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Arsenite biotransformation and bioaccumulation by *Klebsiella pneumoniae* strain SSSW7 possessing arsenite oxidase (*aioA*) gene

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Abstract Arsenite oxidizing *Klebsiella pneumoniae* strain SSSW7 isolated from shipyard waste Goa, India showed a minimum inhibitory concentration of 21 mM in mineral salts medium. The strain possessed a small supercoiled plasmid and PCR amplification of arsenite oxidase gene (*aioA*) was observed on plasmid as well as chromosomal DNA. It was confirmed that arsenite oxidase enzyme was a periplasmic protein with a 47% increase in arsenite oxidase activity at 1 mM sodium arsenite. Scanning electron microscopy coupled with electron dispersive X-ray spectroscopic (SEM–EDS) analysis of 15 mM arsenite exposed cells revealed long chains of cells with no surface adsorption of arsenic. Transmission electron microscopy combined with electron dispersive X-ray spectroscopic (TEM-EDS) analysis demonstrated plasma membrane disruption, cytoplasmic condensation and periplasmic accumulation of arsenic. The bacterial strain oxidized 10 mM of highly toxic arsenite to less toxic arsenate after 24 h of incubation. Fourier transformed infrared (FTIR) spectroscopy confirmed the interaction of arsenite with functional groups present on the bacterial cell surface. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of 5 mM arsenite exposed cells demonstrated over-expression of 87 kDa and 14 kDa proteins of two subunits aioA and aioB of heterodimer arsenite oxidase enzyme as compared to control cells. Therefore, this bacterial strain might be employed as a potential candidate for bioremediation of arsenite contaminated environmental sites.

Keywords Arsenite · *AioA* gene · Bioremediation · Biotransformation

Introduction

Extensive anthropogenic activities such as mining, combustion of fossil fuels, arsenical pesticides, herbicides, paints, ceramic, glass and pharmaceutical industries have resulted in the release of highly toxic metalloid arsenic in the environment which poses serious threat to all living organisms (Welch et al. 2000; Smedley and Kinniburgh 2002; Cheng et al. 2009; Stolz et al. 2010). Although WHO (1993) has set the permissible limit of 10 µg/l arsenic in drinking

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water, many countries still exceed this permissible limit (Chowdhury et al. 2000; Anawar et al. 2002; Mitra et al. 2002; Smedley and Kinniburgh 2002; Mukherjee et al. 2006).

Arsenic usually exists in four oxidation states such as − 3 (arsine), 0 (elemental arsenic), + 3 (arsenite) and + 5 (arsenate) with arsenite and arsenate being the most common forms of arsenic in the environment (Oremland and Stolz 2005). Arsenite is 100 times more toxic than arsenate and acts by interacting with thiol groups of proteins and enzymes inhibiting their functions (Hughes 2002; Rosen 2002; Rai et al. 2011). Arsenic also causes mutagenic and genotoxic effects on humans (Mandal and Suzuki 2002; Chen et al. 2002).

The ubiquity of arsenic in the environment has led microorganisms to develop various transformation mechanisms such as arsenite oxidation, arsenate reduction and arsenite methylation governed by *aio*, *arr, arsC* and *arsM* genes respectively which are located either on chromosomal or plasmid DNA (Silver and Phung 1996; Páez-Espino et al. 2009; Arsene-Ploetze et al. 2010; Bahar et al. 2013; Goswami et al. 2015). These mechanisms are commonly employed by various microorganisms to carry out detoxification or energy generation for their cellular growth and metabolism. The oxidation of highly toxic arsenite to less toxic arsenate encoded by arsenite oxidase enzyme is a key step of detoxification mechanism by microorganisms (Qin et al. 2006; Andreoni et al. 2012; Rauschenbach et al. 2012).

In recent years various bacterial strains capable of arsenite oxidation by arsenite oxidase (*aioA*/*aoxB*) gene have been reported in the genomes of *Acinetobacter junii*, *Acinetobacter baumannii, Geobacillus stearothermophilus, Thiomonas* sp. 3As, *Herminiimonas arsenicoxydans* and *Pseudomonas stutzeri* strain GIST-BDan 2 (Muller et al. 2007; Arsene-Ploetze et al. 2010; Chang et al. 2010; Majumder et al. 2013). In case of *Acinetobacter calcoaceticus* and *Brevibacillus* sp. KUMAs2 the *aioA* gene was present only on plasmid DNA whereas in *Acinetobacter soli,* the *aoxB* gene was located on genomic as well as plasmid DNA (Mallick et al. 2014; Goswami et al. 2015). The *aoxAB/aioAB* genes encode an arsenite inducible periplasmic protein which catalyzes the oxidation of highly toxic arsenite to less toxic arsenate (Silver and Phung 2005; Branco et al. 2009). It consists of two subunits, a small iron-sulfur cluster containing subunit *aoxA/aioB* and a large molybdopterin containing catalytic subunit *aoxB/aioA* (Silver and Phung 2005; Oremland et al. 2009). The *aoxB/aioA* gene acts as a genetic marker for arsenite oxidation (Hamamura et al., 2008; Quemeneur et al. 2008). Two families of arsenite transporters (ArsB and Acr3p) are known in bacteria (Rosen 1999) and Acr3p is divided into two subsets, Acr3(1)p and Acr3(2)p (Achour et al. 2007). Although these transporters have similar sizes and functions, they differ in mechanisms, as well as have different metalloid specificity. ArsB confers resistance to arsenite and antimonite, however Acr3p is highly specific to arsenite (Rosen 1999).

