1999) in culture medium. In our present study, As(III) and As(V) resistance in isolated bacterial strains was evaluated using MIC tests. Aliquots of 1.0 mL of overnight bacterial cultures were incubated in 99.0 mL of LB medium supplemented with either As(III)as NaAsO2 (1–50 mM) or As(V) as Na2HAsO4·7H2O (1–500 mM) and incubated at 30◦C with shaking (170 rev/min) for48 h. The optical density of the cultures, as a measure of bacterial growth, was detected at a wavelength of 600 nm (OD600) byan UV–vis spectrophotometer (BIO-RAD Smart SpecTM3000, USA); a blank with only the culture medium without bacteria was also analyzed. These Experiments were carried out in triplicate.

Oxidation and reduction of arsenic by the isolates

Arsenic-oxidising bacterial strains were screened using the standard silver nitrate (AgNO3) method (Lett *et al*., 2001). The bacteria were cultured on solidified CDM (chemically defined medium) that was supplemented with 1 mM As(III) for 48 h at 30 °C. The plates were inoculated with 0.1 M AgNO3 solutions and the colour changes of the respective colonies were recorded. AgNO3 reacts with As(III), producing a bright yellow silver orthoarsenite (Ag3AsO3) precipitate, whereas the brownish silver ortho arsenate (Ag3AsO4) precipitate is produced at by the reaction of AgNO3 with As(V). Arsenic-oxidising ability was confirmed using a modified microplate technique (Simeonova *et al*., 2004). Each assay was performed in triplicate, and the bacterial isolates that produced brownish coloured precipitates were confirmed as As-oxidising strains.

Arsenite oxidase assay

The bacterial strains exhibiting As-oxidising activity were grown in MMS in the presence of 30 mM of As(III). Cells at late log-phase were harvested by centrifugation at 10,500 ×g rpm for 3 min. The collected cells were washed with 50 mM Tris–HCl buffer (pH 8.0) and suspended in 2 mL buffer containing 0.5 mM phenyl methyl sulfonyl fluoride (PMSF) and 1mg/ml lysozyme. Cell suspensions were incubated for 2 h with occasional stirring. The cells were lysed using sonication and centrifuged at 10,500 × g at 4◦C for 30 min. Cell debris was removed by centrifugation at 10,500 × g rpm for 30 min (Bachate *et al*., 2012). Protein concentrations in the supernatants were determined by Bradford assay (Bradford, 1976) using bovine serum albumin (Sigma) as a standard. The arsenite oxidase assay was performed using a method previously described by Anderson *et al*. (1992)

Nodule formation

The bacterial culture were inoculated in sterilized soil in a pot. The seeds of lentil also inoculated in this pot. An control bacterial culture were also inoculated soil with lentil seeds were also kept as a positive control.Finally we selected the three bacterial isolates which were able to produce the nodule and the further characteristics of those are experimented. A control strain (SAR-1, *E.coli*) were selected for negative control.

Bioremediation test

These three arsenic resistant nodule forming bacteria bacteria were inoculated in the LB liquid medium prepared in 100 ppm arsenate and arsenite solution and incubated at 30ºC. After 24 h, 48 h and 72 h of incubation the solution were centrifuged at 10,000 rpm for 10 min to separate the bacterial biomass from the culture media and the arsenic concentration of the medium were also measured (Ellis *et al*., 2003).

Quantitative determination of PGP traits

The As-resistant isolates were tested for their ability to solubilize phosphate, and IAA-like molecules.

Screening for phosphate-solubilization

The ability of As-resistant bacterial isolates to solubilize phosphate was tested by growing the strains in modified Pikovskaya's medium (Gihring and Banfield., 2001) (suplimented with 1mg/kg As^{V}) with 0.5% of tri calcium phosphate (TCP) at 30◦Cfor 5 days at 170 rev/min in order to reach a stationary phase (determined by measuring absorbance at 600 nm). The cultures supernatants were collected by centrifugation at 6,500 × g for10 min. The soluble phosphate in the culture supernatant was estimated according to the method of Zaidi *et al*.,(Glick and Penrose., 1998.).

