Heavy metal-induced oxidative stress and alteration in secretory proteins in yeast isolates

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Abstract
In the recent years, yeasts have evolved as potent bioremediative candidates for the detoxification of xenobiotic compounds found in the natural environment. Candida sp. are well-studied apart from Saccharomyces sp. in heavy metal detoxification mechanisms. In the current study, Candida parapsilosis strain ODBG2, Candida sp. strain BANG3, and Candida viswanathii strain ODBG4 were isolated from industrial effluents and contaminated ground water, and were studied for their metal tolerance. Among these three isolates, the metal tolerance was found to be more towards Lead (Pb 2 mM), followed by Cadmium (Cd 1.5 mM) and Chromium [Cr(VI), 1 mM]. On further exploring the involvement of primary defensive enzymes in these isolates towards metal tolerance, the anti-oxidative enzyme superoxide dismutase was found to be prominently high (25% with respect to the control) during first 24 h of metal–isolate interaction. The Catalase enzyme assay was observed to have increased enzyme activity at 48 h. It also triggered the activity of peroxidases, which lead to the increase in reduced glutathione in the organism by 0.87–1.9-fold as a metal chelator and also as a second-line defensive molecule. The exoproteome profile showed the early involvement (exponential growth phase) of secreted proteins (low-molecular-weight) of about ~40–45 kDa under Cd and Pb stress (0.5 mM). The exoproteome profiling under heavy metal stress in Candida parapsilosis strain ODBG2 and Candida viswanathii strain ODBG4 is the first report.

Keywords Candida sp. · Exoproteome · Secretory proteins · Glutathione · Superoxide dismutase · Low-molecular-weight proteins

Abbreviations
Pb  Lead
Cd  Cadmium
mM  Millimolar
SOD  Superoxide dismutase
CAT  Catalase
GSH  Glutathione
YPDA  Yeast peptone dextrose agar

HM  Heavy metal (s)
MIC  Minimal inhibitory concentration
ITS  Internal transcribe sequences
PCR  Polymerase chain reaction
TBE  Tris–borate EDTA
BLAST  Basic local alignment search tool
MEGA  Molecular evolutionary genetics analysis
PMSF  Phenylmethylsulfonyl fluoride
H2O2  Hydrogen peroxide
DTNB  5, 5′-Dithiobis(2-nitrobenzoic acid)
SDS-PAGE  Sodium dodecyl sulphate polyacrylamide gel electrophoresis
CHAPS  3-Cholamidopropyl dimethylammonio 1-propanesulfonate
DTT  Dithiothreitol

