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Evaluation of heavy metal resistance profile of *Candida parapsilosis*

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Heavy metals have caused a lot of imbalance in the environment due to its widespread ill effects. There have been tremendous efforts to reduce the levels of these metals from the environment, but demerits of existing methods exceed over the merits in most of the cases and hence there is a need for more effective mechanisms. In the present study, resistance profile of *Candida parapsilosis* was studied against various heavy metals. A time based tolerance study was performed using up to 12 mM concentrations of heavy metal salts such as zinc sulfate (Zn), cupric sulfate (Cu), lead acetate (Pb), mercury chloride (Hg), nickel sulfate (Ni), and potassium chromate (Cr). It was observed that tolerance against heavy metal depends upon its concentration as well as time of exposure. The obtained pattern of resistance for each individual heavy metal was Ni > Zn > Cu > Cr > Pb > Hg. Maximum growth of 57.6% was obtained for Ni salt and least for Hg with 26.9% cell viability at 12 mM concentration. Cell viability decreased as time of exposure was increased. After 72 h only 16.4% cell growth was obtained for Hg as compared to Ni showing cell viability of 37.5% up to 12 mM concentration. Significant resistance to other salts such as Zn, Cu, Cr and Pd have also been shown by *C. parapsilosis*.

Keywords: Resistance, heavy metal salts, *Candida parapsilosis*, cell viability, exposure time

Introduction

Rapid industrialization and growing population have led to a huge load on our natural resources. The water streams, groundwater, and even the soil have been polluted due to industrial pollution and expansion. The main load is caused by heavy metals. Heavy metals comprise of 40 elements and have a density greater than 5 gm/cm^3. Although essential for growth, these metals can cause toxicity if taken in excess[1]. These heavy metals include metals such as zinc, copper, lead, mercury, tin, arsenic and so on. These metals form complexes with the organic matter and cause disruption in the cellular functioning. Even minute quantities of these cause massive toxicity to the cells[2]. They exert their toxicity on the cells by mechanisms yet not fully known to us. The current methods of their removal include the use of chemicals which further causes toxicity and also not cost effective. Hence, clean and green methods are required for effective and efficient remediation of these metals. Bioremediation is one such approach. This approach makes use of micro organisms cellular processes to bring down the level of toxins in the environment[3]. Bioremediation mainly exploits cellular machinery for remediation of toxic substances. Many bacteria and fungi particularly yeast has been exploited for bioremediation strategies. Single species of bacteria is not enough to bring about the degradation of the heavy metals and hence a consortium is used to bring about effective remediation process. *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46, and *E. cloacae* KJ-47 have been reported to be used in a bacterial consortium for degradation of lead, cadmium, and copper showing good remedial properties[4]. Sulfate-reducing bacteria have been employed for bioremediation of heavy metal contaminated soil. These have been effective against cadmium[5]. There are attempts to optimize activated sludge bacteria for effective bioremediation of heavy metal-contaminated effluent. Researchers have found *Enterobacter* sp., *Stenotrophomonas* sp., *Providencia* sp., *Chryseobacterium* sp., *Comamonas* sp., *Ochrobactrum* sp. and *Delftia* sp. to play a vital role in this process[6]. Marzan *et al* had isolated *Gemella* sp., *Micrococcus* sp. and *Hafnia* sp. from tannery effluents for remediation of lead, chromium, and cadmium[7].

However, use of bacteria is quite limited due to low levels of metal resistance to toxic metals as compared to that of fungi or yeasts. Fungi and yeasts have gained popularity in the field of bioremediation due

