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ORIGINAL PAPER

Physiological adaptations and tolerance towards higher concentration of selenite (Se^{+4}) in *Enterobacter* sp. AR-4, *Bacillus* sp. AR-6 and *Delftia tsuruhatensis* AR-7

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Abstract Environmental contamination with selenium is a major health concern. A few bacterial strains have been isolated that can transform toxic selenite to non-toxic elemental selenium only at low concentrations (0.001–150 mM) in recent past. We have previously reported isolation and characterization of few selenite-tolerant bacterial strains. These strains were found to be resistant to selenite at (300–600 mM) concentrations. In the present study we have characterized some physiological adaptations of strains *Enterobacter* sp. AR-4, *Bacillus* sp. AR-6 and *Delftia tsuruhatensis* AR-7 during exposure to higher concentration of selenite under aerobic and anaerobic environments. Adaptive responses are largely associated with alteration of cell morphology and change in total cellular fatty acid composition. Interestingly, electron microscopy studies revealed substantial decrease in cell size and intracellular deposition of Se^0 crystals when reduction is carried out under aerobic conditions. On the other hand, cell size increased with adhesion of Se^0 on cell surface during anaerobic reduction. Fatty acid composition analysis demonstrated selective increase in saturated and cyclic fatty acids and decrease in unsaturated ones during aerobic transformation. Changes observed during anaerobic transformation were in surprising contrast as indicated by total absence of saturated and cyclic fatty acids. Results presented here provide evidences for putative occurrence of two distinct mechanisms involved in tolerance towards higher concentrations of selenite utilization under aerobic and anaerobic conditions. Further, prior exposure to higher concentration of Se^{+4} enabled rapid adaptation indicating role of inducible system in adaptation.

Keywords Selenite · Toxicity · Tolerance · Physiological adaptations · FAMEs analysis · SEM and TEM

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Introduction

Selenium (Se) is a naturally occurring widely distributed element that constitutes an essential component of all living systems including single cellular prokaryotes (at trace levels) for synthesis of essential amino-acids i.e. selenocysteine and selenomethionine. However, it is very toxic to nearly all living forms at higher concentrations (even at μM concentrations) (Wade et al. 1993; Chan et al. 1998; Lenz and Lens 2008). Selenium exists in a number of oxidative states i.e. selenate (Se^{+6}; SeO_4^{2-}), selenite (Se^{+4}; SeO_3^{2-}), organic selenide (Se^{2-}) and elemental selenium (Se^0) in the environment (Fan and Kizer 1990). Selenium toxicity has been postulated to be a direct function of aqueous solubility/biological availability which is significantly influenced by the chemical forms and oxidative state. In general, the Se^0 is least toxic among all the characterized oxidative forms because of its low water solubility and low bioavailability (Wilber 1980). A number of studies for selenium reduction by abiotic methods e.g. cycling and volatilization have been reported both in aqueous and in terrestrial ecosystems (Frankenberger and Arshad 2001). Reduction of selenium oxyanions has also been reported to

occur in the presence of Fe(II)–Fe(III) hydroxide mineral green rust (Chen et al. 2008). However, such abiotic methods are expensive and insufficient to restore the naturally contaminated environments. Conversely, microbial reduction of environmental pollutants has been accepted as a cost effective and environment-friendly approach and therefore, microorganisms with ability to transform selenite (Se^{+4}) into elemental selenium (Se^{0}) are of great ecological significance for remediation of sites contaminated with Se^{+4}.

A few microorganisms have been isolated and characterized for their ability to enzymatically reduce Se^{+4} and Se^{+6} into non-toxic elemental form under aerobic and anaerobic environments (Oremland et al. 1994; Sabaty et al. 2001; Watts et al. 2005; Narasingarao and Haggblom 2007). In some bacterial species Se^{+4} reduction may serve functions of detoxification and maintenance of redox component of the electron transport system by cytoplasmic reductase enzymes (Yamada et al. 1997; Belzile et al. 2006; Narasingarao and Haggblom 2007). Alternatively, microbial communities of selenium-rich environments have also been reported for Se^{+6} or Se^{+4} reductions (Maiers et al. 1988; Siddique et al. 2005; Knotek-Smith et al. 2006; El-Ghawi et al. 2007; Pollard et al. 2007; Liy et al. 2008). Most of the above reports of microbial reduction of selenium with isolated bacterial strains show the reduction only at substantially lesser concentrations than reported at some sites of the source contamination. Therefore, these microbial potentials may not be useful for the restoration of environmental sites that are polluted with high concentration of Se^{+4}. It is important to isolate and characterize bacterial strains that are capable of reducing high concentration of Se^{+4} in order to restore ecological niches.

Earlier studies for bacterial tolerance towards organic solvents and aromatic compounds have indicated tolerance to be mainly associated with alteration of bacterial cell morphology and composition of total cellular fatty acid (Isken and De Bont 1998; Sardessai and Bhosle 2002; Na et al. 2005; Zahir et al. 2006). A few other studies have shown the role of bacterial membrane associated proteins e.g. 'efflux pumps' for the above tolerance (Lix et al. 1998; Isken and De Bont 2000; Tokunaga et al. 2004). However, there is very limited information available till date pertaining to physiological adaptation/response for bacterial tolerance towards such high concentrations of heavy metals and metalloids in either aerobic or anaerobic environment.

Previously, we have isolated and identified eight bacterial isolates from soil and water samples collected from different selenium contaminated sites in India (Ghosh et al. 2008). Importantly, the Se^{+4} minimum inhibitory concentration (MIC) values for all of these isolates were significantly higher as compared to earlier reports. The present study describes detailed and conclusive analysis of physiological adaptation in three of the previously isolated Se^{+4} tolerant bacterial strains (viz. *Enterobacter* sp. AR-4, *Bacillus* sp. AR-6 and *Delftia tsuruhatensis* AR-7). A comparison of adaptive responses, such as growth kinetics, alteration in cell morphology, localization and SEM-EDX identification of the elemental selenium crystals and changes in total cellular fatty acid composition under both aerobic and anaerobic conditions has also been reported. The results obtained suggest that the adaptive response towards alteration in cell morphology and membrane composition for Se^{+4} tolerances is dependent on two distinct mechanisms.