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Introduction

Metals play an important role in the homeostasis of the biological systems. Among the metals, few are considered as hazardous to living biota and characterized as heavy metals (HMs) and metalloids. The HMs has become a part of the environment due to geochemical cycles and as well as anthropogenic activities such as usage of pesticides in excess, industrial effluents, chemical manufacturing unit sites, etc. (Sapatpute et al. 2019). The presence of hazardous metals in the nature exerts various kinds of deformities in the biological systems in all, ranging from microbes to human at the level of biochemical to molecular sites (Lin et al. 2019; Amoakwah et al. 2020). In the global scenario, the scarcity of portable water is high due to contaminants in fresh water system and also the ground water. This is due to geogenic origin as well as the percolating water from industries, especially with arsenic (As). The HMs like mercury (Hg), lead (Pb), cadmium (Cd), and fluoride (F) account as major contaminants (Mohankumar et al. 2016; Shaji et al. 2021). The HMs such as Cd, Pb, Cr, and Hg majorly impact the plants by altering the photosynthesis, yield, and bioaccumulation, leading to HMs entering into the food chain (Khosropour et al. 2019). In animals and human beings, the HMs disturb various cells leading to neurological disorders, decreased immunity, altered endocrine hormones, various tissue damages, and breast cancer (Itoh et al. 2014; Mostafa et al. 2016; Lin et al. 2017). The HMs cause deliberative effects on microbial community in the soil, damaging cell wall structures, enzyme activities, along with inhibiting mechanism on quorum sensing, etc. (Thornhill et al. 2017; Chu 2018). Certain microbes like bacteria and fungi form a natural habitat in these contaminated sites by developing strong biochemical defense mechanisms to thrive under harsh conditions (Bhavya et al. 2015; Geetha et al. 2020). Among the microbes, filamentous fungi and yeasts have evolved strongly, portraying as potent bioremediative candidates for xenobiotic compounds (Dey et al. 2020). Recent studies have reported Candida tropicalis, Candida albicans, Candida parapsilopsis, Candida etchellsii, and Candida lipolytica as a few HM-resistant yeasts (Kachiprath et al. 2019). The fungi/yeast possess a versatile group of mechanisms such as biosorption, bioaccumulation, and sequestration which aids in the detoxification of HMs (Congeevaram et al. 2007), during the process leads to the production of reactive oxygen species (ROS). ROS are byproducts of metabolic pathways. ROS includes hydroxyl ions, hydrogen peroxide, super oxide radicals, etc., which plays a vital role in homeostasis. Elevated ROS causes lipid peroxidation, damage to cell membrane, nucleus, proteins, and other biomolecules. On the other hand, ROS directly acts on activators of transcriptional factors and helps the organism to acclimatize for oxidative stress environment (Schieber and Chandel 2014). The excess ROS in the system needs neutralization to overcome the damages by the production of antioxidant enzymes such as catalase (CAT), peroxidases (PO), glutathione peroxidases (GPx), superoxide dismutase (SOD), glutathione s-transferases (GSTs), etc., (Bandyopadhyay et al. 1999). Antioxidative enzymes reduce the toxicity by converting ROS into water, meanwhile helps in the activation of series of enzymatic pathway leading to the production of low molecular compound like GSH, Metallothiones, oxalic acid, secondary metabolites, etc. (Lazarova et al. 2014). These molecules interact with the HMs through -SH groups, lead to the inactivation of heavy metals, and render the homeostasis maintenance of the cell. The detoxification process is a combinatorial strategy during which involvements of various extra-cellular enzymes like lipase, DNAase, and laccase are identified or observed (Santos et al. 2017). During stress and infectious state, yeast secretes various kinds of metabolites to its surrounding environment to regulate its growth and maintenance. The secretary molecules are not only encompassed of primary and secondary metabolites, exopolysaccharides, but also possess various sets of proteins known as exoproteome/extra-cellular proteins. These metabolites and exoproteins help the fungi to adapt to the harsh changes in its living habitat (Selvam et al. 2015). The profiling of exoproteins in fungi under heavy metal stress helps in understanding the stress response systems.

Based on this knowledge, the current work was targeted to study the role of SOD and CAT, antioxidant enzymes and reduced GSH levels at different time intervals and metal concentrations. Furthermore, as there is a lacuna in the field of exoproteome stress biology, preliminary work was carried out to know the secretary proteome profiles on SDS-PAGE. This part of work has been reported for the first time in Candida parapsilosis and Candida viswanathii strains isolated from heavy metal contaminated water source from the prevailing study.

Materials and methods

Isolation of yeasts from industrial effluents

The textile effluent samples and HM-contaminated ground water samples were collected from Bengaluru and Odisha industrial areas, respectively, and brought to the laboratory, stored at ambient temperature till further experimentation. Isolation of yeasts was carried out by spread plate technique using 100 µl of each sample plated separately on yeast extract dextrose agar media (YPDA: yeast extract 1%, peptone and dextrose 2% each, agar 1.5%; pH 6.2) and
incubated at 35 °C for 48 h. The colonies were selected and pure cultures were used for further studies.

Preparation of metal stock solutions

The metal salts are the source of heavy metal (HM) ions. Currently, cadmium chloride (CdCl₂), lead nitrate [Pb(NO₃)₂], and potassium dichromate (K₂Cr₂O₇) were used as a source of Cd, Pb, and Cr ions, respectively. Each HM stock solution (50 mM) was prepared in deionized water and stored at room temperature. Furthermore, for the experimental purpose, HM concentrations of 0.5, 1.0, 1.5, and 2 mM were chosen.

Determination of minimum inhibitory concentration (MIC)/metal tolerance test

The MIC towards the HMs was determined at different metal ion concentrations for the yeast isolates at a constant temperature of 35 °C in YPD broth media. The actively growing cells (optical density 1.5 at 600 nm) were transferred into YPD broth amended with HM salts (i.e., 0.5 mM, 1.0 mM, 1.5 mM, and 2.0 mM) and the growth was recorded at 24 h interval till 120 h. The isolates growing in non-HM-amended media were considered as a control. The concentration at which the cultures exhibited minimal growth was considered as MIC.

