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Full Length Research Paper

Study of a lead tolerant yeast strain BUSCY1 (MTCC9315)

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The present study highlights isolation, characterization and bio-adsorption strategy of a lead tolerant yeast strain designated BUSCY1 from industrial effluent of Chittaranjan Locomotive Workshop, Burdwan, West Bengal, India. The isolate was found to grow best on yeast extract peptone sucrose (YEPS) medium (2% sucrose and 0.2% yeast extract found to be optimum for growth) with profuse production of extra cellular polymeric substance and a reddish pink pigment at 30°C, pH 5.5. The strain could tolerate up to 8 mM lead nitrate. Preliminary analysis revealed that the exopolysaccharide may have some role in lead tolerance. Laboratory scale studies on removal of lead demonstrated that after 72 h of incubation amount of lead ions decreased in supernatant whereas the amount of lead ions increased in the yeast cell wash which indicated bio-adsorption by the yeast strain. The strain was identified as *Rhodotorula mucilaginosa* based on morphological, physiological, biochemical tests and molecular phylogenetic analysis of partial 26S rDNA molecule.

Key words: *Rhodotorula mucilaginosa,* lead tolerant, pigment, BLAST analysis, phylogenetic analysis, exopolysaccharide.

INTRODUCTION

Contamination of water and sediments with heavy metals is a major environmental problem all over the world (Baldrian and Gabriel, 2000; Gavrilescu, 2004; Malik, 2004; Srivastava and Thakur, 2006). The inorganic micropollutants are released by effluents generated from various industries such as electroplating and metal finishing industries, metallurgy, tannery, battery manufacturing industries etc. Introduction of heavy metal compounds into the environment and generally induces morphological and physiological changes in the microbial communities (Vadkertiova and Slavikova, 2006), hence exerting a selective pressure on the microorganisms (Verma et al., 2001).

Yeast are ubiquitous unicellular microorganisms in the natural environments (including aquatic systems) as well as industrial effluents where they are exposed to a variety of conditions with respect to nutrient availability, temperature, pH, osmotic pressure, access to oxygen and water activity, all of which induce stress responses (Hohmann and Mager, 1977). They offer the advantages of having cell wall materials which show excellent metal binding properties (Gupta et al., 2000). Generally microbial biomasses have evolved various measures to respond to stresses via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules, as well as precipitation and

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Abbreviations: YEPS, Yeast extract peptone sucrose; **PDA,** potato dextrose agar; **NA,** nutrient agar; **YEPD,** yeast extract peptone dextrose medium; **YEPS,** yeast extract peptone sucrose medium; **YEPM,** yeast extract peptone maltose; **EPS,** the extracellular polymer; **SEM,** scanning electron microscope.

transformation of metals (Malik, 2004). Vadkertiova and Slavikova (2006) have studied the metal tolerance property of yeast isolated from polluted environments and found that there exist some intraspecific and interspecific variations in the metal tolerance amongst the tested strains.

The heavy metal lead (II) is highly toxic as its presence in drinking water above the permissible limit (5 µg/ml) causes adverse health effects such as anemia, encephalopathy, hepatitis and nephritic syndrome (Lo et al., 1999). A large number of food and natural products have been found to be contaminated with lead. The 27 nation EU has banned imports of Indian honey alleging that consignments of natural nectar from the country are contaminated with lead (The Economic Times, New Delhi, 2010). Considering the ecological benevolence of microorganisms for bioremediation of heavy metals, present work was designed for the study of heavy metal resistant micro-organisms. The manuscript reports identification and characterization of a lead tolerant yeast strain BUSCY1 which is capable of bioadsorption of lead and grows in presence of 1% NaCl belonging to *Rhodotorula mucilaginosa* for the first time to the best of our knowledge.

MATERIALS AND METHODS

Isolation and characterization

Water samples were collected from Chittaranjan Locomotive Workshop, Burdwan in sterilized glass containers and were plated for isolation of microorganisms following serial dilution on potato dextrose agar (PDA) and nutrient agar (NA) supplemented with 5 mM lead nitrate at 30°C for fungal cultures and at 37°C for bacterial cultures respectively. Several bacterial and fungal (including yeast) isolates were purified and preserved as -20°C glycerol stock. Yeasts were taken for study first. The colony morphology of yeast isolates were studied after growing them on PDA, yeast extract peptone dextrose medium (YEPD), yeast extract peptone sucrose medium (YEPS), yeast extract peptone maltose (YEPM). Cell morphology was determined under compound microscope. Microscopic images were recorded with Leica Microscope (DMLB, Japan) (Figure 8) and Scanning electron microscope (Nikon S530 Hitachi, Japan) (Figures 12, 13, and 14). Out of several isolates, one namely BUSCY1 showed significant tolerance to lead. This isolate was taken for further study. The isolate was identified as *R. mucilaginosa* based on morphological, physiological, biochemical tests (Table 1) and molecular phylogenetic analysis of partial 26SrDNA sequence (continuous stretch of 629 nucleotides) carried out at MTCC, IMTECH, Chandigarh, India. The strain was deposited at MTCC (MTCC9315) and the accession number of its partial 26S rDNA sequence is GU074381.

Determination of optimum temperature, pH for yeast growth

The isolate BUSCY1 was grown on YEPS broth at different temperature: 20, 25, 30, 35 and 40°C, and at different pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 for 72 h on an orbital shaker at 120 rpm. After incubation period, growth was monitored spectrophotometrically (UV-Vis 1700 Pharmaspec, Shimadzu) at 540 nm and also by measuring increment in dry biomass.