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to their higher resistance levels towards the heavy metals. Fungi and yeasts have provided robust platforms for remediation purposes due to (i) increased secretion of extracellular metal chelators, (ii) overproduction of intracellular metal chelators and (iii) overproduction of elements of the anti-oxidative defense system[8]. The microbe-metal interaction is multimodal and can follow these routes such as biosorption, biotransformation, bioaccumulation, biomineralization, microbially enhanced chemisorption of metals, biodegradation of chelating agents and bioleaching. Biosorption is chosen as the most accepted model for bioremediation studies[9]. *Micrococcus* sp. and *Aspergillus* sp. was able for the removal of chromium and nickel from industrial wastewater through biosorption route[10]. A yeast strain ES10.4 isolated from activated sludge has been used as biosorbent of copper, mercury, cadmium, and lead[11]. *Cryptococcus* sp. AH-13 isolated from tea soil was more resistant to Cd, Cu, Zn, Co, Hg, Ag, Fe, Mn, Ni (except Pb) than *Candida palmioleophila* KB-6 as reported by Chau *et al*[12]. Moreover, some *Saccharomyces* species isolated from orange, pineapple, and palm wine were found to be resistant to metals such as cadmium, copper, manganese, zinc, and silver in the range of 1 to 20 mmol/L[13]. Microorganisms are known to produce substance responsible for remediation of metals. Extracellular polymeric substances (EPS) of microbial origin are a complex mixture of biopolymers comprised of polysaccharides, proteins, nucleic acids, uronic acids, humic substances, and lipids are responsible for heavy metal remediation[14]. Metal-binding proteins and peptides are responsible for bioremediation of heavy metals particularly for cadmium[15]. This was shown by a lipoprotein isolated from *Candida lipolytica* (UCP 0988) and used that for remediation of zinc, copper, lead, cadmium, and iron was reported. Results signified that a reduction of 96% was achieved for Zn and Cu and concentration of Pb, Cd, and Fe was also decreased[16]. In the current study, we have reported the use of *C. parapsilosis* for heavy metal remediation of metals such as zinc, copper, lead, mercury, nickel, and chromium.

Materials and Methods

Microorganism and Culture Conditions

The fungal culture of *Candida parapsilosis* was isolated from environmental source. The potato dextrose broth (PDB) was purchased from HiMedia, Mumbai, India. Culture was grown in PDB at 37°C for 48 hrs[17].

Preparation of Heavy Metal Stock Solution

The metal salts were purchased from Sigma Aldrich, USA and were used for the present study. The heavy metals studied were cupric sulfate, mercury chloride, nickel sulfate, potassium dichromate, zinc sulfate and lead acetate. One molar (1M) of a stock solution of each metal was made using the appropriate quantities of each salt. The stock solution was filtered using 0.22 micron filter (Pall Co., MI, USA). Working solutions of each heavy metal were freshly prepared according to desired concentration/ molarity.

Determination of the Heavy Metal Resistance Profile

Heavy metal-resistance potential of culture was determined by supplementing each flask with its respective heavy metal salt. Then gradually salt concentration in each flask was increased up to 12 mM. Positive control, consisting of a metal salt free media inoculated only with microorganism, was also employed. The inoculum of culture was 2%. All cultures were incubated at 37°C and time based study for checking heavy metal resistance for 48 hrs and 72 hrs was performed. After incubation, the optical density of the culture was taken at 600 nm against its respective control, percentage cell viability corresponding to each salt was calculated and graphs were plotted.

Statistical Analysis

Statistical analysis of results was performed by the Student's t-test in Microsoft excel. Results corresponding to P value of <0.05 were considered statistically significant.

Results

Microbial Resistance to Zinc Sulfate

Results presented in Figure 1A depicted the resistance profile of our culture for Zn salt. A good visible growth of 40.9% and 32% was observed with 12 mM salt concentration within 48 hrs and 72 hrs of incubation, respectively.

Microbial Resistance to Mercury Chloride

The fungal isolates were highly sensitive to Hg, when compared to other salts. Maximum inhibition of cells has been observed as time of exposure to salt increases, this could be seen from Figure 1B. Cell viability was decreased from 64.3% to 49.4% within 24 hrs for 2 mM salt concentration. At 12 mM, least cell viability of only 26.9% and 16.4% was obtained after 48 hrs and 72 hrs of exposure.

Microbial Resistance to Lead Acetate

A change of 32.1% was observed when concentration was increased from 2 to 12 mM. This could be seen in Figure 1C. This change was 43.5% for 72 hrs incubation period. Based on results, it could be seen that after Hg the culture was very sensitive to Pb.

Microbial Resistance to Nickel Sulphate

As depicted in Figure 1D, analysis of relative resistance in Ni revealed, maximum tolerance and less reduction in growth with increasing salt concentration. 57.6% of cells were able to grow at 12 mM Ni, whereas only 37.5% cell is alive after 72 hrs of incubation.

Microbial Resistance to Potassium Chromate

Figure 1E represents the chromium salt resistance profile. Percentage decrease in cell viability is very high. At 2 mM concentration 71.3% cell are there but only 33.7% cells present up to 12 mM with 48 hrs of incubation. Similarly for 72 hrs cell growth decreased from 67.4% to 27.1%.