Screening for IAA-like molecules production

For the analysis of the production of indole acetic acid (IAA), Selected three strains were grown in LB broth supplemented with L-tryptophan (0.5 µg L−1) and in presence of 1 mg L−1 As(V) .The cells were harvested after 5 days of incubation at 30 °C by centrifugation at 5000 rpm for 5 min. An inoculum of 2 mL of the supernatant was mixed with 100 µl of 10 mM orthophosphoric acid and 4 ml of Salkowski's reagent (2% of 0.5 M FeCl3 in 35% HClO4 (S. Das *et al*., 2014). The absorbance of the pink color developed after 30min was recorded at 530 nm and concentration of IAA was determined from the standard curve of pure IAA as a standard following regression analysis (S. Das *et al*., 2014).

Siderophore production

Siderophore production was also quantitatively determined. Production.The ability of As-resistant isolates to produce siderophores was detected by using the Chrome Azural S (CAS) method of Schwynand Neilands (1987).

Identification and phylogenetic analysis of arsenic resistant plant growth promoting strains

Bacterial isolates showing considerable As tolerance plant growth promoting capacity were selected for molecular identification by 16S rDNA sequence analysis. Total genomic DNA was extracted (Sambrook 2001), and PCR amplification of 16S rRNA gene with forward primer

27F 5′-AGA GTT TGA TCM TGG CTC AG-3′ and the reverse primer 1492R 5′-GGY TAC CTT GTT ACG ACT-3′ (Chroumous Biotech Private Limited, India) were carried out.

The reaction mixture composed of 1X PCR buffer, 0.2 mM dNTPs, 10 pmole of each primer, 60 ng of DNA template, 2 units of Taq DNA polymerase, and sterile deionized water to a final volume of 25 µL. Before amplification cycle, DNA was denaturation at 94 °C. Then, a final extension step (10 min at 72 °C) was performed. The cycling parameters consisted of 35 cycles initiated through denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min, and extension at 72 °C for 5 min. The PCR products were purified and held at 4 °C until verification through agarose gel electrophoresis (1 %).

The amplified and gel-eluted PCR fragments of the rDNA were sequenced in ABI 3100 Genetic Analyzer with primers 27F. Sequencing reaction was performed by using the Big Dye terminator cycle sequencing Kit

V3.1 (Applied Biosystems, Foster City, USA) following the manufacturer's protocol. The partial 16S rDNA sequences of the isolated strains were compared with the available in the Gen Bank database by BLASTN algorithm to identify the selected bacterial sequences.

III. Results

Analyasis of soil samples.

The physico-chemical characteristics of the experimental agricultural soil samples are described in Table-1. In this study, soil samples were collected from lentil field of Chakdaha block in Nadia district West Bengal , where As-contaminated ground water has been used for irrigation for years. Total and extractable As concentrations of the soils varied from 12 to 17.2 mg kg−1 and from 1.3 to 2.3 mg kg−1 respectively. The experimental soils were neutral in reaction (pH 6.9 to 7.6),. Total microbial count (6.3–6.4 og CFU) , As resistant microbial count (3.5–3.6 log CFU) of the experimental soils did not exhibit significant differences.

Table 1:- . Physiochemical and microbiological properties of experimental soils

Soil parameters	**Site-1**	**Site-2**	**Site-3**	**Site-4**	**Site-5**	**Site-6**
Soil PH	7.5 ^{ab}	6.9^{b}	7.4^{ab}	7.6 ^{a}	7.7^{a}	7.2 ^{ab}
Available arsenic	2.2^{b}	1.5 ^{ab}	1.3 ^{ab}	1.8 ^{ab}	1.7 ^{ab}	2.3^{a}
Total arsenic (mg/kg)	17.1 ^{a}	17.2 ^{a}	16.7 ^{ab}	13.2 ^{ab}	12 ^{b}	17.0 ^{a}
Total bacterial population (log CFU)	6.4 ^{a}	6.3 ^{a}	6.3 ^{a}	6.4 ^{a}	6.3 ^{a}	6.4 ^{a}
Total bacterial population (log CFU)	6.4 ^{a}	6.3 ^{a}	6.3 ^{a}	6.4 ^{a}	6.3 ^{a}	6.4 ^{a}

Values with different alphabets are significantly different from each other according to post hoc Tukey's HSD(honest significant difference) ($P < 0.05$). Each value is a mean of three replicates.