Keeping in view the potential of arsenic toxicity in humans and other life forms it is imperative to remove arsenic present in the environment. The traditional methods to remove arsenic from contaminated environmental sites are expensive, time-consuming and hazardous (Mahimairaja et al. 2005). Therefore, bioremediation of arsenic holds a great potential since it is an eco-friendly method involving microorganisms.

In the present investigation, we characterized one potential arsenite oxidizing bacterial strain from shipyard waste of Goa, India with reference to presence of arsenite oxidase gene, enzyme activity, arsenite uptake, morphological changes, presence of arsenic deposits, protein expression induced by arsenite stress using PCR, SEM, TEM, EDS and SDS-PAGE analysis.

Materials and methods

Isolation of arsenite oxidizing bacteria

Environmental samples were collected from shipyard waste, from Bicholim, Goa, India, in sterile zip-lock bags. Appropriate dilutions of the soil samples were made in 0.85% saline and plated on mineral salt medium (MSM) agar (Mahtani and Mavinkurve 1979) supplemented with 10 mM of sodium (meta) arsenite along with 0.2% glucose as a carbon source. Plates were incubated at 28 °C for 24 h and morphologically distinct bacterial colonies were selected for further studies.

Determination of minimum inhibitory concentration (MIC) of arsenite

Bacterial isolates were spot inoculated on MSM agar plates amended with increasing concentrations of 0–46 mM sodium arsenite along with 0.2% glucose. The plates were checked for visible bacterial colonies after incubation at 28 °C for 24–48 h. The bacterial strains showing highest MIC values were selected for determining MIC in MSM broth. Selected bacterial strains were inoculated in MSM broth supplemented with different concentration of arsenite (0–25 mM) and flasks were incubated at 28 °C, 150 rpm for 24 h. Growth was monitored by recording the absorbance at 600 nm using Biospectrometer (Eppendorf, Germany). The lowest concentration of arsenite which completely inhibited bacterial growth was considered as its MIC value.

Growth behavior of the selected bacterial isolate in presence of sodium arsenite

The selected bacterial strain was inoculated in MSM broth amended with different concentrations of sodium arsenite viz. 5 mM, 10 mM, 15 mM, 20 mM and 21 mM, whereas flask without sodium arsenite was maintained throughout the experiment as control. The flasks were incubated at 28 °C, 150 rpm for 24–30 h and absorbance at 600 nm was recorded after every 2 h using Biospectrometer (Eppendorf, Germany).

Identification of arsenite oxidizing bacterial isolate

The identification of selected bacterial strain was performed by extracting its genomic DNA using Dneasy Blood and Tissue Kit (Qiagen, Hilden, Germany) followed by amplification of 16S rRNA gene using universal eubacterial primers: 27F and 1495R (Studholme et al. 1999). The PCR was carried out using Nexus Gradient Mastercycler (Eppendorf, Germany) and the resulting PCR product was analyzed on 1% agarose gel. The PCR product was purified using PCR clean-up kit (Promega, USA) and sequenced. The nucleotide sequence obtained was subjected to BLAST (tblastn) search analysis using National Center for Biotechnology Information (NCBI) database. The sequence was submitted to GenBank (accession number: MG430351) and its taxonomical relatedness to closely associated genera was determined using the neighbor-joining method with MEGA 7 package (Kumar et al. 2016).

Plasmid profile

The plasmid DNA of the selected bacterial strain was extracted using Gen Elute Plasmid Miniprep kit (Sigma-Aldrich, USA) and was analyzed using 0.8% agarose gel electrophoresis. After electrophoresis gel was visualized under G:BOX gel documentation system (Syngene, UK).

PCR amplification of arsenite oxidase (*aioA*) and transporter (ACR3) genes

The large molybdopterin containing catalytic subunit (aioA) and one of the arsenite transporter (ACR3) genes were PCR amplified with gene-specific primers (Supplementary Table S1) using chromosomal and plasmid DNA separately as templates. The thermal cycler program comprised of an initial denaturation of 5 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. The PCR products were analyzed on 1% agarose gel and visualized under G: BOX gel documentation system (Syngene, UK).

