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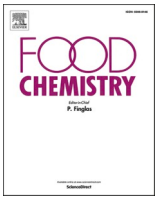

Proteomics and physiologic analysis reveal different response strategies to cadmium stress in *Lentinula edodes*

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ABSTRACT

Lentinula edodes (*L. edodes*) is the second most widely cultivated edible mushroom worldwide. However, it has the ability to accumulate cadmium (Cd), which poses significant health risks. Despite its significance, the protein-level response mechanisms to Cd stress remain insufficiently understood. This study aims to investigate the differential responses of the low-Cd-accumulating strain Le4606 and the high-Cd-accumulating strain Le4625 under Cd stress by biochemical and proteomic methodologies. The results indicate that Le4625 exhibits enhanced Cd absorption, proline accumulation, and vacuolar sequestration for detoxification, with ZRC1 detected exclusively at 7 h. Conversely, Le4606 demonstrates proficiency in glutathione-mediated detoxification, thioredoxin antioxidant activity, tricarboxylic acid cycle activity, autophagy, and Cd extrusion. Overall, vacuolar sequestration and glutathione-mediated detoxification are important for the differences in Cd accumulation. The distinct response strategies offer valuable insights into the underlying mechanisms of Cd accumulation. This research establishes a theoretical foundation for the breeding of low-Cd-accumulating cultivars, benefiting human health.

1. Introduction

Lentinula edodes (*L. edodes*), commonly known as Xiang'gu, is the second most extensively cultivated edible mushroom worldwide. China is the leading producer and exporter of L. *edodes*, with production reaching 11 million tons in 2019 (Gao et al., 2022). *L. edodes* dominates a rapidly expanding global market projected to grow at a compound annual growth rate of 8.7 %, reaching USD 2.29 billion by 2029 (Bin et al., 2024). This surge is driven by increasing consumer demand for nutrient-dense foods and medicinal fungi. *L. edodes* is a significant edible mushroom, renowned for its high nutritional value and distinctive aroma, which is favoured by consumers. It is rich in proteins, essential amino acids, vitamins, and minerals. Additionally, *L. edodes* contains a variety of bioactive compounds, including polysaccharides and ergosterol, which have been associated with antitumor, antioxidant, and hypocholesterolemic properties, among others (Bugajewski et al., 2025). Furthermore, *L. edodes* holds untapped potential as a critical protein supplier, since global climate change is exacerbating insecurity in the food system (Rahman et al., 2025; Yu et al., 2023).

However, *L. edodes* is susceptible to cadmium (Cd) accumulation, posing significant health risks to humans through the food chain. A previous study demonstrated that the Cd content in L. *edodes* samples exceeded national standards by 18.33 % (Yu et al., 2021). Cadmium is highly toxic and mobile in the environment (Kubier et al., 2019). Cadmium can be ingested by humans through dietary intake, leading to its accumulation in various organs, resulting in kidney disease, osteoporosis, cardiovascular diseases, and cancer (Fatima et al., 2019; Suhani et al., 2021). Additionally, Cd adversely affects reproductive health in

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both males and females, impairing hormone synthesis and regulation, and negatively impacting pregnancy rates and outcomes, even at low doses (Kumar & Sharma, 2019).

Currently, several methods are primarily used to reduce Cd content in crops. The first involves the removal of Cd from substrates using physical or chemical methods (Xin, 2024). The second strategy involves adding exogenous substances, such as potassium, to reduce Cd absorption (Huang et al., 2023). The third strategy involves screening and cultivating low-Cd-accumulating cultivars, which is more effective, highly feasible, and easier to implement. For the breeding of low-Cd-accumulating cultivars, understanding the physiological processes and molecular mechanisms of Cd uptake and transport in L. *edodes* is crucial.

Several studies have aimed to elucidate the mechanisms underlying cadmium resistance in L. *edodes* to date. Transcriptomic analyses have identified genes related to glutathione transfer, transmembrane transport, and cytochrome P450 that are involved in Cd resistance in L. *edodes* (Yu et al., 2020). Furthermore, analyses of mRNA and miRNA-like RNA (milRNA) indicate that genes involved in cell wall remodeling, heavy metal chelation, redox homeostasis, signal transduction, transcription regulation, lipid and carbohydrate metabolism, transport, proteolysis, DNA repair, and the cell cycle may contribute to the Cd stress response in L. *edodes* (Shen et al., 2022). Antioxidant enzymes, including superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, mitigate reactive oxygen species (ROS) by enhancing their activity to maintain appropriate cellular ROS levels under Cd stress (Dong et al., 2023). Glutathione is integral to detoxification processes due to its cellular protective properties and metal-binding capabilities (Mittra et al., 2024).

