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Proteomics of *Penicillium chrysogenum* for a Deeper Understanding of Lead (Pb) Metal Bioremediation

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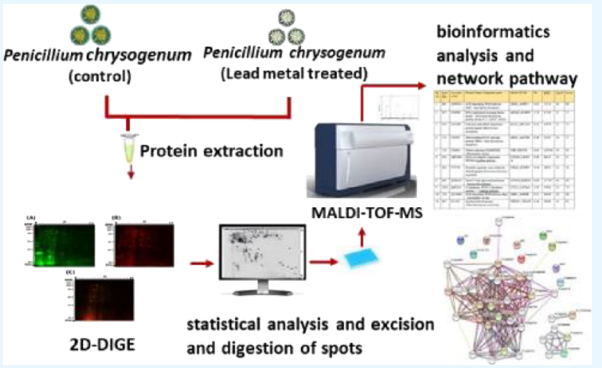
ABSTRACT: *Penicillium chrysogenum* (*P. chrysogenum*), a ubiquitous filamentous fungus, has demonstrated remarkable potential in the bioremediation of lead-contaminated environments. Its inherent tolerance and bioaccumulation capacity for lead (Pb), coupled with its relatively rapid growth rate, make it an attractive candidate for bioremediation applications. This study aims to identify the proteomic changes in *P. chrysogenum*induced by Pb metal stress and unravel the roles of identified proteins in molecular mechanisms and cellular responses. Untargeted proteomic analysis was carried out using a two-dimensional difference in gel electrophoresis (2D-DIGE) coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). This study reported the identification of 43 statistically significant proteins (24 upregulated and 19 downregulated, ANOVA, $p \leq 0.05$; fold change ≥ 1.5) in *P. chrysogenum* as a consequence of Pb treatment. Proteins were grouped according to their function into 18 groups from which 13 proteins were related to metabolism, 11 were related to cellular process and signaling, and 19 proteins were related to information storage and processing. The current study is considered the first report about the proteomics study of *P. chrysogenum* under Pb stress conditions, where upregulated proteins could better explain the mechanism of tolerance and Pb toxicity removal. Our research has provided a thorough understanding of the molecular and cellular processes involved in fungal−metal interactions, paving the way for the development of innovative molecular markers for heavy metal myco-remediation. To the best of our knowledge, this study of *P. chrysogenum* provides valuable insights toward growing research in comprehending the metal−microbe interactions. This will facilitate development of novel molecular markers for metal bioremediation.



1. INTRODUCTION

Environmental contamination by heavy metals has recently been linked to rising ecological and global public health concerns. Heavy metals are defined as naturally occurring elements that may cause toxicity at low exposure levels due to their high atomic weights and densities that are five times greater than those of water.[1] Additionally, due to an exponential rise in their applications in various industrial, agricultural, domestic, and technological fields, human exposure has increased significantly.[2] One of the most toxic heavy metals with high capacity for environmental pollution is lead (Pb) due to its low mobility and nonbiodegradability; therefore, it tends to accumulate in soils and sediments.[3] Due to its nonbiodegradability, Pb has a harmful effect on the environment and therefore affects global health in general.[4] Pb occurs in soil, air, and water, which may enter the environment as a consequence of human activities such as fertilizers, pesticides, urban soil waste, additives in pigments and gasoline, metal plating, chimney of factories, exhaust of automobiles, melting and smelting of ores, and effluents from the storage battery industry.[5]

Remediation of heavy metals, due to their negative impact, is needed, which can be achieved by conventional treatment processes such as thermal treatment, precipitation, chlorination, adsorption, biosorption, ion exchange, bioleaching, filtration, chemical extraction, coagulation, cementation, and stabilization.[1,6] Most of these physical and chemical heavy metal remediation approaches have significant drawbacks, including the destruction of nearby ecosystems and high costs.[7,8] Due to the observed negative impact, alternatives to chemical and physical methods for heavy metal removal need to be addressed. An environmentally friendly remediation

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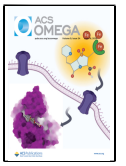

method, bioremediation, has recently gained great concern due to no possible expected drawbacks. Since it relies on microorganisms to remove contaminants from wastewater and soil, bioremediation is regarded as a safe, sustainable technology and a more affordable process.[9]

Microorganisms like bacteria, archaebacteria, yeasts, algae, and fungi showed high ability in the bioremediation process.[10] Among the bioremediation techniques, myco-remediation is a promising technology that has the potential to ameliorate these hazardous chemicals from various environments. Myco-remediation is a form of bioremediation in which fungi are used to decontaminate polluted areas.[11,12] Varied types of fungi and their ability in contaminant removal were studied. From these, filamentous fungi like *Aspergillus* sp., *Curvularia* sp., *Acrimonium* sp., and *Pythium* sp. have been studied for their metal tolerance ability. Sánchez demonstrated the degradation ability of *Aspergillus niger*, *Rhizopus* sp., *Candida* sp., *Penicillium* sp., and *Mucor* sp. on petroleum products and crude oil.[13] Dharmasiri et al. have reported substantial removal of petroleum hydrocarbons from soil contaminated with petrol and diesel in the presence of *A. niger* and *Phanerochaete chrysosporium* at short incubation periods as indicated by total organic carbon (TOC) removal. The unique capacity of fungi to degrade the pollutants is its application of a variety of extracellular and intracellular enzyme systems for detoxification and biodegradation.[14] Furthermore, the biosorption, bioaccumulation, and sequestration mechanisms that fungi possess help in the detoxification of heavy metals.[15] Heavy metals may also create stress conditions for fungi; however, their antioxidant enzymes mitigate such stress and increase their defense mechanism. The detoxification process is a combinatorial strategy that involves various extracellular enzymes such as lipase, DNase, and laccase.[16] The current study applied *P. chrysogenum* as a bioremediator for Pb since it showed high abundance in various soils from contaminated areas in Riyadh city such as the Riyadh industrial city, the Aldiriyah agricultural area, and the Riyadh landfill. However, the *P. chrysogenum* investigated was isolated from Althumamah (north of Riyadh city) from a soil with 1.07 mg/L Pb concentration. *P. chrysogenum* is a filamentous fungus (ascomycete) that has been utilized as a commercial source of -lactam antibiotics. Currently, *P. chrysogenum* is a thoroughly studied model for secondary metabolite production and regulation.[17] A recent study by Xu et al. showed the ability of *Penicillium polonicum* to make Pb(II) immobilized since Pb oxalate precipitates outside the fungal cell, bound with phosphate, nitro, halide, hydroxyl, amino, and carboxyl groups on the cell wall, precipitates as pyromorphite [$Pb_5(PO4)_3Cl$], and is reduced to Pb(0) inside the cell.[18]

The current study was designed to investigate the mechanism that may be used in *P. chrysogenum* for survival in high Pb concentrations using a protein profile. Protein expression and post-translational modifications (PTMs) in a variety of biological systems could be good indicators for mechanisms used for adaptation.[19] Previous studies analyzed fungi and bacteria using proteomics to elucidate microbial survival mechanisms in extreme habitats, such as heavy metal pollution.[20] Therefore, proteome research on filamentous fungi, such as *Aspergillus* sp. and *P. chrysogenum*, indicated the cellular physiology that allows understanding the cellular protein expression alterations in response to various stresses, as well as the proteomics capacity to yield definitive information on protein identity, localization, and post-translational modification.[21] Furthermore, Blachowicz et al. found out that the expression of proteins involved in ribosome biogenesis, translation, and carbohydrate metabolic processes was observed when conidia were studied under simulated mars conditions.[22] This study aims to investigate the tolerance level of *P. chrysogenum* by exposing it to varying concentrations of Pb and to identify the proteomic changes in *P. chrysogenum* induced by Pb metal stress using 2D-DIGE and MALDI-TOF mass spectrometry and to unravel the molecular and cellular mechanisms governing fungal-metals interactions.

2. MATERIALS AND METHODS

Penicillium chrysogenum (OP164653) was isolated from the Ath Thumamah area and subjected to proteomics study after it has been treated with Pb.

2.1. Soil Sample Collection. For soil collection, five points from the Ath Thumamah area (Ath Thumamah desert park located on the northern edge of Riyadh city), the ground surface was cleaned by removing elements that are not part of the soil (stones, salt crust, organic matter such as branches, leaves, and any other nonrelated materials). A hole was made with the shovel at a depth of 10−15 cm. Then, a section of the wall was scraped from the hole with a plastic knife to remove the soil surface that was in contact with the shovel. Sterilized tools were used for soil samples, which were then transferred into sterile plastic ziplocked packets labeled with the collection date and location. The samples were taken and kept at 4 °C in the laboratory.

