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ORIGINAL ARTICLE

Resistance of extremely halophilic archaea to zinc and zinc oxide nanoparticles

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Abstract Industrialization as well as other anthropogenic activities have resulted in addition of high loads of metal and/or metal nanoparticles to the environment. In this study, the effect of one of the widely used heavy metal, zinc (Zn) and zinc oxide nanoparticles (ZnO NPs) on extremely halophilic archaea was evaluated. One representative member from four genera namely *Halococcus, Haloferax, Halorubrum* and *Haloarcula* of the family *Halobacteriaceae* was taken as the model organism. All the haloarchaeal genera investigated were resistant to both $ZnCl_2$ and ZnO NPs at varying concentrations. *Halococcus* strain BK6 and *Haloferax* strain BBK2 showed the highest resistance in complex/minimal medium of up to 2.0/1.0 mM $ZnCl_2$ and 2.0/1.0–0.5 mM ZnO NP. Accumulation of $ZnCl_2$/ZnO NPs was seen as *Haloferax* strain BBK2 (287.2/549.6 mg g^{-1}) > *Halococcus* strain BK6 (165.9/388.5 mg g^{-1}) > *Haloarcula* strain BS2 (93.2/28.5 mg g^{-1}) > *Halorubrum* strain BS17 (29.9/16.2 mg g^{-1}). Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM–EDX) analysis revealed that bulk $ZnCl_2$ was sorbed at a higher concentration (21.77 %) on the cell surface of *Haloferax* strain BBK2 as compared to the ZnO NPs (14.89 %).

Keywords Halophilic · Archaea · *Halococcus* strain BK6 · *Haloferax* strain BBK2 · *Halorubrum* strain BS17 · *Haloarcula* strain BS2 · $ZnCl_2$ · ZnO nanoparticles · Metal tolerance · Growth kinetics

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Introduction

Rapid industrialization and other anthropogenic activities have resulted in drastic environmental pollution. Natural processes like surface runoffs, weathering and/or erosion and anthropogenic activities like mining, industrial effluents, agricultural runoffs and sewage have led to the accumulation of toxic metals and their derivatives like nanoparticles in the environment (Paula et al. 2013; Zhao et al. 2012). Zinc and zinc oxide nanoparticles (ZnO NPs) are of high concern because of their increasing demand in electroplating, galvanization, cosmetics, sunscreens, paints, food industry, anticancer drugs, antimicrobials, ceramics, and semiconductors (Monteiro et al. 2011; Li et al. 2011).

ZnO NPs have been extensively studied for their antifungal (*Candida albicans*), antibacterial (*Escherichia coli*) and antiviral (bacteriophages MS2) effect (Lipovsky et al. 2011; You et al. 2011). There are numerous reports on resistance and/or tolerance of metals by bacterial strains either individually or in consortium (non halophilic) (Gadd 2009). Various mechanisms of Zn resistance such as physical bioadsorption/sorption, ion exchange, bioprecipitation and intracellular accumulation in microorganisms such as bacteria (Gram-positive and Gram-negative), cyanobacteria (*Microcystis aeruginosa*) archaea (*Halobacterium saccharovorum*) and eukarya (diatoms) have been revealed (Gadd 2009; Green-Ruiz et al. 2008; Zeng and Wang 2009; Guine et al. 2006; Mangold et al. 2013; Williams et al. 2013; Gelabert et al. 2006).

Li et al. (2011) studied the antibacterial activity of ZnO NPs on bacterial cells and found that Gram-negative bacteria (*Pseudomonas putida* and *E. coli*) are more resistant than Gram-positive bacteria (*Bacillus subtilis*). On the other hand, Sinha et al. (2011) investigated the toxic effect of silver and zinc oxide nanoparticle on mesophilic and

halophilic bacterial cells and concluded that Gram-positive halophiles were able to withstand the toxicity better than their Gram-negative counterparts. Choudhury and Srivastava (2001) compared the data of microorganisms resisting/combating high Zn by extracellular accumulation or intracellular sequestration by metallothioneins (MT) and efflux by Zn-effluxing ATPases (P-type ATPases) such as ZiaA in *Synechocystis* and ZntA in *E. coli*.