Materials and methods

Bacterial strains and growth conditions

Bacterial strains used during this study were previously isolated from soil and water samples collected from different selenium contaminated sites in India and were identified as *Enterobacter* sp. strain AR-4, *Bacillus* sp. AR-6 and *Delftia tsuruhatensis* AR-7 belonging to *γ-proteobacteria*, *bacilli* and *β-proteobacteria*, respectively. These organisms were found to be resistant to very high concentration of Se^{+4} and Se^{+6} (Ghosh et al. 2008). During the present study the organisms were grown in nutrient broth (NB) or minimal medium (MM). The composition of MM was as follows (per liter): Na_2HPO_4, 4.0 g; KH_2PO_4, 2.0 g; $MgSO_4$, 0.8 g; $(NH_4)_2SO_4$, 0.8 g; yeast extract, 0.02 g; trace element solution, 1.0 ml. The trace element solution contained (per liter): $Al(OH)_3$, 0.1 g; $SnCl_2$, 0.05 g; KI, 0.05 g; LiCl, 0.05 g; $MnSO_4{\cdot}4H_2O$, 0.08 g; H_3BO_3, 0.5 g; $ZnSO_4{\cdot}7H_2O$, 0.1 g; $CoCl_2{\cdot}6H_2O$, 0.1 g; $NiSO_4{\cdot}6H_2O$, 0.1 g; $BaCl_2$, 0.05 g; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.05 g. The pH of the medium was adjusted to 7.2. The MM was supplement with 10 mM sodium succinate and desired concentration of sodium selenite (ranging from 50 to 500 mM). The incubations were carried out at 30°C with aeration. Incubations were also carried out under anaerobic conditions by eliminating the oxygen and lowering the redox potential of the medium by addition of a reducing agent viz. cysteine sulphide. Oxygen was removed by boiling the medium under a stream of nitrogen and carbon dioxide in an anaerobic workstation (Modular Atmosphere Controlled System, D.W. Scientific Ltd., England). Subsequently, the medium was cooled to 40°C and distributed in the tubes under anaerobic conditions. In order to remove the oxygen from the headspace of the culture tube and achieve strict anaerobic conditions, each tube was purged with nitrogen flux, sealed with stoppers and autoclaved (Feio et al. 1998). Bacterial growth was determined by monitoring the OD_{600} spectrophotometrically (Lambda EZ-201 UV–Vis Spectrophotometer, Perkin- Elmer, USA).

Quantitation of selenite (Se^{+4}) reduction

The quantitation of selenite (Se^{+4}) reduction was carried out according to the method described earlier (Suvardhan et al. 2006). The method is based on oxidation of 2,4-dinitrophenylhydrazine hydrochloride (2,4-DNPH) by Se^{+4} in hydrochloric acid followed by coupling reaction with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) to pink coloured derivative with an absorbance maximum at 520 nm. The Se^{+4} reduction plots for the three organisms were plotted using Sigma Plot version 2.0. Reduction of Se^{+4} was also observed visually by development of red colour in the colourless growth medium indicating transformation of (Se^{+4}) to elemental selenium (Se^{0}); this observation was consistent with earlier reports for such reductions (Oremland et al. 2004; Klonowska et al. 2005).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Bacterial cell samples collected from different incubations were subjected to SEM for assessing the alteration in cell morphology as the adaptive response towards higher concentration (500 mM) of Se^{+4}. This was carried out using the method as described earlier (David et al. 1973; Sharma et al. 2007) with minor modifications. Cells were harvested at log phase, washed twice with ice-cold phosphate buffer (10 mM, pH 7.0) and fixed with mixture of 2.5% glutaraldehyde and 2% *p*-formaldehyde (prepared in phosphate buffer; 0.1 mM, pH 7.4) for 6 h at 4°C. The cells were then washed again twice with ice-cold phosphate buffer to remove excess of the fixative reagents and suspended in same buffer for examination. The SEM analysis was carried out using electron microscope (Carl Zeiss NTS, GmbH, Germany). The cytological features of the cells were examined by TEM analysis. The samples were processed as above (for SEM analysis) and thin sections were embedded in Epon after fixation with 1% glutaraldehyde and 1% osmium tetroxide according to the method described earlier (Kellenberger and Ryter 1958; Kellenberger et al. 1958) and analysed by using a Jeol 1210 Transmission electron microscope (Jeol USA Inc., MA. USA).

Elemental composition analysis with energy dispersive X-ray (EDX)

To ascertain the reduction of Se^{+4} to elemental selenium (Se^{0}) the samples were processed by a method similar to that used for SEM analysis. The selected areas within SEM were subjected to elemental composition analysis using EDX (Bruker AXS Inc. USA, Quantax-200) micro-analysis system coupled with electron microscope. Sample collected from the incubation without Se^{+4} was taken as negative control.

Total cellular fatty acid composition analysis

The alteration in total cellular fatty acid composition as an adaptive response was determined by fatty acid methyl esters (FAMEs) analysis following exposure of test organisms to different concentrations of sodium selenite (50 mM and 500 mM) according to the method described previously (Sharma et al. 2007). Cells were harvested at mid-log phase and fatty acid methyl esters were prepared sequentially by saponification, methylation and extraction. The extracted fatty acid methyl esters were analysed by gas chromatography using a Microbial Identification System (MIDI, Inc. Newark, NJ. USA). The instrument consisted of a Helwett Packard Model 5890A gas chromatograph equipped with 5% phenylmethyl silicon column (0.2 m × 25 m) and a flame ionization detector. The analysis was carried out with a constant injector and detector temperatures of 300 and 250°C, respectively, whereas the oven temperature increased from 170 to 270°C at a linear increment of 5°C min^{-1}.

Chemicals

Sodium selenite, sodium succinate, 2,4-dinitrophenylhydrazine hydrochloride (2,4-DNPH) and *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) were procured from Sigma–Aldrich Corp. (St. Louis, MO. USA). All other chemicals used were of highest purity grade available locally.