2.2. Determination of Soil Pb Content. Approximately 0.2 g of pooled soil was weighed for Pb detection using inductively coupled plasma mass spectrometry (ICP-MS). A mixture of 6 mL of nitric acid, 2 mL of hydrochloric acid, and 2 mL of hydrofluoric acid was employed to extract the metal from the soils. The solution was digested using Microwave Digestion equipment (CEM, MARS 6, USA) as follows: Samples were heated to 120 °C for 8 min and held for 3 min; increased to 150 °C for 5 min; and then increased to 190 °C for 35 min. After cooling, 2 mL of H_2O_2 was added to the digested mixture, which was then placed in a heating block at 140 °C until the residual solution was reduced to about 1 mL. Finally, the solution was transferred into 50 mL volumetric flasks, filled with water, and mixed well. ICP-MS was used to determine Pb using the internal standard technique and the standard addition method.

2.3. Fungal Isolation Protocol. About 10 g of soil samples from the study location was placed in a sterilized bottle, and then 1.5 mL of $PbSO_4$ (lead sulfate) was added for 14 days in a trial to detect and isolate the most Pb-tolerant fungal strain. The soil plate method was used by transferring 0.5 g of the treated soil to a sterile Petri dish, and then 15 mL of the sterilized media (potato dextrose agar, at 45 °C) was added, rotated gently to disperse the soil particles in the medium, and incubated at 26 °C for 4−5 days. Thereafter, fungal growth was observed, and strains were isolated and purified. Pure cultures were subjected to molecular analysis for identification.

2.4. Molecular Identification Using the 18S rRNA Gene. To extract fungal DNAs, the InstaGene Matrix Genomic DNA Kit (Macrogen Inc., Seoul, South Korea) was used, and the manufacturer's instructions were followed. A PCR test for the fungal 18S rRNA genes was performed using a DNA template and the primers NS1 F (5′ GTAGTCA-TATGCTTGTCTC 3′) and NS24 R (5′ AAACCTTGT-

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TACGACTTTTA 3′). 10× Taq PCR buffer, 2 L; 2.5 mM dNTP mixture, 1.6 L; F and R primers (10 pmol/L), 1.0 L; KOMATaq (2.5 U/L), 0.2 L; DNA template (20 ng/L), 2 L; and HPLC-grade distilled water to adjust the reaction volume to 20 L comprise the PCR reaction mixture.

The PCR was performed as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 2 min, extension at 68 °C for 1.5 min, and final extension at 68 °C for 10 min. Electrophoresis was used to validate the PCR products. The Montage PCR Cleanup Kit was then used to perform the PCR purification.

2.5. Sequencing and Analysis of the Amplified DNA. The purified PCR products were sequenced at Macrogen, Inc. (South Korea), using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and a 3730xl DNA Analyzer automated DNA sequencing machine (Applied Biosystems, USA). Fungal isolate' 18S rRNA gene sequences were manually modified using Geneious Prime software (Geneious Prime Version 2020.1.2). Forward and reverse sequences were used to construct consensus sequences. Using the nucleotide BLASTn platform, the sequences acquired in this work were compared to closely related reference strains in the National Center for Biotechnology Information (NCBI) database.

2.6. Fungal Treatment. The identified fungal strain *P. chrysogenum* (OP164653) was used for proteomic study after treatment with a medium supplemented with 1500 ppm of Pb in PDB (1 mL/9 mL media). Media without Pb were used as control. After the growth was observed, *P. chrysogenum* masses were taken for protein extraction from both treated and untreated tubes.

2.7. Protein Extraction. Protein was extracted from *P. chrysogenum* by centrifugation for 5 min at 3000*g*/4 °C, as mentioned previously.[23] The supernatants were discarded, and the resulting pellets were washed with phosphate-buffered saline (PBS) and washed two times by centrifugation for 5 min at 3000*g*/4 °C. After that, the protein pellets were suspended in lysis buffer (0.5 mL; pH 8.8; 30 mM Tris buffer containing 7 M urea, 2 M thiourea, 2% 3-((3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate(CHAPS), and the protease inhibitor cocktail; GE Healthcare, Chicago, Illinois, USA) and 500 μm of glass beads on a mixer for 1 h. It was followed by sonication for 1 min three to four times, and then the glass beads were taken out and centrifuged for 30 min at 16,300*g*/20 °C. Next, 4× ice cold acetone was added to the supernatant volume and kept overnight at −20 °C. The day after, the samples were centrifuged for 10 min at 12,000*g*, the supernatant was discarded, and the pellet was washed again with 400 μL of ice-cold acetone and centrifuged again for 5 min at 12,000*g*. Then, the supernatant was removed and the pellet was dried by a speed vacuum. Each protein pellet was resuspended in labeling buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris) with the pH adjusted to 8.5. According to the manufacturer's guidelines, the protein concentrations were measured using 2-D Quant Kit (GE Healthcare, Sweden) and experiments were done in triplicates.

2.8. Cye Dye Labeling, Two-Dimensional (2D) Electrophoresis, and Image Scanning. According to the manufacturer's guidelines, the proteins were labeled with CyDye DIGE Fluor minimum dye (400 pmol/50 g) (GE Healthcare, Sweden), as previously described by our group.[23] In brief, 50 g of protein was treated (30 min on ice in the dark) with 400 pmol of freshly dissolved Cy3 or Cy5 in anhydrous dimethylformamide (DMF) for each sample. By adding lysine (1.0 L, 10 mM, and 10 min on ice in the dark), the process was stopped. Each sample was covalently labeled with either a Cy3 or a Cy5 fluorophore. A pooled internal standard containing 50 μg of total protein from each sample was labeled with Cy2. As previously stated, the labeled samples were mixed according to the experimental design and ran on the same gel for comparison (Table S1). Analytical gel electrophoresis in the first dimension was carried out as previously described.[23−25]

Passively (30 V, 12 h) rehydrated Immobiline DryStrips (24 cm, pH 3−11; GE Healthcare, Sweden). Following that, isoelectric focusing was performed with an Ettan IPGphor IEF device (GE Healthcare). The following stages and hold sequence were used to focus at 20 °C and 50 A per strip: (1) 500 V for 1 h, (2) 1000 V for 1 h, (3) 8000 V for 3 h, and (4) 8000 V for 4 h. The IPG strips were then held at 80 °C until the second-dimension separation was done.

The IPG strips were equilibrated (15 min, RT, moderate stirring, 5 mM Tris−HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, and 65 mM dithiothreitol (DTT) prior to the second-dimension separation. The strips were equilibrated in the same solution with 250 mM iodoacetamide (IAA) for a further 15 min. On low-fluorescence glass, polyacrylamide gradient gels (12%) were made. SDS-PAGE was done on second-dimension sodium dodecyl sulfate polyacrylamide gels (Ettan DALTsix vertical units, GE Healthcare, Sweden; 15 °C, 1 W per gel for 1 h, and then 2 W per gel until the bromophenol blue dye front reached the bottom of the gel).

The gels were scanned with a Sapphire Biomolecular Imager (Azure Biosystems, Dublin, Ohio, USA) and digitalized using the image analysis software Sapphire Capture system (Azure Biosystems, Dublin, Ohio, USA) after SDS-PAGE electrophoresis. Total protein (1 mg) obtained from a pool of equal protein from all samples from both groups, treatment and controls (T: 3 samples, C: 3), was used to make preparative gels. The stained gels were washed quickly with Milli-Q water and kept until the spots could be selected and identified by MS, as previously described.[23−25]

2.9. Protein Identification Using Matrix-Assisted Laser Desorption/Ionization−Time-of-Flight (MALDI-TOF) Mass Spectrometry. Coomassie-stained gel spots were excised manually, washed, and digested according to previously described methods.[24−26] A mixture of tryptic peptides (0.8 μL) derived from each protein was spotted onto a MALDI target (384 MTP AnchorChip; 800 μm AnchorChip; Bruker Daltonics, Bremen, Germany). MALDI-TOF-MS spectra were obtained using an UltrafleXtreme TOF mass spectrometer equipped with a LIFT-MS/MS device (Bruker Daltonics) at reflector and detector voltages of 21 and 17 kV, respectively.

Peptide mass fingerprinting (PMFs) were calibrated against a standard (Bruker Daltonics' peptide calibration standard II). The flexAnalysis program (version 2.4, Bruker Daltonics) was used to evaluate the PMFs. The MS data were analyzed using BioTools version 3.2 (Bruker Daltonics). The Mascot search algorithm (v2.0.04, last updated on 09/05/2023; Matrix Science Ltd., UK) was used to find the peptide masses. If a protein's Mascot score was more than 56, it was considered as accurate. Protein score = −10*Log(P), where P is the likelihood that the observed match is a random occurrence; a protein score more than 56 was judged significant ($p \leq 0.05$).

