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ORIGINAL PAPER

Mechanism of zinc resistance in a plant growth promoting *Pseudomonas fluorescens* strain

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Abstract Bacterial systems have evolved a number of mechanisms, both active and passive, to manage toxic concentrations of heavy metals in their environment. The present study is aimed at describing the zinc resistance mechanism in a rhizospheric isolate, *Pseudomonas fluorescens* strain Psd. The strain was able to sustain an external Zn^{2+} concentration of up to 5 mM in the medium. The strategy for metal management by the strain was found to be extracellular biosorption with a possible role of exopolysaccharides in metal accumulation. The attainment of equilibrium in biosorption reaction was found to be dependent on initial Zn^{2+} concentration, with the reaction reaching equilibrium faster (50 min) at high initial Zn^{2+} concentration. Biosorption kinetics of the process was adjusted to pseudo-first order rate equation. With the help of Langmuir and Freundlich adsorption isotherms, it was established that Zn^{2+} biosorption by the bacterium is a thermodynamically favourable process.

Keywords Zinc · Biosorption · Pseudo-first order kinetics · Langmuir adsorption isotherm · Freundlich adsorption isotherm · Exopolysaccharides

Introduction

Zinc is an essential trace element in all living organisms but may exert toxic effects when it is present in millimolar concentrations (Barceloux 1999; Choudhury and Srivastava 2001a). Therefore, in order to resist high concentrations of this metal and to survive under metal-contaminated habitats, organisms follow different strategies to regulate intracellular Zn levels. A variety of microbial mechanisms exist for metal resistance, including physico–chemical interactions (adsorption to cell wall and other constituents), efflux, intracellular sequestration and/or extracellular precipitation by the excreted metabolites (Ledin 2000; Choudhury and Srivastava 2001a).

Biosorption of metals onto a microbial surface is dependent on the surface properties of the cell such as charge and orientation of metal-binding functional groups and metal speciation and chemistry in aqueous phase (Ledin 2000). Among the known microbial zinc biosorbents are bacteria such as *Pseudomonas* (Bhagat and Srivastava 1994; Pardo et al. 2003; Chen et al. 2005), *Streptomyces* (Rho and Kim 2002), and *Thiobacillus* (Liu et al. 2004); fungi including *Rhizopus* (Preetha and Viruthagiri 2005), *Penicillium* (Tan and Cheng 2003), and *Aspergillus* (Filipovic-Covacevic et al. 2000); and yeasts incuding *Saccharomyces cerevisiae* (Chen and Wang 2007).

Bacterial cell wall is the first barrier encountered by dissolved metals in the environment, and owing to its anionic nature interacts strongly with the metal cations, thus leading to their immobilization (Vijayaraghavan and Yun 2008). Microbial biomass, either dead or live, has been used as metal biosorbents (Taniguchi et al. 2000; Oh et al. 2009) and, thus, may serve as potential tools for bioremediation of heavy metal contaminated environments (Khan et al. 2009). Application of plant growth promoting rhizobacteria (PGPR) in such environments offers an advantage over other microbial systems since such a biomass has tremendous ability to serve as biofertilizers in metal-deficient soils (Whiting et al. 2001). Alternatively, these bacteria can also be used as bioinoculants in the

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rhizosphere of the plants growing in heavy metal-contaminated soils, thus combating the toxic effects of the metals on plants (Belimov et al. 2004; Zhuang et al. 2007).

The aim of the present communication was to decipher the Zn^{2+} resistance mechanism in *Pseudomonas fluorescens* strain Psd, which has earlier been characterized to possess multiple plant growth promoting and biocontrol potential (Upadhyay and Srivastava 2008, 2010, 2011; Kochar et al. 2011). In this study, we report a high level of Zn^{2+} resistance in strain Psd, following biosorption as the mechanism. The non-metabolic nature of Zn^{2+} accumulation was shown with the help of cellular distribution of accumulated Zn^{2+} and effect of growth-inhibitory conditions on metal accumulation. In addition, the process of adsorption was found to be kinetically and thermodynamically favourable. The possible involvement of extracellular polysaccharides (EPS) in Zn^{2+} accumulation process is also indicated. The metal accumulation potential of the strain can be utilized in situ soil bioremediation of Zn^{2+} and provide, inturn, this important micronutrient to the plants, thus contributing towards a better plant growth.

Materials and methods

Organism, culture conditions and chemicals

The strain used in the study, *P. fluorescens* strain Psd, was originally isolated from the roots of *Vigna mungo* and has been characterised earlier by Upadhyay and Srivastava (2008). The strain was maintained on gluconate minimal medium (GMM; Gilotra and Srivastava, 1997) with and without $ZnSO_4 \cdot 7H_2O$. As per the experimental requirement, the medium was supplemented with the appropriate concentration of autoclaved metal salt solution ($ZnSO_4 \cdot 7H_2O$) and/or inhibitor solution (2,4-dinitrophenol and sodium azide) filter-sterilized using 0.2 µ membrane filters (mdi Membrane Technologies, USA). The liquid cultures were raised on controlled environment shaker incubator (Kühner, Switzerland) at 200 rpm at 30 °C for the required period of time. $ZnSO_4 \cdot 7H_2O$ was purchased from Himedia, India, and metal standard used for analysis was purchased from Merck, India. All other chemicals used in the study were purchased from Himedia, India and were of analytical grade. The environmental samples used for the accumulation experiments included Zinc-contaminated soil procured from the area surrounding a zinc industry at Meerakot, Punjab, India (total available Zn^{2+} = 141.67 - mg/L, pH 7.0) and an industrial effluent procured from Common Effluent Treatment Plant at Wazirpur industrial area, New Delhi (Zn^{2+} = 329.67 mg/L, pH 10.5).

