Characterization of Arsenic-Resistant Bacteria and their ars Genotype for Metal Bioremediation

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ABSTRACT

The estuarine and coastal waters of Goa situated on the West coast of India, are known to be highly contaminated with arsenic. The rivers Mandovi and Zuari flowing through the iron and manganese mining areas are daily used for the transportation of over 300 barges of iron-ore to the harbour. However, studies related to the identification of *ars* genotype in arsenic-resistant bacteria inhabiting these estuarine waters are not available. This has prompted us to isolate diverse arsenic-resistant bacteria from the Mandovi and Zuari estuarine systems and characterize the *ars* genotype among the isolates. Additionally, we report the cloning of arsenic detoxification genes (*arsB* and *arsC*) from the arsenic-resistant isolates. These genes, in particular *arsC* gene could be successfully utilized in designing potential arsenic remediation strategies.

A total of 50 heterotrophic bacteria were isolated through serial dilutions on agar plates from marine and estuarine (Mandovi and Zuari) waters of Goa, India that were prone to heavy metal pollution. These bacterial isolates were screened to determine their tolerance to arsenate and arsenite. PCR analysis was performed using genomic DNA and plasmid DNA of arsenic resistant bacteria with primers specific for *ars* genes (*arsA*, *arsB* and *arsC*). With the primers used in the present study, no amplification of the *ars* genes was observed with genomic DNA as the template, but plasmid DNA resulted in the amplification. Hence, it is evident that *ars* genes are anchored to plasmid rather than to chromosomal DNA. Out of the 36 arsenic-resistant bacteria, only 17 harboured the *ars* genes on the plasmid DNA. Phylogenetic analysis based on 16S rRNA sequence analysis showed that these isolates belonged to the phyla *γ-Proteobacteria*, *Actinobacteria* and *Firmicutes*. The *ars* genotype characterization in 36 bacterial isolates (resistant to 100 mM of sodium arsenate) revealed that only 17 isolates harboured the *arsA*, *B* and *C* genes on the plasmid DNA. The *arsA*, *B* and *C* genes were individually detected using PCR in 16, 9 and 13 bacterial isolates respectively indicating the prevalence of *arsA* > *arsC* > *arsB* in the isolates. Molecular identification of the 17 isolates bearing the *ars* genotype was carried using 16S rDNA sequencing. A 409 bp fragment of *arsC* gene coding for arsenate reductase and a 1300 bp full length *arsB* gene encoding arsenite efflux pump were isolated from the genera *Halomonas* and *Acinetobacter*. While full length (1300 bp) *arsB* gene was isolated from the strain *Halomonas* species SPK23 (Acc.No.EU024298), a putative *arsC* comprising 409 bp was obtained from *Acinetobacter* species VKPM 14 (Acc.No. EF570879) and *Halomonas* species SPK 23 (Acc.No. EF583905). The full length *arsB* gene isolated from the strain *Halomonas* sp. SPK23, showed 98% homology with *E.coli* chromosomal *arsB* gene, 82% with plasmid R773 arsenical resistance operon genes, 81% with *Acidiphilium multivorum* plasmid pKW301 and *Klebsiella oxytoca* plasmid pMH12, and 79% with *Enterobacter cloacae arsB* gene. It showed 97% homology with partial *cds* of *Pseudomonas putida* strain RS-17 *arsB* gene, 96% with *Acinetobacter* sp. VKPM45 plasmid arsenite/antimonite transporter gene, and 95% with *Vibrio cholerae* arsenite/antimonite transporter gene. Phylogenetic analysis of *arsB* and *C* genes indicated their close genetic relationship with plasmid borne *ars* genes of *E.coli* and arsenate reductase of plant origin. The putative arsenate reductase gene isolated from *Acinetobacter* species complemented arsenate resistance in *E.coli* WC3110 and JM109 validating its function. Hence, this study dealing with isolation of native arsenic resistant bacteria and characterization of their *ars* genes will be useful for development of efficient arsenic bioremediation strategies. The naturally occurring arsenic-resistant isolates are more environmentally acceptable and safe for detoxification of arsenic. Hence, isolation of such arsenic-resistant species has considerable ecological advantage. However, characterization of arsenic metabolizing genes is required for their successful exploitation in *in situ* arsenic bioremediation. The arsenite/antimonite efflux pump and arsenate reductase encoding genes isolated in the present study could be used to engineer either bacteria or plants which can in turn, help in remediation of arsenic polluted sites. Also, arsenate reductase gene could be co-expressed along with genes encoding metallothionein proteins for developing an efficient bioremediation/phytoremediation technology. Further, we report the isolation of a 1459 bp full-length cDNA sequence encoding a phytochelatin synthase (PCS) from subabul (*Leucaena leucocephala*), designated as *LlPCS*1. The SDS PAGE analysis resulted in a recombinant protein of molecular weight 66 kDa. The deduced 485 amino acid sequence of *LlPCS*1contains three Cys-Cys motifs and 13 single Cys residues, but only 4 of them (Cys-56, Cys-90/91, and Cys-109) in the N-terminal half of the LlPCS1 protein are conserved unlike in other known PCS polypeptides. The relative level of heavy metal tolerance imparted by *AtPCS1* (isolated from *Arabidopsis thaliana*) and *LlPCS1* to *E.coli* was analyzed. When bacterial cells expressing *LlPCS*1 were grown in the presence of heavy metals such as arsenite, arsenate and cadmium, cellular metal