Microbial Resistance to Cupric Sulfate

Figure 1F depicted the tolerance pattern for Cu salt. Almost 50% cell viability was obtained for Cu salt upto 8 mM for 48 hrs exposure. However, up to 12 mM salt concentration 37.2% and only 31.4% cell viability was observed as time increases further by 24 hrs. This showed that resistance for Cu salt was there in our fungal culture.

Discussion

Determination of the Heavy Metal Resistance Profile

The positive control containing only fungal culture had shown much higher growth than culture incubated with heavy metal salts. This could be visualised from Figure 1. We have studied tolerance of fungal culture to some heavy metals like Cu, Zn, Ni, Pb, Hg and K. Percentage of tolerance/ resistance was evaluated for each heavy metal quantitatively by spectrophotometric analysis. Based on the ability of fungus to grow on media containing heavy metals, and its varying concentration, a time based study was performed and results were compared with positive control for evaluation of tolerance for heavy metals[18].

In this study we have observed that bacterial growth was concentration and time dependent. With increase in heavy metal concentration and time of exposure, decrease in absorbance was observed[7,18-20]. This change could be attributed from the stressed

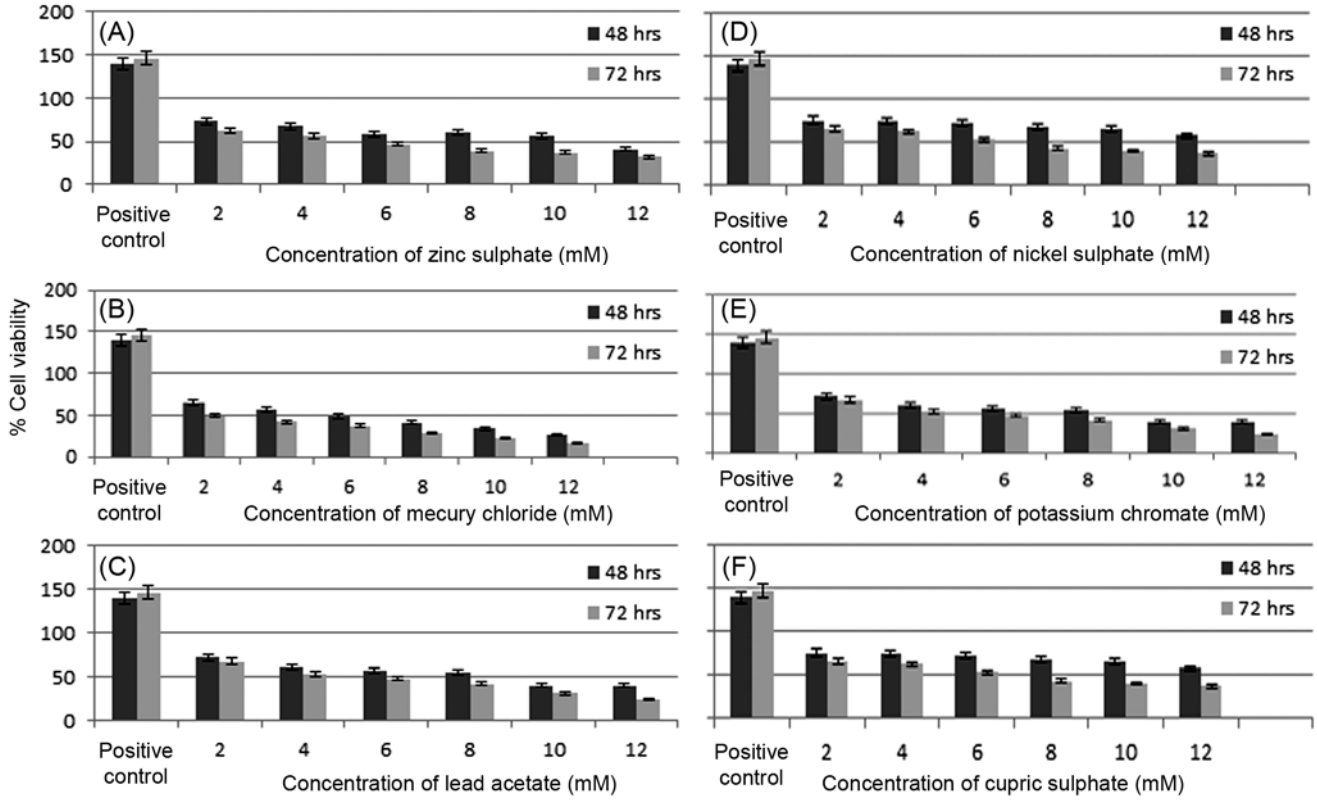

Fig. 1 — Effect of heavy metal salt (up to 12 mM concentration) on cell viability after 48 hrs and 72 hrs exposure.