Arsenite oxidase enzyme assay

Preparation of cell-free extract

The bacterial cells were grown in MSM broth in presence of 15 mM sodium arsenite. Late log phase cells were harvested by centrifugation at 8000 rpm at 4 °C for 10 min. The cell pellet was washed thrice with washing buffer (20 mM Tris–HCl, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.6 mM EDTA with pH 8.4 and 0.9% NaCl with pH 8.4) and the pellet was resuspended in 10 ml 20 mM Tris–HCl buffer (pH 8.0) containing 0.6 mM PMSF and 0.6 mM EDTA. The cell suspension was incubated with 1 mg ml^{-1} lysozyme at 28 °C for 2 h with occasional stirring. Magnesium sulfate (20 mM), magnesium acetate (100 mM), DNase (100 µg) and RNase (500 µg) (Bangalore GeNei) were added to the cell suspension and incubated at 28 °C for 30 min. The cell suspension was sonicated thrice with 2 min bursts

and 10 min cool-down intervals followed by incubation at 60 °C for 1 min in water bath. Subsequently, the suspension was cooled on ice, followed by centrifugation at 8000 rpm for 10 min and the pH of the clear supernatant was adjusted to 8.4 with 2 M NaOH (Prasad et al. 2009).

Preparation of periplasmic and spheroplast fractions

Bacterial cells grown in MSM broth were harvested by centrifugation at 8000 rpm for 10 min and cell pellets suspended in 20 mM Tris–HCl buffer, 0.1 mM PMSF, 10 mM EDTA pH 8.4 along with 20% sucrose. The outer membrane was lysed using lysozyme (0.5 mg ml^{-1}) at 28 °C for 40 min followed by centrifugation at 8000 rpm for 10 min. The supernatant was collected in a fresh centrifuge tube and cell pellet containing spheroplast was washed twice in buffer containing 20 mM Tris–HC1, 0.1 mM PMSF, 10 mM EDTA (pH 8.4), 20% sucrose and assayed for arsenite oxidase activity.

Enzyme assay

The arsenite oxidase enzyme activity was determined in cell free extract, periplasmic and spheroplast fractions following standard method (Anderson et al. 1992). The enzyme sample was mixed with 1 ml of assay buffer containing 60 µM 2,6-dichlorophenol-indophenol (DCIP), 200 µM sodium arsenite and 50 mM morpholino ethelene diol sulfonic acid (MES) buffer (pH 6.0). The change in absorbance due to reduction of DCIP per minute was monitored at 600 nm for 5 min using Biospectrometer (Eppendorf, Germany). The specific activity of the enzyme was expressed as µmol of DCIP reduced min^{-1} mg^{-1} of protein. Similarly, the effect of arsenite (0.5 and 1 mM) on periplasmic protein was also studied. The protein concentration in the supernatants was determined by Folin Lowry method (Lowry et al. 1951) using bovine serum albumin (Himedia, Mumbai, India) as standard.

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopic (SEM-EDS) analysis

The bacterial isolate was grown in MSM broth supplemented with 15 mM sodium arsenite (test) and without sodium arsenite (control). The flasks were incubated at 28 °C, 150 rpm for 8–20 h and bacterial cells in exponential growth phase (8 and 20 h) were harvested from control and test samples by centrifugation at 8000 rpm, 4 °C for 10 min (Eppendorf, Germany). The pellet obtained was washed thrice with 0.1 M phosphate buffer saline (PBS) with pH 7.4. The washed bacterial cells were evenly spread on a clean grease-free cover slip and fixed overnight using 2.5% glutaraldehyde. After incubation, cells were washed with PBS and were subjected to ethanol gradient of 30%, 50%, 70%, 90% and 100% by incubating for 10 min at each concentration. The samples were analyzed by SEM–EDS (Carl-Ziess, Germany).

Transmission electron microscopy coupled with energy dispersive X-ray spectroscopic (TEM-EDS) analysis

The TEM analysis of the bacterial strain was carried out to evaluate intracellular morphological changes and metal uptake by the cells. Cells grown with 15 mM sodium arsenite were harvested in the exponential growth phase (8 and 20 h) by centrifugation at 8000 rpm for 10 min followed by washing with 0.1 M sodium phosphate buffer (pH 7.2). The pellets obtained were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde prepared in 0.1 M sodium phosphate buffer (pH 7.2) for 2–3 h at 4 °C. The fixed bacterial cells were further incubated for 1 h in 1% OsO_4 and propylene oxide followed by graded series of dehydration in ethanol. The samples were then embedded in Epon 812 resins and ultra-thin sectioning (60 nm) was performed. This was followed by examining the samples using transmission electron microscope (TEM-JEOL 2100F, Germany) which were further analyzed for elemental content by EDS. A control without arsenite exposure under similar conditions was also maintained.

Arsenic transformation assay

The arsenite oxidizing ability of bacterial strain was determined qualitatively by silver nitrate test with minor modifications (Lett et al. 2001). The bacterial cells were grown in MSM broth with 15 mM sodium arsenite (test) and without sodium arsenite (control) at 28 °C, 150 rpm for 24 h. One ml culture suspension

was mixed with one ml of 0.1 M $AgNO_3$ and observed for colour change from colourless to light brown.