Growth response to Zn^{2+}

A loopful of culture from GMM Agar plate was inoculated into 10 mL GMM and grown overnight. An inoculum at OD_{600} of 0.01 was subcultured into 10 mL GMM and GMM containing varying concentrations of Zn^{2+} (0–5 mM of $ZnSO_4 \cdot 7H_2O$). The cultures were grown at 30 °C for 24 h at 200 rpm. Growth was determined after 24 h of growth turbidometrically at 600 nm (Biorad SmartSpec™ Plus, USA) and by performing the viable cell count (cfu/mL) by plating the cell suspension on LB agar medium.

Zn^{2+} estimation

Zn^{2+} content was estimated using the atomic absorption spectrophotometer (Perkin Elemer model AAnalyst400) at 219.86 nm, as described by Choudhury and Srivastava (2001b).

Zn^{2+} accumulation from the medium

Cells exposed to varying concentrations of $ZnSO_4 \cdot 7H_2O$ (0–5 mM) in the GMM for 24 h were harvested by centrifugation at 6,000×*g* for 10 min. The pellet obtained was washed thrice with 0.85 % saline solution and dried at 80 °C till a constant wieght was obatined. The dry biomass was then acid-digested using a mixture of nitric acid and perchloric acid (6:1 v/v) and the Zn^{2+} accumulation was determined as described above. The values are represented as µg Zn^{2+}/mg dry weight.

Cellular distribution of Zn^{2+}

In order to look for the cellular distribution of Zn^{2+} taken up by the cells grown at varying concentrations of Zn^{2+}, the cells were fractionated to separate the cytoplasmic and membrane fractions after 24 h of exposure as per the protocol described by Cha and Cooksey (1991). Precisely, the bacterial biomass was harvested by centrifugation at 6,000×*g* for 10 min followed by washing with phosphate buffered saline (PBS). The suspension was then subjected to pulses of 100 W for 20 s in an ultrasonicator (Sonics and material Inc., USA), and centrifuged at 14,000×*g* for 30 min to separate cytoplasmic fraction in the supernatant from the pellet of cell membranes. The two fractions were streaked on LB agar to ensure complete cell destruction. The fractions were then acid-digested and their respective Zn^{2+} contents were determined by atomic absorption spectrophotometry.

Zinc accumulation from environmental samples

Ten milliliters of ddH_2O was added to 5 g of autoclaved industrial soil and the solution was incubated at 30 °C, 200 rpm for 24 h. Afterwards, this solution was filtered through 0.2 μ filter and was used for Zinc accumulation study alongwith the autoclaved industrial effluent. Bacterial biomass was generated by transferring 1 % of an overnight grown culture of strain Psd in 50 mL GMM followed by incubation at 30 °C, 200 rpm for 8 h. The biomass obtained (87.4 mg fresh weight) bacterial biomass was added to these solutions and kept at 30 °C, 200 rpm. After 24 h, the biomass was harvested by centrifugation and analyzed for total Zn^{2+} accumulation. Also, the cellular distribution of accumulated Zn^{2+} was studied by the method described above. Biomass suspended in autoclaved ddH_2O was used as control.

Zn^{2+} accumulation under growth inhibitory conditions

Accumulation by heat-killed cells

One percent of an overnight grown culture was transferred to fresh medium (10 mL) and incubated at 30 °C, 200 rpm for 8 h for subculturing. The bacterial biomass was harvested by centrifugation at $6{,}000\times g$ for 10 min, suspended in 0.85 % saline solution (10 mL) and incubated at 100 °C water bath for 1 h. Loss of viability was confirmed by streaking a loopful of this material on LB agar plates. Live cells were used as control. The biomass (dead/live) was then centrifuged and re-suspended in 10 mL GMM + 2 mM Zn^{2+} and Zn^{2+} accumulation was estimated after 24 h.

Accumulation by carbon-starved cells

The overnight grown culture was subcultured for 8 h by transferring 1 % of the inoculum to fresh medium. The bacterial biomass was harvested by centrifugation and was suspended in GMM devoid of gluconate for 16 h. Thereafter, the cells were again harvested by centrifugation and suspended in GMM (lacking the carbon source) + 2 mM Zn^{2+} for 24 h, after which the whole-cell Zn^{2+} accumulation was measured. Accumulation by cells grown in presence of carbon source was kept as control.

Accumulation in presence of metabolic inhibitors

Metal accumulation was also checked in presence of two metabolic inhibitors, 2,4-dinitrophenol (2,4 DNP) which is an uncoupler of oxidative phosphorylation; and sodium azide (NaN_3), an inhibitor of cytochrome oxidase. The inhibitors, at their respective IC_{50} (2 mM for 2,4 DNP and 2.5 mM for NaN_3) were added in the medium containing 2 mM Zn^{2+}. The accumulation was determined after 24 h of incubation at 30 °C, 200 rpm. Cells grown in the absence of inhibitor were used as control.