Isolation of Arsenic-resistant strains

In this study, ten isolates were screened out as potential arsenic tolarent bacterial strains. Out of ten, The all strains showing high tolerance to Arsenic (minimum inhibitory concentration ranging from 31 Mm to 56 mM and 475 mM to 350 Mm for As(III) and As(V), respectively)). Three were selected based on their nodule formation and selected for the evaluation of PGP traits (Table 2**)**.

Screening of arsenic oxidising bacteria

Three bacterial isolates were screened as As-oxidising bacteria using a standard silver nitrate (AgNO3) method, which produced a brown precipitate as a reaction product between As(V) and AgNO3 after 3 days of incubation. Arsenite transforming ability of the isolates was confirmed by microplate screening assay. The COSAR-1,CSAR-2,GSAR-6,CSAR-1,GSAR-3,CSAR-5,GOSAR-2 strains emerged as the most efficient strains to oxidise As(III) to As(V) in the experimental media (data not shown).

Arsenite oxidase assay

The specific As(III)-oxidase activity in the cellular lysates of the As(III)-oxidizing bacterial isolates varied from 1.74 to 6.68 nM min−1mg−1of protein. COSAR-1 exhibited the highest As(III)-oxidase enzyme activity (6.68 nM min−1mg−1protein).CSAR-2,CSAR-1,CSAR-5,COSAR-2,GSAR-6 are also shown a significant lavel of arsenic oxidizing capacity. It was noted that isolates having higher As(III) oxidation capacity also exhibited higher levels of As(III)-oxidase enzyme activity (Table 2).

Table 2:- Arsenic tolerance and As(III) oxidase enzyme activity by selected bacterial isolates

Treatment	MIC of As^{V} (mM)	MIC of As^{III} (mM)	Enzyme activity (Nmmin−1mg−1protein),
COSAR-1	475.33± 57.79^{a}	56.03 ± 0.06^{a}	6.68 ± 0.16^{a}
CSAR-2	456.77± 57.65^{a}	47.93 ± 5.74^{b}	6.26 ± 0.02^{b}
GSAR-6	382.17 ± 0.15^{bc}	31.57 ± 0.06^{e}	4.80 ± 0.10^{e}
CSAR-1	408.13 ± 0.06^{b}	43.57 ± 0.06^{c}	5.68 ± 0.16^{c}
GSAR-3	350.13 ± 0.15^{d}	37.03 ± 0.06^{f}	2.70 ± 0.10^{g}
NSAR-1	386.67 ± 0.06^{bc}	38.90 ± 0.00^{de}	1.78 ± 0.03^{d}
CAR-11	350.20 ± 0.10^{c}	36.77 ± 0.06^{e}	1.74 ± 0.03^{f}
CSAR-5	390.20 ± 0.10^{bc}	41.27 ± 0.06^{cd}	4.83 ± 0.12^{d}
COSAR-1	355.17 ± 0.12^{c}	36.13 ± 0.06^{e}	1.50 ± 0.20^{f}
COSAR-2	408.37 ± 0.38^{b}	43.67 ± 0.21^{c}	5.78 ± 0.10^{c}

SEm (±)	**14.904**	**1.050**	**0.068**
CD (P=0.05)	**43.967**	**3.097**	**0.201**

Values with different alphabets are significantly different from each other according to post hoc Tukey's HSD(honest significant difference) ($P < 0.05$). Each value is a mean of three replicates.

Nodule formation

We selected the Six strains to check the nodule formation of lentil plant. Among of them only three (CSAR-1,GSAR-6,NSAR-1) were able to produce nodule.A control strain (SAR-1,*E.coli*) were selected for negative control (Table:-6).

Bioremediation of arsenate and arsenite by the isolates

The isolate resistant against high concentration of both As(III) and As(V). It also exhibit the potentiality to reduce arsenic concentration from nutrient broth media containing 100 ppm of arsenate and arsenite (Table:-3,4). It removed 29 % arsenite. However, removal of arsenate of CSAR-1 (29%) was observed from the media after 72 h of incubation (**Table 3, Table 4**).