Quantitative determination of oxidized arsenite (i.e. arsenate) was performed using molybdene blue method with some modifications (Lenoble et al. 2003; Cai et al. 2009). Cells were harvested at 8000 rpm for 10 min and resulting cell pellet was disrupted by sonication (three times for 2 min with 10 min cool-down intervals). The supernatant (0.3 ml) obtained after centrifugation was added to a mixture of 4 ml Milli Q water, 0.4 ml 50% H_2SO_4 (v/v), 0.4 ml of 3% Na_3MoO_4 (w/v) and 0.2 ml of 2% ascorbic acid (w/v). The tubes were incubated at 90 °C in water bath for 20 min. The samples were cooled and final volume was adjusted to 10 ml using Milli Q water. The same protocol was also followed for control sample and absorbance of the samples was measured at 838 nm using Biospectrometer (Eppendorf, Germany). The standard curve of arsenate was used to determine the concentration of arsenate in the test sample.

Fourier transformed infrared (FTIR) spectroscopy

The FTIR samples were prepared using bacterial cells grown with and without 15 mM sodium arsenite. The cell suspension was harvested at 8000 rpm for 10 min followed by washing with 0.1 M PBS (pH 7.4). The cell pellet was dried at 45 °C for 48 h. The dried pellet was subjected to fine grinding in presence of KBr. The IR spectrum was recorded on IR prestige-21 instrument (Shimadzu, Japan) in the region of 4000–400 cm^{-1}.

SDS-PAGE

The bacterial cells were grown with and without 5 mM sodium arsenite and protein profile of extracted protein was studied using standard protocol (Laemmli 1970). Whole cell proteins were extracted and analyzed on 15% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) at a constant voltage of 90 V using BIORAD Mini-PROTEAN Tetra System (BIO-RAD, USA). The gel was stained overnight using freshly prepared 0.05% (w/v) Coomassie Brilliant blue R250 and destained using destaining solution (Sambrook et al. 1989).

Statistical analysis

All the experiments were carried out in triplicates and their mean, as well as standard error were calculated and incorporated as ± in the manuscript.

Results

Isolation of arsenite resistant bacterial strain and determination of MIC of arsenite

Among ten morphologically different arsenite resistant bacterial isolates, strain SSSW7 showed the highest MIC of 46 mM and 21 mM on MSM agar and in MSM broth respectively. The growth pattern of the bacterial strain SSSW7 exposed to sodium arsenite interestingly revealed an extended lag phase at higher concentrations of arsenite. A prominent shift in lag phase with increasing concentrations of sodium arsenite (10, 15, 20 mM) was observed compared to control (Supplementary Fig. 1).

Identification of arsenite oxidizing bacterial isolate

Strain SSSW7 was Gram-negative, non-motile rod which showed oxidase negative and catalase positive reaction. Based on BLAST analysis of 16S rDNA sequence the bacterial strain SSSW7 has been identified as *Klebsiella pneumoniae* (Supplementary Fig. 2) and the sequence has been submitted to Genbank (accession number: MG430351).

Plasmid profile and PCR amplification of arsenite oxidase and arsenite transporter genes

Klebsiella pneumoniae strain SSSW7 possessed a plasmid of > 10 kb in size (Fig. 1a). PCR amplification of *aioA* gene using plasmid and chromosomal DNA as template clearly revealed the presence of arsenite oxidase gene with amplicon size of 1100 bps (Fig. 1b). There was no PCR amplification of ACR3 gene encoding arsenite transporter using genomic as well as plasmid DNA as a template.

Arsenite oxidase assay

Klebsiella pneumoniae strain SSSW7 exhibited highest specific arsenite oxidase activity in the periplasmic

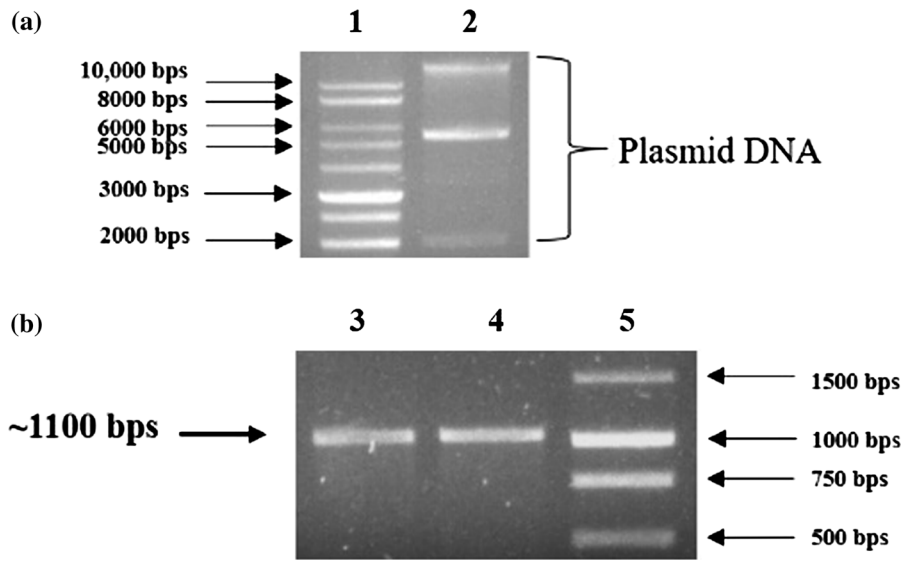

Fig. 1 Plasmid profile and PCR amplification of *aioA* gene of *Klebsiella pneumoniae* strain SSSW7. Lane 1a and 5b: 1 kb DNA markers. Lane 2a: Plasmid DNA of *K. pneumoniae* strain SSSW7. Lane 3b: PCR amplicon of *aioA* gene using chromosomal DNA as template. Lane 4b: PCR amplicon of *aioA* gene using plasmid DNA as template