Determination of salt stress tolerance of the yeast

It has been reported that the yeast cells having salt stress tolerance generally possess uronic acids or negatively charged molecules in the exopolymerics [the extracellular polymer (EPS)] layer and the number of uronic acids is directly proportional to the heavy metal chelation (Dae and Eui, 2003b). So, in present study, trials were being made to determine the salt tolerance capacity of the yeast strain, for which 1 ml (10^8 cells/ml) of BUSCY1 was inoculated in YEPS medium supplemented with different concentrations of NaCl (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%) at 30°C and incubated for 72 h with shaking at 120 rpm. After incubation cells were centrifuged at 8000 rpm for 10 min, the pellet was collected, dried and dry biomass weighed. At the same time yeast growth was measured spectrophotometrically(UV-Vis 1700 Pharmaspec, Shimadzu) at 540 nm (Abdel Nassera and El Moghaz, 2010).

Exopolymer extraction

For exopolymer extraction freshly grown culture of BUSCY1 was inoculated into YEPS broth pH 5.5 at 30°C for 72 h with constant shaking at 120 rpm. Culture was centrifuged at 8000 rpm for 10 min. Pellet was then treated with boiling water and 1 N HCl, followed by centrifugation at 8000 rpm for 10 min. Pellet was discarded and to the supernatant double volume of 96% (v/v) ethanol was added followed by incubation for 24 h at 4°C to precipitate exopolymer. The precipitate was collected by centrifugation and was dialyzed for 3 days and freeze dried (Doughlas et al., 1988).

Determination of heavy metal (lead) tolerance of the yeast

For determining lead tolerance capacity, the BUSCY1 was grown in YEPS broth with different concentrations of lead-nitrate (1, 2, 4, 6 and 8 mM) at 30°C and incubated for 72 h with shaking at 120 rpm. Cells were collected by centrifugation at 8000 rpm for 10 min, After incubation period, growth was monitored spectrophotometrically (UV-Vis 1700 Pharmaspec, Shimadzu) at 540 nm and also by measuring increment in dry biomass (Johncy et al., 2010).

Biosorption of lead (II) ions

For biosorption BUSCY1 cells were grown at lead nitrate containing 30 ml of YEPS broth taken in each of the four conical flasks containing different concentrations of lead nitrate (2, 4, 6 and 8 mM) at 30°C on an orbital shaker for 72 h. Control flasks without lead nitrate were maintained for each concentration of lead nitrate. After incubation, 30 ml of culture broth was taken from each of the conical flasks, centrifuged at 8,000 rpm for 10 min at 4°C (Remi, C 24 BL). The supernatant was filtered through Whatmann no.1 filter paper and atomic absorption spectrophotometric assay was carried out using GBS Avanta AAS. Pellet was washed for 3 times with MiliQ water, centrifuged at 8000 rpm for 10 min. The cell pellet was digested with 1N HCl and followed by centrifugation at 8000 rpm for 10 min. The supernatant (washed solution) was assayed by Atomic absorption spectrophotometry (GBS Avanta) (Rabei et al., 2009).

In-silico approaches used for the analysis of 26S r DNA partial sequence

The partial 26S rDNA gene sequence of this strain (Accession no GU074381) was used as query to search for close relatives in GenBank by using BLAST (Altschul et al., 1990). From the preliminary output of BLAST (having more than 90 homologous

Table 1. Test.

Source	Growth
D-Glucose fermentation	(-)
D-Galactose fermentation	(-)
Maltose fermentation	(-)
Me-alpha-D-glucoside fermentation	(-)
Sucrose fermentation	(-)
Alpha, alpha trehalose fermentation	(-)
Melibiose fermentation	(-)
Lactose fermentation	(-)
Cellobiose fermentation	(-)
Melezitose fermentation	(-)
Raffinose fermentation	(-)
Inulin fermentation	(-)
Starch fermentation	(-)
D-Galactose fermentation	(+)
Growth on L-sorbose	(+)
Growth on D-ribose	(+)
Growth on D-xylose	(+)
Growth on D-arabinose	(+)
Growth on sucrose	(+)
Growth on maltose	(+)
Growth on cellobiose	(+)
Growth on salicin	(+)
Growth on raffinose	(+)
Growth on melezitose	(+)
Growth on Me-alpha-D-glucoside	(-)
Growth on inulin	(+)
Growth on starch	(-)
Growth on glycerol	(-)
Growth on erythritol	(-)
Growth on ribitol	(+)
Growth on XYLITOL	(+)
Growth on L-arabinitol	(-)
Growth on D-glucitol	(+)
Growth on D-mannitol	(+)
Growth on galactitol	(-)
Growth on myo-inositol	(-)
Growth on D-glucono-1,5-lactone	(+)
Growth on D-gluconate	(-)
Growth on D-glucuronate	(-)
Growth on succinate	(+)
Growth on citrate	(-)
Growth on methanol	(-)
Growth on ethanol	(+)
Growth at 25°C	(+)
Growth at 30°C	(++)
Growth at 35°C	(+)
Growth at 37°C	(+)
Growth at 42°C	(-)
Acetic acid production	(-)
Urea hydrolysis	(+)
Pink colonies	(+)

Table 1. Contd.