contents increased as measured by inductively coupled plasma spectrometer by 175, 14.7 and 50-folds respectively compared to their corresponding controls. Bacterial cells expressing *LlPCS1* exhibited 1.49, 1.54 and 1.23-folds enhanced accumulation of AsO_2, AsO_4 and $CdCl_2$ than the cells harboring *AtPCS*1, implying that *LlPCS*1confered enhanced heavy metal tolerance compared to *AtPCS*1. Hence, cloning of the *PCS*1 gene from subabul provides information that will help further our understanding of the genetic basis underlying toxic metal tolerance in this species and its probable use for bioremediation.

INTRODUCTION

Arsenic (As), a major environmental pollutant is released into the environment on global scale as a result of geogenic, anthropogenic and biogenic activities and on local scale as a result of industrialization. Arsenic toxicity has become a global concern owing to the ever-increasing contamination of water, soil and crops in many regions of the world (Matilda et al. 2010). High concentrations of arsenic in ground water have been reported from several countries – Argentina, Austria, Bangladesh, Northern China, Chile, Ghana, Greece, Hungary, India, Japan, Mexico, Mangolia, Nepal, Poland, Taiwan and some parts of United States of America. The situation of arsenic toxicity in India is alarming with reports of severe health problems among the populations of states - West Bengal, Bihar, Assam, Chhattisgarh, Goa. The arsenic content was reported to be in the range of 0.11–0.78 ppb in water, 5.84–9.72 in Mandovi and 5.07–10.20 ppb in Zuari sediment samples (Nair et al. 2003; Nagvenkar and Ramaiah 2010). Arsenic toxicity inactivates - the enzymes involved in DNA replication, DNA repair, nucleic acid and phospholipid synthesis and also inhibits energy flow (Hughes 2002). Inorganic arsenic species cause skin lesions, lung, kidney and liver cancer and also damage to the nervous system (Ng et al. 2003). These incidents serve as a reminder of the need for efficient removal of excess of As from polluted soils and aquatic systems. In the present scenario, *in situ* bioremediation appears to be most efficient, cost effective, environmentally friendly and safe mechanism to detoxify the arsenic. This involves the successful exploitation of native arsenic resistant bacteria and their genes associated with arsenic detoxification. However, studies related to the identification of *ars* genotype in arsenic-resistant bacteria inhabiting these estuarine waters are not available. This has prompted us to isolate diverse arsenic-resistant bacteria from the Mandovi and Zuari estuarine systems and characterize the *ars* genotype among the isolates.

Leucaena leucocephala (subabul), a leguminous tree with high biomass, has metal tolerance and survival ability in metal-contaminated areas. Recent studies by Shweta and Rai (2011) reported that *L. leucocephala* with adaptive potential for toxic metals like Zn and Cd, led to their removal by 20% and 30%. Hence, it has been suggested to be used for the remediation of metal contaminated sites and their fertility restoration by improving microbial functionalities and nitrogen pool. Phytochelatins (PCs) play a crucial role in the detoxification and homeostasis of heavy metals and metalloids in plants. Phytochelatins are synthesized post translationally in the presence of heavy metal ions by PC synthase (PCS). However, in many plant species metal (loid) tolerance has not been well correlated with the accumulation of PCs. However, molecular mechanisms of metal tolerance and phytochelatin accumulation in *L. leucocephala* are not completely understood. So, there is a need to characterize *PCS* genes in metal tolerant leguminous trees like *L. leucocephala*. In the present study, we report cloning and characterization of phytochelatin synthase gene (*LlPCS1*) from *L. leucocephala*. The *LlPCS1* was expressed in *E. coli* to enhance tolerance to different toxic metals. This study can help us in better understanding of the toxic metal tolerance ability of *L. leucocephala* for its successful usage in phytoremediation technologies. The *in-situ* application of technologies using plants for remediation of toxic metals is economical rather than other *ex-situ* non-biological remediation techniques.