fraction as the activity was recorded 1.328 µmol DCIP min^{-1} mg^{-1} protein, followed by cell free extract and spheroplast fraction with enzyme activity of 0.58 µmol DCIP min^{-1} mg^{-1} protein and 0.059 µmol DCIP min^{-1} mg^{-1} protein respectively. This clearly shows that arsenite oxidase enzyme is predominant in the periplasmic space. Interestingly, 12% and 47% increase in enzyme activity was observed in presence of 0.5 mM and 1 mM sodium arsenite indicating a high K_m.

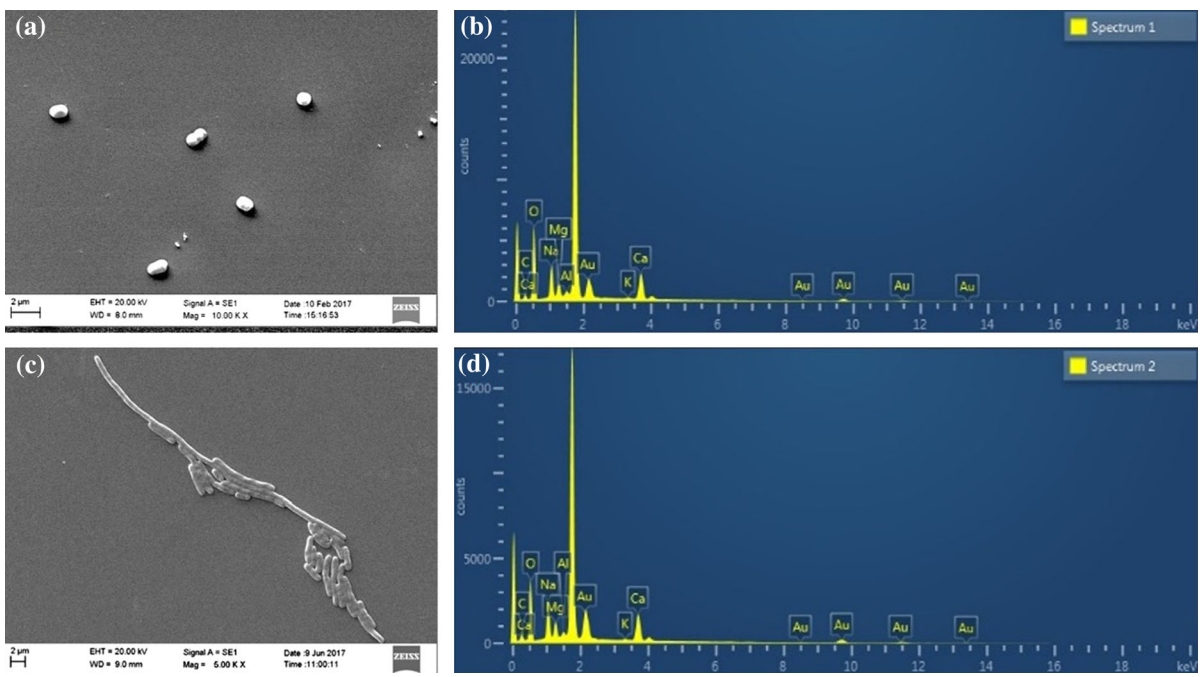

Fig. 2 SEM-EDS micrograph of *Klebsiella pneumoniae* strain SSSW7. **a** Bacterial cells in exponential growth phase (8 h) without exposure to arsenite showing rod shape morphology (control). **b** EDS micrograph of bacterial cells in exponential growth phase (8 h) without arsenite exposure (control). **c** Bacterial cells exposed to 15 mM arsenite in exponential growth phase (20 h) showing interconnected chains of cells. **d** EDS micrograph of bacterial cells in exponential growth phase (20 h) exposed to 15 mM arsenite

SEM-EDS analysis

The scanning electron micrograph of *K. pneumoniae* strain SSSW7 exposed to 15 mM arsenite demonstrated altered morphology from rods to interconnected chains of cells (Fig. 2a, c). The EDS spectrum of the cells exposed to 15 mM arsenite did not reveal any surface adsorption of arsenite (Fig. 2b, d).

TEM-EDS analysis

The intracellular structural analysis of *K. pneumoniae* strain SSSW7 by TEM clearly revealed that arsenite caused disruption of the plasma membrane, condensation of cytoplasm and presence of electron dense deposits throughout the periplasm (Fig. 3a, c). The presence of an arsenic peak in EDS spectrum of cells treated with 15 mM arsenite further confirmed intracellular accumulation of arsenic which was absent in control (Fig. 3b, d).