Budding cells	(+)
Splitting cells	(-)
Filamentous	(-)
Pseudohyphae	(-)
Septate hyphae	(-)
Arthroconidia	(-)
Ballistoconidia	(-)
Symmetric ballistoconidia	(-)
Ascospores	Round, oval, conical, reniform
Polar budding	(-)
Carotenoid production	(+)

(+), Good growth; (++), better growth; (-), no growth.

sequences from various strains of *R. mucilaginosa)* sequences of 16 strains (having greater similarities with the query) along with the 26S rDNA sequence of *R. mucilaginosa* type strain (AF070432) were taken for phylogenetic analysis. *Rhodotorula glutinis* 26S rDNA sequence (Acc no EU441897) was taken as outgroup. The sequences were aligned using CLUSTAL_X (Thompson et al., 1997) and the alignment was manually edited. Phylogenetic tree was constructed by TREECON (Van de Peer and Wachter, 1997). The evolutionary distance was calculated using Neighbor-joining method (Saitou and Nei, 1987) according to Kimura's two parameter model (Kimura, 1980). Bootstrap value of 100 was taken to assess the confidence limit of the branching (Felsenstein, 1985).

RESULTS AND DISCUSSION

The Yeast strain which has been identified as *R. mucilaginosa* from 26S rDNA partial sequencing (Accession no. GU074381, NCBI) exhibited optimum growth on YEPS medium containing 2% sucrose suggests sucrose play a significant role in the increment of biomass (Figure 9). Increasing concentration of sucrose above 2% (3, 4 and 5%) decreases the biomass content but otherwise increases the EPS (exopolymer) production (Figures 4 and 6). YEPS medium supports the good growth of the yeast isolate. YEPS medium was also found to support the synthesis of a reddish pink colour pigment (Figure 9). The optimum pH and temperature for the growth of the yeast was recorded to be 5.5 and 30°C respectively (Figures 1 and 2) which suggest that pH and temperature also play a vital role for the growth of *R. mucilaginosa*. In YEPS medium highest yeast biomass was recorded at the pH 5.5 and at temperature 30°C (Figures 1 and 2). Fermentation of different substrates gives mixed result (Table 1). It was evident from the results that the yeast strain has capacity to produce extra polymeric substance against the salinity stress (NaCl) and heavy metal stress (lead nitrate). Higher concentration of lead nitrate was proved to EPS production by the yeast (Figure 7). The exopolysaccharide structure determination is in progress (whether uronic acids or any other negatively charged groups are present or not). NaCl stress at 1% concentration and lead nitrate stress at 1 mM concentration supplied with yeast extract peptone sucrose medium were appeared to be the best for optimum biomass production (Figures 3 and 5) and pigment production (Figures 10 and 11) by the yeast, after the previously mentioned concentration of biomass production decreases and pigment biosynthesis inhibited which also suggest that lead nitrate and NaCl plays a significant role in growth and pigment production (investigation is in progress) of (Figures 10 and 11). 8 mM of lead nitrate (Figures 13) and 10% NaCl (Figures 14) stress may impart some effects on the morphological changes of the yeast cells which were observed under the scanning electron microscope (SEM). It was clearly revealed from the bioadsorption experiment that after 72 h of incubation of the yeast in lead nitrate containing YEPS medium amount of lead ions, decreases in supernatant as the amount of lead ions increases in the yeast cell wash (Table 2 and Figure 16). Under experimental conditions described previously, higher levels of lead nitrate could be entrapped into the binding sites present in the exopolysaccharide of *R. mucilaginosa* by biosorption (Das et al., 2008). Generally microbial biomasses have evolved various measures to respond to stresses via processes such as transport across the cell membrane entrapment in extracellular capsules, precipitation and transformation of metals enzymatic reduction (Malik, 2004), in this communication we have tried to find out the biosorption capability of *R. mucilaginosa*. Cho and Kim (2003a) reported lead biosorption by *R. glutinis* but lead biosorption by biomass of *R. mucilaginosa* another approach of bioremediation. Biosorption of copper, uranium, cadmium, mercury by biomass and extracellular polymeric substances of *Saccharomyces, Aspergillus, Ulva, Pichia, Rhizopus, Rhodotorula* etc. are the modern way of treating industrial effluent.

The partial 26S rDNA gene sequence of the strain BUSCY1 (Acc No GU074381) showed high level of sequence similarity with that of other strains belonging to *R. mucilaginosa*. The preliminary result (having more

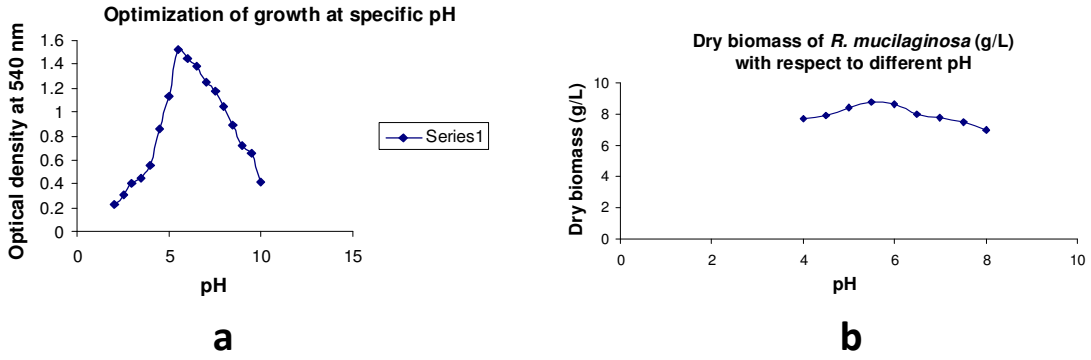

Figure 1. pH optimum for growth of *R. mucilaginosa* (MTCC 9315).