Extremely halophilic archaea (order *Halobacteriales*, family *Halobacteriaceae*, phylum *Euryarchaeota*, domain *Archaea*) inhabit hypersaline regions. (Ma et al. 2010; Mani et al. 2012). They are also reported to have survived approximately 250 millions of years by getting entrapped in salt crystals (Legat et al. 2013; Schubert et al. 2010; Bragança and Furtado 2009). They are polyextremophilic surviving extreme conditions such as high salinity, high temperatures, neutral to alkaline pH, low water activity (a_w), and extreme gamma radiations (Kottemann et al. 2005). Studies on Zn resistance in halophilic archaea are scarce. Kaur et al. (2006) proved that P1 ATPases (ZntA and YvgX) are responsible for active efflux of the Zn(II) along with other metals like Co(II), Ni(II) and Cu(II) in *Halobacterium salinarum* NRC-1. This study aimed at evaluating the resistance of extremely halophilic archaea to Zn and ZnO NPs.

Materials and methods

Zinc and zinc oxide NPs solutions

Zinc chloride ($ZnCl_2$) was obtained from Sd fine chem. Ltd, Mumbai and Zinc oxide nanoparticles (ZnO NPs) with average particle size < 100 nm were obtained from Sigma-Aldrich, USA. Stock solution of ZnO NPs (40.695 gL^{-1}) was prepared in deionised water, sonicated at pulse rate of 3–5 Hz for 15 min (Microson^{TM} Sonicator) and autoclaved. Stock solutions of 0.5 M $ZnCl_2$ (68.145 gL^{-1}) were prepared in sterile deionized water.

Extremely halophilic archaeal strains and growth medium

Haloarchaeal strains belonging to four different genera used in this study were isolated from salterns of Goa (Mani et al. 2012) and deposited in Microbial Culture Collection (MCC), National Centre for Cell Science, Pune, India. They are *Haloferax volcanii* strain BBK2 (GenBank/EMBL/DDBJ accession number AB588756; MCC2589), *Halococcus salifodinae* strain BK6 (GenBank/EMBL/DDBJ accession number AB588757; MCC2602), *Haloarcula japonica* strain BS2 (GenBank/EMBL/DDBJ accession number HQ455798; MCC2588), and *Halorubrum* spp. strain BS17 (GenBank/EMBL/DDBJ accession number AB971753; MCC2603). Depending upon the strain, two types of complex media were used for optimum growth of the cultures. BBK2 and BK6 were grown in NaCl–Tryptone Yeast Extract (NTYE) medium, while BS2 and BS17 were grown in NaCl–Tryptone (NT) medium, both containing 25 % (w/v) NaCl. The metal studies were carried out in minimal media (referred as NGSM media) with 0.2 % glucose (w/v) as carbon source supplemented with 0.1 % yeast extract (NGSM) (Salgaonkar et al. 2012).

Minimum inhibitory concentration (MIC) studies

MIC of the heavy metal and metal nanoparticles was determined by growing the cultures in complex (NTYE/NT) and minimal media (NGSM) incorporated with $ZnCl_2$ and ZnO NPs. The MIC was noted by gradually increasing the concentration of metal and metal NPs in the media until the culture ceased to grow. The concentrations of $ZnCl_2$ and ZnO NPs employed for MIC studies were 0.1, 0.5, 1.0, 2.0 and 4.0 mM. The growth was recorded after 5–15 days of incubation.

Growth kinetics in presence of Zn and ZnO NPs

The growth kinetics were studied by culturing the selected strains in NGSM with Zn and ZnO NPs, concentrations nondetrimental to their growth. They were BK6 (0.5 mM), BBK2 (0.5 mM), BS2 (0.1 mM) and BS17 (0.1 mM). Respective controls were maintained without the Zn and ZnO NPs for all experiments. All the flasks were incubated at 37° C and 110 rpm. The culture growth was monitored after every 24-h interval at 600 nm by UV–Vis spectrophotometer (UV-2450 Shimadzu, Japan). The growth rate (μ) and lag phase time (λ) were calculated from plot of optical density (OD_{600} nm) against time using the curve-fitting DMFit programme (http://www.ifr.ac.uk/safety/DMfit) (Baranyi and Roberts 1994).

Pigments analysis of haloarchaeal strains grown in presence of Zn and ZnO NPs

Pigments were extracted from stationary phase culture (8–10 days old). The cells were harvested by centrifugation at 8000 rpm for 10 min. To one set of the cell pellet, acetone was added and to the second set chloroform: methanol in the ratio of (2:1 v/v) was added. The mixture was vortexed for 5 min until entire pigment (orange–red) was extracted in the solvent. The solvent fraction containing the pigment was separated from the cell debris (colorless or white) by centrifugation at 8000 rpm for 10 min. The

supernatant was then scanned between 190–800 nm using UV–visible spectrophotometer (Shimadzu UV-2450, Japan).