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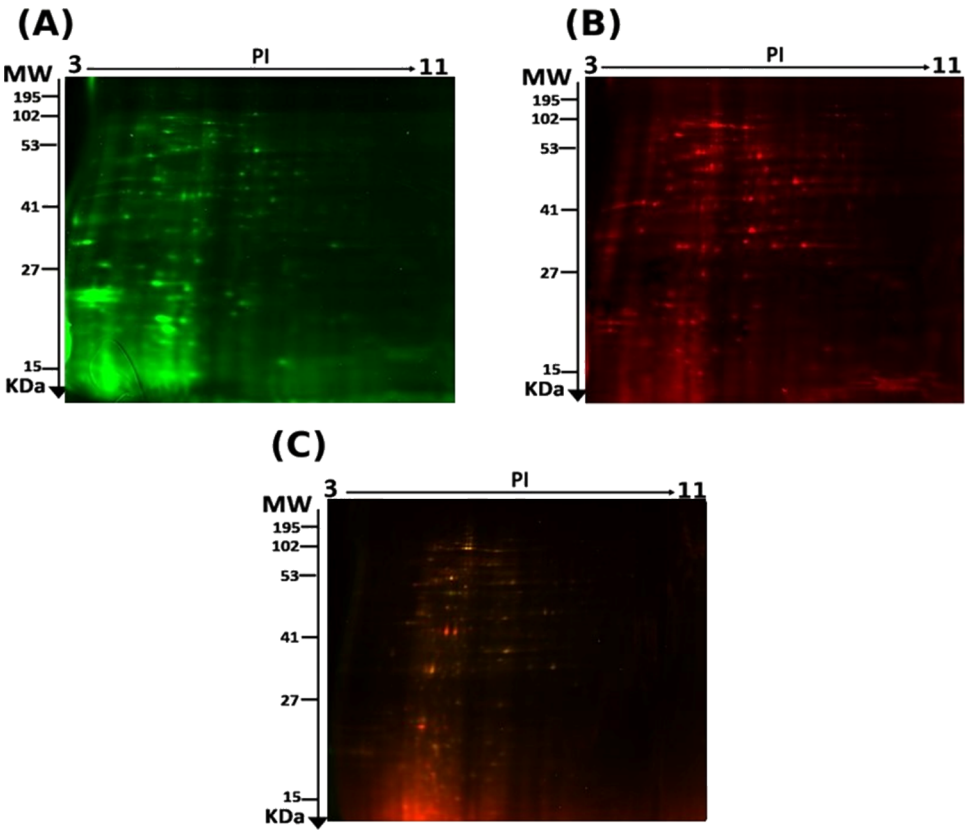

Figure 1. Representative fluorescent protein profiles from a 2D-DIGE containing: (A) *P. chrysogenum* tagged with Cy3, (B) control labeled with Cy5, and (C) representative overlay of treatment and control Cy3/Cy5 images. Proteins were separated in the first dimension using the IPG strip (pH 3−11), and then in the second dimension using 12% PAGE. The images were taken with a Typhoon 9400 Variable Mode camera.

Low-scoring ID proteins were eliminated since they were primarily random matches and negligible ($p > 0.05$). Some proteins were in low quantity and did not produce sufficiently powerful mass fingerprints, thus not all areas of interest could be recognized; other spots were combinations of various proteins.[23−25]

2.10. Bioinformatics Analysis. The interaction network of the variably expressed proteins was discovered by bioinformatic analysis using STRING v11.0. Furthermore, using the PANTHER (protein analysis through evolutionary relationships) categorization method (http://www.pantherdb.org/), the detected proteins were grouped into several groups based on their molecular function and biological activity.

2.11. Statistical Analysis. An automatic spot recognition approach was employed to examine 2D-DIGE gel images using the Progenesis SameSpots program (Nonlinear Dynamics, UK). Gel warping, DIGE normalization, and comparison modules were provided in the package. To guarantee that no data were lost, all gel images were aligned with the reference gel and superimposed. From the Cy3 to Cy5 spot volume ratio, the program determined the normalized volume (NV) of each spot on each gel. To create the normally distributed data, the spot volumes were log transformed. Differential expression was measured using log-normalized volume (LNV). The control and treatment groups were directly compared, and fold difference values and *p*-values were obtained by using one-way ANOVA.

Before using statistical criteria (ANOVA, $p \leq 0.05$), all locations were prefiltered and manually examined. For statistical processing, normalized spot volumes were employed instead of spot intensities. Only locations that met the statistical criteria were submitted to MALDI-TOF-MS analysis.

3. RESULTS

The level of Pb was low in the soil from which *P. chrysogenum* (OP164653) was isolated (1.12 ± 0.04 mg/kg); however, it tolerated higher concentration when treated in the laboratory. Proteomic analysis of *P. chrysogenum* treated with Pb (1500 ppm) was carried out and compared with the untreated control to find out the mode of action that enables the tested strain to tolerate the Pb toxicity in terms of protein expression. For the determination of significant changes in *P. chrysogenum*protein abundance, 2D-difference gel electrophoresis (2D-DIGE) and MALDI-TOF mass spectrometry were used. Therefore, better knowledge about variations in *P. chrysogenum* metabolism between the Pb-treated strain and control is expected.

3.1. 2D-DIGE Evaluation and Proteomic Analysis. 2D-DIGE gel results showed fluorescent protein profiles for Pb-treated *P. chrysogenum*that was labeled with Cy3 (Figure 1A), untreated control that was labeled with Cy5 (Figure 1B), and an overlay of Cy3/Cy5 images of treatment and control (Figure 1C). A substantial shift in protein abundance levels (fold change >1.5) was detected in Pb-treated *P. chrysogenum*among the recognized spots on the gels when compared to the control, according to the ANOVA test ($p \leq 0.05$). After mapping all spots on the gels, a total of 1173 protein spots matched across all of the gel images and Progenesis SameSpot statistical software identified 145 spots from the preparative gel that indicated a significant increase or decrease in protein expression for further protein identification by MS (Figure 2).

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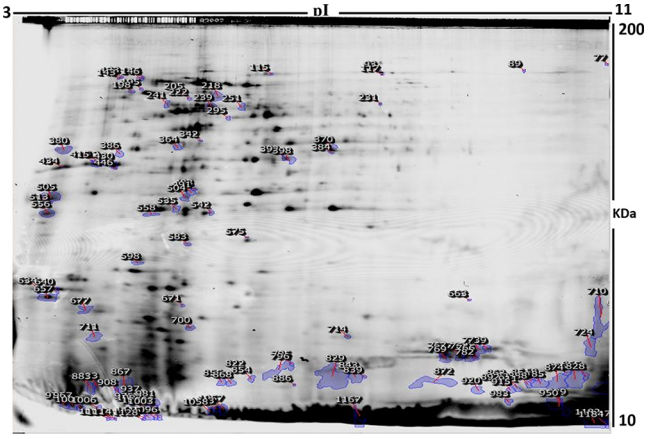

Figure 2. Representative 2D-DIGE image of protein spots from the samples. The numbered spots represent those that were found to be differently expressed between the control and treatment groups (fold change (FC) > 1.5; $p \leq 0.05$) and effectively detected using MALDI-TOF-MS. MW, protein molecular weight; pI, isoelectric point.

Using MALDI-TOF mass spectrometry, PMFs were successfully employed to identify 71 out of the 145 protein spots excised from the preparative gel. Out of the 71 identified proteins matched to entries in the SWISS-PROT database by Mascot with high confidence scores, 43 spots were found to be unique protein sequences. The sequence coverage of the proteins identified using PMFs ranged from 10 to 56% (Table 1, Table S2).

All 145 spot traits were subjected to principal component analysis (PCA), which revealed substantial variations ($p \leq 0.05$ by ANOVA) in their abundance from the preparative gel. In addition, PCA identified two distinct groups based on various proteins, using 79.84% as the cutoff value (Figure 3). Furthermore, as shown in Figure 4, differentially abundant locations showed clusters of expression patterns based on hierarchical clustering analysis. The clustering pattern revealed that the variance in protein abundance for chosen sites differed considerably between control and Pb-treated samples (Figure 4A,B).

Pb-treated *P. chrysogenum* revealed that only 24 proteins were upregulated and 19 were downregulated out of the total of 43 proteins discovered in the pooled sample. The majority of the downregulated proteins were found in the cytoplasm (5), mitochondria (2), nuclear (5), plasma membrane (2), cytosol (1), multivesicular body (1), and endoplasmic reticulum lumen (3). The majority of the elevated proteins were found in the cytoplasm (9), mitochondria (2), nuclear (12), and cytosol (1).

The STRING analysis exhibited the network of protein−protein interactions that could be linked to the *P. chrysogenum* mode of action to tolerate and resist Pb (Figure 5).