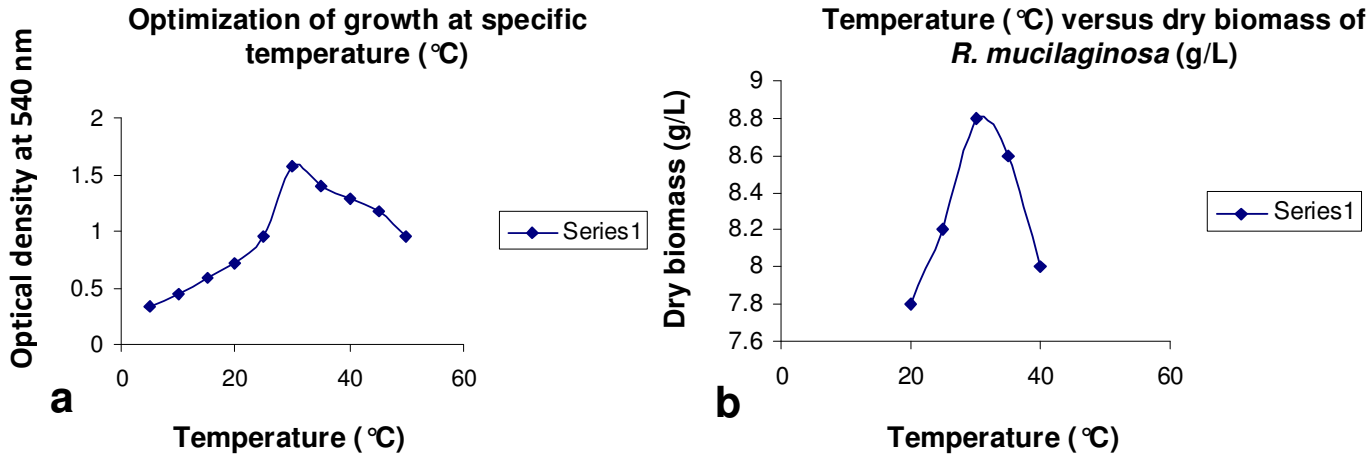

Figure 2. Temperature optimum for growth of *R. mucilaginosa* (MTCC 9315).

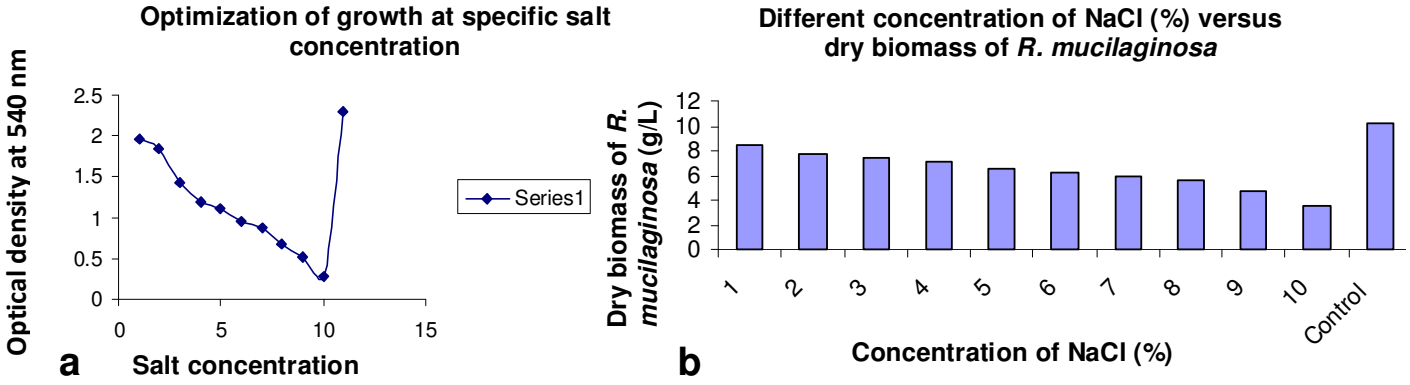

Figure 3. Effect of salt stress on growth of *R. mucilaginosa* (MTCC 9315).

than 90 hits) showed that most of them were either identical or differ by only few nucleotides. In order to have a meaningful conclusion, sequences that were moderately different were chosen for phylogenetic analysis. The strain BUSCY1 (GU074381) formed a distinct cluster along with strain MZKI K453 of *R. mucilaginosa* (Figure 15). The analysis firmly proves that the strain BUSCY1 belongs to *R. mucilaginosa* lineage.

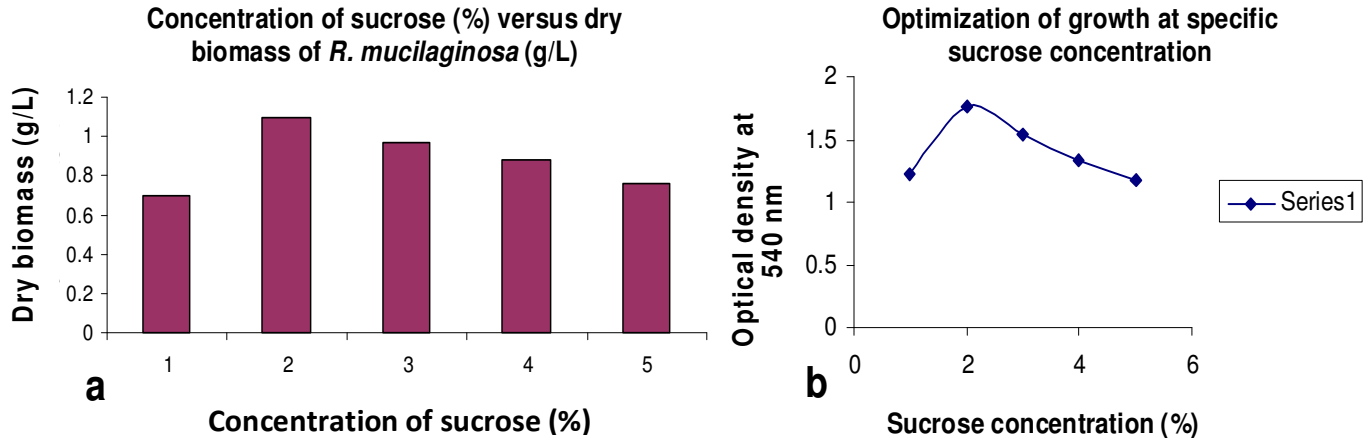

Figure 4. Effect of different concentrations of sucrose on growth of *R. mucilaginosa* (MTCC 9315).