Comparative analysis with the *S. rugosoannulata* Cd stress proteome reveals both evolutionarily conserved pathways and lineage-specific innovations in fungal metal detoxification (Dong et al., 2023). While L. *edodes* has been characterized at transcriptomic, milRNA-regulatory, and physiological levels (Chen et al., 2025), the absence of proteomic data until this study created a critical knowledge gap. This gap is particularly consequential given the limited correlation between mRNA abundance and functional protein dynamics—a discrepancy attributable to translational buffering and post-translational modificationsthat modulate protein activity without altering transcript levels.

Tandem Mass Tag (TMT)-based quantitative proteomics is a powerful tool for analyzing physiological changes at the protein level. Proteomics offers distinct advantages over other 'omics' approaches, as proteins are central to most cellular processes. In our previous study, *L. edodes* strains with different Cd tolerance were obtained. Specifically, after 7 h of Cd exposure, the Cd content in strain Le4625 was significantly higher than in strain Le4606 (Yu et al., 2020). The molecular mechanisms underlying this difference have not been identified clearly. Employing two distinct varieties with markedly different capacities for Cd absorption to thoroughly investigate the coping mechanisms under cadmium stress represents an efficient and innovative research strategy. Variability in cadmium accumulation mechanisms among other cultivars has been well documented (Huang et al., 2019; Yin et al., 2022).

In this study, TMT-based quantitative proteomics, combined with biochemical analyses of two varieties with significantly different capacities for cadmium absorption was employed. Investigating the physiological responses of L. *edodes* to Cd stress is of considerable theoretical and practical importance. To reduce human Cd absorption from L. *edodes*, the most effective strategy is to identify and cultivate varieties with low Cd accumulation. Our findings offer valuable insights that will inform future research on the mechanisms underlying Cd resistance in L. *edodes* and facilitate the breeding of low-Cd-accumulating L. *edodes* strains.

2. Material and methods

2.1. Cadmium stress treatment and L. edodes mycelium harvest

L. edodes strains Le4606 and Le4625 were obtained from the National Engineering Research Center of Edible Fungi, Shanghai, China. Freshly cultured colonies were inoculated into Potato Dextrose Broth (PDB) and incubated at 25 °C in the dark for 12 h. After an 8-day incubation, the cultures were exposed to varying concentrations of cadmium (0, 0.1, 1, 5, and 10 mg/L) for different durations (0, 0.5, 2, 7, 12, and 17 h). Subsequently, the mycelia were harvested using four layers of gauze, washed twice with distilled water, ground in liquid nitrogen, and stored at −80 °C.

2.2. Determination of Cd content

The samples were oven-dried at 50 °C and ground to pass through an 80-mesh sieve. The determination followed the method outlined in GB/T 5009.15–2014. Approximately 0.5 g of each sample was weighed into digestion flasks, and 5 mL of concentrated HNO_3 and 2 mL of H_2O_2 were added. After digestion, the samples were rinsed three times with 1 % HNO_3 and then transferred to volumetric flasks. Cd content was determined using graphite furnace atomic absorption spectrometry (GFAAS). For GFAAS, Cd was atomized at 1600 °C after a heating phase at 110 °C and 1308 °C, with pyrolysis at 500 °C. The wavelength for Cd was 228.80 nm. All experiments were performed with three biological replicates, and each sample was analyzed in triplicate.

2.3. Determination of mycelial growth rate and agronomic traits of fruiting bodies

The culture medium, supplemented with Cd at concentrations of 0 and 5 mg/L, was inoculated with two fungal strains. Mycelial growth was measured at 3, 6, 9, 12, 15, 18, and 21 days post-inoculation by drawing and measuring lines. The average mycelial growth rate was then calculated in millimetres per day (mm/d). Agronomic traits were assessed at different developmental stages: primordial, growth, mature, and parachute stages. All experiments were performed in triplicate, with each biological replicate containing three technical replicates.