Results and discussion

Growth profile and quantitation of Se^{+4} reduction

The growth characteristics of strains *Enterobacter* sp. AR-4, *Bacillus* sp. AR-6 and *Delftia tsuruhatensis* AR-7 were studied on different concentrations of Se^{+4} by growing them on MM supplemented with 10 mM of sodium succinate and different concentrations of sodium selenite (50 mM and 500 mM). The growth rate of the organisms AR-4 and AR-7 were higher and lag phase significantly shorter as compared to the organism AR-6 at 50 mM and 500 mM Se^{+4} concentrations under aerobic condition. However, a prior exposure/induction to Se^{+4} reduced the lag phase to ~4 h following growth of cells on 50 mM, and to ~8 h when grown on 500 mM of the Se^{+4} salt. This was accompanied by concurrent reduction of Se^{+4} to the non-toxic elemental form. The quantitative reduction of Se^{+4} was found to be fastest at 50 mM and 500 mM in strains AR-4 and AR-7, respectively under induced aerobic condition. The strain AR-6 reduced Se^{+4} comparatively at a slower rate than strains AR-4 and AR-7. However, the rates of reduction

under un-induced condition were found to be ~25% slower than the induced condition (Figs. 1a, b, 2a, b, 3a, b). Cell growth in the presence of Se^{+4} salt under anaerobic (induced and un-induced) condition was observed only with strains AR-4 and AR-7. The growth characteristics and quantitative reduction of Se^{+4} were found to be similar to those observed under aerobic conditions, but at comparatively slower rate (Figs. 1c, d, 2c, d).

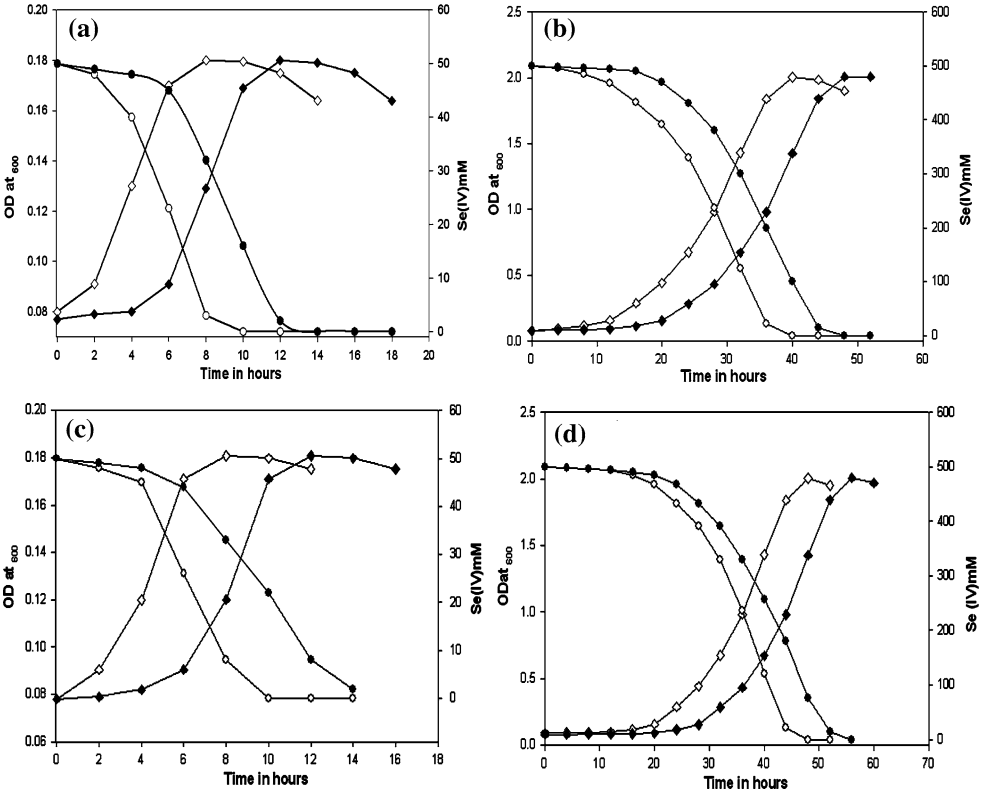

Fig. 1 The values represented are the average of triplicates: **a** Growth profile and Se^{+4} reduction by strain AR-4 in aerobic condition (induced and un-induced) at 50 mM selenite. **b** Growth profile and Se^{+4} reduction by strain AR-4 in aerobic condition (induced and un-induced) at 500 mM. **c** Growth profile and Se^{+4} reduction by strain AR-4 in anaerobic condition (induced and un-induced) at 50 mM. **d** Growth profile and Se^{+4} reduction by strain AR-4 in anaerobic condition (induced and un-induced) at 500 mM. *Diamond* Growth profile (induced); *filled diamond* growth profile (un-induced); *open circle* Se^{+4} reduction (induced); *filled circle* Se^{+4} reduction (un-induced)