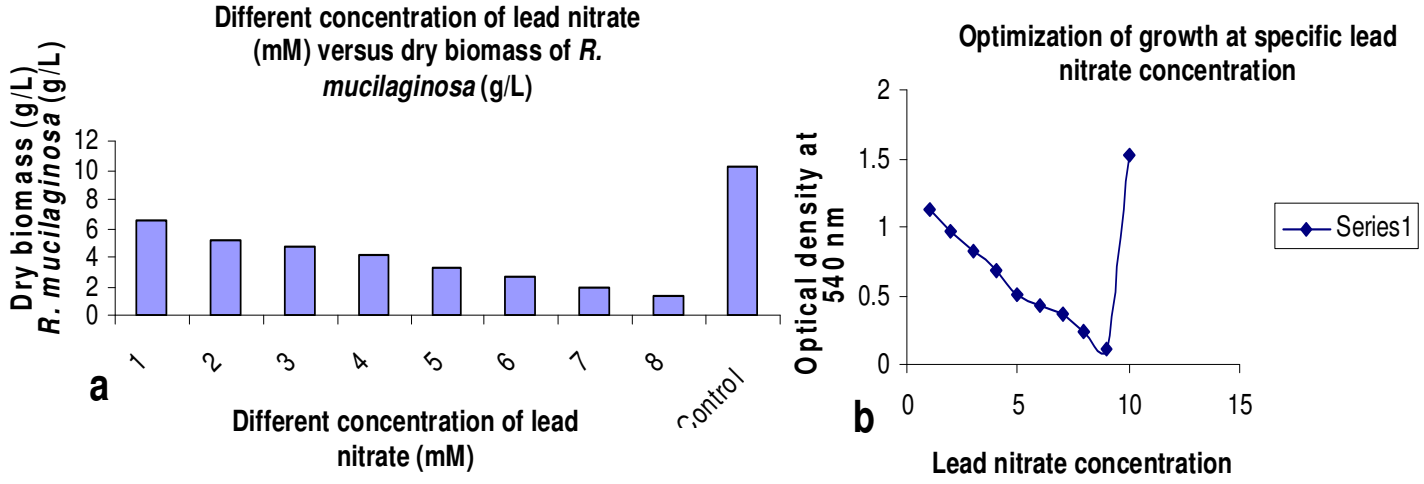

Figure 5. Effect of lead nitrate on growth of *R. mucilaginosa* (MTCC 9315).

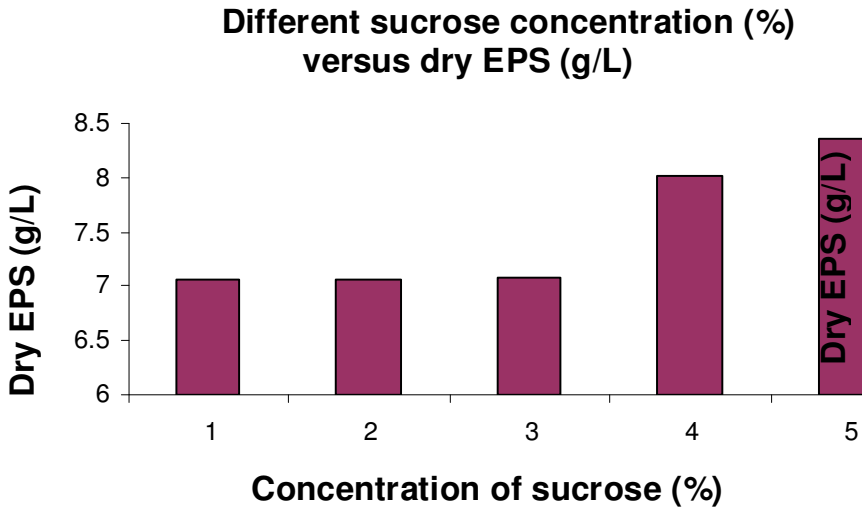

Figure 6. Effect of different sucrose concentration.

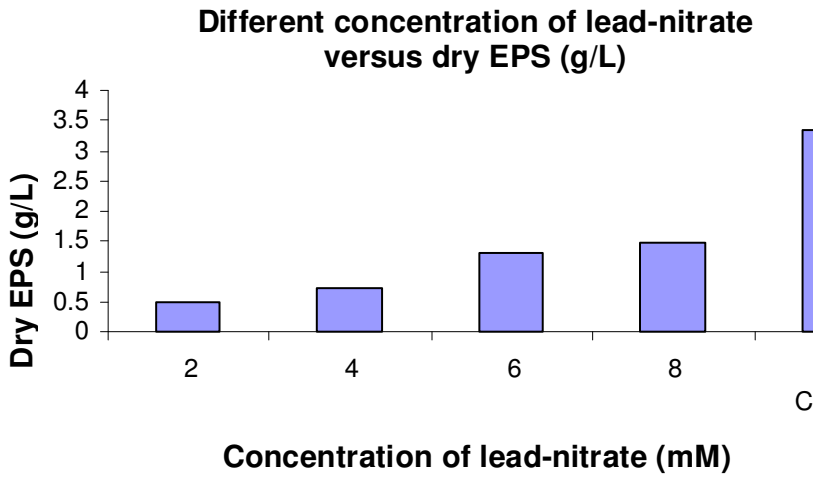

Figure 7. Effect of different concentrations of lead nitrate on dry EPS production by *R. mucilaginosa* on dry EPS production by *R. mucilaginosa* (MTCC 9315) (MTCC 9315).