Zn accumulation study

1. Atomic absorption spectroscopy (AAS)

10 ml of respective haloarchaeal culture, grown in the presence of $ZnCl_2$ and ZnO NPs (0.1 and/0.5 mM), was centrifuged at 10,000 rpm for 15 min. The cell pellets were hydrolysed with concentrated nitric acid: sulphuric acid in the ratio of 3:1 (v/v). The mixture was further completely digested in a sand bath at 100 °C for 3 h till clear solution was obtained (Das et al. 2014). The solutions were analyzed for Zn content by GBC atomic absorbtion spectrophotomer (AAS) after appropriate dilution. Standard curve was obtained using a stock of 10 mg/l Zn^{2+} solution. The cell dry weight (CDW) of the haloarchaeal cultures were determined by centrifuging the culture broth at 10,000 rpm for 15 min and drying overnight at 80 °C.

2. SEM–EDX analysis

Late log phase cells of all four haloarchaeal isolates *Haloferax s*train BBK2, *Halococcus s*train BK6, *Haloarcula s*train BS2 and *Halorubrum s*train BS17 grown in the presence of Zn/ZnO NPs were smeared onto glass slides/coverslips, air dried and desalted with 2 % acetic acid. The cells were fixed for 10 h with 2 % glutaraldehyde. Samples were dehydrated by exposing to a series of increasing acetone concentrations (10, 30, 50, 70, 90 %), each for 10 min and finally with 100 % acetone for 30 min. The samples were mounted onto stubs followed by gold coating for SEM–EDX analysis (JEOL-5800 LV SEM). Cobalt stub was used as the standard for EDX analysis.

3. X-ray diffraction studies (XRD)

100 ml of cells of *Haloferax* strain BBK2 grown in the presence of 0.5 mM $ZnCl_2$/0.5 mM ZnO NPs was harvested by centrifuging at 8,000 rpm for 20 min. The supernatant was discarded and the cell pellet obtained was dialysed against distilled water for 20 h with regular changes of water after every 3–4 h. The dialysed samples were dried at 80 °C in a hot air oven for 24 h. With the help of mortar and pestle, the dried cells were pulverized and the X-ray diffraction of the powderized samples was carried at 5°–75° with scanning speed of 2° min^{-1} using the Rigaku Mini-Flex II powder X-ray diffractometer. Cells of *Haloferax* strain BBK2 grown in absence of metal prepared in the same way were used as control.

Results and discussion

MIC of Zn and ZnO NPs

The minimal inhibitory concentration (MIC) of Zn and ZnO NPs on extremely halophilic archaea was assessed as the minimum Zn concentration that inhibits the growth. The MIC results in NTYE/NT and NGSM are represented in Table 1. The $ZnCl_2$ resistance in complex/minimal media was seen as *Halococcus* strain BK6 (2.0/1.0 mM) > *Haloferax* strain BBK2 (2.0/1.0 mM) > *Halorubrum* strain BS17 (0.5/0.5 mM) = *Haloarcula* strain BS2 (0.5/0.1 mM) whereas for ZnO NPs resistance was BK6 (2.0/1.0 mM) > BBK2 (2.0/0.5 mM) > BS17 (0.5/0.5 mM) > BS2 (0.1/0.1 mM).

Zinc (Zn) is essential for proper functioning of large number of metalloproteins (Zn-binding proteins) and is required by organisms of all three domains of life (archaea, bacteria and eukaryote) thereby making it one of the key metal of life (Andreini et al. 2006). However, metals in excess are detrimental and cause cellular damage (Bini 2010). Zn is toxic to cells due to the formation of reactive oxygen species (ROS) there by inhibiting some of the vital enzymes like endonucleases, DNA glucosylases, etc. Acosta et al. (2011) and Zhao et al. (2013) investigated that increase in ionic strength (salinity) increases the concentration of metal (Pb, Cu, Cd, Zn and Mn) released. High concentrations of $MgCl_2$ and NaCl led to an increased release of Zn from the sediments there by increasing its mobility and bioavailability.