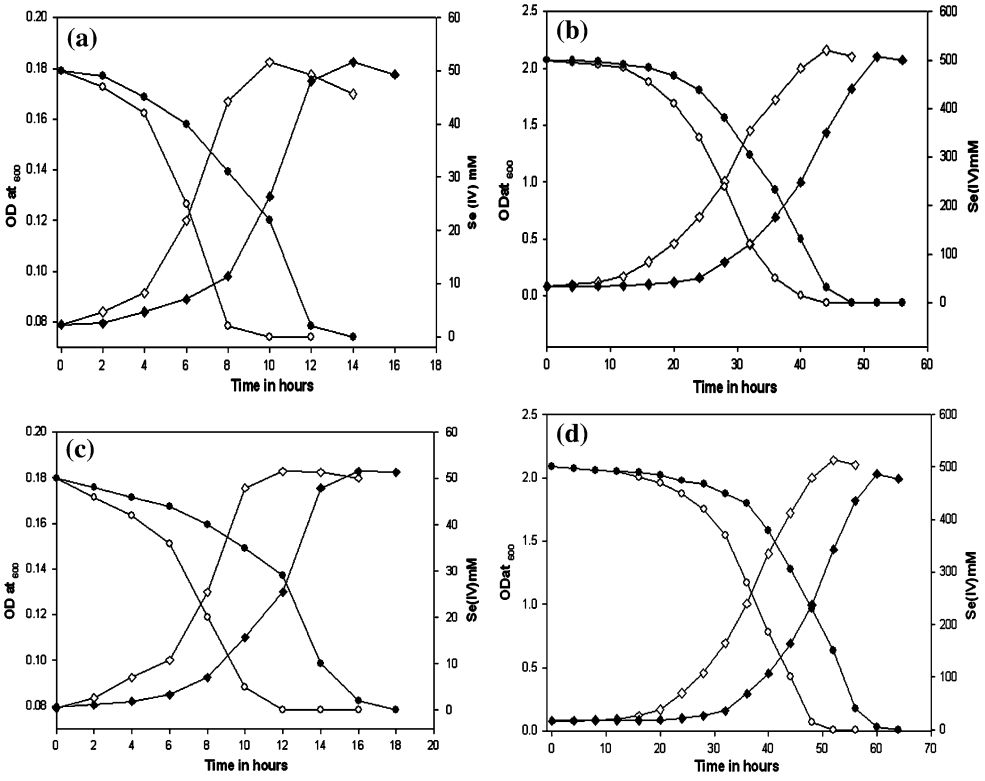

Fig. 2 The values represented are the average of triplicates: **a** Growth profile and Se^{+4} reduction by strain AR-7 in aerobic condition (induced and un-induced) at 50 mM. **b** Growth profile and Se^{+4} reduction by strain AR-7 in aerobic condition (induced and un-induced) at 500 mM. **c** Growth profile and Se^{+4} reduction by strain AR-7 in anaerobic condition (induced and un-induced) at 50 mM. **d** Growth profile and Se^{+4} reduction by strain AR-7 in anaerobic condition (induced and un-induced) at 500 mM. *Diamond* Growth profile (induced); *filled diamond* growth profile (un-induced); *open circle* Se^{+4} reduction (induced); *filled circle* Se^{+4} reduction (un-induced)

The above results indicate that reduction/tolerance of Se^{+4} by the organisms is faster under aerobic condition and prior exposure/induction with Se^{+4} significantly reduces the lag phase. This observation of relative fast reduction under aerobic condition suggests that selenite reduction process is highly dependent upon the oxygen concentration in the culture medium as reported earlier (Dungan et al. 2003). Slow reduction under anaerobic conditions might be due to the inactivation of some electron carriers, or enzymatic activities due to the less oxygen availability. This suggests that various physiological adaptations might be associated upon initial exposure to selenite, and once adapted, cells can grow rapidly even at high concentrations of selenite.

Cell morphological alterations

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed to assess the morphological changes and internal cellular structures during the adaptive responses. The major morphological change observed in case of these organisms during aerobic growth in presence of higher concentration of Se^{+4} salts (500 mM) was sequential decrease in cell size (~3 times) to 0.55–0.66 μm as compared to the cell sizes of 1.95–2.01 μm when cells were grown in absence of any Se^{+4} salt (Figs. 4a, b, 5a, b, 6a, b). The decrease in the cell size(s) mediated mechanism has been recently reported for bacterial tolerance towards higher concentration of toxic organic compounds earlier e.g. *n*-butanol (Veeranagouda et al. 2006) and *o*-nitrobenzoate/*p*-hydroxybenzoate (Sharma et al. 2007). In contrast, when strains AR-4 and AR-7 were grown under anaerobic conditions in presence of higher concentration of Se^{+4} salts, the cells were found to have an increased cell size (~2 times) with filamentous appearance having an average size of 4.21–4.28 μm as compared to 1.96–1.98 μm (Figs. 4c, d, 5c, d). Table 1

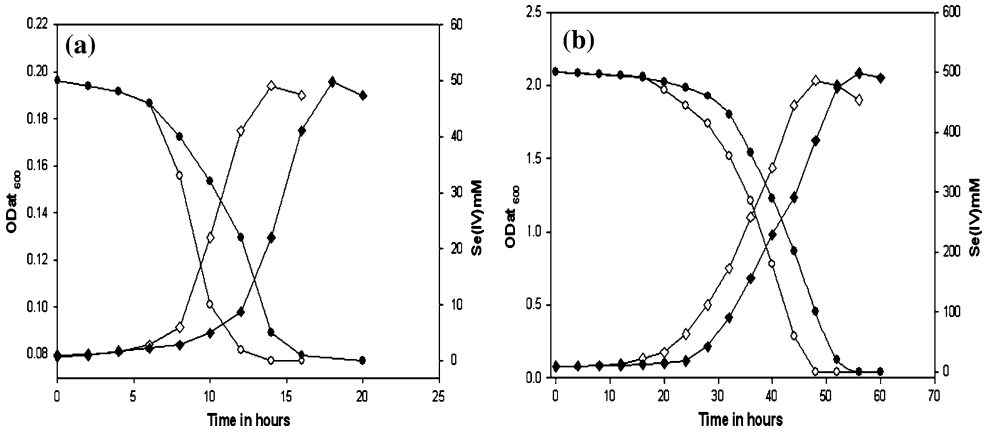

Fig. 3 The values represented are the average of triplicates: **a** Growth profile and Se^{+4} reduction by strain AR-6 aerobic condition (induced and un-induced) at 50 mM. **b** Growth profile and Se^{+4} reduction by strain AR-6 aerobic condition (induced and un-induced) at 500 mM. *Diamond* Growth profile (induced); *filled diamond* growth profile (un-induced); *open circle* Se^{+4} reduction (induced); *filled circle* Se^{+4} reduction (un-induced)

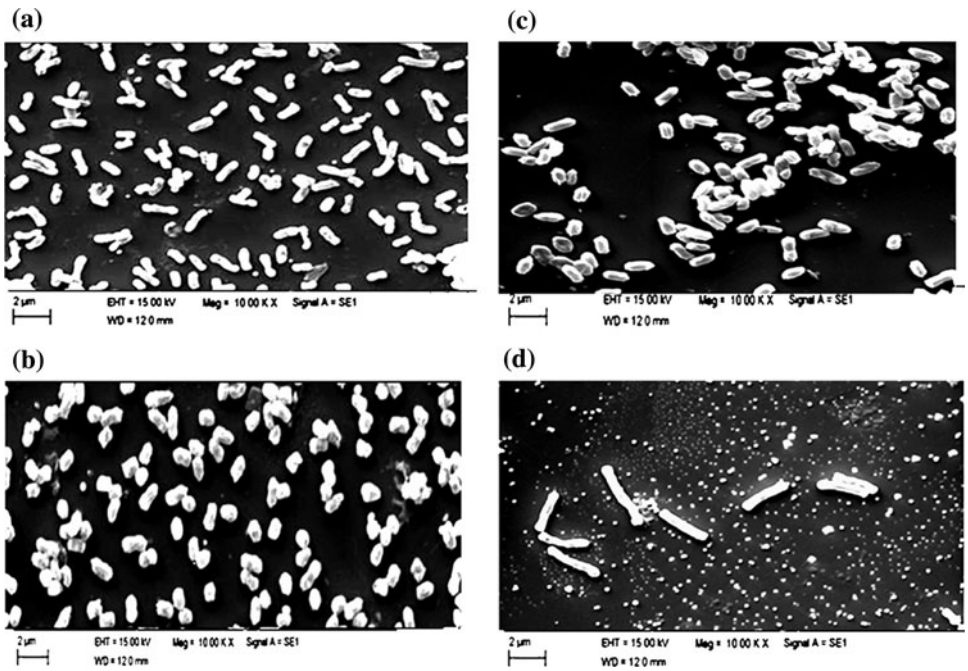

Fig. 4 SEM images of strain AR-4: **a** Se^{+4} free medium under aerobic condition; **b** 500 mM concentration of Se^{+4} under aerobic condition; **c** Se^{+4} free medium under anaerobic condition; **d** 500 mM concentration of Se^{+4} under anaerobic condition