MATERIALS AND METHODS

Screening for arsenic-resistant bacteria and determination of maximum tolerance concentrations

In the present study, arsenic-resistant isolates were isolated by enrichment isolation technique from estuarine water samples collected from various sites of Mandovi and Zuari estuarine systems. They were initially characterized in terms of colony morphology and basic microscopic observations and the maximum tolerance concentration of arsenic was evaluated to determine the resistance of the bacterial isolates. The concentration of the metal in the medium that does not inhibit the growth of the isolate was defined as the maximum tolerance concentration (MTC). The isolates were grown in Luria-Bertani (LB) broth incorporated individually with different concentrations of sodium arsenate (1–650 mM) and sodium arsenite (100 µM–10 mM) at 28 ± 2°C for 7 days. The optical density of the cultures, as a measure of microbial growth, was detected at a wavelength of 600 nm by an UV–visible spectrophotometer.

Characterization of *ars* genotype in arsenic-resistant bacteria and their molecular identification

Characterization of *ars* genotype among the arsenic-resistant isolates was carried out by PCR analysis according to Saltikov and Olson (2002). Both plasmid and genomic DNA of the bacterial isolates which exhibited tolerance were used individually as templates in PCR. Few of the respective amplicons of *arsA*, *arsB* and *arsC* were cloned into pTZ57 R/T cloning vector (MBI-

Fermentas, USA) and were later sequenced using genetic analysis system model CEQ-800 (Beckman, Coulter Inc., Fullerton, CA, USA). The sequences of *arsA*, *B* and *C* genes were confirmed based on homology analysis by using NCBI BLAST software (Altschul et al. 1990). The gene sequences were submitted to Genbank and accession numbers were obtained. For molecular identification, genomic DNA extracted from the arsenic-resistant bacterial isolates was used for PCR with universal 16S rRNA gene primers according to Marchesi et al. (1998). The 16S rRNA gene amplicons obtained from different arsenic resistant bacterial isolates were sequenced to identify and confirm the genera of these isolates.

Isolation and phylogenetic analysis of *arsB* and *arsC* genes

Plasmid DNA of the bacterial isolates containing *arsB* and *arsC* genes were used as template for PCR. The primer sets for the isolation of full length *arsB* gene— *arsB* F1: 5¹-CCCTGTCAGGAGGTTTTATGTTA-3¹, *arsB* R1: 5¹-GCAGGCTGGGTTATGATAAATAG-3¹ and *arsB* F2: 5¹-AGGTTTTATGTTACTGGCAGGAG-3¹, *arsB* R2: 5¹-TCATTACAATGTGACAGAGAGACG-3¹, *arsC* gene— *arsC* F1: 5¹-GCTACGTCTCTCTCTGTCACATTGTA-3¹ and *arsC* R1: 5¹-CTGCTTCATCAACGACTTTTTC-3¹ were designed using Primer 3 software (Rozen and Skaletsky 2000). The PCR protocol for each primer set consisted of an initial denaturation step (94°C for 5 min) followed by 30–35 cycles of 94°C for 1 min, 50-52°C for 30 s, 72°C for 1 min. A final extension was carried out for 5 min at 72°C. The *arsB* and *arsC* amplicons were eluted and sequenced. The gene sequence was assembled with Chromas Lite software (version 2.01) and BLAST analysis was conducted to identify the most similar sequence. Phylogenetic trees were constructed with the TreeView software (Page 1996).

Arsenic resistance assay of *arsC* deletion and *ars* sensitive *E. coli* strains

In order to perform the arsenic resistance assay, *arsC* gene was cloned into pUC18a vector. The pUC18a—*arsC* plasmid construct was then individually transferred into *E. coli arsC* deletion strain WC3110 and *E. coli ars* sensitive strain JM109. Arsenate resistance assays were carried out in low phosphate medium (LPM) as described by Shi et al. (1999).