condition generated by heavy metals for cell growth[18]. The pattern of resistance of individual heavy metal could be seen in Figure 1. Heavy metal tolerance directly corresponds to % cell viability. We have observed the pattern of tolerance upto 12 mM concentration for heavy metals as Ni > Zn > Cu > Cr > Pb > Hg. After 48 hrs, percent of cell viability of 40.9%, 33.7% and 37.2% was achieved for Zn, Cr and Cu, respectively. Hg and Pb has shown approximately similar level of tolerance with 26.9% and 39.4% of cell growth. Maximum growth of 57.6% was obtained after Ni exposure upto 12 mM for 48 hrs. Our results are similar to what was reported in previous studies by Hassan *et al*, toxicity order of *P. aeruginosa* for heavy metal was Pb > Cu > Ni > Zn for 48 h of exposure[18]. It was already reported as time of exposure to heavy metals increases, cell viability rate decreases. This is well supported by our results too. Cell viability decreased to 32% and 27.1% after 72 hrs with Zn and Cr salts respectively. Pb and Hg showing little toxicity and only 23.6% and 16.4% of cell growth was obtained. Whereas Cu and Ni have shown some tolerance upto 12 mM concentration with survivality of 31.4% and 37.5% was achieved. The pattern of tolerance for 72 hrs exposure was same as that was obtained for 48 hrs incubation i.e. Ni > Zn > Cu > Cr > Pb > Hg. Pal *et al* has observed 6 mM Ni and 2 mM Cr tolerance of its fungus on 5 days incubation with metals[21]. These results were in agreement with our results of fungal culture with tolerance of Ni > Cr. It was also interpreted by Hassan *et al* that resistant to Ni was more as compared to Hg and Pb[18]. The pattern of tolerance for 48 hrs of metal exposure was demonstrated as Ni > Pb > Zn > Cu > Hg[22]. Hg to be the most toxic and Ni to be least among all metals. These results are also similar to results reported by us for similar time of exposure.

Some researchers have different observation to us, Marzan *et al* had shown bioremediation potential of bacterial culture was more for Pb was more as compared to Cr[7], But some bacterial culture have also shown same resistances pattern as observed by us i.e. Ni > Cr[21]. Our fungal culture has shown slightly different pattern as observed for *P. aeruginosa* up to 10 mM salt concentration. The pattern of resistance was Zn > Pb > Cu > Hg > Ni > Cr for 24-48 hrs of exposure. Complete inhibition of growth at 10 mM concentration, with the exception of $ZnSO_4$ and $PbCl_2$ showing 67% and 15% growth, respectively, was observed[23]. Heavy metal resistance pattern in *Pseudomonas* sp. was observed as Cu > Pb = Cr > Zn and Cu = Pb > Zn = Cr in *Aeromonas* sp. for 24 hrs of exposure with heavy metals[24]. Heavy metal toxicity profile of *Candida tropicalis* and *C. glabrata* against metals such as lead, cobalt, and cadmium was studied. Varying concentrations ranging from 0.072-0.41 mM for lead and 0.25-1.44 mM for cobalt and 0.062-0.687 mM for cadmium respectively were used[25]. It was observed that both *C. tropicalis* and *C. glabrata* were able to grow in cadmium salts. But for lead or cobalt salt, there was a significant decrease in growth of *C. glabrata* and no effect on growth for *C. tropicalis* was observed. In comparison to these studies, our *Candida.* sp. has shown better results against heavy metals[25].

Conclusion

The current study was based on heavy metal resistance profile of *Candida parapsilosis.* It was evident from the present study that fungal culture was able to resist heavy metals of nickel, zinc, chromium and copper up to the concentration of 12 mM though it is sensitive towards mercury and lead. The obtained pattern of resistance for individual heavy metal was Ni > Zn > Cu > Cr > Pb > Hg after exposure. The culture was highly sensitive to the increasing concentration of the heavy metal as well as exposure time. This was shown by decrease in growth after 48 hrs and 72 hrs of exposure to salt as compared to control. It can be inferred from the study that the present culture has the potential for effective and efficient remediation of heavy metals from the environment. However, environment contains many other types of heavy metal that were not included in our study. So, further research should undertake for detail understanding of bioremediation potential of *Candida parapsilosis*.

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