Table 3:- Percentage of arsenate (As^V) removal

Name of the isolates	**24 hr**	**48 hr**	**72 hr**
	Percentage of arsenate(As^V) removal		
control	0.00 ± 0.00	0.20 ± 0.034	0.10 ± 0.036
CSAR-1	23.00 ± 1.106	27.00 ± 0.844	29.00 ± 1.008
GSAR-6	7.00 ± 0.198	7.50 ± 0.045	7.80 ± 0.042
NSAR-1	4.00 ± 0.077	5.00 ± 0.079	5.00 ± 0.081
	Hours (H)	**Isolates (I)**	**H × I**
SEm (±)	**0.150**	**0.173**	**0.300**
CD (P=0.05)	**0.440**	**0.507**	**0.880**

value are mean of three replicates ± standard deviation

Table 4:-

Percentage of arsenite (As^{III}) removal

Name of the isolates	24 hr	48 hr	72 hr
	Percentage of arsenite (As^{III}) removal		
control	0.00 ± 0.00	0.20 ± 0.04	0.10 ± 0.041
CSAR-1	20.00 ± 0.615	27.00 ± 0.981	29.0 ± 0.755
GSAR-6	9.00 ± 0.686	9.50 ± 0.047	9.70 ± 0.886
NSAR-1	5.00 ± 0.043	5.50 ± 0.934	6.00 ± 0.877
	Hours (H)	**Isolates (I)**	**H × I**
SEm (±)	**0.190**	**0.220**	**0.380**
CD (P=0.05)	**0.557**	**0.645**	**1.115**

value are mean of three replicates ± standard deviation

PGP traits of the isolates under Arsenic stress.

The selected three As-resistant nodule forming isolates were assayed for one or more characteristics considered to be important for PGP activity under arsenate stress condition (Table 5, Table 6) and stress free condition . From these three, two isolates are able to carry the phsosphate solubilization, indole acetic acid production,siderophore production and nodulation. Maximum phosphate solubilization was observed with the isolate CSAR-1 in stress condition and stress free condition. CSAR-1 solubilize highest amount of phosphate under stress free and stress condition also. The phosphate solubilization was remain the same in arsenate stress condition also. They(selected three strains) all produce nodule and under the stress free condition and stress condition. But among of them only two CSAR-1,GSAR-6 produce siderophores under stress condition. (Table 6).

So the two strains CSAR-1 and GSAR-6 are the best strain which possess the both character arsenic resistant ability and plant growth promoting treaits.

Table 5:- PGP character under arsenate stress free condition and arsenate stress condition

Isolate	PGP Charecteristics (with out arsenic)			PGP Charecteristics (with arsenic stress condition)		
	IAA production (μml^{-1})	Soluble Phosphate (gL^{-1})		IAA production (μml^{-1})	Soluble Phosphate (gL^{-1})	
CSAR-1	2.11 ± 0.108	2.51 ± 0.305		2.10 ± 0.108	2.50 ± 0.305	
GSAR-6	0.89 ± 0.095	1.00 ± 0.295		0.89 ± 0.095	1.00 ± 0.295	
NSAR-1	2.50 ± 0.282	1.89 ± 0.025		2.50 ± 0.282	1.89 ± 0.025	
Control	0.00 ± 0.000	0.12 ± 0.058		0.00 ± 0.000	0.12 ± 0.058	
	MIC (M)	**Isolates (I)**	**M × I**	**MIC (M)**	**Isolates (I)**	**M × I**
SEm (±)	**0.047**	**0.067**	**0.094**	**0.047**	**0.067**	**0.094**
CD (P=0.05)	**0.143**	**0.203**	**0.285**	**0.143**	**0.203**	**0.285**

value are mean of three replicates ± standard deviation

Table 6:- PGP character under arsenate stress free condition and arsenate stress condition

Isolate	PGP Charecteristics (with out arsenic)		PGP Charecteristics (with arsenic stress condition)	
	Nodulation	Siderophore production	Nodulation	Siderophore production
CSAR-1	+	+	+	+
GSAR -6	+	+	+	+
NSAR-1	+	+	+	-
Control (SAR-1)	-	-	+	-