Isolation, characterization and expression of phytochelatin synthase gene from *Leucaena leucocephala*

Homologous sequences of *PCS* genes from various plant sources collected from the Genbank database and the primers (PCS forward: 5'-CGCATGGCTATGGCGAGTTTATATCGGC -3'; PCS reverse: 5'- ATCTCGCTGCTCCTGCCTATTAGCAC-3') were designed using the PRIMER 3 software tool. Total RNA was extracted from stem tissues of *L. leucocephala* seedlings. RT-PCR was performed and the cDNA was used as the template to perform gradient PCR with the following program: 1 min at 95°C (1 cycle), followed by 30 s at 94°C, 30 s at 55 – 65°C, and 90 s at 72°C (35 cycles), followed by a final 10 min 72°C extension step. The PCR products were cloned into pTZ 57R/T vector and the clones were sequenced. The deduced protein sequence of LlPCS1 was searched using "Structure" navigator in Entrez search in GenBank. Further, the LlPCS1 protein was modeled using Geno3D tool (Combet et al. 2002) and metal binding sites were predicted using Metal Detector tool (Passerini et al. 2011). Later, *LlPCS1* gene was expressed in *E. coli* by cloning it into pET32a expression vector under the control of T7 promoter and the construct was transferred into *E. coli* strain BL21. Both control and the recombinant *E. coli* cells were grown at 37°C in LB broth supplemented with ampicillin (100 mg/ml). Expression of *LlPCS1* was induced by the addition of 0.5 mM IPTG when the optical density (600 nm) reached 0.4 and cultures were further incubated for 3 h. Five milliliters of cultured cells were pelleted, suspended in 300 µl of a 0.1 M Tris-HCl buffer (pH 8.0). The cell lysate was centrifuged at 10,000 g for 10 min at 4°C and the buffer soluble proteins were subjected to SDS-PAGE analysis and were visualized using Coomassie blue. To estimate the metal tolerance ability conferred by *LlPCS1* gene to *E. coli*, both control and recombinant *E. coli* cells with *LlPCS1* gene construct were induced with IPTG and exposed to the toxic metals (arsenate, arsenite, copper, cadmium, cobalt, mercury, zinc and tin respectively). Later, the optical density of *E. coli* cells was estimated by spectrophotometric analysis at 600 nm. The tolerance was considered to be directly proportional to the optical density of the *E. coli* cells. For quantitative determination of the metal content, both control and recombinant *E. coli* cells were harvested after induction and exposure to toxic metals. Metal content was determined using an ICP-OES device (Varian) and standard solutions were supplied by Merck.

RESULTS AND DISCUSSION

Screening for arsenic-resistant bacteria and determination of maximum tolerance concentrations (MTC)

In the present study, 44 arsenic-resistant bacteria were isolated. The MTC range of arsenate and arsenite were 10-650 and 0.05-10 mM respectively. The arsenate and arsenite resistance levels of the bacteria isolated in the present study were found high and these were comparatively equivalent to the arsenic resistance levels of the isolates reported by Escalante et al. (2009). The isolates were grouped into sensitive, moderately tolerant, tolerant and highly tolerant based on the MTC values of the arsenic (Table 1). For sodium arsenate, two isolates were sensitive, six moderately tolerant (100 mM), 26 tolerant (100-250 mM) and 10 were highly tolerant (500-650 mM). For sodium arsenite, two isolates were sensitive, 13 moderately tolerant (1 mM), 21 tolerant (1-5 mM) and eight were highly tolerant (10 mM).

Characterization of *ars* genotype in arsenic-resistant bacteria and their molecular identification