2.4. Determination of antioxidant system

Mycelia exposed to 0.1 mg/L Cd for varying durations (0, 0.5, 2, 7, 12, and 17 h) were collected for analysis. The activities of antioxidant enzymes (CAT, GST, SOD), non-enzymatic antioxidants (T-AOC, ASA, GSH), physiological indicators (PRO, SS, SP), and peroxidation metabolites (MDA, H_2O_2) were measured using kits from the Suzhou Keming Institute of Bioengineering. The total antioxidant capacity (T-AOC) was measured using the DPPH assay. All measurements were performed with three biological replicates, and each sample was analyzed in triplicate.

2.5. TMT-based proteomics analysis

Two mycelial samples, each with three biological replicates, were collected after exposure to 0.1 mg/L Cd stress for different durations (0, 0.5, and 7 h). TMT-based proteomics analysis was performed by Personalbio Company (Shanghai, China). First, proteins were extracted in lysis buffer and quantified using the bicinchoninic acid (BCA) assay. For each sample, 200 μg of protein was taken and digested with trypsin using the Filter Aided Sample Preparation (FASP) method (Wiśniewski et al., 2009). After digestion, peptides were desalted, vacuum-dried, and labeled with a TMT 10-plex Isobaric Label Reagent Set (Thermo Fisher Scientific, San Jose, CA, USA) following the manufacturer's instructions (Li et al., 2020). Finally, high-pH reverse-phase HPLC was used to fractionate the peptides. The resulting raw LC–MS/MS spectra were analyzed with Mascot 2.2 and Proteome Discoverer 1.4 for protein

identification and quantitative analysis.

Differentially expressed proteins were defined as those with a fold change greater than 1.2 with a *p*-value <0.05. Manual corrections were also applied to any zero values in the subsequent calculations. All raw data and output tables have been deposited in the iProX database and are accessible using the accession number IPX0009838001.

2.6. Data analysis and plotting

Data analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) and Pearson correlation, conducted with SPSS 23.0.Figures were generated using GraphPad Prism 9 software. The R package cluster Profiler was used for GO and KEGG pathway enrichment analysis and plotting. The graphical abstract and schematic of the response to Cd stress in L. *edodes* were created using Figdraw.

3. Results

3.1. Effect of Cd stress on Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625 at different developmental stages

To evaluate the Cd accumulation capability of two strains, the Cd content in the mycelia of both strains was measured after exposure to 0.1 mg/L Cd for 7 h. The results show that the Cd concentration in Le4625 (9.86 ± 0.81 mg/kg) is approximately five times higher than that in Le4606 (Fig. 1A).

To investigate the impact of Cd stress on mycelial biomass, we quantified the biomass of two strains after exposure to varying concentrations of Cd (0, 0.1, 1, 5, 10 mg/L) over an 8-day period. The findings show a reduction in the biomass of Le4625, with a more pronounced decrease observed in Le4606 (Fig. 1B). Additionally, mycelial growth rates were assessed, revealing significant inhibition of growth under Cd stress (Fig. 1C). These results suggest that Cd stress inhibits mycelial growth.

To further investigate the effects of Cd stress on Cd accumulation in the fruiting bodies of L. *edodes* at various developmental stages, we quantified the Cd content at the primordial, growth, mature, and parachute stages. The results show a progressive increase in Cd content in individual mushrooms with growth, followed by a decline at the parachute stage (Fig. 1D). Moreover, Cd content in Le4625 was consistently higher than that in Le4606 across all developmental stages.

Regarding the impact of Cd stress on the primary agronomic traits of L. *edodes*, the results show a reduction in the yield of Le4625, whereas an increase in the yield of Le4606 is observed at the mature stage (Fig. 1E).

In this subsection, it can be concluded that Cd stress inhibits mycelial growth. Additionally, the yield of Le4625, with higher Cd enrichment capability, is reduced, while the yield of Le4606,with lower Cd enrichment capacity, is increased. These findings suggest that L. *edodes* may have evolved distinct strategies to respond to Cd stress.

3.2. Effects of Cd stress on biochemical indicators in Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625 over time

To investigate the effects of Cd stress over time, mycelia from two strains were exposed to 0.1 mg/L Cd for varying durations (0, 0.5, 2, 7,