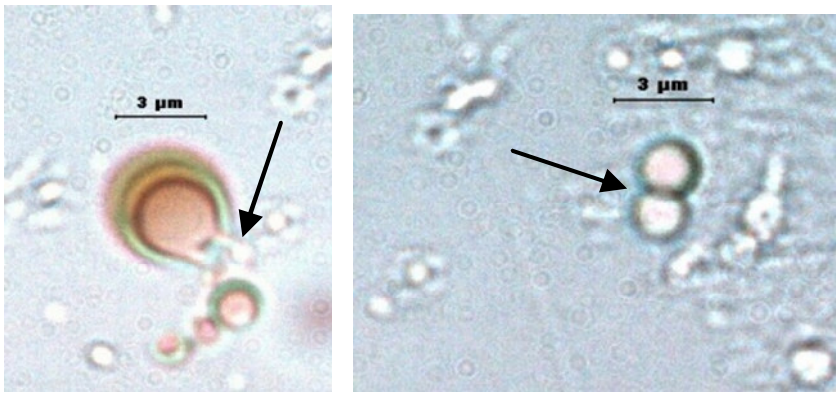

Figure 8. *R. mucilaginosa* under Lica Microscope (DMLB, Japan).

Figure 9. *R. mucilaginosa* grown in yeast extract peptone sucrose agar (2%).

Figure 10. *R. mucilaginosa* grown in yeast extract peptone sucrose broth supplied with 1, 2 and 3 mM lead nitrate.

Figure 11. *R. mucilaginosa* grown in yeast extract peptone sucrose broth supplied with 1, 1.5, 2, 3 ….8, 9 and 10% NaCl.

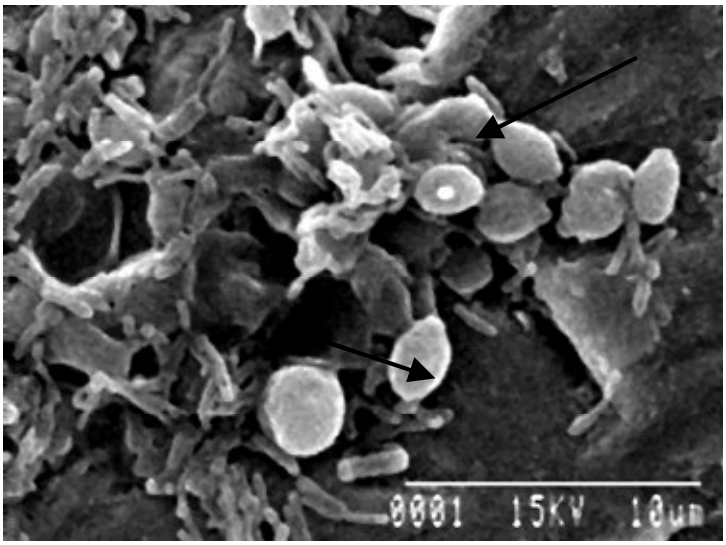

Figure 12. Yeast cells in 2% sucrose supplement.

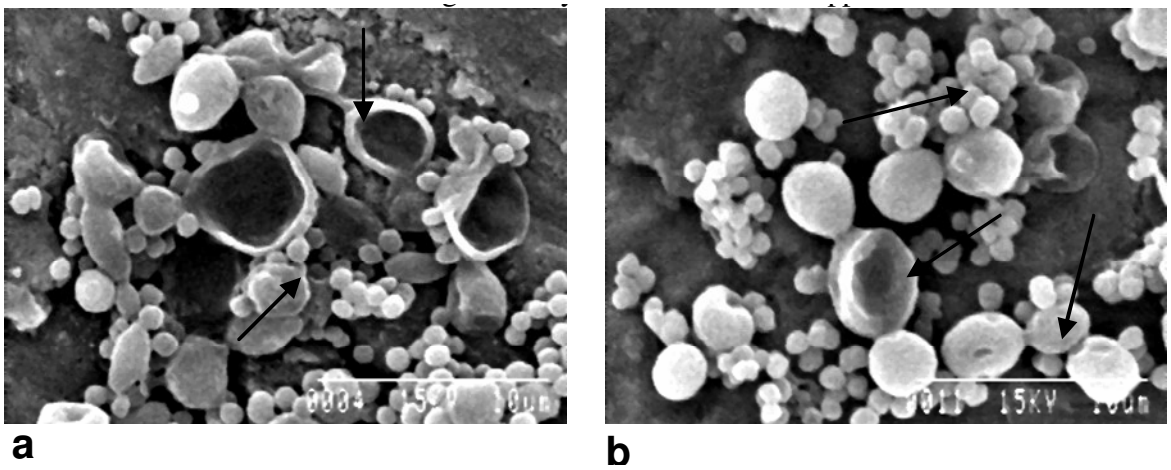

Figure 13. Yeast cells in 8 mM Lead nitrate supplement.