Molecular identification of heavy metal-tolerant yeast isolates/strains

Colony PCR technique was chosen for the amplification of 5.8 s rRNA gene by choosing the universal primers, the forward primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and the reverse primer ITS4 (5′-TCCTC CGG CTATT GATAT GC-3′) (White et al. 1990). In brief, the log-phase growing colonies were selected and dispensed in 50 µl of deionized water. From this, 2 µl was used for amplification. The PCR reaction mixture consisted of ready to use master mix 20 µl (TaKaRa Taq HS perfect Mix), forward primer (2 µl—ITS 1), reverse primer (2 µl—ITS 4), 2 µl cells as template and 14 µl of sterile water. PCR was carried out at 95 °C for 5 min 30 s for initial denaturation. This was followed by denaturation at 94 °C for 45 s, annealing at 56 °C for 55 s, and extension at 72 °C for 60 s, repeated for 30 cycles. Later, a final extension for 10 min at 72 °C was done. PCR products (amplicons) were analyzed on 1.2% agarose gel using 1X Tris borate EDTA buffer (1X TBE). The PCR amplicons were sent for Sanger sequencing to Juniper Life science Pvt Ltd, Bengaluru for sequencing using ITS 1 (forward primer). The obtained sequences were identified using nBLAST from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were deposited in Gene Bank and accession numbers were obtained. The sequence alignment and phylogenetic tree was carried out among the identified strains using MEGA X (Kumar et al. 2018).

Oxidative stress-induced enzyme studies (SOD and CAT)

Enzyme extraction

The protein extraction was carried out according to Ilyas et al. (2016) with slight modification. In brief, cells were harvested by centrifuging at 12,000 rpm and washed thrice in phosphate buffer of pH 7. Cells were suspended in 1.5 ml of extraction buffer containing 50 mM Tris–HCl buffer of pH 7.4, 5 mM EDTA, 0.5 M sucrose, 100 mM KCl, and 1 mM PMSF. Cells were sonicated for 2 min with 5 s pulse on and 9 s pulse off at 25 Hz for three cycles, and later centrifuged at 10,000 rpm for 12 min (4 °C). Protein concentration in the sample was determined by Bradford method and BSA as a standard (Bradford 1976). The extracts were aliquoted and stored at 4 °C until further use.

Enzyme activity assay

Superoxide dismutase (SOD) assay SOD activity was determined based on indirect assay, i.e., inhibition of auto-oxidation of quercetin (Kengaiah et al. 2020). The reaction contained 16 mM sodium phosphate buffer of pH 7, 8 mM TEMED, 200 mM EDTA, 0.1% quercetin, and crude enzyme extract. The reaction was monitored at 406 nm for 1 min. Percentage inhibition of autoxidation of quercetin was determined.

Catalase (CAT) assay The CAT activity was carried out according to Zeng et al. (2012). The reaction mixture consisted of 50 mM potassium phosphate buffer of pH 7, 8 mM hydrogen peroxide (H₂O₂) (30%), and the crude enzyme extract. The decrease in the absorbance at 240 nm was recorded for 2 min. The activity was calculated and expressed as µmoles of H₂O₂ decomposed/min/mg of protein.

Anti-oxidant biomarker assay

Reduced glutathione (GSH) assay The reduced GSH level in the samples was determined according to Ellman’s method (Ellman 1959) with slight modification. The cells were homogenized in extraction buffer consisting of 0.6% sulfosalicylic acid, 0.1% Tritonx100 in 0.1 M potassium phosphate buffer pH 7.5 with 5 mM EDTA. The cell lysate was collected by centrifuging at 8000 rpm for 10 min at 4 °C. For the determination of reduced GSH content, to 100 µl of about extract, Tris–HCl buffer of pH 8.2, 10 mM DTNB was
added and incubated for 10 min at ambient temperature. The reaction concoction was spun at 3000 rpm for 5 min. The absorbance of clear supernatant was measured at 415 nm. The amount of reduced GSH in the sample was determined using standard reduced GSH curve. The amount of reduced GSH was expressed in the terms of µM/mg of protein.