Biosorption kinetics

Time-course kinetics of biosorption was studied by subculturing 1 % of an overnight grown inoculum in 15 mL GMM for 8 h. The biomass obtained, corresponding to 11 mg (dry weight), was suspended in PBS with initial Zn^{2+} concentrations of 155, 233 and 378.3 mg/L respectively. The flasks were incubated at 30 °C, 200 rpm and 1 mL sample was taken out at different time intervals and the value of residual Zn^{2+} in the medium was recorded. Zn^{2+} biosorbed (%) was plotted against time to follow the time-course of Zn^{2+} biosorption. The value of biosorption capacity q (μg of Zn/mg biomass) was calculated from the formula:

$$q = \left[V(C_i - C_f)\right]/M$$

where q = metal uptake (μg of Zn/mg biomass), V = volume of solution in contact flask (mL), C_i = initial Zn^{2+} concentration in solution (μg/mL), C_f = Final Zn^{2+} concentration in solution (μg/mL), M = mass of cells (g).

Further, pseudo-first order kinetics model was applied using the following equation:

$$\log(q_e - q_t) = \log q_e - [(k \cdot t)/2.303]$$

where k is Lagergren rate constant (min^{-1}) (Lagergren 1898) and q_e and q_t signify metal uptake (μg of Zn/mg biomass) at equilibrium and at time (t), respectively.

Adsorption isotherm

For metal biosorption study, 1 % inoculum from an overnight grown culture was subcultured in fresh medium (10 mL) for 8 h. The biomass thus obatined (8 mg dry weight) was suspended in PBS with different concentrations of Zn^{2+} (0, 1, 2, 3, 4, 6, 8, 10 mM) for 24 h. The initial and final Zn^{2+} concentrations of the medium were used to work out two adsorption models, namely, Langmuir and Freundlich isotherms.

Langmuir adsorption isotherm, for adsorption on monolayer surface, can be represented by the following equation:

$$1/q = 1/q_m + (1/q_m \cdot K_L) \cdot \left(1/C_f\right)$$

where q_m is the maximum adsorption capacity (mg/g) and K_L (L/mg) is constant for the model. The applicability of this model was further represented by the equation (Zhou et al. 2009):

$$R_L = 1/(1 + K_L \cdot C_0)$$

where C_o is the highest initial Zn^{2+} concentration used and R_L is a dimensionless constant separation factor. If the value of R_L lies between 0 and 1, Langmuir isotherm is favourable for biosorption.

The Freundlich adsorption isotherm, used for adsorption on heterogenous surfaces is described by the following equation:

$$q = K \cdot C_f^{1/n}$$

The equation for linear fitting is as follows:

$$\log q = \log K + (1/n) \cdot \log C_f$$

where K and n represent the Freundlich's constants signifying adsorption capacity and adsorption intensity, respectively (Zhou et al. 2009).

Exopolysaccharide extraction and estimation

Exopolysaccharide (EPS) extraction was carried from 5 day-spent culture filtrate of the bacterium grown at different Zn^{2+} concentrations (0, 1, 2, 5 mM) in the medium by the method described by Bitton and Freihofer (1978). The concentration of EPS (μg/mL) was derived from a standard curve of D-glucose as per the protocol of Jayaraman (1981).

Statistical analysis

Statistical analysis was performed using Dunnet's *t* test, and data are presented in the form of three replicates with average standard deviation (±SD).

Results

Growth response to $Zn^{2+}$and its accumulation from medium

The differences obtained in net growth at different external Zn^{2+} concentrations are shown in Fig. 1. As is clear from the results, the cells were able to sustain an external Zn^{2+} concentration up to 5 mM both in terms of OD_{600} as well as cfu/mL. The accumulation of Zn^{2+} by whole cells from medium supplemented with increasing $ZnSO_4{\cdot}7H_2O$ concentrations was also analysed. The cells displayed the ability to remove Zn from the medium that was reflected in a net increase in Zn^{2+} content of the cells after 24 h (Fig. 2). The maximum Zn^{2+} uptake of 273 μg/mg dry weight was observed in the cells grown in presence of 5 mM of external Zn^{2+} concentration. Washing with

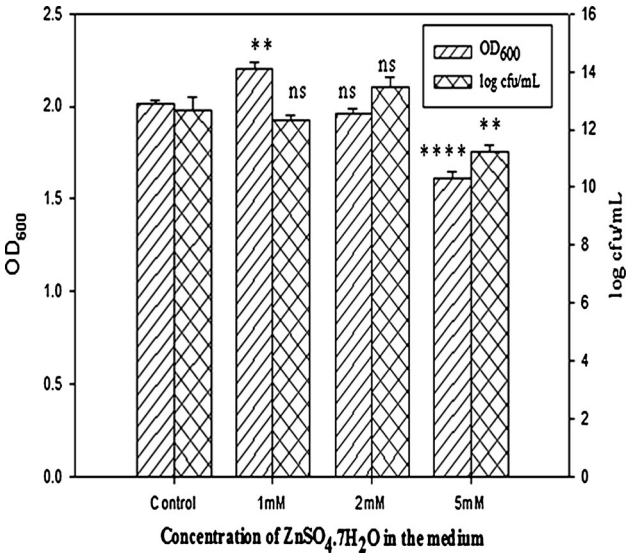

Fig. 1 Net growth response of *P. fluorescens* strain Psd grown in GMM supplemented with different external Zn^{2+} concentrations (1, 2, 5 mM) after 24 h of culture at 30 °C. Cells grown in absence of added $ZnSO_4{\cdot}7H_2O$ were taken as control (**$P < 0.01$; ****$P < 0.0001$; *ns* not significant)