The identification and characterization of *ars* genes may serve as potential molecular biomarkers to monitor the level of arsenic pollution in that environment (Stocker et al. 2003). To our knowledge, this is the first report on the identification and characterization of *ars* genotype in the environmental isolates inhabiting the arsenic contaminated Mandovi and Zuari estuarine surface waters of Goa, India. Out of 36 arsenic resistant bacteria screened, only 17 harboured the *ars* genes on the plasmid DNA. The genotype *ars-ABC* was found in seven and *arsAC* in five resistant bacteria each. Genotype *arsA* was found in three; while *arsBC* and *arsAB* genotypes were present in one resistant bacterium each. Based on the PCR analysis of *ars* genes, more *arsA*-like sequences were identified in the arsenic-resistant bacteria inhabiting these waters. Earlier studies by Saltikov and Olson (2002) revealed the presence of *arsA*-like sequences in isolates obtained from arsenic enriched waters and predominance of *arsBC* genotype in environments with low arsenic concentrations. Thus, this study helps to correlate the occurrence of the *ars* genotype with the level of arsenic pollution in these waters. In the present study, we identified arsenic-resistant isolates of the genera *Brevibacterium* (isolates SK1, SPK05, SPK14, KM14), *Acinetobacter* (isolates SK2, VKPM45, VKM05, VKPM14), *Providencia* (isolates VKPM23 and SP09), *Pseudomonas* (isolates VKM014, SK3 and SP9), *Halomonas* (isolates SPK23 and SP45), *Vibrio* (isolates SK4), *Exiguobacterium* (isolate KM05) and *Staphylococcus* (isolate SK4) representing 3 major phyla- γ-*Proteobacteria* (*Pseudomonas*, *Providencia*, *Acinetobacter*, *Halomonas* and *Vibrio* species), *Firmicutes* (*Exiguobacterium* and *Staphylococcus* species) and *Actinobacteria* (*Brevibacterium* species) from these sites (Table 1). Recently, Nagvenkar and Ramaiah (2010) reported the isolation of arsenite-resistant bacterial species belonging to *Pseudomonas* and *Acinetobacter* from estuarine waters of Mandovi and Zuari. To our knowledge, the present study is the first report on the isolation of bacterial isolates belonging to *Brevibacterium*, *Providencia* and *Halomonas* genera from these waters and about their arsenic-resistance. Among the arsenic-resistant isolates, bacteria tolerant to lethal concentrations of arsenate were predominant than those to arsenite. These results are in agreement with earlier studies by Jackson et al. (2005) who also reported less prevalence of arsenite tolerant species among the environmental isolates. The distribution pattern of the *ars* genotype among the arsenic resistant bacteria is shown in Table 1.

Isolation, phylogenetic analysis of *arsB* and *arsC* genes and confirmation of arsenic resistance in the *E. coli arsC* deletion strain WC3110 and *E. coli ars* sensitive strain JM109

PCR analysis for full length *arsB* gene resulted in an amplicon of 1300 bp only from the *Halomonas* species (isolate SPK23). The deduced amino acid sequence of the amplicon resulted in 433 amino acids with stop codon. Hence, this amplicon was confirmed as full length *arsB* gene sequence and was deposited in the NCBI Genbank with accession number EU024298. Phylogenetic analysis of arsenite efflux pump of *Halomonas* species indicated its close genetic relationship with plasmid borne arsenite efflux pumps of *Enterobacter*, *Acidiphilium*, *Klebsiella*, *Escherichia*, *Salmonella* and *Yersinia* species. Similarly, the isolates which showed the amplification of partial sequence of *arsC* gene were selected for the isolation of full length clone. PCR resulted in an amplicon of 409 bp only from the *Acinetobacter* and *Halomonas* species (isolates VKPM14 and SPK23). Phylogenetic analysis also revealed that these *arsC* gene sequences were grouped along with the arsenate reductases of *Salmonella typhimurium*, *Enterobacter cloacae*, *Acidiphilium multivorum*, *Klebsiella oxytoca*, chromosomal and R773, R46 plasmid borne arsenate reductases of *E. coli* and also arsenate reductases of plants like *Pteris* and *Pityrogramma*. The putative arsenate reductase encoding genes isolated in the present study from isolates VKPM14 and SPK23 were deposited in the NCBI Genbank with accession numbers EF570879 and EF583905, respectively. The *arsC* gene isolated from VKPM14 could complement arsenic resistance in WC3110, *E. coli* arsenate reductase deletion strain and JM109, *E. coli* arsenate sensitive strain (Figure 1). Hence, these bacteria with *ars* genes may play an important role in controlling the mobility of arsenic in environment and its subsequent detoxification by complexation. Therefore, these arsenic-resistant bacteria with *ars* genes could be utilized for *in situ* bioremediation of polluted aquifers in Mandovi-Zuari estuarine network.