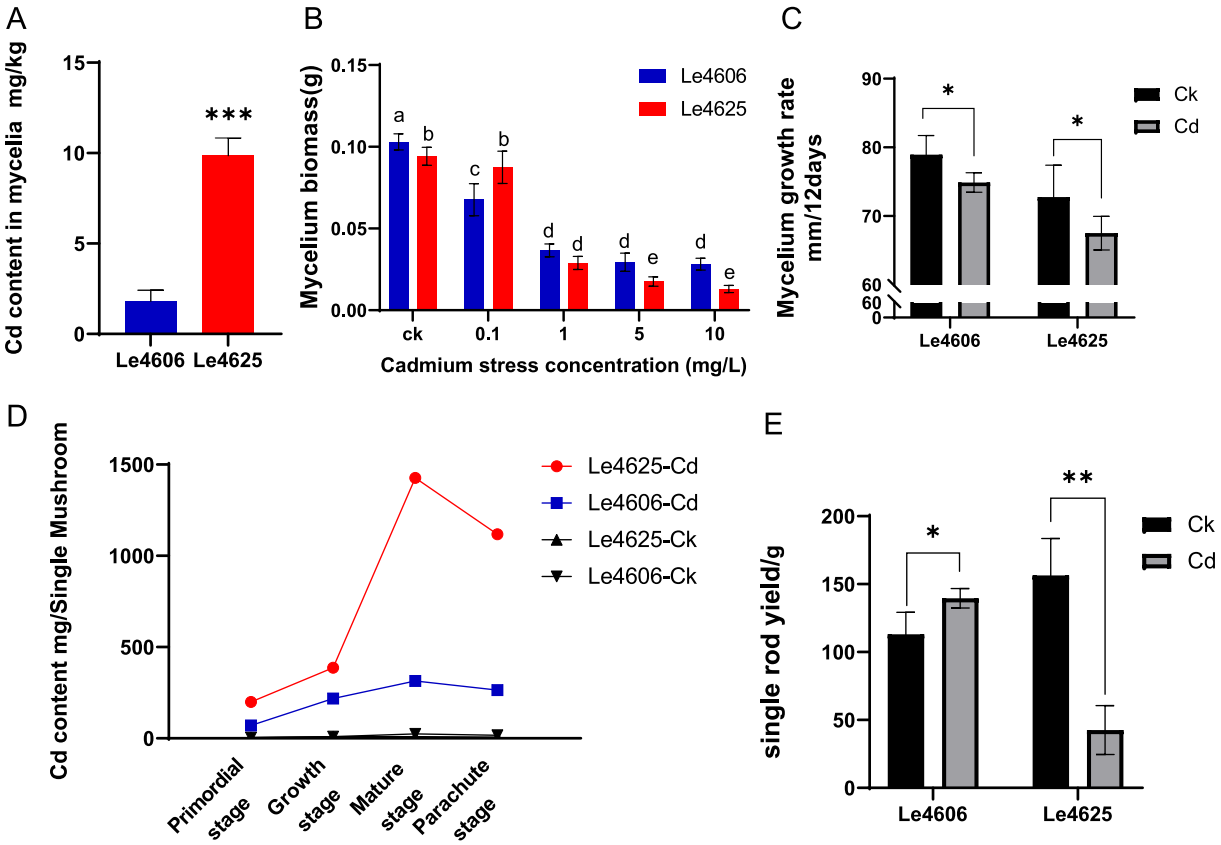

Fig. 1. (A) Cadmium (Cd) content in Le4606 and Le4625 after 7 h of exposure to 0.1 mg/L Cd during the mycelial growth stage, with three biological replicates for each condition. (B) Mycelial biomass of Le4606 and Le4625 following exposure to varying concentrations of Cd (0, 0.1, 1, 5, and 10 mg/L) over an 8-day period. Different letters above the groups indicate statistically significant differences, while shared letters indicate no significant differences. (C) Cd content in individual mushrooms of Le4606 and Le4625 at various developmental stages: primordial, growth, mature, and parachute stages. (D) The mycelial growth rate of Le4606 and Le4625, with or without 0.1 mg/L Cd stress, following a 12-day incubation period in the dark. (E) Single rod yield of Le4606 and Le4625, with or without 0.1 mg/L Cd stress, at the mature stage. Statistical significance is indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

12, and 17 h). Data were normalized using the initial values of Le4606 as the control. The total antioxidant capacity of Le4606 initially increases, followed by a decline, while Le4625 shows an initial decline, followed by an increase (Fig. 2). This difference may be attributed to the distinct response strategies of Le4606 and Le4625 under Cd stress.

Cadmium stress induces oxidative stress in cells. The superoxide ion (O^{2-}), responsible for oxidative stress, is converted to H_2O_2 by SOD. As shown in Fig. 2, SOD levels initially increase, then decrease, while H_2O_2 levels increase and are scavenged up to 7 h in Le4606. In contrast, in Le4625, both SOD and H_2O_2 levels initially decrease, then increase. Notably, the accumulation of H_2O_2 is significantly smaller in Le4625 at 7 h. This discrepancy may be due to the differential degradation pathways of H_2O_2.

