

Full length Research Paper

Isolation and characterization of H₂S producing yeast to detoxify mercury containing compounds

Aatif Amin and Zakia Latif*

Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan

Accepted 05 December, 2011

The aim of the present study was to isolate yeast isolates from local environment for hydrogen sulfide production to detoxify mercury containing compounds. Preliminary twenty two yeast isolates from seventy were screened out by qualitative and quantitative assay having maximum ability to detoxify mercury. Zone of inhibition ranging from 7 - 14mm in well plate method and remediation of mercury in the culture medium ranging from 2 to 19µg/ml out of 20µg/ml HgCl₂ were observed. Mercury resistant isolates were further characterized for hydrogen sulfide production by growing them on Lead acetate (LA) medium. Maximum resistance to 100 µg/ml HgCl₂ yeast isolates showed minimum zone of inhibition (7- 9 mm) gave darker color on LA agar plates as compared to yeast isolates sensitive to mercury. On the basis of these results seven yeast isolates were characterized by 18S rRNA sequencing as *Candida tropicalis* (JF896569), *Candida rugosa* (JF896570-71), *Pichia kudriavzvi* (JF896572-73), *Candida xylopsoci* (JF896574) and *Candida inconspica* (JF896575). It is concluded from our studies that *Candida xylopsoci* and *Pichia kudriavzvi* have potential to detoxify mercury 95% and 94.5% respectively from the medium containing 20 µg/ml HgCl₂ within 36 hrs. Our findings suggest that both strains may have significant biotechnological role in the treatment of contaminants containing mercury before they discharge into the environment to make it friendly for living organisms.

Keywords: *Bioremediation*; *Candida xylopsoci*; Dithizone; Hg⁺² Reduction; *Pichia kudriavzevii*;

INTRODUCTION

Mercury is one of the environmental pollutants of most concern. It is highly toxic heavy metal to living organisms which is still used frequently. The living organisms require traces amount of some heavy metals like Iron, Zinc, cobalt, copper, manganese and molybdenum. Excessive levels can be detrimental to the organisms. Other heavy metals such as mercury, plutonium and lead are toxic metals that have no known vital or beneficial effect on organism and their accumulation over time can cause serious illness. Exposure to mercury and its compounds considered health hazardous are associated with many diseases in human beings. The main pathway for mercury to humans is through the food chain and by inhalation. Once in the body, mercury concentrates in the nerves, liver and especially kidneys. The major source of

environmental contamination of mercury arises from various anthropogenic activities such as chlor-alkali industries, fluorescent lamps, in the recovery of gold, burning coal, natural gas, metal processing and petroleum products, use of mercurial fungicides, etc. These activities contribute about 2190 t of mercury in the environment (Li *et al.*, 2009). All activities have increased mercury levels about doubled or tripled in the atmosphere and the atmospheric burden is increased day by day. Global mercury emission is projected to reach the level of 2390–4860 Mg by the year 2050 (Streets *et al.*, 2009). Heavy metals are not degradable and thus pose a different kind of challenge for remediation. Because of its persistence in the environment and possible toxicity to living organism, mercury has been listed as one of the priority pollutants by many international agencies (Jiang *et al.*, 2006). It is, therefore, urgent need to remove these hazardous pollutants from the environment. Many physico-chemical methods like ion exchange, oxidation, membrane separation, coagulation etc. are available for

*Corresponding author: zakia.mmg@pu.edu.pk and umna123@yahoo.com

the treatment of heavy metals but the drawback of these methods is that most of the techniques which are in practice to remediate metal pollutants are expensive, less efficient to detoxify their activity and generally generate hazardous end products (Manohar *et al.*, 2002; Zhang *et al.*, 2005). As a response to toxic mercury compounds globally distributed by geological and anthropogenic activities, nature has developed remarkable phenomena in microorganisms to detoxify poisonous burden from the environment. Bioremediation of heavy metal is well known phenomena in certain microorganisms which challenged with the environmental pollutants (Thakkar *et al.*, 2010). Microorganisms have been found to have the ability to remediate them into forms that are precipitated or volatilized from solution and hence less toxic and easily disposable.

Different mechanisms exist in nature for the remediation of pollutant in our environment by bacteria (Sinha and Khare, 2011; Rojas *et al.*, 2011) fungi (Das *et al.*, 2007) algae, mosses (Sari and Tuzen, 2009) and higher plants. (Bennicelli *et al.*, 2004) are reported to detoxify mercury pollutants. The ideal process for mercury detoxification will be the one in which it can be trapped as Hg^{2+} or convert it into non toxic form Hg^0 . Bacteria and fungi attack heavy metals molecule. They have special defense mechanisms called resistance genes, produced Hydrogen sulfide (H_2S) gas which binds to the attacker and neutralizes it. It co-precipitate mercury in the form of H_2S resulting the conversion into non-toxic form. So, to overcome the toxic effect of mercury containing compounds, it is necessary to convert it into non toxic form by the H_2S producing microorganisms or by other way. We have screened different mercury resistant yeast isolates from our local environment and checked their ability to detoxify mercury pollutant. Hydrogen sulfide producing yeast isolates having maximum ability of mercury detoxification were characterized by 18S rRNA ribotyping as *Candida tropicalis*, *Candida rugosa*, *Candida inconspicua*, *Candida xylophaci* and *Pichia kudriavzvi*.