Isolation, characterization and expression of phytochelatin synthase gene from *Leucaena leucocephala*

PCR amplification of cDNA template derived from stem tissues of *L. leucocephala* resulted in amplicon of length 1459 bp (GU205821). The deduced amino acid sequence of LlPCS1 was compared with other known PCS polypeptides of legumes and it revealed a high degree of similarity in the N-terminal but extreme variability in the C-terminal domain. Electrostatic and structural studies deciphered that LlPCS1 protein has possible binding sites (77-Lys, 81-Arg, 46 Tyr, 84-Asp and 86-Ser) for the substrate glutathione. Metal detector analysis revealed that *LlPCS*1 has cadmium, zinc and copper binding sites (183-Arg, 184-Phe, 185-Lys, 70-Ser and 64-Val), iron binding sites (142-Glu, 143-Asn, 146-Met, 183-Arg), and manganese binding sites (Asp - 201, Ser - 202, Ile - 203, Asp - 204 and Gln - 205). SDS-PAGE analysis clearly showed the expression of phytochelatin synthase protein band with an expected molecular weight of 66 kDa along with His-tag (Figure 2). The growth assays showed that under inducing conditions (+ IPTG), *E. coli* cells carrying the pET32a-*LlPCS1* construct grew better than the control cells (pET32a vector) on exposure to cadmium, cobalt, copper, arsenite, arsenate and mercury as evident from

the optical density. The recombinant *E. coli* (BL21) cells expressing *LlPCS1* gene when grown on metal-enriched LB broth sequestered significantly higher concentrations of arsenite (199 folds), arsenate (14 folds), copper (50 folds), mercury and cobalt (10 folds of each respectively) cadmium (5 folds), zinc, tin (3 folds of each respectively),when compared to their corresponding control *E.coli* cells containing pET32a vector. The contribution of His-tag to metal tolerance and accumulation were nullified by using the *E. coli* cells bearing the pET32a vector as the control. Hence, it is concluded that expression of *LlPCS1* mediates heavy metal tolerance in bacterial cells. These results are in agreement with the earlier reports by Sauge-Merle et al. (2003) and others. Their studies clearly indicated significant intracellular sequestration of metals like cadmium, copper and arsenic in the recombinant bacteria harboring *PCS* construct compared to that of control strains. Hence, in the present scenario, isolation and characterization of *LlPCS1* helps in better understanding of *PCS* role in the metal accumulation.

Conclusions

The naturally occurring arsenic-resistant isolates are more environmentally acceptable and safe for detoxification of arsenic. Hence, isolation of such arsenic-resistant species has considerable ecological advantage. However, characterization of arsenic metabolizing genes is required for their successful exploitation in *in situ* arsenic bioremediation. The arsenite/antimonite efflux pump and arsenate reductase encoding genes isolated in the present study could be used to engineer either bacteria or plants which can in turn, help in remediation of arsenic polluted sites. Further, arsenate reductase gene could be co-expressed along with genes encoding phytochelatin synthase for developing an efficient metal bioremediation.

ACKNOWLEDGEMENTS

Sri Lakshmi Sunita would like to thank Council of Scientific and Industrial Research-University Grants Commission, New Delhi for providing JRF and SRF fellowships and to the UGC, New Delhi for awarding Dr. D.S. Kothari post-doctoral fellowship. We gratefully acknowledge Dr. Rita Mukhopadhyay, and Prof. B. P. Rosen, Wayne State University, Detroit, USA, for gifting *E. coli arsC* deletion strain WC3110. PBK is thankful to the University Grants Commission for the award of UGC-BSR fellowship, and the Department of Biotechnology, New Delhi for the financial assistance in the form of DBT-OU-ISLARE program.

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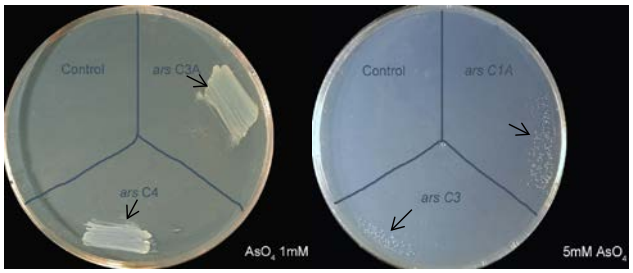

Fig. 1. Complementation of *arsC* gene in WC3110 in LPM agar. Growth of *E. coli* WC 3110 containing pUC18-*arsC* construct (ars C3A, arsC4, arsC1A and arsC3) in the presence of arsenate. Arsenate resistance assays were performed in low-phosphate medium (LPM agar).