Generated H_2O_2 can be scavenged by CAT or ASA-GSH cycle. The change in CAT activity was greater in Le4625, suggesting that CAT plays a more important role in regulating H_2O_2 concentration in Le4625. In the ASA-GSH cycle, ASA and GSH serve as primary antioxidants involved in H_2O_2 scavenging (Han et al., 2021). As shown in Fig. 2, ASA and GSH levels are reduced, and the enzymatic activity of glutathione S-transferase (GST) decreases more significantly in Le4606. These findings suggest that the ASA-GSH cycle plays a crucial role in H_2O_2 scavenging in Le4606.

Proline and soluble sugars are common osmolytes that protect cells. Proline hyperaccumulation contributes to stress responses by increasing cellular osmolarity and mitigating oxidative stress injury by reducing membrane oxidative damage(Sorkheh et al., 2012). As shown in Fig. 2, proline levels do not change significantly in Le4606, but increase significantly in Le4625. Proline hyperaccumulation in Le4625 may help reduce Cd-induced H_2O_2 accumulation. Additionally, the change in soluble sugars is considerably greater in Le4625 at 7 h. These results suggest that Cd stress influences cellular osmolyte dynamics.

Cd stress induces lipid peroxidation and the accumulation of MDA. Lipid peroxidation serves as a marker for ROS-induced oxidative damage (Li et al., 2022). As illustrated in Fig. 2, MDA content increased significantly in both strains under Cd stress, with Le4625 exhibiting markedly higher MDA content than Le4606. This elevated MDA accumulation suggests that Cd exposure causes greater ROS-induced oxidative damage in Le4625.

Overall, the Cd high-accumulation strain Le4625 maintains redox balance mainly through CAT and proline, with greater ROS-derived oxidative damage. The GSH-ASA cycle helps maintain cellular redox balance more efficiently in Cd low-accumulation strain Le4606.

3.3. *Proteomic analysis between Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625 upon Cd treatment*

To elucidate the impact of Cd stress on Le4606 and Le4625 at the protein level, a TMT-based proteomics analysis was conducted following exposure to 0.1 mg/mL Cd stress for 0.5 and 7 h. A total of 3235 proteins were identified across the groups L_0, L_0.5, L_7, H_0, H_0.5, and H_7. Differentially expressed proteins (DEPs) were defined as those exhibiting a fold change greater than 1.2 with a p-value <0.05. Moreover, zero