Arsenic transformation assay

The *K. pneumoniae* strain SSSW7 demonstrated arsenite oxidizing ability since a light brown coloured precipitate of silver-orthoarsenate was formed, indicating oxidation of arsenite to arsenate (Supplementary Fig. 3). Quantitative estimation of arsenate through molybdene blue method revealed that the bacterial strain SSSW7 internalized 10 mM of arsenate within 24 h.

Fourier transformed infrared (FTIR) spectroscopy

The FTIR spectrum analysis of 15 mM arsenite exposed bacterial cells of *K. pneumoniae* strain SSSW7 showed shifting as well as sharpening of many peaks which could be assigned to various functional groups responsible for arsenite accumulation (Fig. 4; Table 1). Arsenite exposed bacterial cells showed spectral changes in the region of

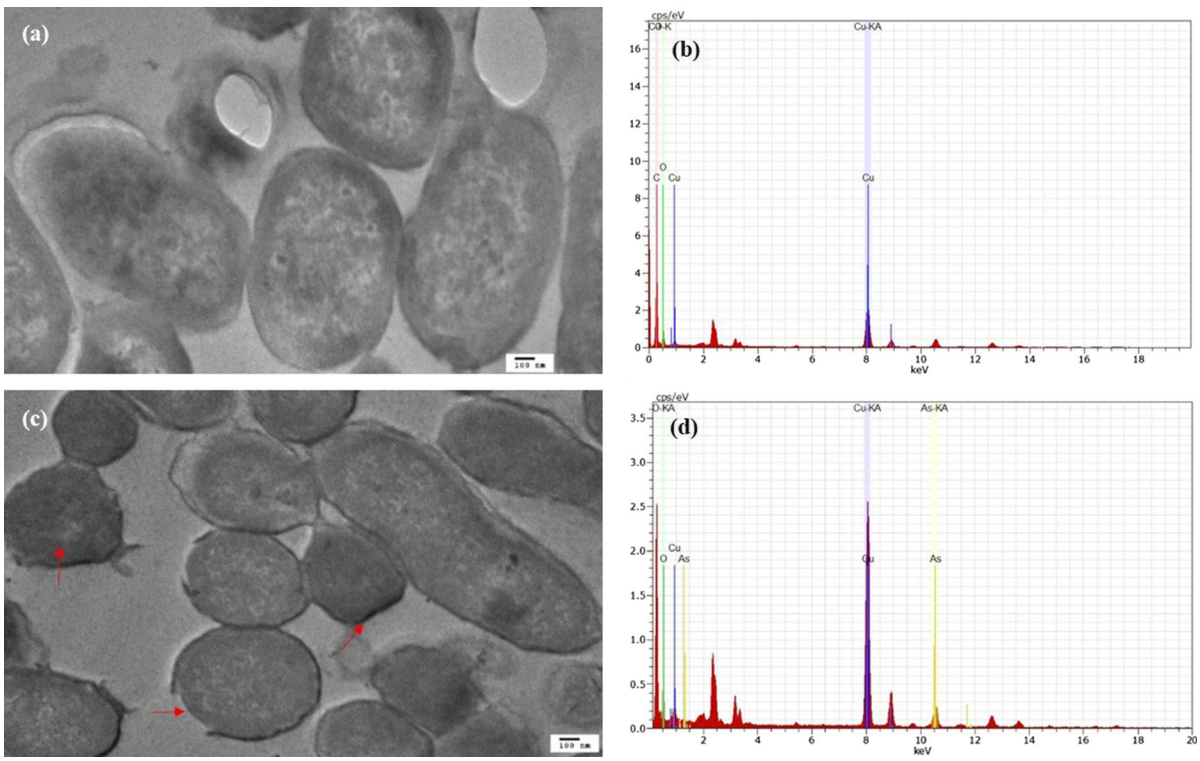

Fig. 3 TEM micrograph of *Klebsiella pneumoniae* strain SSSW7. **a** Bacterial cells in exponential growth phase (8 h) without arsenite exposure showing intact plasma membrane, clear cytoplasm and periplasm (control). **b** EDS micrograph of bacterial cells in exponential growth phase (8 h) without arsenite exposure (control). **c** Bacterial cells exposed to 15 mM sodium arsenite in exponential growth phase (20 h) showing disrupted plasma membrane, condensed cytoplasm and dark periplasm. **d** EDS micrograph of bacterial cells exposed to 15 mM arsenite in exponential growth phase (20 h)