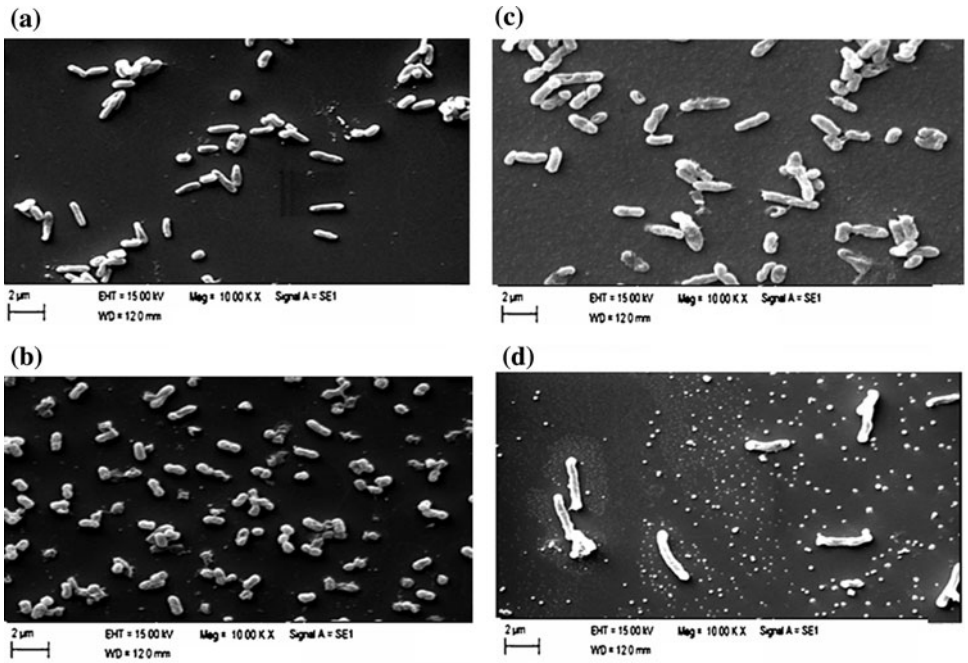

Fig. 5 SEM images of strain AR-7: **a** Se^{+4} free medium under aerobic condition; **b** 500 mM concentration of Se^{+4} under aerobic condition; **c** Se^{+4} free medium under anaerobic condition; **d** 500 mM concentration of Se^{+4} under anaerobic condition

presents the comparative cell sizes of the above organisms grown under different conditions. Possibly, the changes in cell morphology could be explained by the surface/volume ratio. During aerobic condition, the organisms reduced their cell size and increased their relative surface area for better uptake of the nutrients for survival in environmental stress conditions. Conversely, during anaerobic conditions, the organisms increased their cell size and reduced their relative surface area to reduce toxic effects and this might be the putative adaptive mechanism for tolerance.

Localization and identification of elemental (Se^0) crystals formation

TEM analysis demonstrated that during aerobic reduction of Se^{+4} there is an accumulation of elemental selenium (Se^0) as an intracellular crystal in strains AR-4 and AR-7 (Fig. 7a, c). Such accumulation of Se^0 crystals is in accordance with earlier reports for bacterial reduction of Se^{+4} (Roux et al. 2001; Rathgeber et al. 2002). However, interestingly, when strains AR-4 and AR-7 transformed Se^{+4} under anaerobic conditions the Se^0 crystals were observed adhering to bacterial cell membrane extracellularly (Fig. 7b, d). Adherence of Se^0 crystals to the bacterial cell membrane during anaerobic reduction was recently reported in case of Se^{+4} reductions by *Shewanella oneidensis* (Klonowska et al. 2005). Similarly, another report suggested that the reduction of selenite occurs close to the membrane, possibly due to a membrane-associated reductase, and that the particles are rapidly expelled by a membrane efflux pump (Losi and Frankenberger 1997).

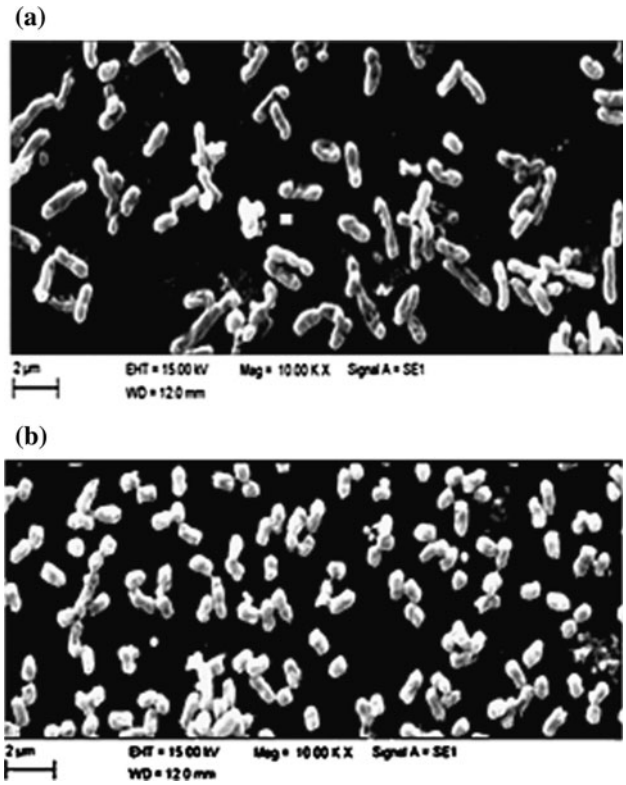

Fig. 6 SEM images of strain AR-6: **a** Se^{+4} free medium under aerobic condition; **b** 500 mM concentration of Se^{+4} under aerobic condition