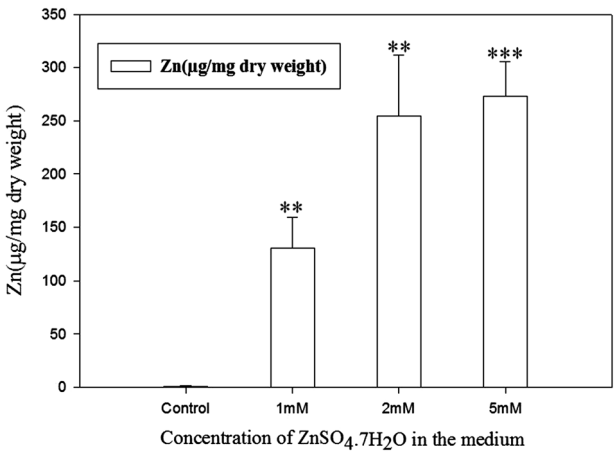

Fig. 2 Net accumulation of Zn^{2+} by whole cells of *P. fluorescens* strain Psd after 24 h of exposure to different Zn^{2+} concentrations (1, 2, 5 mM). Accumulation by cells grown in absence of added $ZnSO_4{\cdot}7H_2O$ was taken as control (**$P < 0.01$; ***$P < 0.001$)

0.85 % saline solution did not lead to any loss of accumulated Zn^{2+} (data not shown).

Cellular distribution of Zn^{2+}

On the basis of higher Zn^{2+} accumulation by the cells, it became mandatory to know its cellular distribution in order to understand the mechanism employed by bacteria to tolerate such high metal concentrations. In this study, when

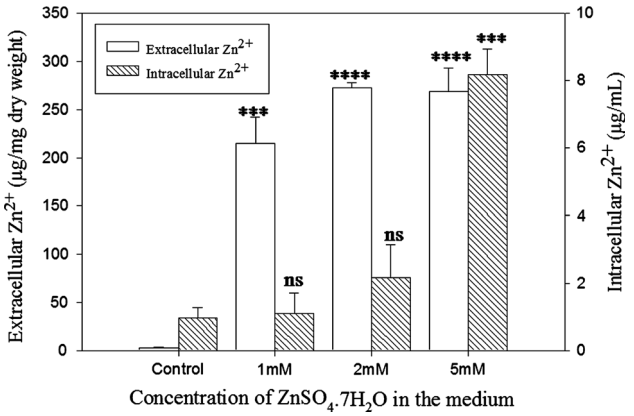

Fig. 3 Cellular distribution of Zn^{2+} in *P. fluorescens* strain Psd grown in GMM substituted with different external Zn^{2+} concentrations (1, 2, 5 mM) after 24 h of culture at 30 °C. Cellular distribution in the cells grown in absence of added $ZnSO_4{\cdot}7H_2O$ was taken as control (***$P < 0.001$; ****$P < 0.0001$; *ns* not significant)

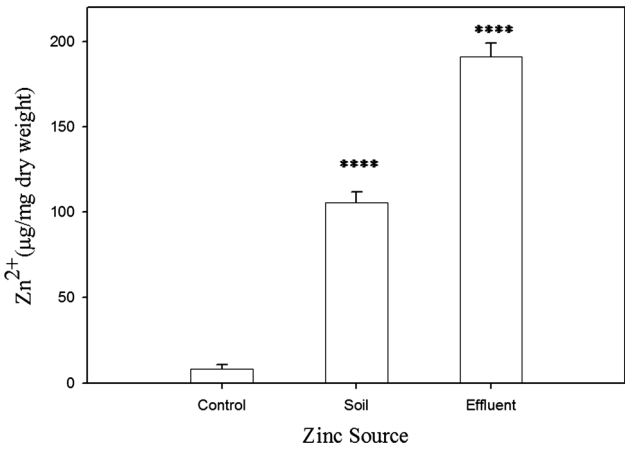

Fig. 4 Whole-cell zinc accumulation by *P. fluorescens* Psd biomass (87.4 mg fresh weight) under simulated environmental conditions after 24 h of exposure at 30 °C. Accumulation by biomass suspended in ddH_2O was taken as control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$)

the cells were grown in increasing Zn^{2+} concentrations, the Zn^{2+} content in the extracellular and intracellular fractions suggested that the maximum accumulation was confined to the outer membrane. The intracellular environment, on the other hand, displayed a very low metal concentration (Fig. 3). It was also checked that washing with PBS did not lead to any loss of accumulated Zn^{2+} (data not shown).