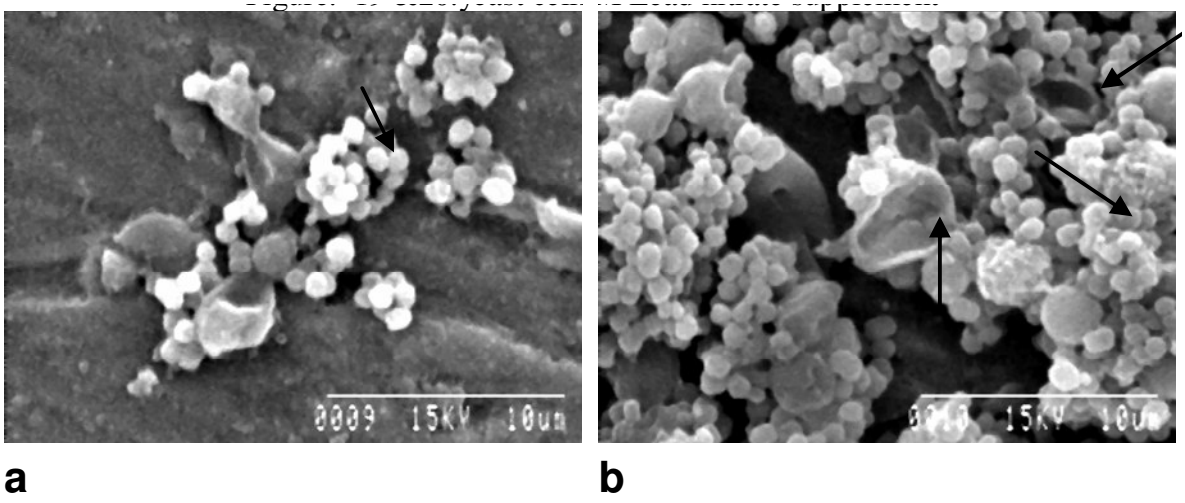

Figure 14. Yeast cells in 10% NaCl supplement.

Table 2. Uptake of Lead (%) = $\frac{(C_0-C_f)}{C_0} \times 100$.

Type of sample	Concentration of lead (mM)	C_0 (mM) initial concentration	C_f (mM) final concentration after 72 h	% uptake of lead
Cell wash with MiliQ water and with 1N HCl (3 times)	2	0.92	0.45	51.08±0.50
Do	4	1.25	0.33	73.6±0.45
Do	6	1.61	0.005	99.68± 0.21
Do	8	1.80	0.002	99.88± 0.13
				Amount of lead remains in the supernatant
Supernatant	2	0.92	0.002	0.918
Do	4	1.25	0.003	1.247
Do	6	1.61	0.004	1.606
Do	8	1.80	0.006	1.794

C_0 - Initial metal concentration, C_f - Final metal concentration.