MATERIALS AND METHODS

Collection of samples

Samples from different sources such as tenaries, poultry waste, sewage water, poultry feed, commercial yeasts, yogurt, rotten fruits, sunflower, rose, mango tree bark, garden soil from different areas of Lahore - Pakistan were collected and stored at 4°C in airtight bags.

Isolation and purification of yeast isolates

Ten fold dilution of 1% sample were prepared in autoclaved distilled water and 100 µl from each dilution

was spreaded on YEPD agar plates. The plates were incubated for overnight at 30°C (Singh and Sherman, 1974). Morphologically different colonies appeared on YEPD agar plates were selected and streaked on new YEPD agar plates. The plates were incubated at the same conditions as before to get pure single colonies.

Preliminary screening of mercury resistant yeast isolates

All purified yeast isolates were grown on YEPD agar plates containing different conc. of $HgCl_2$ (10, 25, 50 and 100 µg/ml). After incubation at 30°C for 24 hrs. mercury resistant strains were selected by observing growth.

Screening of mercury resistant yeast isolates by disc/well plate method

Selected mercury resistant yeast isolates were grown in YEPD liquid medium at 30°C for overnight at 200 rpm on continuous shaking incubator. The optical density at 600 nm was measured by spectrophotometer and fixed 0.1 by diluting with autoclaved YEPD liquid medium. The same concentration of yeast cultures was used in all experiments. Diluted overnight culture (100 µl) was spreaded on YEPD agar plates and four sterilized filter paper discs and wells (5 mm in diameter) were prepared on each plate separately. Filter sterilized mercuric chloride solution (50, 75, 100 µg/ml) prepared in autoclaved distilled water was used (15 µl) in both disc plate and well plate method (Zeroual *et al.*, 2001). Zone of inhibition was measured in mm after incubation at 30°C for 24 hours.

Hydrogen sulfide production by yeast isolates

Two microlitre (2 µl) overnight cultures having 0.1 optical density at 600 nm was spotted on LA agar plates (0.3% peptone, 0.5% yeast extract, 4% glucose, 0.02% ammonium sulfate, 0.1% lead acetate, and 2% agar). The plates were incubated for 3 days at 30°C for qualitative analysis of hydrogen sulfide production by selected yeast strains (Ono *et al.*, 1991).

Growth kinetics

Two hydrogen sulfide producing (Z-HS 33, Z-HS 51) and two non-hydrogen sulfide producing (Z-HS 13 and Z-HS 26) yeast isolates having relatively same no. of cells were inoculated in 50 ml of YEPD liquid medium to observe growth kinetics. Optical density of overnight culture at 600 nm was measured and adjusted to 0.4 by diluting with fresh YEPD liquid medium. Cultures (50 ml) were

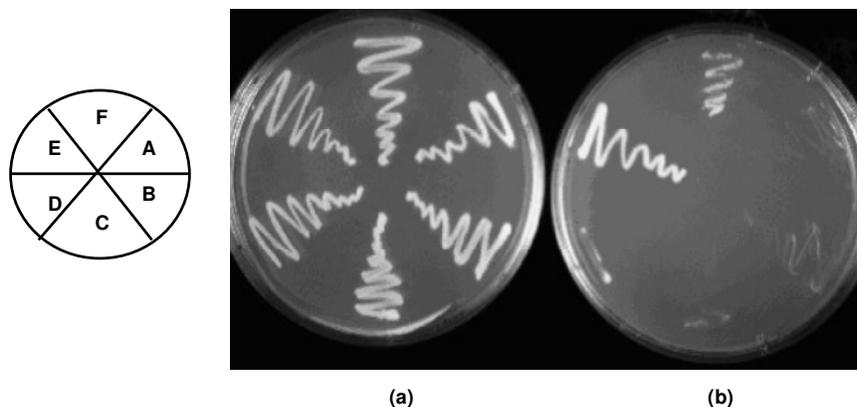


Figure 1. Growth of yeast isolates after 24 hrs. incubation at 30°C on YEPD agar plates: a) Δ HgCl₂ (b) supplemented with 50 μ g HgCl₂/ml, as i: Z-HS 51, ii: Z-HS 05, iii: Z-HS 13, iv: Z-HS 25, v: Z-HS 57 and vi: Z-HS 62.

again incubated at the same conditions. After 3 hrs. of incubation, the O.D₆₀₀ of each isolate was measured and stress of HgCl₂ (20 μ g/ml) was given in three flasks and fourth flask was considered as control (without HgCl₂). After every 1.5 hrs of incubation, the growth of each isolate was (stress and control) monitored by measuring optical density at 600 nm upto 10.5 hrs of incubation.