The differential abundance of highly regulated proteins in *P. chrysogenum*in the presence of 1500 ppm of Pb was divided into 18 groups, as shown in Figure 6. In the first group, seven proteins were identified for post-translational modification, protein turnover, and chaperones-related proteins; six of them were upregulated, and one was downregulated (Table 1,1.1). For amino acid transport and metabolism, three proteins were identified where one of them was downregulated and two were upregulated (Table 1,1.2). One protein involved in carbohydrate transport and metabolism was identified for the third group, which was upregulated (Table 1,1.3). Three proteins were responsible for energy production and conversion; two of them were downregulated, and one was upregulated (Table 1,1.4). Four proteins involved in translation, ribosomal structure, and biogenesis were detected. Of them, one was downregulated and three were upregulated (Table 1,1.5). Two proteins with unknown functions were expressed as downregulated (Table 1,1.6). One downregulated protein involved in the cytoskeleton was expressed (Table 1,1.7). Four proteins were involved in nucleic acid binding, two of them expressed as upregulated, and two were downregulated (Table 1,1.8).

Two proteins involved in pyridoxamine-phosphate oxidase activity were downregulated (Table 1,1.9). One protein involves one protein in nuclear structure, intracellular trafficking, secretion, and vesicular transport (Table 1,1.10). Two proteins involved in transcription were downregulated (Table 1,1.11). One protein responsible for signal transduction mechanisms was upregulated (Table 1,1.12). Seven proteins involved in replication, recombination, and repair were expressed where five of them were upregulated and two were downregulated (Table 1,1.13).

Furthermore, one protein involved in the actin cytoskeleton-regulatory complex was identified as an upregulated protein (Table 1,1.14). One protein involved in synthesis inhibitor proteins was expressed as an upregulated protein (Table 1,1.15). One protein involved in catalytic activity was upregulated (Table 1,1.16). One involved in guanyl-nucleotide exchange factor activity is downregulated (Table 1,1.17). One involved in lipid transfer protein is downregulated (Table 1,1.18).

3.2. Classification of the Identified Proteins. The detected proteins were classified into three groups, as shown in Figure 7.

3.2.1. Metabolism-Related Proteins. Thirteen proteins were those involved in amino acid transport and metabolism (7%), carbohydrate transport and metabolism (2%), energy production and conversion (7%), pyridoxamine-phosphate oxidase activity (5%), nuclear structure, intracellular trafficking, secretion, and vesicular transport (2%), synthesis inhibitor proteins (2%), catalytic activity (2%), and those involved in lipid transfer (2%).

3.2.2. Proteins Involved in the Cellular Process and Signaling. Eleven proteins were those involved in post-translational modification, protein turnover, chaperone-related proteins (16%), cytoskeleton (2%), signal transduction (2%), and actin cytoskeleton-regulatory complex proteins (2%) besides those involved in guanyl-nucleotide exchange factor activity (2%).

3.2.3. The Last Identified Protein Groups Were Those Related to Information Storage and Processing. Nineteen proteins were involved in replication, recombination, and repair proteins (16%), transcription (5%), nucleic acid binding protein (9%), translation, ribosomal structure, and biogenesis (9%) besides the proteins with unknown function (5%).

4. DISCUSSION

The ability of fungi to adapt to and survive heavy metal treatment is critical in providing a means for myco-remediation. Proteomics analysis can be used to better understand how fungi respond to heavy metal cytotoxicity and sheds light on the mode of action. Furthermore, identifying the proteomics profile of microbes treated with heavy metals versus untreated controls aids in the identification of their mode of action, regulated proteins, active genes, and

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Table 1. Identified Proteins, with Changes in Abundance of Significantly Differentially Abundant Proteins between *P. chrysogenum*-Treated and Control Samples[a]

	protein name/organism name	accession no.[b]	MASCOT ID	cellular location	fold change treated/control[c]	exp[d]
1.1 Proteins involved in post-translational modification, protein turnover, and chaperone						
1	pre-mRNA-splicing factor CWC21	Q751G9	CWC21_ASHGO	nucleus	3.41	UP
2	FK506-binding protein 1	Q754 K8	FKBP_ASHGO	nucleus	4.09	UP
3	pre-mRNA-splicing factor CWC21	Q751G9	CWC21_ASHGO	nucleus	2.76	UP
4	pre-mRNA-splicing factor CWC21	Q751G9	CWC21_ASHGO	nucleus	2.07	UP
5	pre-mRNA-splicing factor CWC21	Q751G9	CWC21_ASHGO	nucleus	4.12	UP
6	pre-mRNA-splicing factor CWC21	Q751G9	CWC21_ASHGO	nucleus	4.0	UP
7	pre-mRNA-splicing factor CWC2 ·	Q6CT50	CWC2_KLULA	nucleus	−4.0	DOWN
1.2 Proteins involved in amino acid transport and metabolism						
1	probable aspartate-semialdehyde dehydrogenase	P78780	DHAS_SCHPO	nucleus	4.11	UP
2	sorbitol dehydrogenase 1e	P35497	DHSO1_YEAST	cytosol	4.65	UP
3	protein FMP27, mitochondrial	Q06179	FMP27_YEAST	mitochondria	−3.72	DOWN
1.3 Proteins involved in carbohydrate transport and metabolism						
1	sterol 3-beta-glucosyltransferase	Q5B4C9	ATG26_EMENI	cytoplasm	4.04	UP
1.4 Proteins involved in energy production and conversion						
1	V-type proton ATPase catalytic subunit A	P17255	VATA_YEAST	membrane	−1.5	DOWN
2	ATPase GET3	C0NV23	GET3_AJECG	endoplasmic reticulum	−4.48	DOWN
3	actin/Thermomyces lanuginosus	P10365	ACT_THELA	cytoplasm	3.38	UP
1.5. Proteins involved in translation, ribosomal structure, and biogenesis						
1	54S ribosomal protein L23, mitochondrial	Q12487	RM23_YEAST	mitochondria	1.50	UP
2	cytoplasmic tRNA 2-thiolation protein 2	Q6FLE5	CTU2_CANGA	cytoplasm	3.59	UP
3	SVP1-like protein 2	Q5B464	HSV2_EMENI	cytoplasmic	−2.89	DOWN
4	eukaryotic translation initiation factor 3 subunit A	Q0CUP6	EIF3A_ASPTN	cytoplasmic	2.3	UP
1.6. Proteins with unknown function						
1	exocyst complex component EXO84	Q6FM00	EXO84_CANGA	cytoplasmic	−6.79	DOWN
2	uncharacterized protein YJL049W	P47048	YJE9_YEAST	multivesicular body	−2.0	DOWN
1.7 Protein involved in cytoskeleton						
1	protein ABC1 homologue, mitochondrial	Q92338	ABCI_SCHPO	mitochondria	−2.06	DOWN
1.8 Proteins involved in nucleic acid binding						
1	mitochondrial RNA-splicing protein MRS1/	P41905	MRS1_SACDO	mitochondria	1.89	UP
2	DNA damage-binding protein cmr1	Q2UUT4	CMR1_ASPOR	cytoplasm	−2.3	DOWN
3	nucleolar protein 16	A6RC73	NOP16_AJECN	nucleus	2.60	UP
4	autophagy-related protein 2	Q9HFR4	ATG2_PICPA	endoplasmic reticulum	−3.19	DOWN
1.9 Proteins involved in pyridoxamine-phosphate oxidase activity						
1	pyridoxamine 5′-phosphate oxidase C1952.08c homologue/	Q9UUK0	PDXH_SCHPO	cytoplasm	−4.77	DOWN
2	mannitol-1-phosphate 5-dehydrogenase	B6HRL5	MTLD_PENRW	cytosol	−3.30	DOWN
1.10 Protein involved in nuclear structure, intracellular trafficking, secretion, and vesicular transport						
1	protein MSN5/	P52918	MSN5_YEAST	cytoplasm	−2.83	DOWN
1.11 Proteins involved in transcription						
1	RuvB-like helicase 2	Q5AGZ9	RUVB2_CANAL	nucleus	−2.71	DOWN
2	transcription and mRNA export factor SUS1	P0CS73	SUS1_CRYNB	nucleus	−2.79	DOWN
1.12 Protein involved in signal transduction						
1	calcium/calmodulin-dependent protein kinase/	O14408	KCC1_METAN	cytoplasm	5.06	UP
1.13 Proteins involved in replication, recombination, and repair						
1	ATP-dependent RNA helicase ded1	Q4WP13	DED1_ASPFU	cytoplasm	8.09	UP
2	DNA replication licensing factor mcm3	P30666	MCM3_SCHPO	nucleus	2.56	UP
3	DNA repair protein REV1	P12689	REV1_YEAST	nucleus	−3.79	DOWN
4	ATP-dependent RNA helicase DRS1	A5DKW3	DRS1_PICGU	nucleus	3.95	UP
5	ATP-dependent RNA helicase DBP7e	Q4HZ68	DBP7_GIBZE	nucleus	−3.07	DOWN
6	ATP-dependent RNA helicase dbp7	Q2UE66	DBP7_ASPOR	nucleus	1.83	UP
7	replication factor C subunit 2	P0C7N7	RFC2_PHANO	nucleus	2.54	UP