Selenite reduction by microorganisms may be the multiple detoxification process because elemental Se^0 has been found as deposits in the cytoplasm and in the periplasm

Table 1 The comparative cell sizes of strains AR-4, AR-6 and AR-7 at mid-log phase in presence of 500 mM of Se^{+4}

Strain	Aerobic growth		Anaerobic growth	
	Length (μm)		Length (μm)	
	SS (Control)	Se^{+4} (500 mM)	SS (Control)	Se^{+4} (500 mM)
Enterobacter sp. (AR-4)	1.95 ± 0.270	0.55 ± 0.087	1.96 ± 0.29	4.21 ± 0.41
Delftia tsuruhatensis (AR-7)	1.96 ± 0.102	0.66 ± 0.067	1.98 ± 0.48	4.28 ± 0.43
Bacillus sp. (AR-6)	2.01 ± 0.161	0.58 ± 0.124	NA	NA

The experiments were carried out in triplicates and the average values for cell size were calculated with arithmetic mean. The data were also analysed with Student's *t* test and the *P* values of ≤0.05 were considered statistically significant

SS sodium succinate, *NA* not applicable

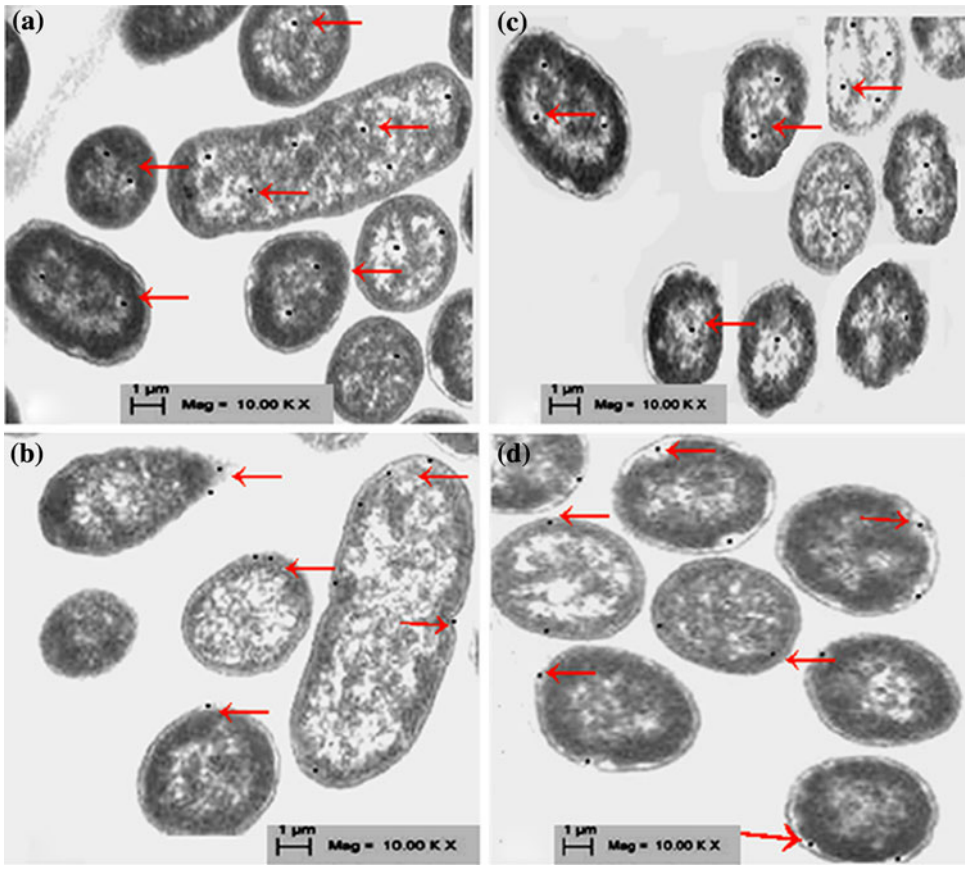

Fig. 7 TEM images of localization of selenium crystals (Se^0) indicated by *arrows*: **a** intracellular localization of selenium crystal (Se^0) in strain AR-4 under aerobic condition; **b** adherence of selenium crystal (Se^0) to cells in strain AR-4 under anaerobic condition; **c** intracellular localization of selenium crystal (Se^0) in strain AR-7 under aerobic condition; **d** adherence of selenium crystal (Se^0) to cells in strain AR-7 under anaerobic condition

(Gerrard et al. 1974; Yamada et al. 1997). Therefore, it would be a challenge to elucidate the mechanism of selenium reduction.

The identity of Se^0 crystal formation under different incubation conditions was ascertained by energy dispersion X-ray (EDX) analysis (Fig. 8). Since the EDX analysis identifies only the elemental crystalline compounds, we concluded that the red coloured precipitate was specific selenium absorption peak at 1.50 keV (peak Se Lα) and 11.25 keV (peak Se Kα) was crystalline Se^0. These observations pertaining to difference in localization of Se^0 crystals under aerobic and anaerobic conditions clearly demonstrate that Se^{+4} reductions in strains AR-4 and AR-7 follow two independent mechanisms. The Se^{+4} reduction under aerobic and anaerobic conditions is putatively carried out by cytosolic and membrane associate reductase respectively. Furthermore, the membrane-associated reductase may be oxygen sensitive, whereas the cytosolic reductase is non-sensitive to the presence of oxygen (Dungan et al. 2003).