Kinetic studies and detoxification of HgCl₂

Two yeast isolates, one H₂S producing (Z-HS 51) and other non H₂S producing (Z-HS 13), were inoculated in four flasks containing 50 ml YEPD liquid medium supplemented with 20 μ g/ml HgCl₂ and incubated at 30°C. After every 12 hrs interval, 5 ml medium from each flask was taken out and Hg was measured upto 48 hrs. by dithizone method (Elly, 1973; Khan et al., 2007). One flask containing YEPD supplemented with HgCl₂ was used as negative control without inoculation of yeast.

Molecular characterization of yeast isolates (18S rRNA)

Seven yeast isolates showing promising results (Z-HS 01, Z-HS 05, Z-HS 13, Z-HS 25, Z-HS 33, Z-HS 51, and Z-HS 62) were selected for their molecular characterization through 18S rRNA sequencing. Yeast isolates were sent to Macrogen Sequencing facility at Korea for sequencing. The data (sequences) obtained from Macrogen Sequencing facility at Korea were BLAST and submitted to NCBI for accession numbers.

Phylogenetic relationship between the isolates

Phylogenetic analysis was done with MEGA 4. The

method used in this study was distance-based tree built with Neighbor-Joining on the TN93 distance With Bootstrapping value 1000 (No. of data sets).

RESULTS

Yeast isolation from different sources

Approximately seventy yeast isolates named Z-HS 01.....Z-HS 70 were selected from YEPD agar plates and stored as glycerol stocks for further screening.

Screening of mercury resistant yeast isolates

YEPD agar plates supplemented with different concentration of mercuric chloride (10-100 μ g/ml) were used for preliminary screening of yeast isolates. After 24 to 48 hrs of incubation at 30°C, about 24 yeast isolates were selected by observing their growth on different HgCl₂ concentrations. Most promising results were observed on 50 and 100 μ g/ml HgCl₂ plates (Figure1) for the selection of mercury resistant yeast isolates.

Selection of HgCl₂ resistant yeast isolates by disc and well plate methods

It was observed that yeast isolates with maximum zone of inhibition showed greater resistance to HgCl₂. It is clear from Figure 2a and b that well plate method gave the most promising and clear results. Zone of inhibition in disc plate method ranges from 5-10 mm while 7-14 mm in well plate method against different conc. of HgCl₂. Maximum zone of inhibition (14 mm) was observed in well plate method on YEPD agar plates supplemented with 100 μ g/ml HgCl₂. So for further screening of yeast

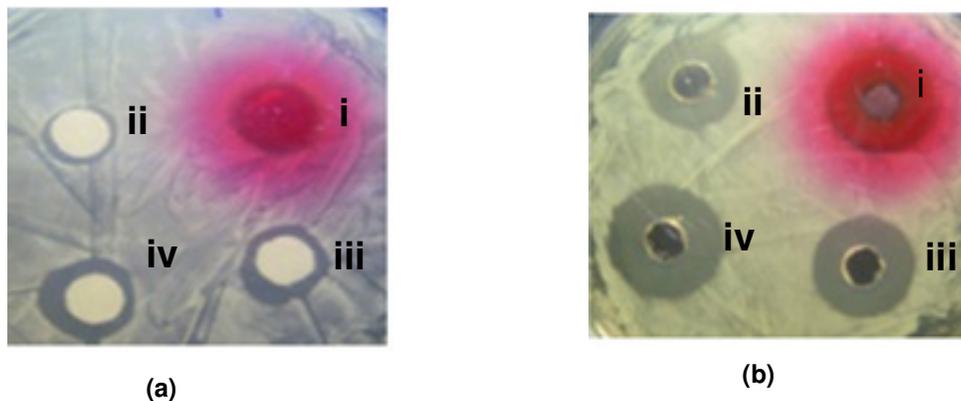


Figure 2. Comparison of disc plate (a) and well plate (b) method for measuring inhibition zone (mm) of yeast isolates by different concentrations of HgCl₂, (ii) 50, (iii)75, (iv)100 µg/ml,(i) safranin was used to observe the diffusion efficiency in the medium.

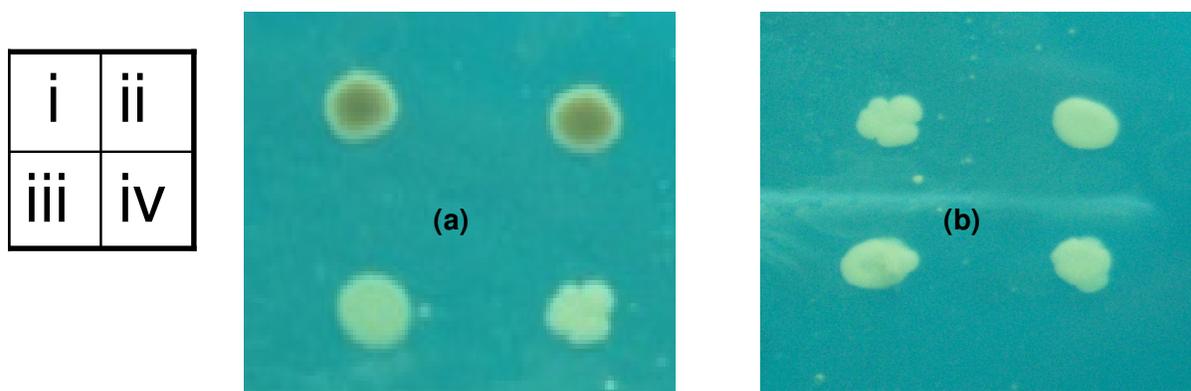


Figure 3. Dark color colonies by mercury resistant yeast isolates (i: Z-HS 33 and ii: Z-HS 51on LA medium (a) indicate the release of H₂S whereas white color colonies sensitive to mercury (iii: Z-HS 26 and iv: Z-HS 13) are non-hydrogen sulfide producers (sensitive to Hg). No color was developed by the same isolates of yeast when grown on control plates (b) without lead acetate

isolates well plate method was used.