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Table 1. continued

	protein name/organism name	accession no.[b]	MASCOT ID	cellular location	fold change treated/control[c]	exp[d]
1.14 Protein involved in actin cytoskeleton-regulatory complex						
1	actin cytoskeleton-regulatory complex protein end3	A1D2B8	END3_NEOFI	cytoplasm	2.09	UP
1.15 Protein involved in synthesis inhibitor						
1	Cap-associated protein CAF20	Q6BLT5	CAF20_DEBHA	cytoplasm	2.17	UP
1.16 Proteins involved in Catalytic activity						
1	enolase	Q76KF9	ENO_PENCH	cytoplasm	5.41	UP
1.17 Protein involved in guanyl-nucleotide exchange factor activity						
1	Bud site selection protein 3	P25558	BUD3_YEAST	endoplasmic reticulum	−3.77	DOWN
1.18 Protein involved in lipid transfer						
1	prospore membrane adapter protein SPO71	Q03868	SPO71_YEAST	membrane	−2.30	DOWN

[a]Table 1 shows values for the average ratio between the two states, with their corresponding levels of fold changes and one-way ANOVA ($p \leq 0.05$) using 2D-DIGE. Analysis type: MALDI-TOF-MS; database: SWISS-PROT; taxonomy: *Penicillium chrysogenum*. [b]Protein accession number for SWISS-PROT Database. [c]Ratio between the groups. [d]Protein expression between the groups.

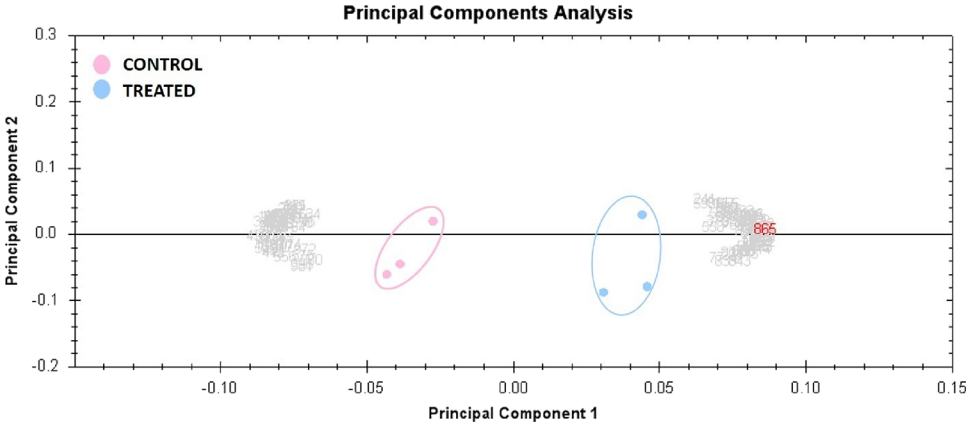

Figure 3. Principal component analysis of the control samples is indicated in pink dots, whereas blue dots represent Pb-treated samples. Both accounted for 79.84% of the variability in the chosen location.

transcriptional regulatory mechanisms during treatment, which may aid in a better understanding of their mode of action. LC-MS/MS analysis for proteomics studies to get fungal insight mechanisms in metal resistance was reported.[11,27,28] Proteomics analysis may enhance recognition of protein biomarkers that monitor and identify heavy metal pollution, which can be identified as a protein form besides their copy numbers, which are assessed by the regulation of the translational process.[29]

Metal ion accumulation in the microbial cell besides ions and metal-containing compounds could be the main reasons affecting the microbial growth and metabolism. However, proteins related to Pb toxicity and those related to defense mechanisms are rarely investigated. Therefore, currently, the Pb-treated *P. chrysogenum* proteomics profile was investigated. From the total of 43 proteins identified, only 24 proteins were upregulated and 19 were downregulated. Fungal resistance against heavy metals might be detected by the upregulated proteins, and the toxicity level is expressed by the downregulated ones.

The separated and identified proteins were grouped according to their function into 18 groups. Fungal protein excretion is highly dynamic and affected by varied environmental conditions, as suggested by Cherrad et al. when they investigated *Botrytis cinerea* under cadmium, nickel, zinc, or copper stress since the oxidoreductase was accumulated under Cu, Ni, and Cd.[30] It has been earlier stated by Gadd and White that the resistance of fungi might be approved by their incidence at the heavy metal-polluted areas; therefore, current strains were isolated from varied locations with varied levels of accumulated heavy metals.[31] Metal bioaccumulation, biomineralization, biotransformation, biosorption, and extracellular precipitation are the known strategies for the process of fungal detoxification.[32] Metal ion chelation in the cytoplasm is a way of mitigating the negative impact on metabolic processes of heavy metal inside the cell. Therefore, they are stored or inactivated.[33]

For all organisms, the critical factor in cell multiplication and viability is the protein homeostasis, which highly influences cellular signaling under varied environmental conditions so they can be proper biomarkers.[11] It could be noticed that Pb may lead to degradation of some proteins, lipid, DNA, and other biological materials in the cell, which might be linked to its ability in enhancing ROS production; thus, oxidation of cellular component appeared.[34] Some proteins related to carbohydrate metabolism were overproduced, which might be a form of resistance to Pb since carbohydrate participates in the synthesis of cell wall. Similar findings were also reported by as an influence of Cu^{2+} on yeast.[35] Three of the identified proteins involved in energy production and conversion were overexpressed, which might suggest high tolerance of *P. chrysogenum* to Pb. A similar observation was earlier noted by Li et al. when they studied *P. polonicum* under Pb stress conditions.[36]

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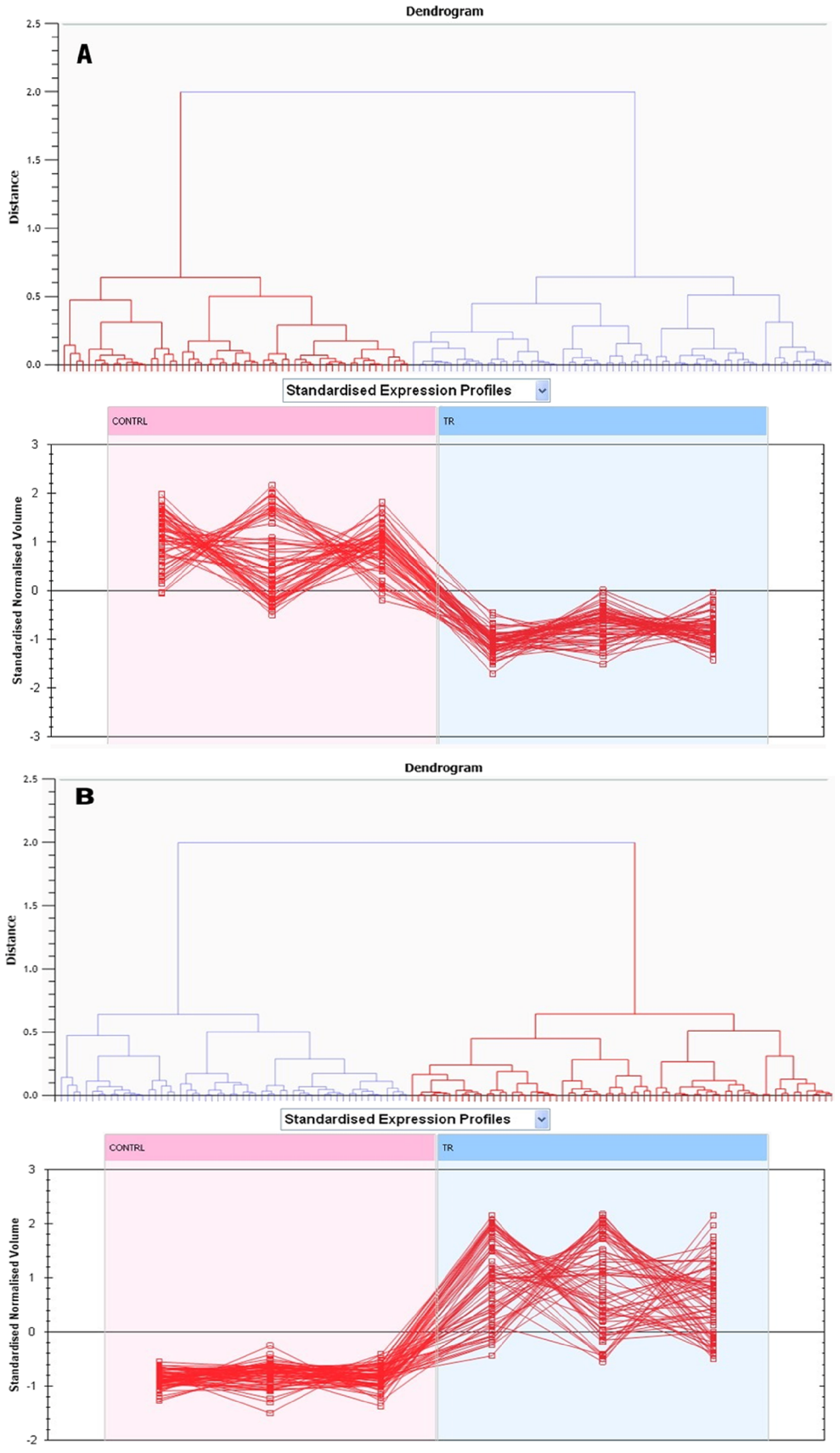

Figure 4. Expression profiles of detected proteins are divided into clusters of expression patterns, with the number of spots for each cluster indicated. Each line indicates the standardized abundance of a spot overall gels and is associated with one of the clusters produced by hierarchical cluster analysis. The spots with higher abundance represent the elevated proteins (A). The spots with lower abundance represent the downregulated proteins in Pb-treated (B) samples (Progenesis SameSpots).