Total cellular fatty acid analysis

Strains AR-4, AR-6 and AR-7 were subjected to analysis of some of the putative physiological adaptations required

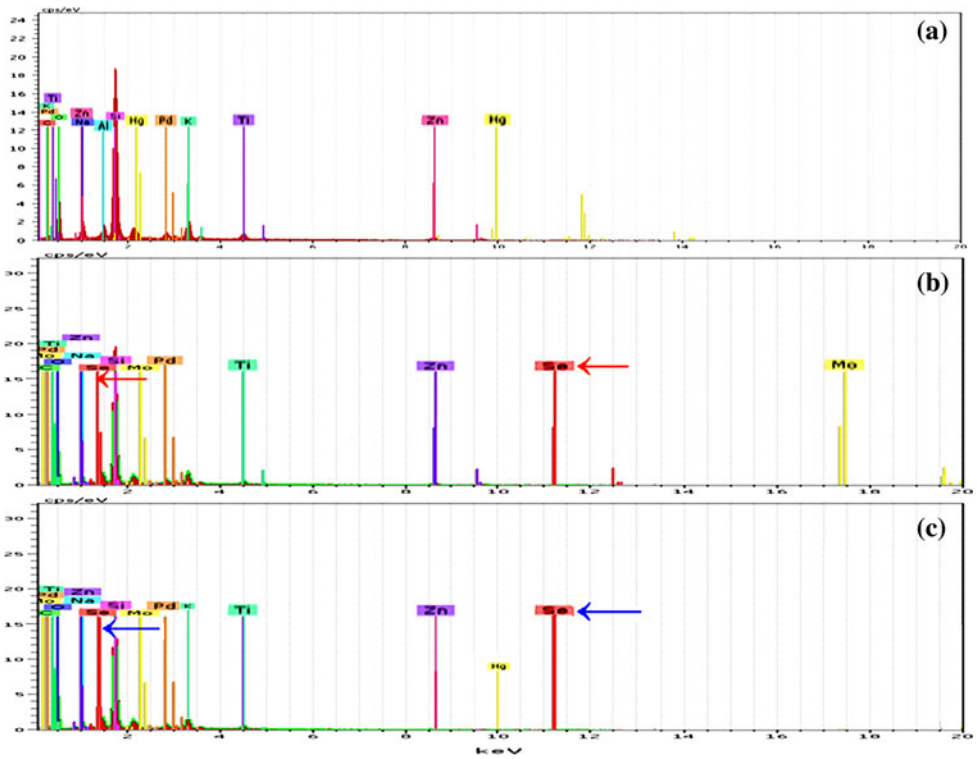

Fig. 8 **a** EDX in Se^{+4} free medium as negative control; **b** identification of selenium crystal (Se^{0}) formation indicated by *red arrows*; **c** selenium standard (Se^{0}) indicated by *blue arrows*

for the resistance/tolerance towards higher concentration of Se^{+4}. Earlier studies have demonstrated the bacterial adaptations to be articulated via alteration in cell morphology and changes in the total cellular fatty acid composition (Junker and Ramos 1999; Sharma et al. 2007). For the fatty acid analysis organisms were grown on 10 mM sodium succinate with different concentrations of sodium selenite (50 mM and 500 mM) and they showed significant alterations in the fatty acid composition. The major fatty acids found were C12:0, C14:0, C16:0, C17:0, C17:0 cyclo, C18:1w7c and C18:1w9c in the organism AR-4. The organism AR-7 had C18:0 as an additional major fatty acid. However, strain AR-6 showed the presence of C12:0, C12:03OH, C14:0, C16:0, C16:1W11, C17: cyclo, C18:0 and C18:1w7c as the major fatty acids. During growth on higher concentration of Se^{+4} (500 mM) under aerobic environment the relative % concentration of total saturated fatty acid and cyclic fatty acid increased significantly whereas the amount of total unsaturated fatty acids decreased (Table 2). The adaptation towards the higher concentration is also marked by synthesis of branched fatty acids e.g. C14:0 iso, C15:0 iso, C16:0 iso, C17:0 iso and C15:0 anteiso only at 500 mM of selenite. The statistical analysis of these results obtained in comparison with control clearly demonstrated that most of these alterations were significant. Interestingly, the alterations observed in total fatty acid composition of strain AR-4 and AR-7 during growth under anaerobic environments were in contrast to those observed during aerobic environments. In case of anaerobic incubations the relative % concentration of saturated fatty acid decreased whereas unsaturated fatty acid increased (Table 3). However, the pattern for increase in relative abundance of the branched fatty acids (total iso fatty acids: total anteiso fatty acids) followed a similar pattern during aerobic condition.

The above observations clearly indicate a potentially important role of saturated, unsaturated and branched fatty acids in determining the bacterial adaptation towards heavy metal induced toxicity (Tables 4, 5). These fatty acids may regulate the membrane fluidity under metal stress conditions during adaptation (Hazel and Williams 1990; Pennanen et al. 1996). An earlier report has shown nearly exclusive role of branched fatty acids for adaptations towards environmental toxicity (Chattopadhyay and Jagannadham 2007) where the ratio of anteiso/iso fatty acid was used as one of the most important determinant for bacterial cell membrane fluidity and consequently their adaptation towards environmental stress. In case of *Bacillus subtilis* an increase in anteiso/iso ratio was observed during its growth at extremely low temperatures (Klein et al. 1999). Sharma et al. (2007) also reported a similar observation for adaptive response of *Arthrobacter protophormiae* RKJ100 during growth on higher concentrations of benzoates. The observation for putative role of saturated fatty acid and unsaturated fatty acid can regulate adaptive response. The present report may serve as a model for similar future studies.

Table 2 Comparative analysis of cellular fatty acids composition of strains AR-4, AR-6 and AR-7 in presence of increasing concentration of Se^{+4} under aerobic conditions