Zinc accumulation from environmental samples

Under simulated environmental conditions, the cells were able to remove 74 and 58 % Zn^{2+} from contaminated soil and an industrial effluent, respectively after a period of 24 h. The respective Zn^{2+} content of the cells after 24 h of exposure to contaminated soil and effluent was 105.29 µg/mg dry weight and 191.08 µg/mg dry weight (Fig. 4). The residual Zn^{2+} concentrations of soil and effluent after 24 h were 38.23 and 139.46 ppm, respectively. Further, from the pattern of cellular distribution of accumulated zinc by the cells, it was observed that membrane fraction accounted for the majority of accumulated zinc [84.7 % (89.48 µg/mg dry weight) in contaminated soil and 62.3 % (119.37 µg/mg dry weight) in effluent] as shown in Fig. 5.

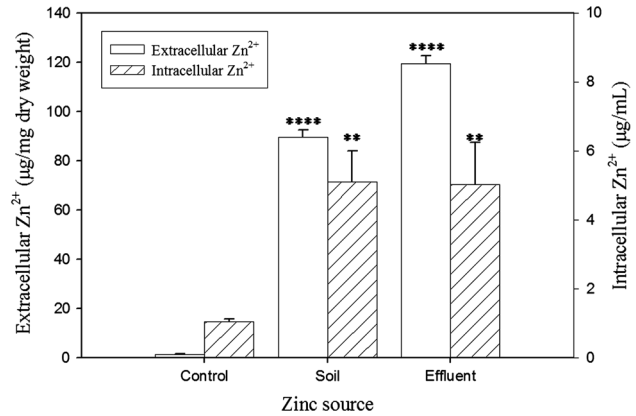

Fig. 5 Cellular distribution of accumulated zinc in *P. fluorescens* Psd exposed to zinc-contaminated soil and industrial effluent for 24 h at 30 °C. Cellular distribution in biomass suspended in ddH_2O was taken as control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$)

Zn^{2+} accumulation under growth inhibitory conditions

In order to look for the involvement of energy requiring mechanisms for Zn^{2+} accumulation in strain Psd, Zn^{2+} content of the cells under various inhibitory conditions was looked at. Figure 6a shows the accumulation of Zn^{2+} by heat-killed cells which is approximately 45 % more than the live cells.

Similarly, the accumulation profile of the strain under other growth-limiting conditions, like carbon source starvation and metabolic inhibitor treatment (Fig. 6b, c) did not vary significantly compared to untreated control, indicating towards the extracellular biosorption of the metal onto the outer membrane. Keeping these preliminary observations in mind, the Zn^{2+} adsorption kinetics was worked out.

Biosorption kinetics

The time taken for biosorption of Zn^{2+} on *P. fluorescens* strain Psd varied with initial Zn^{2+} concentration. At $C_i = 378.3$ mg/L, equilibrium was attained in 50 min as compared to 80 min in case of C_i values of 155 and 233 mg/L (Fig. 7). The concentration of Zn^{2+} at these time points were taken as equilibrium concentration and the