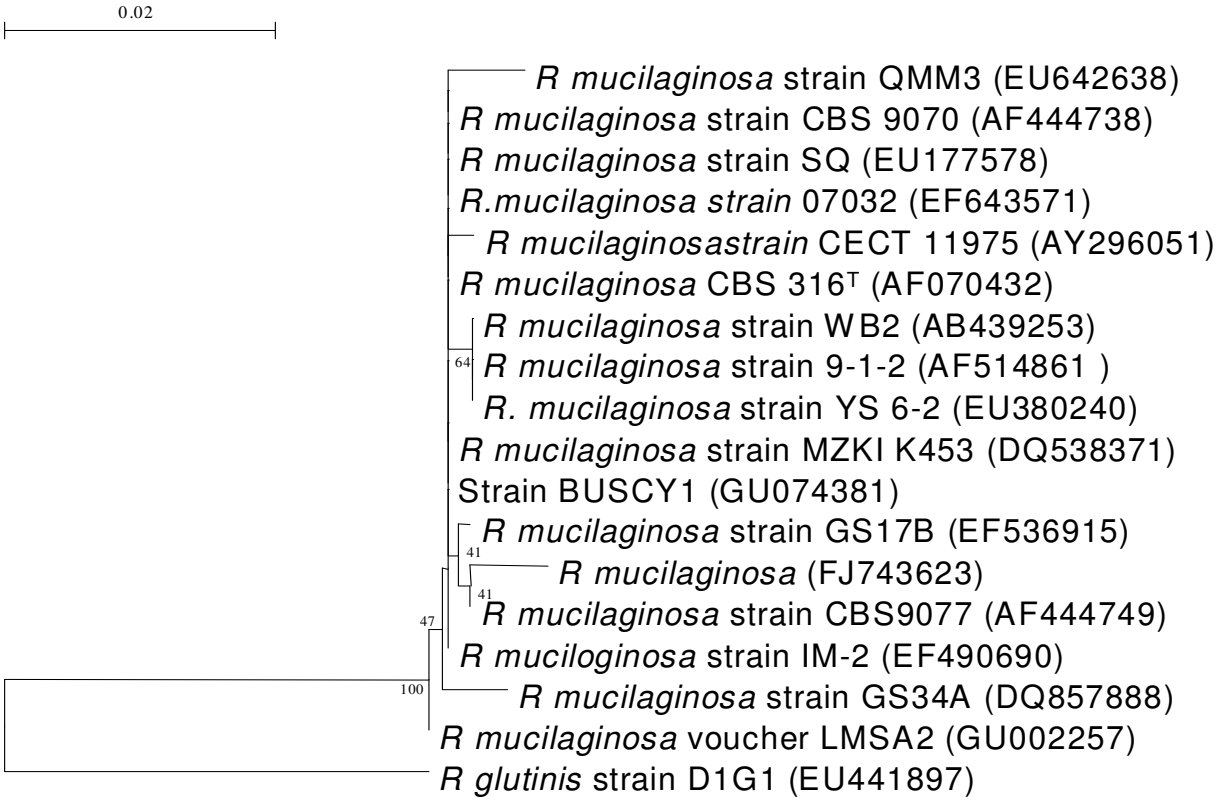

Figure 15. Phylogenetic tree based on 16s rDNA sequence analysis, showing relationship of strain BUSCY1 with other *R. mucilaginosa* strain (including type strain). Numbers at the node indicate boostrap value (more than 40 are displayed) of 100 replicate. The tree was generated using TREECON (Van de Peer and Watcher De) with Jukes and Cantor's (1969) parameter. Bar. 0.02 substitution per sites.

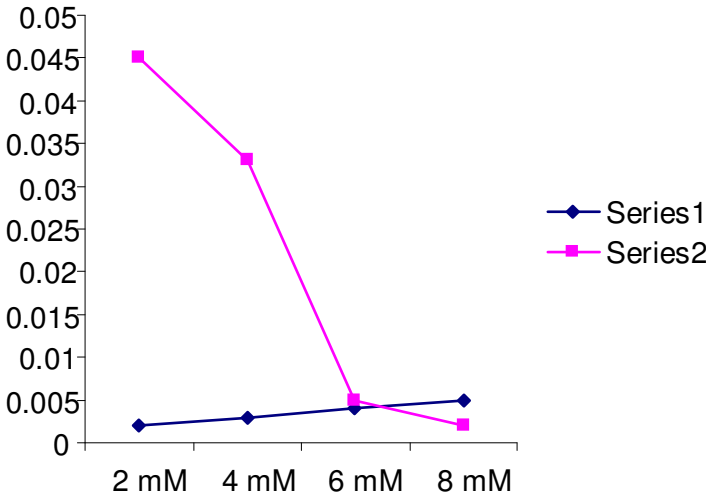

Figure 16. It shows that after 72 h the concentration of Lead ions remains in the supernatant (series 1) was less than the lead ions remains in the cell wash (series 2).

R. mucilaginosa biomass may have useful applications for the removal of heavy metals from the industrial effluents or the biomass with exopolysaccharide may be used as a water purifier for heavy metals containing drinking water owing to having their ability of bioaccumulation of heavy metals (Das et al., 2008).

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REFERENCES

Abdel NA, El-Moghaz (2010). Comparative study of salt tolerance in *Saccharomyces cerevisiae* and *Pichia pastoris* yeast strains. Adv. Biores., 1(1):170-177.

Altschul SF, Warren G, Webb M, Eugene WM, David JL (1990). Basic local alignment search tool. J. Mol. Biol., 215:403-410.

Baldrian P, Wiesche C, Gabrie J, Nerud F, Zadrazil F (2000). Influence of Cadmium and Mercury on activities of lignolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil. J Appl. Environ. Microbiol., 66:2471-2478.

Cho HD, Kim YE (2003a). Charaterization of Pb^{+2} biosorption from aqueous solution by *Rhodotorula glutinis*. Bioprocess Biosyst Eng., 25:271-277.

Das N, Vimala R, Karthika P (2008). Biosorption of heavy metals-an overview. Indian J. Biotechnol., 7:159-169.

Dae HC, Eui YK (2003b). Characterization of Pb^{+2} bioadsorption from aqueous solution by *Rhodotorula glutinis*. Bioprocess Biosyst Eng., 25(5):271-277.

Doughlas HB, Miriam EW, Michael S (1988). Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement. Proc. Natl. Acad. Sci., 85:3923-3927.

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39:783-791.

Fourest E, Roux CJ (1992). Heavy metal biosorption by fungal mycelial by- products: mechanisms and influence of pH. Appl. Microbiol. Biotechnol., 37(3):399-403.

Gavrilescu M (2004). Removal of Heavy metals from the Environment by Biosorption. Eng. Life Sci., 4(3):219-232.

Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000). Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. Curr. Sci., 78(8):967-973.

Hohmann S, Mager PWH (1977).(Eds.) Yeast stress Responses. Topics in Current Genetics Vol 1.

Johncy RM, Hemambika B, Hemapriya J, Rajesh KV (2010). Comparative Assessment of heavy metal removed by immobilized and dead bacterial cells. A biosorption approach., Afr. J. Environ. Sci. Technol., 4(1):23-30.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16:111-120.

Lo W, Chua H, Lam KH, Bi SP (1999). A comparative investigation on the biosorption of lead by filamentous fungal biomass. Chemosphere, 39:2723-273.

Malik A (2004). Metal bioremediation through growing cells. Environ. Int., 30:261–278.

Saitou N, Nei M (1987).The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Bio. Evol., 4:406-425.

Srivastava S, Thakur IS (2006).Biosorption Potency of *Aspergillus niger* for Removal of Chromium (VI). Curr. Microbiol., 53:232–237.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. Nucleic Acids Res., 25:4876-4882.

Vadkertiova R, Slavikova E (2006). Metal tolerance of yeasts isolated from water, soil and plant environments. J. Basic Microbiol., 46:145-152.

Van de Peer Y, Wachter De R (1997).Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Appl. Biosci., 13:227-230.

Verma T, Srinath T, Gadpayle RU, Ramteke PW, Hans RK, Garg SK (2001). Chromate tolerant bacteria isolated from tannery effluent. Bioresour. Technol., 78:31-35.