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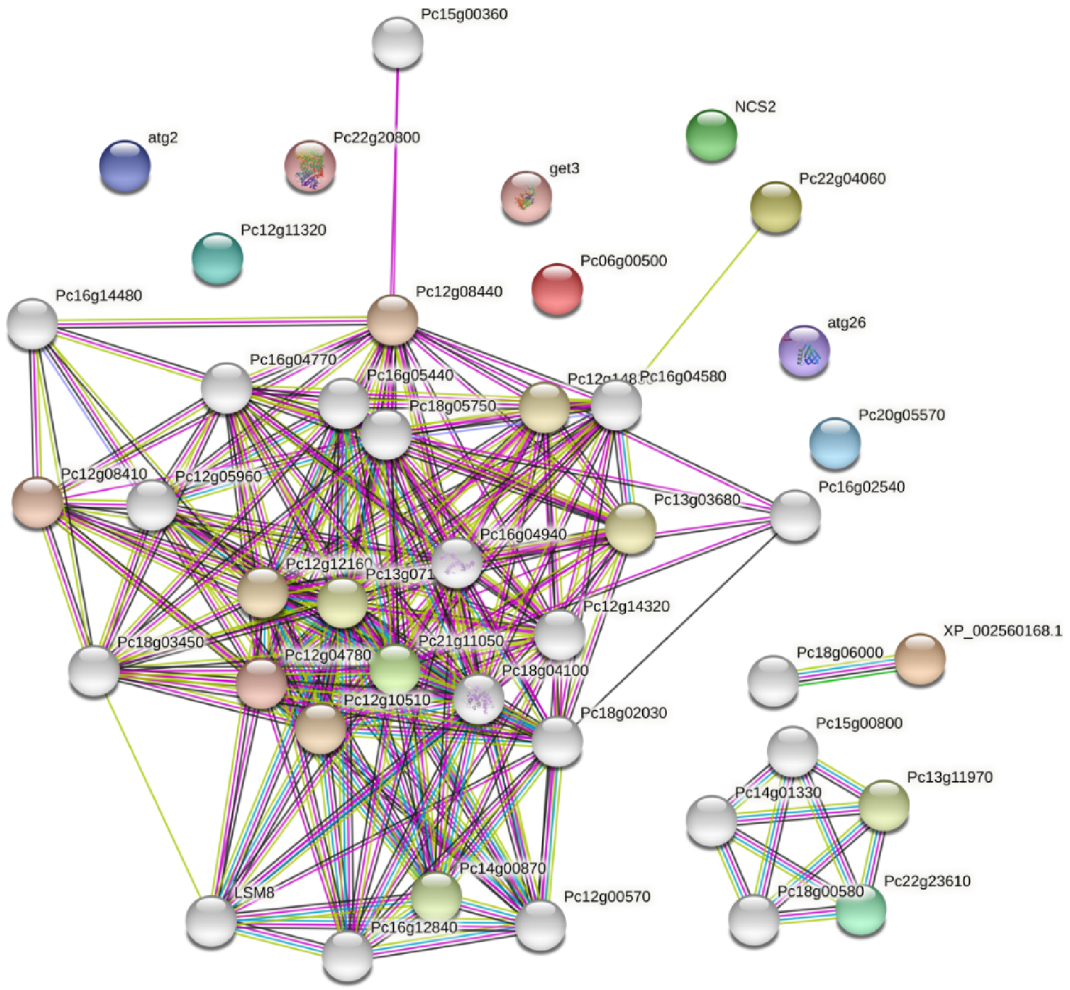

Figure 5. Protein−protein interaction network of the differentially expressed proteins in treated samples using STRING v11.0 (https://string-db.org/). Many lines show a higher number of interactions, and a single line indicates one interaction.

The activity of ATPase was downregulated, which might suggest the low growth rate, as consequences of Pb stress, due to their role in energy production. However, about 71% of the detected proteins involved in replication, recombination, and repair proteins were upregulated, which might suggest the trial on *P. chrysogenum* to grow and replicate in Pb conditions. It was also confirmed by the upregulation of the actin protein that is responsible for cell growth and development.[37] Proteins involved in post-translational modification are important in a variety of biological processes, including cell growth and development as well as environmental adaptation to a variety of stress conditions.[38] Such information might explain the overregulation of all detected proteins related to PTM to help and enhance *P. chrysogenum* survival in Pb stress conditions. 80% of the detected proteins involved in translation, ribosomal structure, and biogenesis were upregulated, which might suggest that Pb-treated *P. chrysogenum* had damaged proteins and tried to produce new proteins. Okay et al. stated similar findings when *Phanerochaete chrysosporium* treated with Cu[39]

Overexpression of calcium/calmodulin-dependent protein kinase is expected due to their role in fungal growth regulation and tolerance to varied environmental conditions. Li et al. indicated its role in different cellular processes and stress tolerance in *P. italicum*.[36] Furthermore, some key enzymes in TCA (tricarboxylic acid cycle) were upregulated such as enolase that mediate oxalic acid production, which is an important secondary metabolite produced by some fungi as consequences to heavy metal conditions to enhance their fixation to fungal cells.[40]

Transporters go through several regulated turnovers in any physiological or stressful environment. Thus, transporters briefly interact dynamically with multiple proteins during the process.[41] In this study, we discovered that one protein involved in intracellular trafficking, secretion, and vesicular transport was downregulated. This protein aids in physiological processes such as the intercellular exchange of proteins and RNA.[42]

The nitrogen cycle is critical for nitrogen assimilation and transformation as well as stress tolerance. Heavy metals have an effect on enzymes involved in nitrogen metabolism.[43] Three proteins responsible for amino acid transport were identified, two of which were unregulated and one of which was downregulated in response to Pb stress. This enzyme may play a role in resolving the mitochondrial NAD/NADH imbalance.[11]

At the cellular level, the stress response starts with environmental sensing, where cells perceive external cues. In fungi, sensing occurs through transmembrane receptors and signal reception and transduction at hyphal cells that activate response pathways to prevent damage and restore homeostasis.[44]

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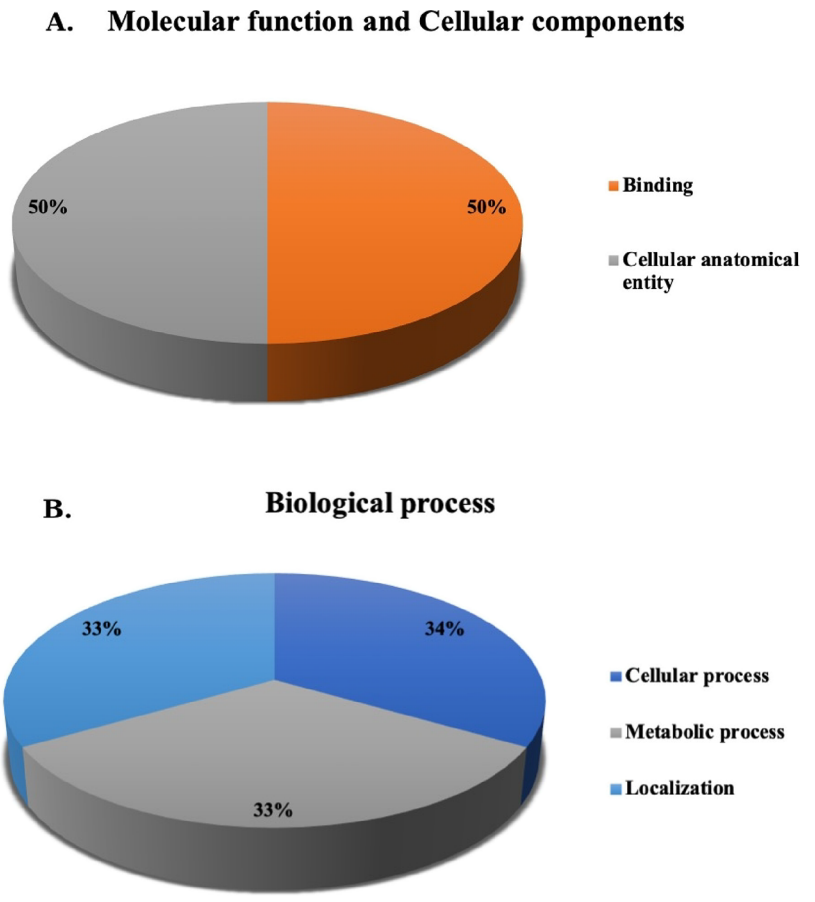

Figure 6. Comparative depiction (%) of identified proteins categorized into groups according to their Molecular Function and Cellular Components (A) and Biological Process (B).