Fatty acids	*Enterobacter* sp. AR-4			*Delftia tsuruhatensis* AR-7			*Bacillus* sp. AR-6		
	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM
C12:0*	3.96 ± 0.09	4.51 ± 0.16	4.75 ± 0.20	3.83 ± 0.08	5.23 ± 0.22	13.21 ± 0.32	2.50 ± 0.06	12.08 ± 0.30	16.24 ± 0.48
C12:03OH	ND	ND	ND	ND	ND	ND	ND	2.58 ± 0.10	2.65 ± 0.18
C13:0 anteiso	ND	ND	ND	ND	ND	ND	ND	ND	1.89 ± 0.12
C14:0*	7.31 ± 0.24	9.38 ± 0.36	10.13 ± 0.38	3.81 ± 0.16	4.53 ± 0.25	6.97 ± 0.30	0.90 ± 0.09	1.55 ± 0.12	2.59 ± 0.20
C14:0 ISO	ND	ND	ND	ND	ND	ND	ND	ND	18.67 ± 0.42
C15:0 iso	ND	ND	8.84 ± 0.54	ND	ND	7.99 ± 0.52	ND	ND	12.16 ± 0.34
C15:0 anteiso	ND	ND	7.90 ± 0.48	ND	ND	9.94 ± 0.42	ND	ND	8.92 ± 0.48
C16:0*	30.61 ± 0.53	36.35 ± 0.65	42.47 ± 0.84	37.45 ± 0.70	46.45 ± 0.76	59.36 ± 0.86	11.74 ± 0.26	28.29 ± 0.32	51.03 ± 0.82
C16:0 iso	ND	ND	2.65 ± 0.18	ND	ND	9.56 ± 0.38	ND	ND	16.35 ± 0.44
C16:1W11C	ND	ND	ND	ND	ND	ND	1.62 ± 0.16	0.90 ± 0.13	ND
C17:0*	0.42 ± 0.06	4.68 ± 0.18	6.12 ± 0.26	ND	ND	ND	ND	ND	ND
C17:0 iso	ND	ND	ND	ND	ND	ND	ND	ND	16.67 ± 0.48
C17:0 cyclo*	9.51 ± 0.16	17.32 ± 0.22	22.56 ± 0.32	23.53 ± 0.32	34.56 ± 0.56	45.70 ± 0.78	1.43 ± 0.12	4.82 ± 0.10	12.59 ± 0.28
C18:0*	ND	ND	ND	0.97 ± 0.12	15.56 ± 0.29	24.38 ± 0.40	1.44 ± 0.14	4.68 ± 0.18	6.53 ± 0.28
C18:1W7C*	19.09 ± 0.38	2.39 ± 0.08	ND	6.69 ± 0.28	2.55 ± 0.16	ND	3.77 ± 0.14	2.12 ± 0.12	ND
C18:1W9C	0.44 ± 0.08	ND	ND	ND	ND	ND	ND	ND	ND

Values are represented as percentage of total fatty acids; all experiments were carried out in triplicate and the average values of fatty acids were calculated with arithmetic mean

SS sodium succinate, *ND* not detected

* The statistically significant differences in fatty acid are indicated by asterisk

Table 3 Comparative analysis of cellular fatty acids composition of strains AR-4, AR-6 and AR-7 in presence of increasing concentration of Se^{+4} under anaerobic condition

Fatty acids	*Enterobacter* sp AR-4			*Delftia tsuruhatensis* AR-7		
	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM
C12:0*	3.65 ± 0.09	1.50 ± 0.04	ND	3.62 ± 0.12	1.22 ± 0.10	ND
C12:03OH	ND	ND	ND	ND	ND	0.95 ± 0.13
C13:0 anteiso	ND	ND	ND	ND	ND	ND
C14:0*	7.01 ± 0.16	2.09 ± 0.08	ND	3.23 ± 0.10	1.39 ± 0.09	ND
C15:0 iso	ND	ND	11.36 ± 0.13	ND	ND	8.15 ± 0.09
C15:0 anteiso	ND	ND	9.65 ± 0.15	ND	ND	13.32 ± 0.13
C16:0*	29.52 ± 0.40	5.35 ± 0.19	ND	37.32 ± 0.32	7.58 ± 0.25	ND
C16:0 iso	ND	ND	5.39 ± 0.20	ND	ND	10.12 ± 0.17
C17:0	0.34 ± 0.06	ND	ND	ND	ND	ND
C17:0 cyclo*	8.22 ± 0.13	7.30 ± 0.09	ND	22.61 ± 0.25	10.05 ± 0.17	ND
C18:0	ND	ND	ND	0.83	ND	ND
C18:1W7C*	0.40 ± 0.02	1.59 ± 0.05	3.94 ± 0.16	7.31 ± 0.16	13.59 ± 0.30	22.19 ± 0.40
C18:1W9C*	21.52 ± 0.32	38.66 ± 0.36	43.21 ± 0.42	ND	ND	ND
19:0 Cyclo*	ND	ND	ND	6.68 ± 0.20	1.93 ± 0.12	ND

Values are represented as percentage of total fatty acids; all experiments were carried out in triplicate and the average values of fatty acids were calculated with arithmetic mean

SS sodium succinate, *ND* not detected

* The statistically significant differences in fatty acids are indicated by asterisk

Table 4 Comparative analysis of total cellular fatty acids composition of strains AR-4, AR-6 and AR-7 under aerobic condition

Total fatty acids	*Enterobacter* sp. AR-4			*Delftia tsuruhatensis* AR-7			*Bacillus* sp. AR-6		
	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 M	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM
Total saturated fatty acids	42.30	54.92	63.47	46.06	71.77	103.92	16.58	46.60	76.39
Total unsaturated fatty acids	19.53	2.39	ND	6.69	2.55	ND	5.39	5.24	2.65
Total cyclo fatty acids	9.51	17.32	22.55	23.53	36.56	45.70	1.43	4.82	12.59
Total anteiso fatty acids	ND	ND	7.90	ND	ND	9.94	ND	ND	10.81
Total iso fatty acids	ND	ND	11.49	ND	ND	17.55	ND	ND	63.85

Values are represented as percentage of total fatty acids

SS sodium succinate, *ND* Not detected

Table 5 Comparative analysis of total cellular fatty acids composition of strains AR-4, AR-6 and AR-7 under anaerobic condition

Total fatty acids	*Enterobacter* sp. AR-4			*Delftia tsuruhatensis* AR-7		
	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM	SS (Control)	Se^{+4} 50 mM	Se^{+4} 500 mM
Total saturated fatty acids	40.52	8.94	ND	45.0	10.19	ND
Total unsaturated fatty acids	21.92	40.25	47.15	7.31	13.59	23.15
Total cyclo fatty acids	8.22	7.30	ND	29.29	11.98	ND
Total anteiso fatty acids	ND	ND	9.65	ND	ND	13.32
Total iso fatty acids	ND	ND	16.75	ND	ND	18.27

Values are represented as percentage of total fatty acids

SS Sodium succinate, *ND* not detected

Conclusion

This work clearly shows that the adaptive mechanisms (changes in the FAMEs profiles and cell sizes) are highly influenced in presence and absence of oxygen in the selenium-resistant strains AR-4, AR-6 and AR-7. The occurrence of alternative adaptive mechanisms towards higher concentration of Se^{+4} under different environmental conditions also indicate the possibility of implementation of strains AR-4, AR-6 and AR-7 (which could tolerate up to 500 mM (85,000 μg ml^{-1} Se^{+4}) for development of a bioremediation process for decontamination of soils and sites that are heavily contaminated with seleniferous pollutants. This study also gives an insight into the molecular basis of such adaptive responses, which could be exploited further to construct genetically modified organisms with vast metabolic potential and unique growth/tolerance to higher concentration of heavy metals.

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