Qualitative test for hydrogen sulfide production by yeast isolates

Since it is known and reported that H₂S helps in precipitation of mercury containing compounds and reduces its toxicity (Ghosh, 1996) and it is clear that isolates Z-HS 51 and Z-HS 62 showing resistance to HgCl₂ are hydrogen sulfide producing and mercury containing compounds in the cell react with H₂S to yield highly insoluble and less toxic HgS. Hydrogen sulfide producing isolates Z-HS 51 and Z-HS 62 developed darker color due to formation of PbS and non-hydrogen sulfide producing Isolates Z-HS 05 and Z-HS 13 showed white colored colonies on the LA medium (Figure 3) as compare to control plates without lead acetate. Twenty two yeast isolates, showing resistance against 100 µg/ml HgCl₂ in well plate method, were selected for this study.

Total seven yeast isolates including H₂S and non H₂S producer were selected for further study.

Relationship between hydrogen sulfide (H₂S) production and zone of inhibition

Hydrogen sulfide producing yeast isolates determined by qualitative test on LA agar plates, were divided into three categories (low, medium and high H₂S producing) according to the color of the colonies. The isolates with smaller zone of inhibition gave darker colonies on LA medium indicate have the ability to produce high H₂S. In contrast, isolates with larger zone of inhibition gave lighter color on LA medium have the ability to produce low H₂S. From the comparative qualitative analysis, it is clear that H₂S production by yeast isolates is directly proportional to the resistance against HgCl₂ or vice versa. Our findings are in good agreement as reported by Ono (1999).

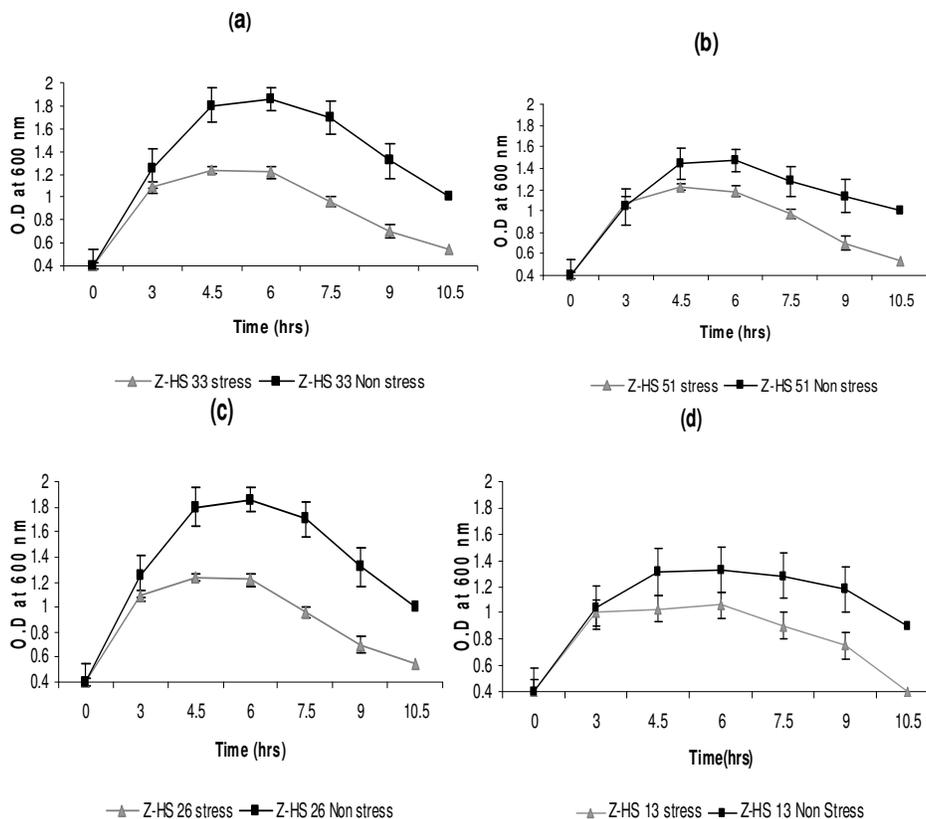


Figure 4. Growth curves of H₂S (a: Z-HS 33; b: Z-HS 51) and non-H₂S (c: Z-HS 26; d: Z-HS 13) producing yeast isolates

Growth kinetics

Growth kinetics of hydrogen sulfide producing (Z-HS 33 and Z-HS 51) and non-hydrogen sulfide (Z-HS 26 and Z-HS 13) producing yeast isolates was checked in the presence of HgCl₂ (20 µg/ml) and without HgCl₂ *in vitro* conditions. All isolates were grown linearly but when the stress was applied, the decline in growth was very rapid and log phase was very short i.e 2 hrs as compared to the growth in without HgCl₂ of yeast isolates (Figure 4).