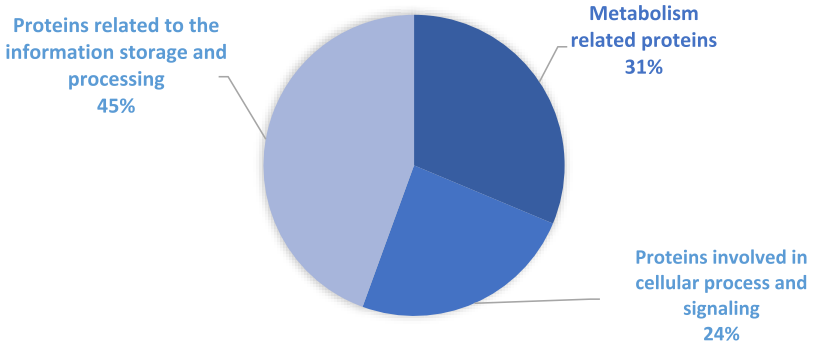

Figure 7. Proteins are identified and classified into three groups according to their functions.

As a response to metal stress, the fungi cell produces a catalytic activity protein such as enolase. This protein is a key glycolytic enzyme in the cytoplasm of prokaryotic and eukaryotic cells and is considered a multifunctional protein. Enolase is expressed on the surface of several cell types, where it acts as a plasminogen receptor, concentrating proteolytic plasmin activity on the cell surface. This protein was upregulated protein and highly expressed in our results. Enolase could be considered as a marker of pathological stress in a high number of diseases, performing several of its multiple functions, mainly as a plasminogen receptor.[11]

In Pb stress, the fungi upregulate a synthesis inhibitor protein as a defense tool. This protein is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that Pb directly to the generation of new proteins. It usually refers to substances that act at the ribosome level, and it acts as an inhibitor of cap-dependent translation. This protein inhibits translation and stabilizes mRNA.[45]

There is a protein that was upregulated and related to actin cytoskeleton-regulatory complex proteins. This protein is the most abundant and multifaceted protein in eukaryotic cells. It is highly conserved and participates in more protein−protein interactions than any known protein. Actin's functions in genome organization and nuclear events like chromatin remodeling, DNA repair, and transcription play a key role. It also plays a role in regulating multiple aspects of cellular behavior, including the control of cell morphology and motility. Moreover, the interaction of filamentous actin with myosin forms the basis of muscle contraction. With so many cellular functions depending on the actin cytoskeleton, it is not

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surprising that abnormal regulation or functioning of cytoskeletal components is often a result of stress.[46]

5. CONCLUSIONS

The proteomic study of *P. chrysogenum* under Pb stress conditions reported upregulated proteins that could better explain the way of tolerance and Pb toxicity removal. This study provides new insights into fungal metal interactions, which could lead to the development of new molecular biomarkers for pollutant myco-remediation. To the best of our knowledge, this is the first study investigated the fungus *P. chrysogenum* and its metal tolerance means. The findings of this study could facilitate the development of novel molecular markers for contaminant bioremediation.

■ ASSOCIATED CONTENT

Data Availability Statement

Upon reasonable request, the data sets used and/or analyzed in this study are available from the corresponding author.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02006.

(Table S1) Experimental design: six samples run on 2D-PAGE gels, samples labeled randomly with Cy3 and Cy5; (Table S2) mass spectrometry list of significant differentially abundant proteins between treated and control states identified in samples, using 2D-DIGE with protein name, accession number, Mascot score, MS % coverage, protein MW, and pI values according to UniProt database listed; and (Table S3) canonical pathways for each protein interaction network (PDF)

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Author Contributions

A.A. and A.E.M. conceived and designed the study. H.B., S.S.J., I.O.A., and A.A.A. performed the proteomic lab work; H.B., S.S.J, A.A., D.A.-s., and A.E.M. conducted the data analysis and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Notes

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■ REFERENCES

(1) Masindi, V.; Muedi, K. L. Environmental contamination by heavy metals. *Heavy metals* **2018**, *10*, 115−132.

(2) Ali, H.; Khan, E.; Ilahi, I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *J. Chem.* **2019**, *2019*, No. 6730305.

(3) Shi, T.; Ma, J.; Zhang, Y.; Liu, C.; Hu, Y.; Gong, Y.; Wu, X.; Ju, T.; Hou, H.; Zhao, L. Status of lead accumulation in agricultural soils across China (1979−2016). *Environ. Int.* **2019**, *129*, 35−41.

(4) Bhat, S. A.; Hassan, T.; Majid, S. Heavy metal toxicity and their harmful effects on living organisms−a review. *Int. J. Med. Sci. Diagn. Res.* **2019**, *3* (1), 106−122.

(5) Thakur, D.; Venkatesh, T. Ubiquity of lead in our lives—lead in food. *J. Krishna Inst. Med. Sci. Univ.* **2015**, *4*, 4−9.

(6) Selvi, A.; Rajasekar, A.; Theerthagiri, J.; Ananthaselvam, A.; Sathishkumar, K.; Madhavan, J.; Rahman, P. K. S. M. Integrated remediation processes toward heavy metal removal/recovery from various environments-a review. *Front. Environ. Sci.* **2019**, *7*, 66.

(7) Li, C.; Zhou, K.; Qin, W.; Tian, C.; Qi, M.; Yan, X.; Han, W. A review on heavy metals contamination in soil: effects, sources, and remediation techniques. *Soil and Sediment Contamination: An International Journal* **2019**, *28* (4), 380−394.

(8) Nleya, Y.; Simate, G. S.; Ndlovu, S. Sustainability assessment of the recovery and utilisation of acid from acid mine drainage. *Journal of Cleaner Production* **2016**, *113*, 17−27.

(9) Bwapwa, J., Factors affecting the bioremediation of industrial and domestic wastewaters. In *Microbial biodegradation and bioremediation*; Elsevier: 2022; pp 461−472.

(10) Bala, S.; Garg, D.; Thirumalesh, B. V.; Sharma, M.; Sridhar, K.; Inbaraj, B. S.; Tripathi, M. Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. *Toxics* **2022**, *10* (8), 484.

(11) Dey, P.; Malik, A.; Singh, D. K.; Haange, S.-B.; von Bergen, M.; Jehmlich, N. Insight into the molecular mechanisms underpinning the mycoremediation of multiple metals by proteomic technique. *Frontiers in Microbiology* **2022**, *13*, No. 872576.