**Secretory protein analysis by SDS-PAGE—a preliminary study**

The cells were separated from the culture broth by centrifuging at 12,000 rpm for 20 min at 4 °C. The filtrate was filtered through 0.22 µm membrane to remove the cell debris. The proteins were precipitated by TCA-acetone precipitation method with modification (Wu et al. 2014 and Chen et al. 2015a). Briefly, the filtrate was added with 10% TCA followed by overnight incubation at 4 °C. The precipitated proteins were separated by centrifuging at 12,000 rpm, 15 min at 4 °C. Then, the proteins were suspended in 80% ice-cold acetone encompassed of 0.07% β-mercaptoethanol and incubated for 2 h at 4 °C. The contents were centrifuged; pellet was washed thrice with methanol and acetone, respectively. Finally, the protein pellets were air-dried and solubilized in buffer containing urea (7 M), thiourea (2 M), CHAPS (1%), and 65 mM DTT. The Bradford method (Bradford 1976) was adapted for determination of protein concentration in the samples. The 60 µg of protein was suspended in SDS-sample buffer (Tris–HCl buffer of pH 6.8, SDS, β-mercaptoethanol, glycerol, and bromophenol blue) and heated at 95 °C for 5 min. Equal amount of protein (60 µg) was loaded along with the marker; separated on 10% SDS–polyacrylamide mini gel on constant voltage of 70 V for 30 min, followed at 80 V till the complete run. The gel was stained by CBB staining technique and differences in the banding pattern were noted (Laemmli 1970).

**Results and discussion**

**Isolation of yeasts from industrial effluents and contaminated ground water**

The industrial effluent collected from Bengaluru and the contaminated ground water from Gajapathi area in Odisha lead to the isolation, growth and axenic culturing of one and two yeast colonies, respectively. The isolates were coded as BANG3, ODBG2, and ODBG4 for further studies and molecular identification. The microscopic observation exhibited the single-cell structure ovoid in shapes with various budding stages along with clear and distinguished cell wall structure. Figure 1 represents the macroscopic and microscopic views of the yeast isolates. There were not many changes in the morphological and microscopic observations; thus, the exact identification of the isolates was not revealed. This indicated the necessity for molecular-based identification.

**Fig. 1** Macroscopic view of the yeast isolates A ODBG2, B BANG3, and C ODBG4 on yeast extract peptone agar media. The respective microscopic views at 40X in a, b, and c showing the typical yeast cell structure

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Table 1 Minimal inhibitory concentration of yeast isolates to different heavy metal concentrations

| Isolates code | MIC in mM Pb (II) | Cd (II) | Cr (VI) |
|---------------|-------------------|---------|---------|
| ODBG2         | 2                 | 1.5     | 0.5     |
| BANG3         | 2                 | 1       | –       |
| ODBG4         | 1.5               | 1       | 1       |

Determination of MIC (minimum inhibitory concentration)/metal tolerance test

The metal tolerance test/MIC revealed the capability of the isolate ODBG2 later identified as Candida parapsilosis strain ODBG2, to utilize the heavy metals for its growth and tolerated up to the concentration of 2.0 mM Pb, 1.5 mM Cd, and 0.5 mM Cr. The significant difference in the growth pattern was observed after 48 h of metal–isolate interaction with respect to Cd and Pb, after 24 h under hexavalent Cr stress. In the second isolate BANG3; Candida sp. strain BANG3; exhibited the metal tolerance up to 2 mM of Pb and 1.0 mM Cd, with significant growth difference with respect to control (without HM amended) was observed after the incubation period of 72 h and 24 h, respectively, whereas zero tolerance towards Cr(VI). The isolate ODBG4; Candida viswanathii strain ODBG4 was adaptive to grow under Pb at 1.5 mM followed by Cr and Cd at 1.0 mM with a significant difference after the time interval of 48 h. The growth curve of yeast isolate ODBG2, BANG3, and ODBG4 with and without heavy metal amended is shown in the supplementary Fig S1, and the summarization of MICs of different isolates explained above is represented in Table 1. The recent study by Bansal et al. (2019) on Candida parapsilosis, an environmental isolate, and its metal tolerance have indicated the survival capability and utilization under heavy metal such as Ni, Pb, and Cd at the concentration of 12 mM. Similarly, the various studies have shown the ability of Candida sp. to tolerate and to grow under various heavy metals which were isolated from contaminated sources like soil, water and industrial effluent system. The difference in metal tolerance might be ascribed to the level of HM contamination in the water samples, the contact, and incubation time (Azcon et al. 2010; Muneer et al. 2013; Moreno et al. 2019).