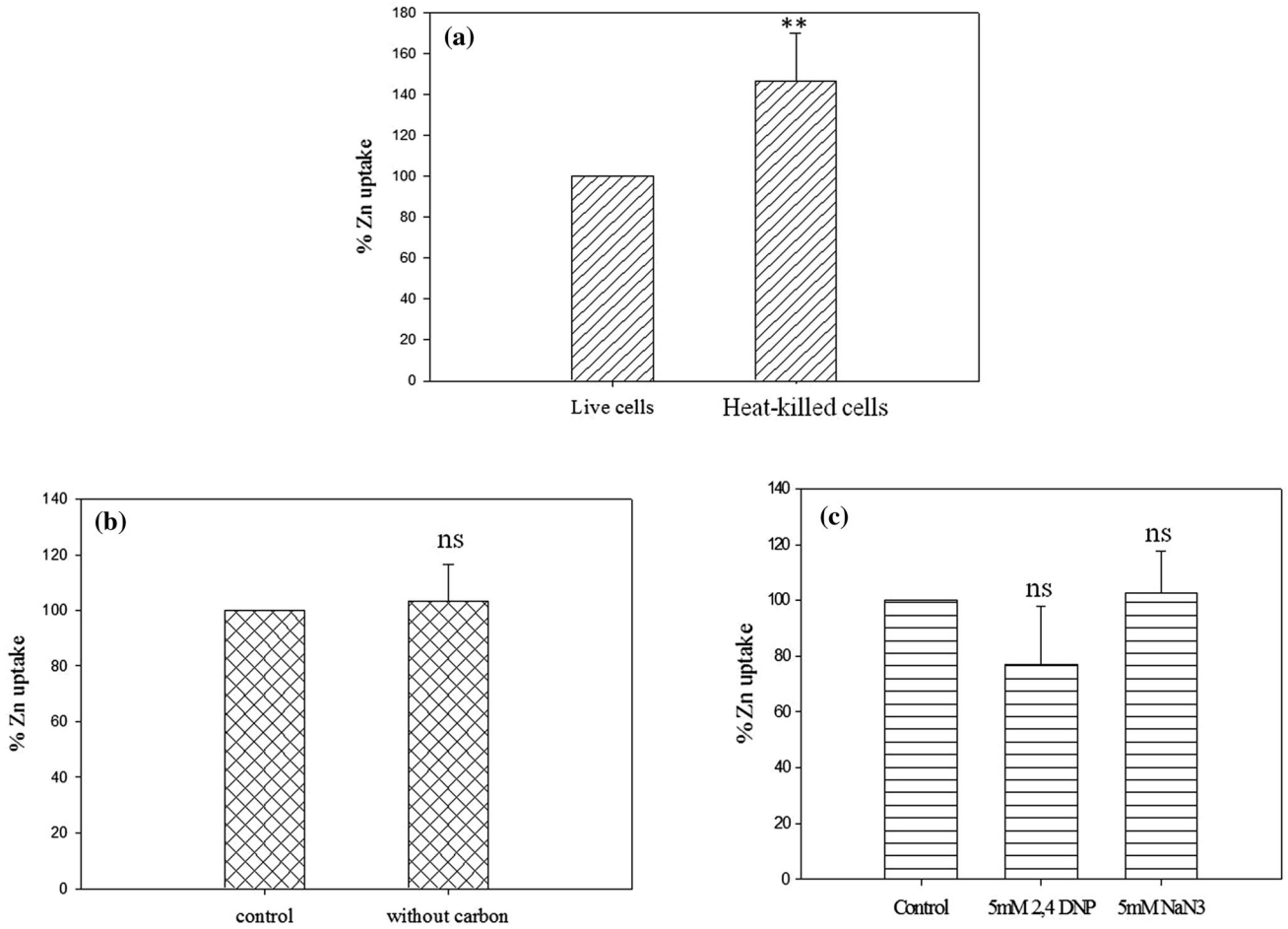

Fig. 6 Whole-cell Zn^{2+} accumulation by *P. fluorescens* strain Psd exposed to external Zn^{2+} concentration of 2 mM $ZnSO_4{\cdot}7H_2O$ for 24 h at 30 °C under different growth inhibitory conditions. **a** Heat-killing, **b** carbon-starvation and **c** treatment with metabolic inhibitors (2,4 DNP and NaN_3). The accumulation is shown as percent Zn^{2+} uptake relative to the control cells where no such treatment was given. Accumulation by control cells is taken as 100 % and the relative Zn^{2+} uptake (%) under various treatments is calculated (**$P < 0.01$; *ns* not significant)

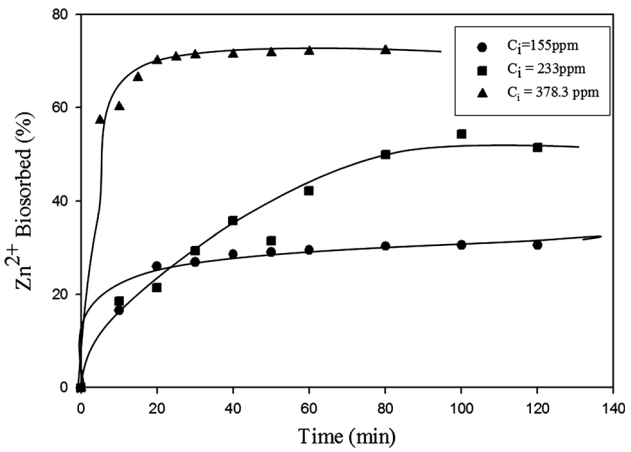

Fig. 7 Zn^{2+} biosorption (%) by *P. fluorescens* strain Psd grown at 30 °C, pH 7.0 with different initial concentrations (C_i = 155, 233, 378.3 ppm)

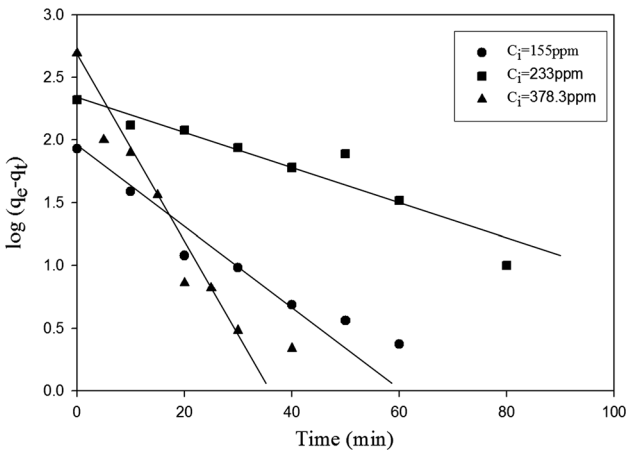

Fig. 8 Pseudo-first order plot for Zn^{2+} biosorption by *P. fluorescens* strain Psd exposed to varying initial Zn^{2+} concentrations (C_i = 155, 233, 378.3 ppm) at 30 °C, pH 7.0

Table 1 Pseudo-first order kinetic parameters for Zn^{2+} biosorption by *P. fluorescens* strain Psd at different initial concentrations

Initial concentration (C_i)	q_e	k	R^2
155	87.09	0.074	0.976
233	229.08	0.036	0.943
378	501.11	0.165	0.960

Table 2 Parameters for Zn^{2+} biosorption by *P. fluorescens* strain Psd obtained from Langmuir and Freundlich models

Langmuir model			Freundlich model		
q_m	K_L	R^2	n	K	R^2
100	1.18×10^{-3}	0.953	1.25	12.58	0.949

value of q (μg of Zn/mg biomass) obtained was designated as q_e. The values when substituted in Lagergren's pseudo-first order rate equation generated a linear plot (Fig. 8), confirming that the process followed pseudo-first order kinetics. These values of pseudo-first order kinetic parameters q_e and k are shown in Table 1.