Kinetics of HgCl₂ remediation

Hydrogen sulfide producing (Z-HS 51) and non-hydrogen sulfide producing (Z-HS 13) yeast isolates were inoculated in YEPD liquid medium containing 20 µg/ml of mercuric chloride to check the bioremediation of HgCl₂. After an interval of every 12 hours, the amount of mercury was measured by Dithizone method (Elly, 1973; Khan *et al.*, 2007). After 36 hrs of incubation, only 1.3 µg/ml (out of 20 µg/ml) mercury was present in the medium as remainder means 18.7 µg/ml (95 %) was reduced in case of H₂S producing isolate Z-HS 51 but in case of non-hydrogen sulfide producing isolate Z-HS 13,

the reduction of mercury was not significant (Figure 5).

Mercury remediation by yeast isolates

Twenty two yeast strains were divided into three categories; low, medium and high according to their behavior of mercury detoxification. All selected isolates were inoculated in YEPD medium supplemented with 20 µg/ml of HgCl₂ and incubated at 30°C. After 36 hours of incubation, the Hg⁺² concentrations in the medium were measured. The mercury was significantly co-precipitated into non toxic form in the medium by isolates who gave darker color on LA agar plates (H₂S producers). Level of mercury remediation in the medium supplemented with HgCl₂ varied according to the intensity of color reflecting by yeast. White color colonies (low H₂S producing yeasts) have almost no effect on the reduction of HgCl₂ (Figure 6). The high H₂S producing yeast isolates showed small zone of inhibition because they have the ability to co-precipitate the mercury in the non-toxic form (HgS).

Out of 22 yeast isolates, four highly resistant isolates having maximum reduction capability (Z-HS 01, Z-HS 33, Z-HS 51 and Z-HS 62), one medium (Z-HS 25) and two with low (Z-HS 05 and Z-HS 13) were selected for

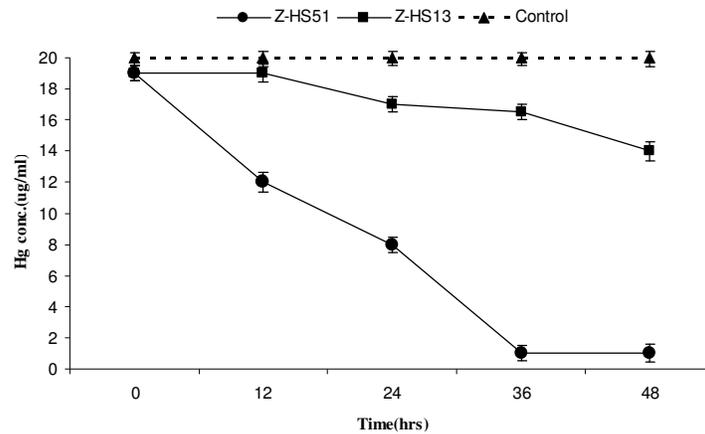


Figure 5. Comparison of mercury reduction from the medium at different time interval between H₂S producing (Z-HS 51) and non- H₂S producing (Z-HS 13) yeast isolates with control.