Presence of high concentrations of NaCl increases the toxicity of Zn due to the formation of $ZnCl^-$ species which is more toxic than the cationic Zn^{2+} (Nieto et al. 1987). Among all haloarchaeal strains screened, the genera *Halococcus* and *Haloferax* showed the best resistance and tolerated highest amount of both Zn and ZnO NPs in complex NTYE medium and the minimal medium NGSM. Nieto et al. (1987) in his study of haloarchaeal susceptibility to different heavy metals found that the MIC of Zn was 0.05–0.5 mM. Williams et al. (2013) reported that haloarchaeal strains *Halobacterium saccharovorum* can tolerate only up to 0.01 mM of Zn. The MIC of Zn of *Haloferax* strain BBK2 in NTYE and NGSM media (2.0/1.0 mM) was much higher as compared to reports by Popescu and Dumitru (2009) which was 0.5–1.0 mM of Zn. Gunalan et al. (2012) and Premanathan et al. (2011) reported MIC values of ZnO NPs for Gram-positive organisms like *Staphylococcus aureus* is in the range of 0.8–1.5 mM where as that of Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa* to be 6.1 mM.

In metal microbe interactions, the cell wall is the first part of microbe which will contact and interact with the metal. Studies on metal resistance in halophilic bacteria

Table 1 Minimal inhibitory concentration (MIC) of $ZnCl_2$ (heavy metal) and ZnO NPs (metal nanoparticles) on four extremely halophilic archaeal genera *Halococcus*, *Haloferax*, *Halorubrum* and *Haloarcula* grown in complex (NTYE/NT) and minimal (NGSM) media

	Extremely halophilic archaeal cultures							
	Halococcus strain BK6		*Haloferax* strain BBK2		*Halorubrum* strain BS17		*Haloarcula* strain BS2	
Growth media	NTYE	NGSM	NTYE	NGSM	NT	NGSM	NT	NGSM
Heavy metal ($ZnCl_2$) mM								
Control	+++	+++	+++	+++	+++	+++	+++	+++
0.1	+++	+++	+++	+++	++	++	++	++
0.5	+++	++	++	++	+	+	+	±
1	++	++	+	+	−	−	−	−
2	+	−	+	−	−	−	−	−
4	±	−	±	−	−	−	−	−
Metal nanoparticles (ZnO NPs) mM								
0.1	+++	+++	+++	+++	++	++	++	+
0.5	+++	++	++	+	+	+	−	−
1	++	++	+	−	−	−	−	−
2	+	−	+	−	−	−	−	−
4	±	−	±	−	−	−	−	−

+++ very good growth, ++ good growth, + growth, ± not sure, − no growth

done by Al-Momani et al. (2007) indicated that the metal was accumulated on the cell wall, plasma membrane as well as in the cytoplasm. Li et al. (2011) studied the susceptibility of Gram-positive (*Bacillus subtilis*) and Gram-negative (*Pseudomonas putida* and *E. coli*) bacteria to ZnO NPs and found that Gram-negative organisms were more resistant to ZnO NPs. The cell wall of Gram-positive bacteria comprises of thicker peptidoglycan as compared to their Gram-negative counterparts which have an outer membrane. The outer membrane acts as impermeable lipid barrier and hence most of Gram-negative bacteria are resistant to most of the toxic substances like antibiotics, metals, etc.

Increased concentrations of zinc results in inhibition of the electron transport chain where as ZnO NPs results in the formation of reactive oxygen species (ROS) and lipid peroxidation resulting in apoptosis in human myeloblastic leukemia cells—HL60 (Premanathan et al. 2011). The toxicity of the ZnO NPs and their bulk counterparts on eukaryotes like zebrafish is found to be in the range of 0.04–0.099 mM (Xiong et al. 2011).

Effect of Zn and ZnO NPs on growth of halophilic archaea

Growth kinetics were studied in NGSM medium (Fig. 1; Table 2) and the concentration of $ZnCl_2$/ZnO NPs was selected based on the MIC results for the respective haloarchaeal strains.

Haloferax strain BBK2 in presence of 0.5 mM of $ZnCl_2$/ZnO NPs reached its maximum OD of 1.31/1.4 in 7 days which was almost same when compared to the control, i.e., 1.36 in 6 days. The culture grew with doubling time of 37.9 h which increased with $ZnCl_2$/ZnO NPs to 47.0/47.5 (Fig. 1).

Halococcus strain BK6 in presence of 0.5 mM of $ZnCl_2$/ZnO NPs reached its maximum optical density (OD at 600 nm) of 1.22/0.69 in 7 days which was little lower when compared to control, i.e., 1.44 in 3 days (Fig. 1). The culture grew with doubling time of 16.1 h which increased with $ZnCl_2$/ZnO NPs to 53.6/72.2.