Phenotypic and biochemical characterization of the isolates

Both the isolates were milky white in color, have a smooth surface, and flat colonies. The colony of isolate CSAR-1 was round in shape and GSAR-6 had also same shape and size. Colony of the both strains are concentric with transparent center and opaque periphery. But CSAR-1 isolates were Gram-negative,rod shaped and GSAR-1 were gram positive, rod shaped strain. Different biochemical properties of these isolates are presented in **Table:-6**

Table:-6 biochemical characterization of the isolates

Name of the isolates	Colour	Shape	Size(μm)	Gram's nature
CSAR-1	milky white	rod	2	negative
GSAR-1	milky white	rod	2	positive

Identification and phylogenetic analysis of arsenic resistant plant growth promoting strains

Based on phenotypic, 16S rRNA and biochemical studies the isolates CSAR-1 and GSAR-6 were identified as *Burkholderia cepacia* and *Bacillus flexus* respectively(Table:-7).

Nucleotide accession number:-The gene bank accession number of CSAR-1 and GSAR-1 are MK747363 and MK747247(Table:-7)..

Table:-7 Identifying bacteria and their gene bank accession numbers

Name of the isolates	Identifying bacteria	Accession no.	Taxon	Closest cultured relative in GenBank (% similarity
CSAR-1	*Burkholderia cepacia*	MK747363	*Firmicutes*	98.72%
GSAR-1	*Bacillus flexus*	MK747247	*Betaproteobacteria*	100%

IV. *Discussion:-*

From the experimental data It can be shown that plant growth promoting bacteria also found arsenic contaminated soil. When a microbial community survives under a selective stress condition like high concentration of arsenic for a very long period, they must develop some mechanism to detoxify it and overcome the restriction for growth (Huang,. *et al* 2010). It is reported that sometimes microbes gain protection against toxic agents through the formation of microbial biofilms (Mah and Toole., 2001). Hence it may be possible that through the formation of biofilms the isolated bacterial strains can resist the toxicity of arsenic. The arsenic resistance mechanism of bacteria can be plasmid associated (Tsai., *et al* 1997) or by *ars*

operon, containing the genes *ars*RBC when it can resist higher concentrations. (Rosen., 2002.). In 2004, Anderson and Cook (Anderson and Cook., 2004) have isolated seventeen bacterial strains including Bacillus licheniformis, Bacillus polimyxa, etc. which were able to resist up to 100 ppm arsenic..The isolate CSAR-1can remove 29% arsenite and arsenate, from the media after 72 h of incubation. Arsenic resistant microbes can reduce the arsenic concentration from the media by developing a number of detoxifying mechanisms including metal reduction, metal efflux, bacterial cell membrane binding, adsorption of heavy metals on to cell surface and complexation of the metal with exopolysacharides (Anyanwu and Ugwu 2010). TheAs-resistant microorganisms might have a selective advantage with regard to survival under As-stressed conditions. In addition to phosphate-solubilization by As-resistant bacteria has also been reported to play an important role in plant growth and survival of bacteria under As-stressed conditions (Srivastava *et al.,* 2013). The ability of As-resistant bacteria to produce IAA has been reported to induce higher shoot length and higher numbers of leaves and total chlorophyll content in inoculated plants (Srivastava *et al.,* 2013). Plant growth-promoting bacteria synthesize IAA utilizing tryptophan excreted by roots in the rhizosphere.

V. *Conclusion:-*

The isolated bacterial isolates *Burkholderia capacia* (CSAR-1, MK747363)and *Bacillus flexus* (GSAR-1, MK747247) are of particular interest because they offer great potential in regard to novel crop production strategies due to their resistance to As and the presence of several potential PGP traits. The isolate CSAR-1 (Burkholderia capacia) which is comparatively more resistant to As, possesses greater As(III)-oxidizing activity and exhibits higher phosphate-solubilization, production of siderophore, IAA-like molecules to other As-resistant isolates. It could therefore be a better choice for potential application in As bioremediation as well as for sustainable agronomic production programs in arsenic contaminated soils.

VI. *References:-*

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