(12) Deshmukh, R.; Khardenavis, A. A.; Purohit, H. J. Diverse metabolic capacities of fungi for bioremediation. *Indian journal of microbiology* **2016**, *56*, 247−264.
(13) Sánchez, C. Fungal potential for the degradation of petroleum-based polymers: An overview of macro-and microplastics biodegradation. *Biotechnology advances* **2020**, *40*, No. 107501.
(14) Dharmasiri, R.; Nilmini, A.; Undugoda, L.; Nugara, N.; Udayanga, D.; Pathmalal, M., Polyaromatic hydrocarbons (PAHs) degradation ability of Pseudomonas stutzeri isolated from phyllosphere of urbanareas in Sri Lanka. 2021.
(15) Priyadarshini, E.; Priyadarshini, S. S.; Cousins, B. G.; Pradhan, N. Metal-Fungus interaction: Review on cellular processes underlying heavy metal detoxification and synthesis of metal nanoparticles. *Chemosphere* **2021**, *274*, No. 129976.
(16) Bhavya, G.; Hiremath, K. Y.; Jogaiah, S.; Geetha, N. Heavy metal-induced oxidative stress and alteration in secretory proteins in yeast isolates. *Arch. Microbiol.* **2022**, *204* (3), 172.
(17) Guzmán-Chávez, F.; Zwahlen, R. D.; Bovenberg, R. A. L.; Driessen, A. J. M. Engineering of the filamentous fungus Penicillium chrysogenum as cell factory for natural products. *Front. microbiol.* **2018**, *9*, 2768.
(18) Xu, X.; Hao, R.; Xu, H.; Lu, A. Removal mechanism of Pb(II) by Penicillium polonicum: immobilization, adsorption, and bioaccumulation. *Sci. Rep.* **2020**, *10* (1), 9079.
(19) Wen, B.; Zeng, W.-F.; Liao, Y.; Shi, Z.; Savage, S. R.; Jiang, W.; Zhang, B. Deep learning in proteomics. *Proteomics* **2020**, *20* (21−22), No. 1900335.
(20) Manikandan, R.; Harish, S.; Karthikeyan, G.; Raguchander, T. Comparative Proteomic Analysis of Different Isolates of Fusarium oxysporum f.sp. lycopersici to Exploit the Differentially Expressed Proteins Responsible for Virulence on Tomato Plants. *Front. Microbiol.* **2018**, *9*, 420.
(21) Barreiro, C.; Garca-Estrada, C.; F., J.; Prasain, J. Proteomics methodology applied to the analysis of filamentous fungi-new trends for an impressive diverse group of organisms. *Tandem Mass Spectrom.: Appl. Princ.* **2012**, 127−160.
(22) Blachowicz, A.; Chiang, A. J.; Elsaesser, A.; Kalkum, M.; Ehrenfreund, P.; Stajich, J. E.; Torok, T.; Wang, C. C. C.; Venkateswaran, K. Proteomic and metabolomic characteristics of extremophilic fungi under simulated mars conditions. *Front. Microbiol.* **2019**, *10*, 1013.
(23) Lau, S. K.; Tse, H.; Chan, J. S.; Zhou, A. C.; Curreem, S. O.; Lau, C. C.; Yuen, K. Y.; Woo, P. C. Proteome profiling of the dimorphic fungus Penicillium marneffei extracellular proteins and identification of glyceraldehyde-3-phosphate dehydrogenase as an important adhesion factor for conidial attachment. *FEBS journal* **2013**, *280* (24), 6613−6626.
(24) Alfadda, A. A.; Benabdelkamel, H.; Masood, A.; Moustafa, A.; Sallam, R.; Bassas, A.; Duncan, M. Proteomic analysis of mature adipo cytes from obese patients in relation to aging. *Experimental Gerontology* **2013**, *48* (11), 1196−1203.
(25) Masood, A.; Benabdelkamel, H.; Ekhzaimy, A. A.; Alfadda, A. A. Plasma-based proteomics profiling of patients with hyperthyroidism after antithyroid treatment. *Molecules* **2020**, *25* (12), 2831.
(26) Alwehaibi, M. A.; Al-Ansari, M. M.; Alfadda, A. A.; Al-Malki, R.; Masood, A.; Abdel Rahman, A. M.; Benabdelkamel, H. Proteomics Investigation of the Impact of the Enterococcus faecalis Secretome on MCF-7 Tumor Cells. *International Journal of Molecular Sciences* **2023**, *24* (19), 14937.
(27) Kraut, A.; Marcellin, M.; Adrait, A.; Kuhn, L.; Louwagie, M.; Kieffer-Jaquinod, S.; Lebert, D.; Masselon, C. D.; Dupuis, A.; Bruley, C.; Jaquinod, M.; Garin, J.; Gallagher-Gambarelli, M. Peptide storage: are you getting the best return on your investment? Defining optimal storage conditions for proteomics samples. *J. Proteome Res.* **2009**, *8* (7), 3778−3785.
(28) Zhang, X.; Fang, A.; Riley, C. P.; Wang, M.; Regnier, F. E.; Buck, C. Multi-dimensional liquid chromatography in proteomics—a review. *Analytica chimica acta* **2010**, *664* (2), 101−113.
(29) Ohno, M.; Karagiannis, P.; Taniguchi, Y. Protein expression analyses at the single cell level. *Molecules* **2014**, *19* (9), 13932−13947.
(30) Cherrad, S.; Girard, V.; Dieryckx, C.; Gonçalves, I. R.; Dupuy, J.-W.; Bonneu, M.; Rascle, C.; Job, C.; Job, D.; Vacher, S.; Poussereau, N. Proteomic analysis of proteins secreted by Botrytis cinerea in response to heavy metal toxicity. *Metallomics* **2012**, *4* (8), 835−846.
(31) Gadd, G. M.; White, C. Microbial treatment of metal pollution—a working biotechnology? *Trends Biotechnol.* **1993**, *11* (8), 353−359.
(32) Wang, Y.; Yi, B.; Sun, X.; Yu, L.; Wu, L.; Liu, W.; Wang, D.; Li, Y.; Jia, R.; Yu, H.; Li, X. Removal and tolerance mechanism of Pb by a filamentous fungus: A case study. *Chemosphere* **2019**, *225*, 200−208.
(33) Avery, S. V.; Codd, G. A.; Gadd, G. M. Replacement of cellular potassium by caesium in Chlorella emersonii: differential sensitivity of photoautotrophic and chemoheterotrophic growth. *Microbiology* **1992**, *138* (1), 69−76.
(34) Sumner, E. R.; Shanmuganathan, A.; Sideri, T. C.; Willetts, S. A.; Houghton, J. E.; Avery, S. V. Oxidative protein damage causes chromium toxicity in yeast. *Microbiology* **2005**, *151* (6), 1939−1948.
(35) Sánchez-Rojas, T.; Espinoza-Culupú, A.; Ramírez, P.; Iwai, L. K.; Montoni, F.; Macedo-Prada, D.; Sulca-López, M.; Durán, Y.; Farfán-López, M.; Herencia, J. Proteomic Study of Response to Copper, Cadmium, and Chrome Ion Stress in Yarrowia lipolytica Strains Isolated from Andean Mine Tailings in Peru. *Microorganisms* **2022**, *10* (10), 2002.
(36) Li, S.-Y.; Yang, X.-Q.; Chen, J.-X.; Wu, Y.-M.; Yang, Y.-B.; Ding, Z.-T. The induced cryptic metabolites and antifungal activities from culture of Penicillium chrysogenum by supplementing with host Ziziphus jujuba extract. *Phytochemistry* **2022**, *203*, No. 113391.
(37) Marks, J.; Hagan, I. M.; Hyams, J. S. Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. *J. Cell Sci.* **1986**, *1986* (Supplement_5), 229−241.
(38) Retanal, C.; Ball, B.; Geddes-McAlister, J. Post-translational modifications drive success and failure of fungal−host interactions. *Journal of Fungi* **2021**, *7* (2), 124.
(39) Okay, S.; Yildirim, V.; Büttner, K.; Becher, D.; Özcengiz, G. Dynamic proteomic analysis of Phanerochaete chrysosporium under copper stress. *Ecotoxicology and environmental safety* **2020**, *198*, No. 110694.
(40) Kolenčík, M.; Urík, M.; Čerňanský, S.; Molnárová, M.; Matúš, P. Leaching of zinc, cadmium, lead and copper from electronic scrap using organic acids and the Aspergillus niger strain. *Fresenius Environ. Bull.* **2013**, *22* (12a), 3673−3679.
(41) Lewandowski, D.; Sander, C. L.; Tworak, A.; Gao, F.; Xu, Q.; Skowronska-Krawczyk, D. Dynamic lipid turnover in photoreceptors and retinal pigment epithelium throughout life. *Progress in Retinal and Eye Research* **2022**, *89*, No. 101037.
(42) O'Brien, K.; Breyne, K.; Ughetto, S.; Laurent, L. C.; Breakefield, X. O. RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nat. Rev. Mol. Cell Biol.* **2020**, *21* (10), 585−606.
(43) Singh, R. L. *Principles and applications of environmental biotechnology for a sustainable future*. Springer: Singapore 2017.
(44) Branco, S.; Schauster, A.; Liao, H. L.; Ruytinx, J. Mechanisms of stress tolerance and their effects on the ecology and evolution of mycorrhizal fungi. *New Phytologist* **2022**, *235* (6), 2158−2175.
(45) Leprivier, G.; Rotblat, B.; Khan, D.; Jan, E.; Sorensen, P. H. Stress-mediated translational control in cancer cells. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms* **2015**, *1849* (7), 845−860.
(46) Arafat, M. Y.; Narula, K.; Nalwa, P.; Sengupta, A.; Chakraborty, N.; Chakraborty, S. Proteomic analysis of phytopathogenic fungus Macrophomina phaseolina identify known and novel mycelial proteins with roles in growth and virulence. *Journal of Proteins and Proteomics* **2022**, *13* (3), 149−157.

https://doi.org/10.1021/acsomega.4c02006
ACS Omega 2024, 9, 26245−26256