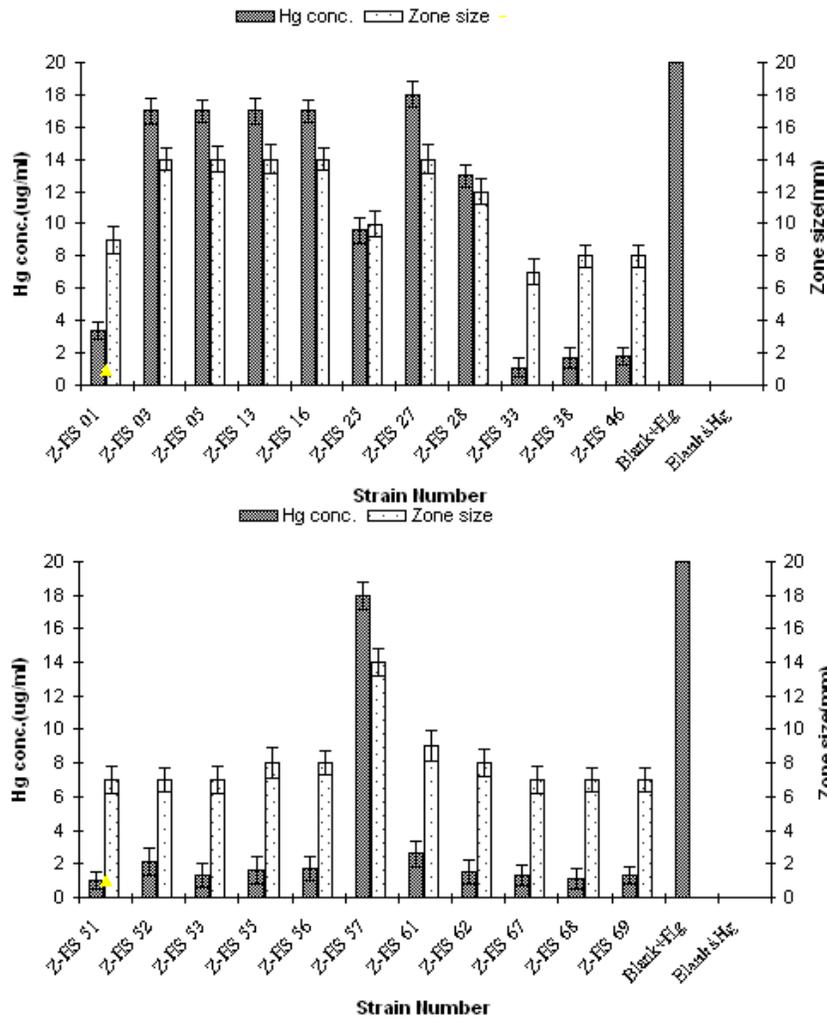


Figure 6. Relationship between zone size and HgCl₂ detoxification by selective yeast isolates. Yeast isolates resistant to mercury with smaller zone of inhibition reduced more mercury whereas isolates sensitive to mercury reduced less mercury which is non significant.

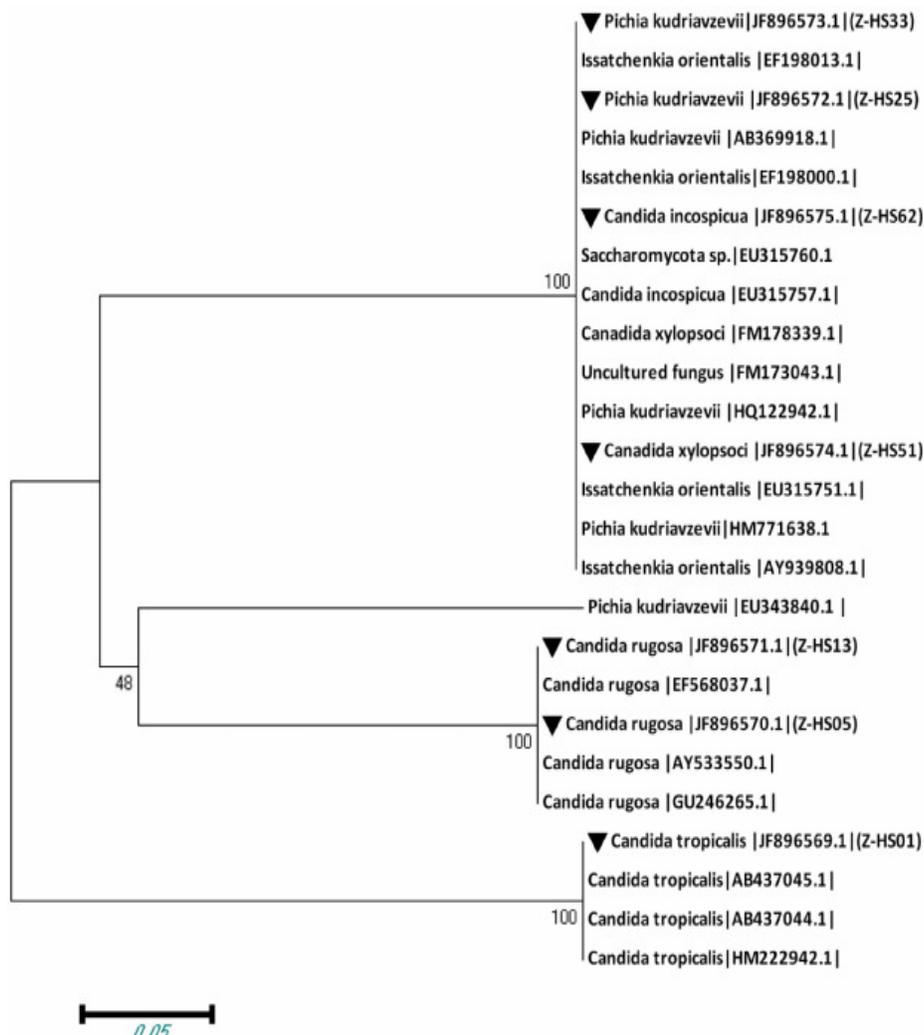


Figure 7. Neighbor-joining tree showing the phylogeny of *Candida* sp. and *Pichia* sp. with other closely related yeast strains. Scale bar represents 0.05 changes per nucleotide position.

molecular characterization.

Identification of yeast isolates /18S rRNA sequencing

Seven yeast strains (Z-HS 01, Z-HS 05, Z-HS 13, Z-HS 25, Z-HS 33, Z-HS 51 and Z-HS 62) were selected on the basis of zone size as well as detoxification of mercury by dithizone method and were characterized through 18S rRNA sequencing. The accession numbers of yeast isolates obtained by NCBI are as given in Figure 7.