Molecular identification of heavy metal-tolerant yeast isolates/strains

The PCR amplicons of the isolates with the codes ODBG2, BANG3, and ODBG4 resulted in the product of ~500 bp size. Furthermore, the sequencing of the region ITS1, 5.8rRNA, and ITS2 using ITS 1 (forward primer) of the amplicons ODBG2, BANG3, and ODBG4 resulted in 493 bp, 438 bp, and 447 bp product. The BLAST search analysis showed high sequence similarity to Candida parapsilosis with respect to our isolate code ODBG2, Candida sp. with respective isolate code BANG3, and Candida viswanathii with respect to isolate code ODBG4. Thus, obtained and identified sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Accession numbers were obtained. The code of isolates and accession no. are represented in Table 2. The evolutionary history was inferred using the Maximum-Likelihood method and Tamura-Nei model (Tamura and Nei 1993). Evolutionary analyses were conducted in MEGA X (Felsenstein 1985; Kumar et al. 2018); Fig S2 (Supplementary Figure) represents the phylogenetic tree.

Oxidative stress-induced enzymatic and non-enzymatic studies (SOD, CAT, and GSH)

Effect of heavy metal-induced oxidative stress on SOD activity

The primary defense antioxidant enzymes in any biological system are attributed towards the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidases (PO). The activity of SOD was detected based on the percentage inhibition of quercetin autoxidation in the control and treated samples. The SOD activity in Candida parapsilosis strain ODBG2 was found to be high in the range of 83.5% (Cd 0.5 mM), 72.5% (Cd 1.0 mM), 77.5% (Pb 0.5 mM), and 75% (Pb 1.0 mM) during first 24 h of stress; however, the gradual decrease in the abundance at 48 h and thereafter (Fig. 2A), which corresponds to 16%, 27.5%, 22.5%, and 25% high activity with respect to control at 24 h. The SOD activity in Candida viswanathii strain ODBG4 was relatively high for Cd (0.5 mM concentration) at 24 h, and for Pb (1 mM), it slightly increased at 48 and 72 h (Fig. 2B), indicating minimal effect on SOD. SOD is the primary defense enzyme found to be active during abiotic stress-induced intracellular ROS in biological systems, which interacts and converts into H2O2 and water molecule, leading to the sequential activation of other primary defense enzymes such as CAT and

Table 2 Details of the identified isolates with their accession number

| Isolate code | Accession no | Identification/deposition name |
|--------------|--------------|--------------------------------|
| ODBG2        | MZ841637     | Candida parapsilosis strain ODBG2 |
| BANG3        | MZ841638     | Candida sp. strain BANG3 |
| ODBG4        | MZ841639     | Candida viswanathii strain ODBG4 |
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PO, subsequently the non-enzymatic small compounds like GSH (Bandyopadhyay et al. 1999). The varied response of SOD with respective different time intervals and concentrations has been observed in fungi such as Pleurotus ostreatus HAU-2 upon Pb (Zhang et al. 2016) and Trichosporon asahii upon different heavy metal and metalloid treatments (Ilyas et al. 2014). SOD was found to be not effective at the higher concentration rate and incubation time, which is indicating the role of CAT and PO in the system.

Effect of heavy metal-induced oxidative stress enzyme—CAT

The CAT showed increased activity of 38 and 40 μmoles/mg of protein/min upon Pb stress at 0.5 mM and 1.0 mM concentration during at 48 h incubation, and subsequently decreased on 72 h of incubation with 20 and 28 μmoles/mg of protein/min in Candida parapsilosis strain ODBG2 (Fig. 3A). Similarly, CAT activity was higher at

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Fig. 2  A Inhibition of quercetin by SOD heavy metal-treated cells in Candida parapsilosis strain ODBG2. The control protein samples were normalized to get 50% inhibition activity during the assay. Based on this inhibition activity, the test samples were assayed. The bar value represents the mean ± SD of triplicates, where \( p < 0.0001 \). Significant difference with respect to control samples is represented by Dunnett’s multiple comparisons test. B Inhibition of quercetin by SOD in heavy metal-treated cells in Candida viswanathii strain ODBG4. The protein sample control was normalized to get the 50% inhibition activity during the assay. Based on this inhibition activity, the test samples were assayed. The bar value represents the mean ± SD of triplicates, where \( p < 0.0001 \). Significant difference with respect to control samples is represented with Dunnett’s multiple comparisons test.
48 h under Pb stress at both the concentrations with 15 and 16 µmoles/mg of protein/min in Candida viswanathii strain ODBG4, whereas the Cd stress at 0.5 mM induced a steady increase with activity of about 16 µmoles and 18 µmoles/mg of protein/min at 48 h and 72 h, respectively (Fig. 3B). The activity of CAT was found to vary based on the metal ion concentration, time, and the isolate. The high CAT activity in the basal cells could trigger other relative enzymes such as PO, which would be strategic in mitigating the toxicity of heavy metal ion such as Cd, thus exhibiting steady increase under Cd stress (Pradhan et al. 2017).