Growth of *Halorubrum* strain BS17 was very slow with maximum OD of 0.87/0.92 in 7 days in presence of 0.1 mM $ZnCl_2$/ZnO NPs which was comparable to the control, i.e., 0.84 in 7 days (Fig. 1). The culture grew with doubling time of 92.4 h which decreased with bulk $ZnCl_2$ to 86.4 h and increased with ZnO NPs to 97.6 h.

Haloarcula strain BS2 in presence of 0.1 mM of $ZnCl_2$/ZnO NPs reached its maximum OD of 1.15/1.19 in 7 days which was almost same when compared to the control, i.e., 1.09 in 7 days. The culture grew with doubling time of 49.9 h which surprisingly decreased with $ZnCl_2$/ZnO NPs to 35.7/35.0 (Fig. 1).

The four haloarchaeal genera *Halococcus*, *Haloferax*, *Haloarcula* and *Halorubrum* when grown in presence of Zn and ZnO NPs showed varying resistance in both complex (NTYE/NT) and minimal medium. The cultures exhibited increased resistance in complex media when compared with the minimal medium. This may be due to the complex formation by the media ingredients with the metal which decreases the availability of metal and/or metal NPs to the microorganisms. Similar observation was made by

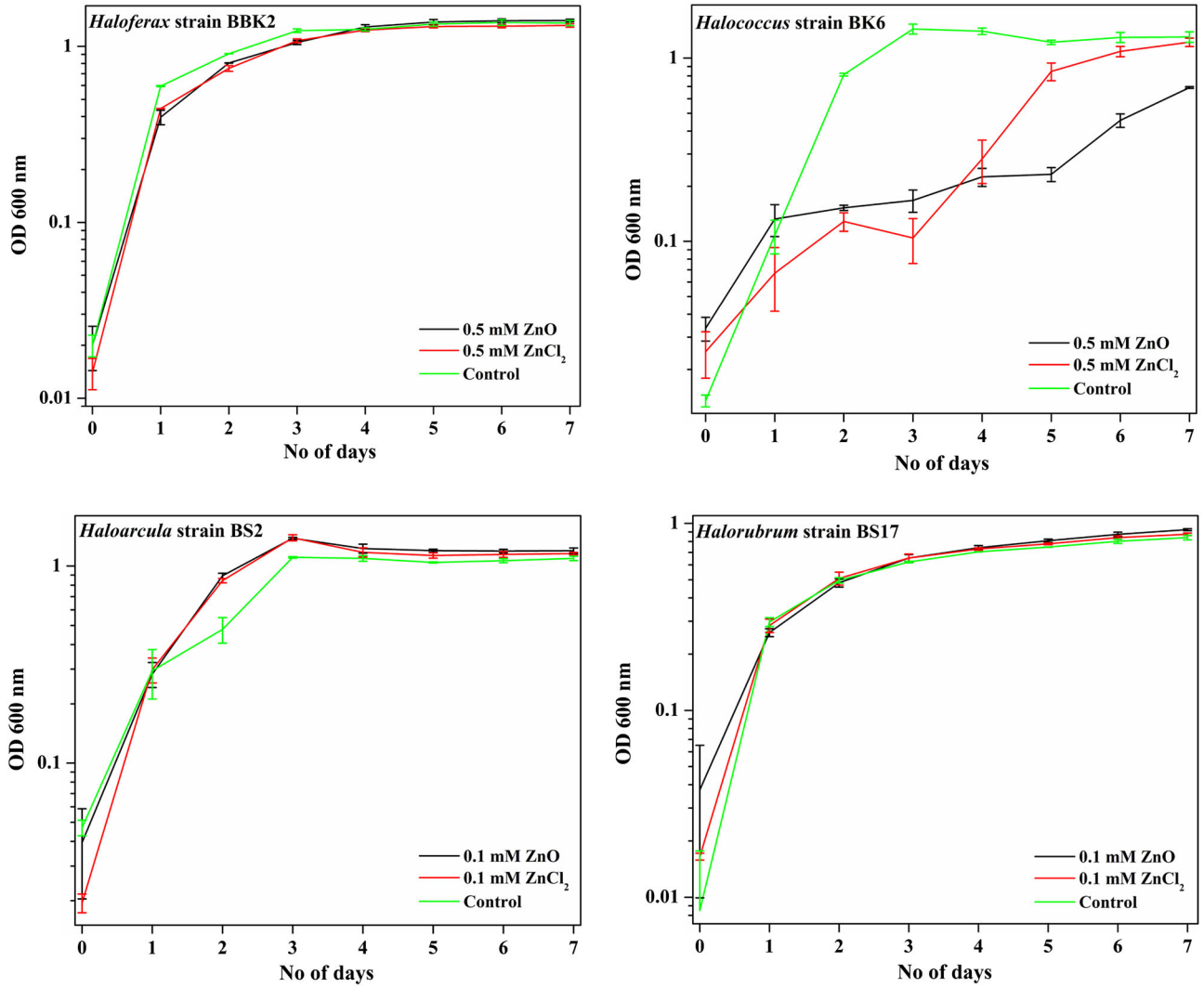

Fig. 1 Growth profile of the extremely halophilic archaeal cultures *Haloferax* strain BBK2, *Halococcus* strain BK6, *Halorubrum* strain BS17 and *Haloarcula* strain BS2 grown in NGSM with $ZnCl_2$ and ZnO NPs

Table 2 Comparative study of the effect of $ZnCl_2$ (heavy metal) and ZnO NPs (metal nanoparticle) on the growth profile of the haloarchaeal genera during growth in NGSM

Haloarchaeal isolates	Control			$ZnCl_2$			ZnO		
	λ (h)	μ (h^{-1})	t (h)	λ (h)	μ (h^{-1})	t (h)	λ (h)	μ (h^{-1})	t (h)
Halococcus strain BK6 (1.0 mM)	29.7	24.7	16.1	70.5	7.4	53.6	110.4	5.5	72.2
Haloferax strain BBK2 (0.5 mM)	–	10.4	37.9	–	8.4	47.1	–	8.3	47.5
Haloarcula strain BS2 (0.1 mM)	–	7.9	49.9	–	11.1	35.7	–	11.3	35.0
Halorubrum strain BS17 (0.1 mM)	–	4.3	92.4	–	4.5	86.4	–	4.0	97.6