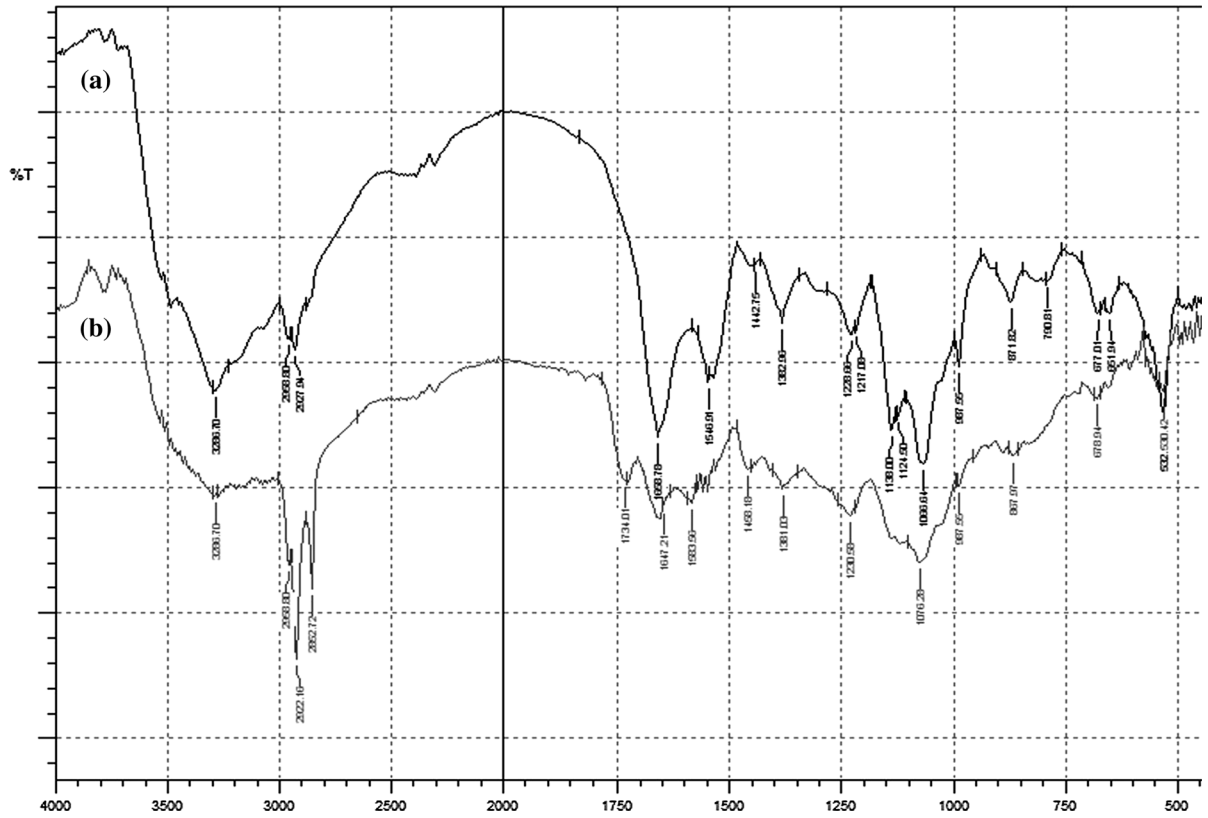

Fig. 4 FTIR spectrum of *Klebsiella pneumoniae* strain SSSW7. **a** Bacterial cells exposed to 15 mM arsenite. **b** Bacterial cells without exposure to arsenite (control)

3300–2800 cm^{-1} which may be attributed to stretching of amide and hydroxyl groups. Shifting of FTIR peaks was observed in the region spanning from 1750–1500 cm^{-1} and 1500–1200 cm^{-1} which showed the interaction of amide linkages from protein and peptides. The sharpening and peak shifts from 1200 to 1000 cm^{-1} was also observed in arsenite exposed cells which may be assigned to C–N stretching of an aliphatic amine and C–O stretching of alcohols, carboxylic acids, esters, and ethers.

SDS-PAGE

The SDS-PAGE analysis of whole-cell proteins of *K. pneumoniae* strain SSSW7 in presence of 5 mM arsenite clearly revealed up-regulation of several proteins as compared to control. Interestingly, two up-regulated proteins of molecular weight 87 kDa and 14 kDa were also observed (Supplementary Fig. 4) which may resemble the two subunits of arsenite oxidase enzyme aioA and aioB respectively.

Discussion

The arsenite resistant bacterial strain SSSW7 isolated from shipyard waste of Goa, India was identified as *K. pneumoniae*. It is interesting to note that *K. pneumoniae* strain SSSW7 exhibited the highest MIC of 21 mM in MSM broth as compared to previously reported bacterial strains. For instance, *K. pneumoniae* strains MNZ4 and MNZ6 tolerated up to 2.3 mM and 2.9 mM sodium arsenite in acetate minimal medium, whereas *K. pneumoniae* strain MR4 showed MIC of 5 mM in Luria–Bertani broth (Daware et al. 2012; Abbas et al. 2014). However, it would be inappropriate to compare the MIC values of present study with previous reports since the media composition alters availability of arsenite in the growth medium. Growth studies revealed extended lag and delayed log phases for this strain at increasing concentrations of arsenite in MSM broth. The slower growth of this bacterial strain exposed to arsenite may be attributed to ensuing

Table 1 Characteristic IR absorption peaks indicating functional groups on the surface of *K. pneumoniae* strain SSSW7