Fig. 3 A Catalase activity in heavy metal-treated cells in Candida parapsilosis strain ODBG2. The bar value represents the mean ± SD of triplicates, where $p<0.0001$. Significant difference with respect to control samples is represented with Dunnett’s multiple comparisons test. B Catalase activity in heavy metal-treated cells in Candida viswanathii strain ODBG4. The bar value represents the mean ± SD of triplicates, where $p<0.0001$. Significant difference with respect to control samples is represented by Dunnett’s multiple comparisons test.
Effect of heavy metal-induced oxidative stress on non-enzymatic antioxidant-reduced GSH

The intracellular GSH plays a key role for maintaining cell homeostasis under normal and stress conditions. In the current study, the levels of reduced GSH in the cells under heavy metal stress were found to be time and concentration-dependent with respect to the treated yeast isolates. In Candida parapsilosis strain ODBG2, the levels of reduced GSH under Cd (0.5 mM) and Pb (0.5 mM and 1.0 mM) stress at 24 h were found to be lesser to that of untreated cells, expect in Cd (1.0 mM) treatment, which was found to be high. However at 48 h, there was an increase by 1.9-fold (Cd 1.0 mM) followed by 0.87-fold in Pb (1.0 mM) at 48 h. In Candida viswanathii strain ODBG4, a maximum of 1.6- and 1.7-fold increased reduced GSH was found at Cd and Pb (1.0 mM) concentration. Increased GSH content at 48 h under Pb stress is similar to the studies by Rehman and Anjum (2011), with Cd stress in Saccharomyces spp. (Fau­chon et al. 2002). Furthermore, decrease in reduced GSH at 72 h under metal treatment is due to the interaction of SH group of GSH with that of heavy metals, the protective ability towards the toxicity, and detoxification of heavy metals (Gharieb and Gadd 2004; Ilyas et al. 2017) or due to the increased intracellular ions induced activation of other pathways, which leads to imbalance in homeostasis (Zhang et al. 2016). The reduced GSH content under different metal stress and at different time intervals is represented in Fig. 4A, B for the Candida parapsilosis strain ODBG2 and Candida viswanathii strain ODBG4, respectively.

Alterations in secretory proteins upon different heavy metal stress in Candida parapsilosis strain ODBG2 and Candida viswanathii strain ODBG4 by SDS-PAGE

Candida parapsilosis strain ODBG2 and Candida viswanathii strain ODBG4 extracted at 48 h of post-heavy metal stress (0.5 mM concentration) of Pb(II), Cr(VI), and Cd (II) showed similar secretory protein profiles on 10% SDS-PAGE (Fig. 5A, B). In the present study, the SDS-PAGE profile showed two (2) over-expressed intense protein bands in the molecular range of ~40–45 kDa under Cd stress and faint polymorphic bands in the range of
~66–116 kDa. The Pb stress also signposted the secretory protein prominently expressed approximately at 43–44 kDa. Further analogous protein profiles under Cd and Pb in Candida isolates attributed to similar kind of signal perception, gene activation, protein, and metabolite production at the species level. The secretory protein of control and Cr (VI)-treated samples shared similarity, even though the growth was observed at 0.5 mM in both the strains. This could probably due to the time requirement for Cr(VI) to induce the secretion and accumulation of secretory proteins in the media (Feldman et al. 2017). Since this work has been carried out for the first time, further protein analysis studies by LC–MS/MS-based approach are required to know and clarify the expressed protein and extra-cellular-mediated detoxification process.

**Conclusions**

The current study is emphasized to understand the differential ability of HM tolerance among Candida sp., i.e., Candida parapsilosis strain ODBG2 and Candida viswanathii strain ODBG4 isolated from industrial effluent-mediated contaminated ground water systems. These isolates have an ability to be used as potent HM detoxifiers in contaminated environment. It can be presumed that the potentiality of tolerance towards Cd and Pb is due to communication in intra and extra-cellular secretory molecules modulated cell signaling during log-phase growth under HM stress. This contemporary work has paved a way for further investigations on exploring exoproteome identification in Candida sp. upon HM stress by LC–MS/MS-based approach. On further note, the application of proteomics and metabolomics is needed to know the biochemical and signaling modulations occur during the HM stress, which helps in designing of the experiment at large-scale application.

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Declarations

Conflict of interest The authors do not have any competing interest in the submitted work.

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