Adsorption isotherm

Langmuir and Freundlich adsorption isotherms for zinc biosorption are shown in Fig. 9. The values of parameters obtained from both the models (Table 2) indicate that there is no significant difference between the two models in terms of correlation coefficients obtained ($R^2_{Langmuir} = 0.953$; $R^2_{Freundlich} = 0.949$). However, the value of R_L from Langmuir isotherm was found to be 0.9823, indicating that the biosorption process is better suited to Langmuir model.

Estimation of exopolysaccharides

When exopolysaccharide synthesis was studied in the cells in presence of increasing Zn^{2+} concentrations, a progressive enhancement in the levels of EPS synthesized was observed (Fig. 10).

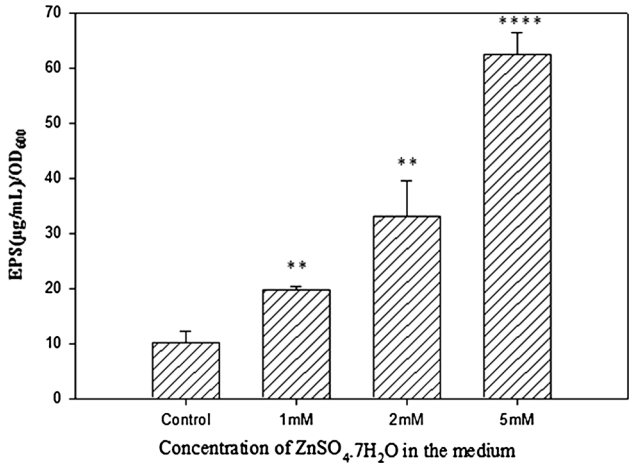

Fig. 10 Exopolysaccharide (EPS) production by *P. fluorescens* strain Psd grown in GMM supplemented with increasing Zn^{2+} concentrations (1, 2, 5 mM) for 5 days at 30 °C. EPS production by the cells grown in absence of added $ZnSO_4 \cdot 7H_2O$ was taken as control (**$P < 0.01$; ****$P < 0.0001$)

Discussion

We have earlier described a rhizosphere isolate, *P. fluorescens* strain Psd possessing multiple plant growth promoting and biocontrol properties (Upadhyay and Srivastava 2008, 2010, 2011; Kochar et al. 2011). In the present study, we have shown that strain Psd has the ability to withstand high concentrations of metal Zn^{2+} as well. The principle mechanism of zinc resistance operating in the strain Psd has been worked out to be biosorption. Bacterial cell's requirement for zinc ranges from 0.5 to 1.0 μM (0.032–0.065 μg/mL) for their optimum growth (Lu et al. 1997). The cells of strain Psd, in contrast, not only displayed the total cellular Zn^{2+} accumulation of up to

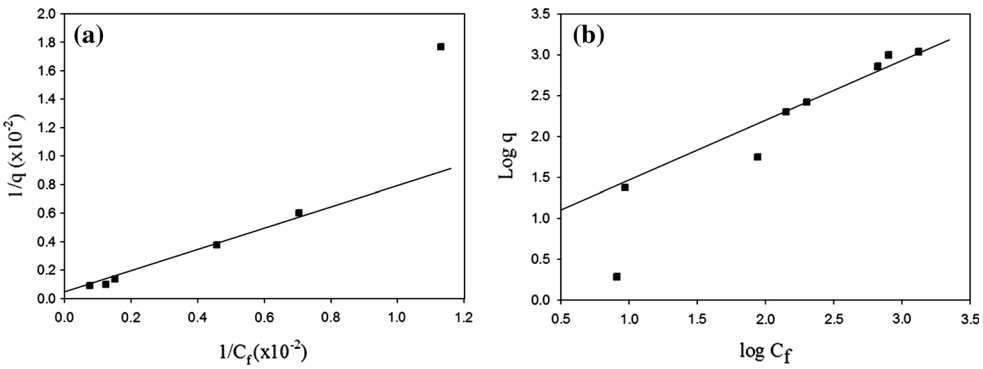

Fig. 9 Zn^{2+} biosorption by *P. fluorescens* strain Psd by **a** Langmuir adsorption isotherm ($R^2 = 0.953$) and **b** Freundlich adsorption isotherm ($R^2 = 0.949$)

273 μg/mg dry weight at 5 mM external Zn^{2+} concentration, but also managed to survive at this concentration which is, generally toxic for the cell. The zinc removal efficiency of the cells could also be extrapolated to two environmental samples i.e. contaminated soil and industrial effluent and it was found that the cells were able to accumulate zinc from these sources. Such a property of a PGPR strain can not only be translated in bioremediation so as protect the plants from a metal stress (Belimov et al. 2004; Zhuang et al. 2007) but should also be able to mediate a Zn exchange cycle benefitting the plants. Further, the distribution of accumulated Zn^{2+} indicated towards the extracellular sequestration of the metal, protecting the intracellular environment from metal toxicity.