The BLAST query revealed that Z-HS 01 18S rRNA gene was homologous to *Candida tropicalis*, Z-HS 05 and Z-HS 13 were homologous to *Candida rugosa* and Z-HS 51 and Z-HS 62 were homologous to already reported gene of *Candida xylopsoci* and *Candida inconspicua*. Other close matches to *Candida tropicalis*

included *C.tropicalis* AB437045 and *C.tropicalis* AB437044 (100% similarity). Likewise, the gene of *C.inconspicua* and *C.xylopsoci* was similar to *C.inconspicua* EU315757 (100% similarity).

Similarly Z-HS 25 and Z-HS 33 were homologous to already reported gene of *Pichia kudriavzevii*. The 18S rRNA gene of *P.kudriavzevii* was homologous to *P.kudriavzevii* HQ122942 (100% similarity).

The yeast isolates also show similarity (18S rRNA gene) among themselves. The maximum similarity is found between *C.inconspicua* and *C.xylopsoci* (100% similarity) whole minimum similarity observed is 48% between *C.rugosa* and *P.kudriavzevii* yeast isolates. Phylogenetic tree based on 18S rRNA gene sequence comparison shows the relationship between members of genus *Candida*, and also with genus to genus between *Candida* and *Pichia* (Figure 7).

DISCUSSION

Mercury is a naturally occurring element that can affect the health of humans and wildlife. The mercury is released into the environment by human activities such as the combustion of fossil fuels, waste disposal and by industry. Throughout the world, a number of sources are increasing the concentration of mercury reaching at alarming situation. It is now urgent need to treat mercury contaminants before they are discharge into the environment. The use of microbes received great attention in recent years as it could be technically feasible and economically viable sustainable technology for the treatment of heavy metal contaminants (Rise- Robert, 1998).

Mercury resistance has been reported so far in limited number of yeast, namely, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis* (Berdicevsky *et al.*, 1993; Wysocki and Tamas, 2010; Yannai *et al.*, 1991), *Candida parapsilosis* and *Debaryomyces hansenii* (Jayasree and Saramma, 1996). Keeping in mind the eradication of poisonous heavy metals from the environment, we have isolated and purified the mercury resistant yeast isolates from different industrial areas of Lahore, Pakistan and qualitatively determined the level of hydrogen sulfide production. Since it is known that H₂S aids the precipitation of mercury containing compounds, detoxification of mercury containing compounds from the cell via such a mechanism is possible. However, it is also possible that mercury containing compounds are converted to Hg²⁺ in the cell and then reacts with H₂S to yield highly insoluble and less-toxic HgS. Studies of the chemical transformation of mercury in the cell and the involvement of H₂S in it are needed for a better understanding of the molecular basis of mercury containing compounds resistance (Ono *et al.*, 1991). Our results are strongly in consistent with Ono *et al.* (1991).

We have quantified the detoxification of mercury and zone of inhibition by different yeast isolates, isolated from different sources. In this biological study, we have concluded that H₂S producing yeast isolates showed minimum zone of inhibition are greater resistance to HgCl₂ as compared to non-H₂S producer isolates. As Z-HS 1, Z-HS 33, Z-HS 51 and Z-HS 62 are high H₂S producer, qualitatively showing dark color on LA agar plate are highly resistant to mercury giving minimum inhibition zone (7.0mm). On the other hand, non H₂S producers, Z-HS 05 and Z-HS 13 are sensitive to mercury exhibited large zone of 14 mm. The molecular characterization of seven selected yeast strains was done by 18S rRNA sequencing and characterized as *Candida tropicalis* (JF896569), *Candida rugosa* (JF896570-71), *Pichia kudriavzvi* (JF896572-73), *Candida xylopsoci* (JF896574), and (JF896572) and *Candida inconspica* (JF896575).

Many *Candida* sp. have the capacity to absorb and accumulate metals from their environment (Podgorski *et*

al., 2004). In the present study yeast isolate Z-HS 51 characterized as *Candida xylopsoci* showed maximum potential to remove mercury from the medium as compare to other yeasts. The percentage of mercury reduction recorded was 40 %, 50 %, 95 % and 95 % after 12 hrs, 24 hrs, 36 hrs and 48 hrs respectively. Most of mercury removal was occurred within 36 hrs incubation. Same pattern of mercury remediation was observed with other *Candida* sp. but potential to remove mercury from the medium was not significant even after 48 hrs incubation at the same conditions.

In order to assess the ability of yeast isolates to detoxify mercury, a lab- scale experiment was conducted and investigated that not only the *Candida* sp. but *Pichia* sp. also have the potential to remediate mercury from the medium. The percentage of mercury detoxification was 94.5 % by *Pichia kudriavzevii* after 36 hrs incubation in the medium supplemented with mercuric chloride. It is concluded from our studies that *Candida xylopsoci* and *Pichia kudriavzevii* have great potential to detoxify maximum mercury from the medium within 36 hrs. It would be suggested that the detoxification/bioremediation ability might be due to the close relationship between *Pichia* and *Candida* species as *Pichia kudriavzevii* has 100% homology with *Candida xylopsoci*. Our finding suggested that both yeast isolates may have useful biotechnological role to detoxify mercury from the environment to make it friendly for living organisms.