λ lag phase time, μ growth rate, t doubling time

Bragança and Furtado (2013) while studying the resistance of the haloarchaeon *Halobacterium* strain R1 to cadmium when grown in minimal medium. The overall doubling time of haloarchaea increased in presence of $ZnCl_2$ and further increased in ZnO NPs. Interestingly, *Haloarcula* strain BS2 showed better growth in presence of bulk as well as Zn NPs.

Pigment analysis

The haloarchaeal strains when grown in NGSM showed pink or mauve pigmentation (Fig. 2). The pigment was unaltered during growth in the presence of both bulk Zn or ZnO NPs. Extraction of the pigments in acetone showed characteristic peaks at 389, 471, 496 and 528 nm corresponding to bacterioruberins. Interestingly, a shift in the peaks (323, 394, 477, 503 and 536) was observed when pigments were extracted in chloroform:methanol (2:1 v/v).

Zn accumulation studies

The amount of Zn accumulated by whole cells of each genus varied when grown in $ZnCl_2$ and ZnO NPs. The $ZnCl_2$ accumulation analyzed by atomic absorption spectroscopy was seen as *Haloferax* strain BBK2 (287.2 mg g^{-1}) > *Halococcus* strain BK6 (165.9 mg g^{-1}) > *Haloarcula* strain BS2 (93.2 mg g^{-1}) > *Halorubrum* strain BS17 (29.9 mg g^{-1}), whereas for ZnO NPs accumulation was *Haloferax* strain BBK2 (549.2 mg g^{-1}) > *Halococcus* strain BK6 (388.5 mg g^{-1}) > *Haloarcula* strain BS2 (28.5 mg g^{-1}) > *Halorubrum* strain BS17 (16.2 mg g^{-1}) (Table 3).

Among the four genera, *Haloferax* showed a higher Zn accumulation although both *Halococcus* and *Haloferax* were grown in 0.5 mM of $ZnCl_2$ and 0.5 mM of ZnO NPs. Interestingly, the amount of ZnO NPs accumulated was more as compared to $ZnCl_2$ as revealed by AAS. However, *Haloarcula* strain BS2 and *Halorubrum* strain BS17 grown in 0.1 mM of $ZnCl_2$ and 0.1 mM of ZnO NPs showed higher accumulation of $ZnCl_2$ than ZnO NPs. Our recent study by Das et al. (2014) indicated that *Haloferax* strain