Biosorption as the basis of bacterial Zn^{2+} resistance has been reported earlier (Gowri and Srivastava 1996; Taniguchi et al. 2000; Pardo et al. 2003; Chen et al. 2005, 2008, 2009; Bautista-Hernández et al. 2012). In *P. fluorescens* strain Psd, this mechanism was further substantiated by the observation that total Zn^{2+} accumulation by the cells under growth inhibitory conditions did not vary significantly from that of untreated control. In fact, the heat-killed cells of strain Psd displayed higher zinc accumulation capacity, which can be explained on the basis of the better exposure of cation-binding sites in dead cells (Vijayaraghavan and Yun 2008). These results are in accordance with the observation that when the majority of the metal accumulated by a strain remains confined to the extracellular surface, none of the energy-demanding processes of metal accumulation, like efflux and intracellular sequestration, are involved in metal resistance (Bhagat and Srivastava 1994). Similar results were obtained by Horikoshi et al. (1981) where Uranium uptake by *Actinomyces levoris* and *Streptomyces viridochromogenes* was not affected by the presence of inhibitors. On the other hand, in *Pseudomonas putida* strain S4, a decline in Cu^{2+} accumulation of up to 60 % on treatment with inhibitors was observed because copper efflux turned out to be the main strategy of Cu^{2+} management (Saxena et al. 2002). Similarly, in *Trichoderma atroviride,* carbon- starvation led to a decreased accumulation of Cu^{2+}, Zn^{2+} and Cd^{2+} (Errasquin and Vazquez 2003).

Time-course kinetics studies reveal the rate of chemical reaction and also the factors affecting it. Sorption processes depend strictly on physico-chemical characteristics of the adsorbent and also on the reaction conditions (Kumar, 2006). In the present study, the biosorption kinetics worked out at different initial Zn^{2+} concentrations revealed that at higher initial metal concentration (i.e. at 378.3 mg/L), sorption process was fast and reaction attained equilibrium earlier as compared to the cases where initial Zn^{2+} concentrations were comparatively low. This is because at higher initial metal concentrations, the ratio of moles of solute to available surface area of the biosorbent may be high, leading to increased rate of biosorption (Vijayaraghavan and Yun 2008). This was in accordance with the other reports (Ho and McKay 1999; Binupriya et al. 2007). Also, the amount of Zn^{2+} uptake increased with increase in external Zn^{2+} concentrations. The maximum Zn^{2+} uptake of 264.8 mg/g dry weight (~70 %) was obtained at the external Zn^{2+} concentration of 378.3 mg/L. In literature, maximal Zn uptake by a bacterial biosorbent (172.0 mg Zn^{2+}/g dry weight of biomass) has been reported for a chemically-modified variant of *Thiobacillus ferrooxidans*, after 2 h of treatment (Liu et al. 2004). The present study, to the best of our knowledge, is the first report showing such a high level of Zn^{2+} uptake by a native bacterium. Other chemically-unmodified bacterial biosorbents with high Zn^{2+} biosorption capacities are *Streptomyces rimosus* and *Brevibacterium* sp. strain HZM-1 with uptake capacities of 30.0 and 42.0 mg Zn^{2+}/g dry weight, respectively (Mameri et al. 1999; Taniguchi et al. 2000). Biosorption of Zn^{2+} on to the outer membrane of *P. fluorescens* strain Psd was found to obey pseudo-first order reaction kinetics (Lagergren, 1898) as also reported by Ho and McKay (1998).

The surface sorption capacity of any biological material can be determined using the adsorption isotherm which relies on the fact that the initial metal ion concentration plays a key role in metal uptake and an increase in this initial concentration leads to an increase in biosorption capacity (Bautista-Hernández et al. 2012). To understand the biosorption kinetics, two important isotherms, Langmuir and Freundlich models, were employed in the present study. From the parameters obtained for both the models, no significant difference was found between Langmuir model ($R^2 = 0.953$) and Freundlich model ($R^2 = 0.949$). The dimensionless separation constant R_L (0.98), however, supported the suitability of Langmuir model. According to Zhou et al. (2009), value of R_L in the range of 0 and 1 indicate towards a favorable biosorption process. As suggested by Babu and Ramakrishna (2003), the value of Freundlich constant n between 0.5 and 10 signifies thermodynamically favourable biosorption process. The value obtained in the present case was 1.25, further proving the efficiency of biosorption process.

Bacterial EPS are known to be key players in metal biosorption, due to their negatively-charged nature and electrostatic interaction with the metal cations (Vijayaraghavan and Yun 2008). Using equilibrium titrations and X-ray absorption fine structure (EXAFS) spectroscopy, Guiné et al. (2006) have demonstrated the role of EPS in Zn biosorption by *P. putida*, *Escheichia coli* and *Cupriavidus metallidurans*. We have shown an increase in EPS content in the culture raised at higher Zn concentrations, indicating towards its possible role in Zn^{2+} biosorption. This aspect is under further investigation.

The results of the present investigation indicate towards the potential of *P. fluorescens* strain Psd in in situ bioremediation of Zn-contaminated environment. Further, being

a PGPR, its beneficial properties should not be affected in such contaminated soils.

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