ACKNOWLEDGEMENT

The authors acknowledge their profound gratitude to the University of the Punjab, Lahore- Pakistan for providing the Research Project Grant No.D/5719/Est.1 to conduct the research work. We are grateful to Prof. Dr. Shahida Husnain for financial support for sequencing to the Macrogen, Korea, and Dr. Abdul Rehman for valuable guidance for manuscript preparation

REFERENCES

- Bennicelli R, Stepniewska Z, Banach A, Szajnocha K, Ostrowski J (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chemosphere*. 55: 141-146.
- Berdicevsky I, Duek L, Merzbach D, Yannai S (1993). Susceptibility of different yeast species to environmental toxic metals. *Envi. Pollu.* 80(1): 41-44.
- Das N, Charumathi D, Vimala R (2007). Effect of pretreatment on Cd²⁺ biosorption by mycelial biomass of *Pleurotus florida*. *Afr. J. Biotechnol.* 6: 2555-2558.
- Elly CT (1973). Dithizone Procedure for Mercury Analysis. *J. (Wat. Pollu. Cont. Fed.)*. 45(5): 940-945.
- Ghosh S, Sadhukhan PC, Chaudhuri J, Ghosh DK, Mandal A (1996). Precipitation of mercury by immobilized mercury-resistant bacterial cells. *J. App. Microbiol.* 81(1): 104-108.
- Jayasree R, Saramma AV (1996). Effect of pesticide and heavy metals on growth of marine yeasts *Candida parapsilosis* and *Debaryomyces hansenii*. *Ind. J. Mar. Sci.* 25(4): 373-375.

- Jiang GB, Shi JB, Feng XB (2006). Mercury pollution in China. *Environ. Sci. Technol.* 40: 3672–3678.
- Khan H, Ahmed MJ, Bhangar MI (2007). A rapid spectrophotometric method for the determination of trace level lead using 1,5-diphenylthiocarbazone in aqueous micellar solutions. *Analy. Sci.* 23(2): 193-199.
- Li P, Feng XB, Qiu GL, Shang LH, Li ZG (2009). Mercury pollution in Asia: a review of the contaminated sites. *J. Hazard Mater.* 168: 591–601.
- Manohar DM, Krishnan KA, Anirudhan TS (2002). Detoxification of mercury (II) from aqueous solutions and chlor-alkali industry wastewater using 2-mercaptobenzimidazole- clay. *Water Res.* 36: 1609–1619.
- Ono BI, Ishi N, Shizu FI, Aoyama I (1991). Role of Hydrosulfide Ions (HS⁻) in mercury containing compounds resistance in *Saccharomyces cerevisiae*. *App. Envi. Microbiol.* 57(11): 3183-3186.
- Ono BI, Hazu T, Yoshida T, Kawato T, Shinoda S, Brzywczy J, Paszewski A (1999). Cysteine biosynthesis in *Saccharomyces cerevisiae*: a new outlook on pathway and regulation. *Yeast* 15: 1365–1375.
- Rise-Roberts E (1998). Remediation of petroleum contaminated soils. Biological, physical and chemical processes. Boca. Raton, Florida: CRC.
- Rojas LA, Yáñez C, González M, Lobos S, Smalla K, Seeger M (2011). Characterization of the metabolically modified heavy metal-resistant *Cupriavidus metallidurans* strain MSR33 generated for mercury bioremediation. *PLoS One* 6(3) : e17555, doi:10.1371/journal.pone.0017555.g001.
- Sari A, Tuzen M (2009). Detoxification of mercury (II) from aqueous solution using moss (*Drepanocladus revolvens*) biomass: equilibrium, thermodynamic and kinetic studies. *J. Hazard. Mater.* 171: 500-507.
- Sinha A, Khare S (2011). Mercury bioremediation by mercury accumulating *Enterobacter* sp. cells and its alginate immobilized application. *Biodetoxification*, pp. 1-10.
- Streets DG, Zhang Q, Wu Y (2009). Projections of global mercury emissions in 2050. *Environ. Sci. Technol.* 43: 2983-2988.
- Wysocki R, Tamas MJ (2010). How *Saccharomyces cerevisiae* copes with toxic metals and metalloids. *Fems Microbiol. Rev.* 34(6): 925-951.
- Yannai S, Berdicevsky I, Duek L (1991). Transformations of inorganic mercury by candida-albicans and *saccharomyces-cerevisiae*. *App. and Environ. Microbiol.* 57(1): 245-247.
- Zeroual Y, Moutaouakkil A, Blaghen M (2001). Precipitation of mercury by immobilized bacteria (*Klebsiella pneumoniae*) in different support by using fluidized bed bioreactor. *Curr. Microbiol.* 43: 322-327.
- Zhang FS, Nriagu JO, Itoh H (2005). Mercury detoxification from water using activated carbons derived from organic sewage sludge. *Water Res.* 39: 389-395.