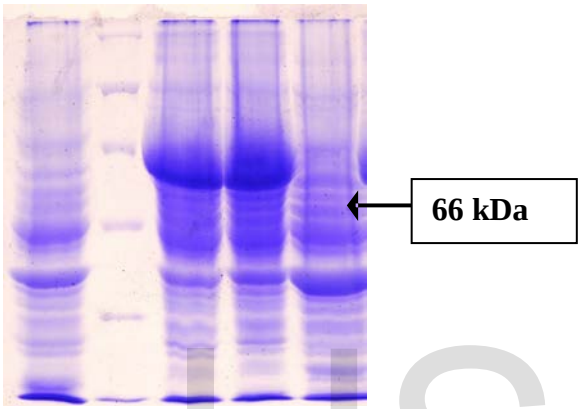

Fig. 2. SDS-PAGE analysis of *LlPCS* gene
Lane 1 and 4: Protein from control *E.coli* (with pET32a)
Lane 2: Protein from recombinant *E.coli* (pET32a-*AtPCS*1)
Lane 3: Protein from recombinant *E.coli* (with pET32a-*LlPCS*1)
Lane M: Molecular weight ladder

Table 1 Tolerance levels to arsenic, *ars* genotype characterization and identification of isolates from Mandovi and Zuari estuaries of Goa

Isolate	Maximum tolerance concentration		*ars* genotype	Isolate accession number	Putative identification based on 16S rRNA
	As(V) mM	As(III) mM			
U01	100	0.05	*ars*⁻	NO	ND
SP9	100	0.1	*arsA*	GQ861585	*Pseudomonas sp.*
VKPM23	250	10	*arsABC*	EF434412	*Providencia sp.*
SP02	100	5	*arsBC*	NO	ND
U02	10	1	NC	NO	ND
U03	10	1	NC	NO	ND
U04	10	1	NC	NO	ND
VKPM45	100	10	*arsABC*	EF434413	*Acinetobacter sp.*
SP09	100	10	*arsABC*	GQ476786	*Providencia sp.*
U05	100	2	*ars*⁻	NO	ND
SPK14	600	4	*arsA*	EF529441	*Brevibacterium sp.*
SPK23	600	10	*arsABC*	EF529442	*Halomonas sp.*
VKM05	500	0	*arsAC*	EF108316	*Acinetobacter sp.*
U06	100	2	*ars*⁻	NO	ND
SPK05	650	10	*arsAB*	EF529440	*Brevibacterium sp.*
U07	100	0	*ars*⁻	NO	ND
U08	100	1	*ars*⁻	NO	ND
U09	50	0.1	NC	NO	ND
SK4	650	0.5	*arsAC*	EU401867	*Vibrio sp.*
U10	10	0.1	NC	NO	ND
VKPM14	100	1	*arsABC*	EF434411	*Acinetobacter sp.*
U11	100	1	*ars*⁻	NO	ND
U12	100	1	*ars*⁻	NO	ND
U13	100	0.1	*ars*⁻	NO	ND
U14	0	0.1	NC	NO	ND
U15	10	0.1	NC	NO	ND
KM14	500	5	*arsABC*	EF570876	*Brevibacterium sp.*
U16	100	10	*ars*⁻	NO	ND
U17	100	10	*ars*⁻	NO	ND
KM05	500	0.5	*arsABC*	EF570875	*Exiguobacterium sp.*
U18	100	1	*ars*⁻	NO	ND
U19	100	0.1	*ars*⁻	NO	ND
SPK4	500	0.1	*ars*⁻	EU350141	*Staphylococcus sp.*
U20	100	2	*ars*⁻	NO	ND
SP45	500	5	*arsA*	GQ861584	*Halomonas sp.*
U21	100	0.1	*ars*⁻	NO	ND
U22	100	5	*ars*⁻	NO	ND
SK1	500	1	*arsAC*	EU401864	*Brevibacterium sp.*
U23	100	5	*ars*⁻	NO	ND
SK3	100	10	*arsAC*	EU401866	*Pseudomonas sp.*
U24	0	0.1	NC	NO	ND
U25	100	0.1	*ars*⁻	NO	ND
SK2	100	5	*arsAC*	EU401865	*Acinetobacter sp.*
U26	100	5	*ars*⁻	NO	ND