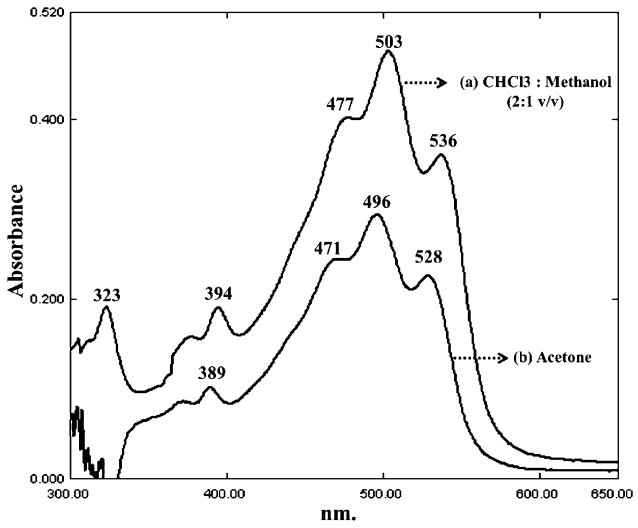

Fig. 2 Spectrophotometric scans of pigments from *Halorubrum* strain BS17 grown in NGSM containing 0.1 mM $ZnCl_2$and extracted using *a* chloroform: methanol (2:1 v/v) and *b* acetone

Table 3 Bioaccumulation of $ZnCl_2$ and ZnO NPs by the haloarchaeal strains

Haloarchaeal strains	Bioaccumulation (mg g^{-1})	
	$ZnCl_2$	ZnO NPs
Haloferax strain BBK2	287.2	549.6
Haloarcula strain BS2	93.2	28.5
Halorubrum strain BS17	29.9	16.2
Halococcus strain BK6	165.9	388.5

BBK2 was also resistant to cadmium (Cd) and accumulated 21.08 and/or 15.19 % of Cd in the presence of 0.5/1 mM Cd. *Haloferax* is known to produce exopolysaccharide (EPS) which protects the cells from direct contact with the metals and hence its resistance (Poli et al. 2011). Halococcal cells resist lysis when suspended in low-osmolarity solutions (3.5 % NaCl) (Mani et al. 2012; Legat et al. 2013). The cell wall of *Halococcus* is composed of heteropolysaccharide with acetylated amino sugars unlike glycoprotein S-layer in genus *Haloferax* (Schleifer et al. 1982; Kandler and Konig 1998). This could be a contributing factor for higher metal resistance of these organisms. On the other hand, Al-Mailem et al. (2011) studied the resistance and mercury (Hg) volatilization (Hg^{2+} to Hg^{o}) and oil consumption capability of haloarchaea viz *Haloferax*, *Halobacterium* and *Halococcus* and found that genus *Halococcus* was the most efficient in Hg volatilization as compared with the other genera.

The haloarchaeal cells grown in NGSM medium with $ZnCl_2$/ZnO NPs showed peaks for Zn on the cell surface when examined by SEM–EDX. However, the amount (percent) of Zn sorbed on the surface of cells of *Haloferax* strain BBK2 grown in the presence of $ZnCl_2$ was greater (21.77 %) than cells grown in presence of ZnO NPs (14.89 %) (Fig. 3). Peaks of K, Mg, Ca, which are components of the growth medium, were also detected in SEM–EDX analysis. Recent study by Williams et al. (2013) investigated the ability of halophilic archaeon *Halobacterium saccharovorum* to tolerate up to 0.01 mM Zn with maximum of 68.6 % biosorption, while at higher Zn concentration (0.1 mM) only 19.5 % of Zn was biosorbed.

Figure 4 depicts the XRD pattern of dialysed cells of *Haloferax* strain BBK2 grown in presence of $ZnCl_2$ and/ZnO NPs. The XRD analysis of the cells showed peaks between 22° and 57° for cells grown in presence of $ZnCl_2$. This indicated the difference in crystallinity due to the sorption of bulk $ZnCl_2$ by the haloarchaeal cell components. At 2θ of 28.8°, weak reflection was seen which could be attributed to the reflection of ZnS sphalerite phase (111) as reported by Dedova et al. 2007 for $ZnCl_2$ sample. The broadening of the peaks indicated the amorphous nature of the sample. However, XRD profile of BBK2 cells grown in