Control (frequency, cm^{-1})	Arsenite-exposed (frequency, cm^{-1})	Band assignment
3296.70	3296.70	N–H stretch of amides and O–H stretch of hydroxyl groups
2922.16	2927.94	C–H stretch of alkanes and O–H stretch of carboxyl acids
2852.72	–	C–H stretch of alkanes, O–H stretch of carboxyl acids
1734.01	1658.78	–C=C– stretch of alkenes
1647.21	1546.91	N–O asymmetric stretch of nitro compounds
1583.56	–	N–H bend of 1° amine
1458.10	1442.75	C–C stretch of aromatics
1381.03	1382.96	–C–H, bend of alkane
1230.58	1228.86	C–O stretch of alcohols, carboxylic acids, esters ethers
–	1138.00	C–N stretch of aliphatic amine and C–O stretch of alcohol carboxylic acids, esters, ethers
1076.28	1066.64	C–O stretch of alcohol carboxylic acids, esters, ethers
987.56	987.56	=C–H bend of alkenes
867.97	871.82	=C–H, bend of alkenes, C–H bend, aromatics
678.94	677.01	C–Br stretch of alkyl halide
651.94	651.94	C–Cl and C–Br stretch of alkyl halide
530.42	532.35	C–Br stretch of alkyl halide

physiological adaptation during extended lag phase leading to increase in doubling time (Paul et al. 2014).

PCR amplification using gene specific primers revealed that *K. pneumoniae* strain SSSW7 possessed *aioA* gene on both plasmid as well as chromosomal DNA. A similar study using *Acinetobacter soli* having *aioA* gene on both plasmid and chromosomal DNA has been reported (Goswami et al. 2015). Many arsenite transforming bacteria possessing arsenite oxidizing gene (*aoxB*) located only on chromosomal or plasmid DNA has been previously reported (Majumder et al. 2013; Mallick et al. 2014; Goswami et al. 2015). Interestingly, the absence of ACR3 gene from the plasmid and chromosomal genome of *K. pneumoniae* strain SSSW7 suggested an intracellular accumulation of arsenite since ACR3 protein has been reported to specifically transport arsenite in bacteria (Wysocki et al. 1997; Achour et al. 2007). Arsenite oxidase assay using different cell fractions further confirmed higher expression of arsenite oxidase enzyme in the periplasmic space. Similarly, arsenite oxidase enzyme is also reported in the periplasm of *Hydrogenophaga* sp strain NT-14, *Rhizobium* NT-26 and *Ochrobactrum triticii* SCII24 (Santini and Vanden Hoven 2004; Vanden Hoven and Santini 2004; Branco et al. 2009).

The exposure of bacterial cells to 15 mM arsenite demonstrated significant morphological alterations which were prominent as compared to control cells (Fig. 2a, c). This could be one of the strategies of bacterial cells to overcome arsenite toxicity since decrease in cell to volume ratio reduces toxicity. Similar morphological alterations have also been observed in arsenite exposed cells of *Acinetobacter lwoffii*, *Pseudomonas resinovorans* and *Acinetobacter calcoaceticus* (Banerjee et al. 2011). The EDS analysis revealed that there was no surface adsorption of arsenite and it may accumulate intracellularly. It was further substantiated by the absence of ACR3 gene which regulates transport of arsenite. Furthermore, TEM analysis of arsenite exposed cells evidently demonstrated structural changes which were similar to previous observations in *Microbacterium oleivorans* strain Ransu-1 and *Acinetobacter* sp. (Goswami et al. 2015). The TEM-EDS analysis of arsenite exposed cells also revealed intracellular accumulation of arsenic in the periplasm which is in agreement with the report of Banerjee et al. (2011). The bacterial strain SSSW7 could oxidize arsenite and showed intracellular accumulation of 10 mM arsenate which was higher than previous reports in bacterial strains (Jain et al. 2014; Naureen and Rehman 2016).

FTIR spectroscopic analysis further revealed interaction of functional groups such as carboxyl, hydroxyl and amino groups on bacterial cell surface with arsenite anions. Similar observations have also been reported in *E. coli* and *Bacillus aryabhattai* strain NBRI014 (Wu et al. 2010; Singh et al. 2016). SDS-PAGE analysis of whole cell proteins of strain SSSW7 exposed to 5 mM arsenite revealed over-expression of 87 kDa and 14 kDa proteins which may resemble with two subunits of arsenite oxidase enzyme. Therefore, it is clear that under stress of arsenite, over-expression of *aioA* gene facilitates transformation of arsenite to arsenate by bacterial cells in order to overcome the arsenite toxicity. This enzyme has been previously reported in *Rhizobium* NT-26 and *Hydrogenophaga* sp. strain NT-14 (Santini and Vanden Hoven 2004; Vanden Hoven and Santini 2004).

Conclusion

The *K. pneumoniae* strain SSSW7 isolated from shipyard waste demonstrated presence of arsenite oxidase gene and periplasmic arsenite oxidase enzyme. It showed high resistance to arsenite and could oxidize 10 mM arsenite to less toxic arsenate within 24 h which was found to be accumulated in the periplasmic space. Therefore, this bacterial strain SSSW7 has potential to bioremediate arsenite present in contaminated environmental sites.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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