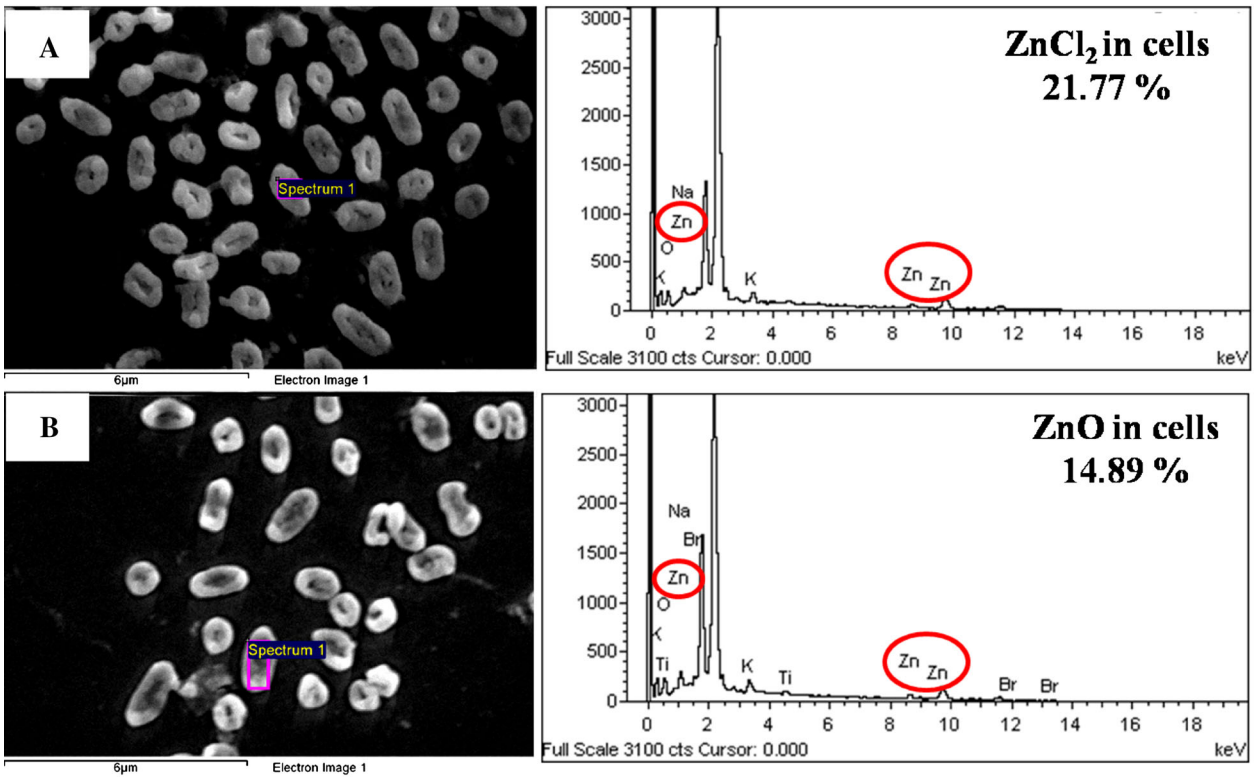

Fig. 3 Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis of haloarchaeon *Haloferax* strain BBK2, grown in NGSM containing 0.1 mM **a** $ZnCl_2$ and **b** ZnO nanoparticles

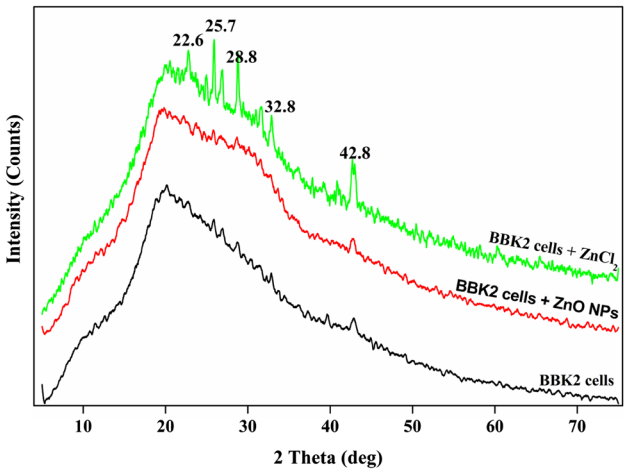

Fig. 4 X-ray diffraction pattern of haloarchaeon *Haloferax* strain BBK2 cells grown in presence of $ZnCl_2$ and ZnO NPs

presence of ZnO NPs and without Zn showed no significant difference.

Conclusions

In conclusion, the four representative genera *Halococcus, Haloferax, Halorubrum* and *Haloarcula* used in the study were able to tolerate and grow in complex as well as minimal media in presence of Zn and ZnO NPs. *Halococcus* strain BK6 and *Haloferax* strain BBK2 showed the best resistance of up to 2.0 and 1.0 mM in both complex and minimal media.

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