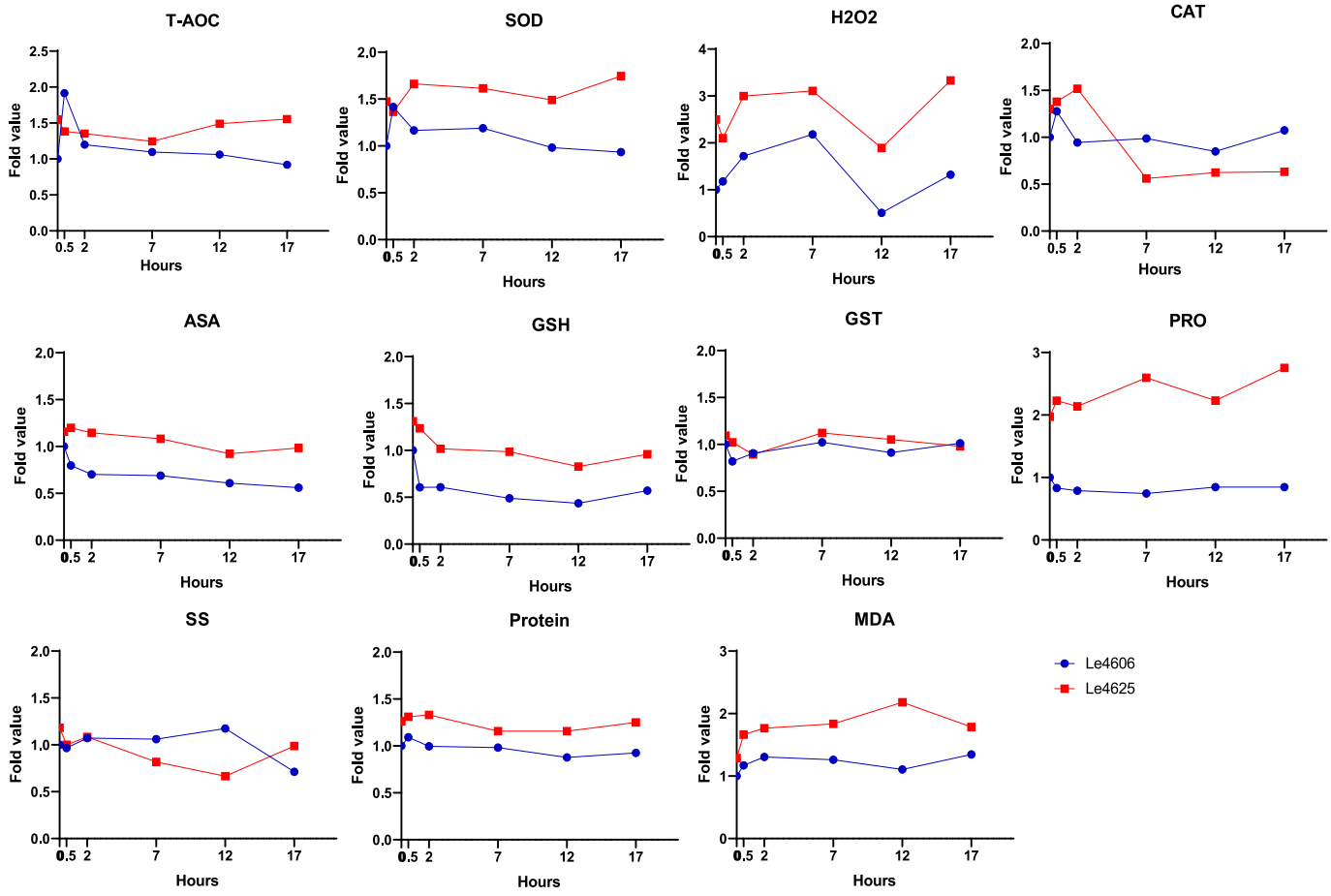

Fig. 2. Effects of Cd stress on biochemical indicators in the Cd low-accumulation strain Le4606 and the Cd high-accumulation strain Le4625 over time. Each sample was analyzed in triplicate, and the results were averaged. Data were normalized using the initial values of the Le4606 strain as the control, set to 1. Mycelia were exposed to 0.1 mg/L Cd for varying durations (0, 0.5, 2, 7, 12, and 17 h) and then collected for analysis. T-AOC, total antioxidant capacity; CAT, catalase; GST, glutathione S-transferases; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; ASA, ascorbic acid; GSH, L-glutathione; MDA, malonic dialdehyde; PRO, proline; SP, soluble protein; SS, soluble sugar.

values were manually corrected in subsequent calculations.

The number and ratio of DEPs in two mycelial samples following exposure to 0.1 mg/L Cd stress for varying durations (0, 0.5 and 7 h) are presented in Fig. 3. As shown in Fig. 3A, 1,066 DEPs were identified in the absence of Cd stress (L_0 vs. H_0), indicating significant initial differences between the two strains. In Le4606, 12.70 % of DEPs were observed after 0.5 h of Cd stress exposure, increasing to 35.39 % after 7 h. In contrast, in Le4625, 26.12 % of DEPs were detected after 0.5 h of Cd stress exposure, increasing further to 46.03 % after 7 h. The results indicate that Le4625 exhibits more DEPs than Le4606 after both 0.5 h and 7 h of Cd stress exposure. This suggests that Cd high-accumulation strain Le4625 is more sensitive and responsive to Cd stress, while the Cd low-accumulation strain Le4606 is more stable.

3.4. Similar Cd responses between Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625

To investigate the overall impact of Cd stress on L. *edodes*, we focused on proteins exhibiting similar trends. A heatmap generated using GraphPad Prism software was used to visualize the temporal changes in the relative abundance of these proteins. Out of the 3235 proteins analyzed, 1091 proteins that did not exhibit significant changes across all groups were excluded from further analysis. The remaining proteins were categorized into four distinct types based on their observed trends, as detailed in Supplementary Table A.1.

As shown in Fig. 3B, a total of 1318 proteins demonstrate similar trends between Le4606 and Le4625. The consistent changes observed in these proteins across both Le4606 and Le4625 imply a general effect of Cd stress on *L. edodes*. To analyze the functional distribution of these 1318 proteins, Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed. The

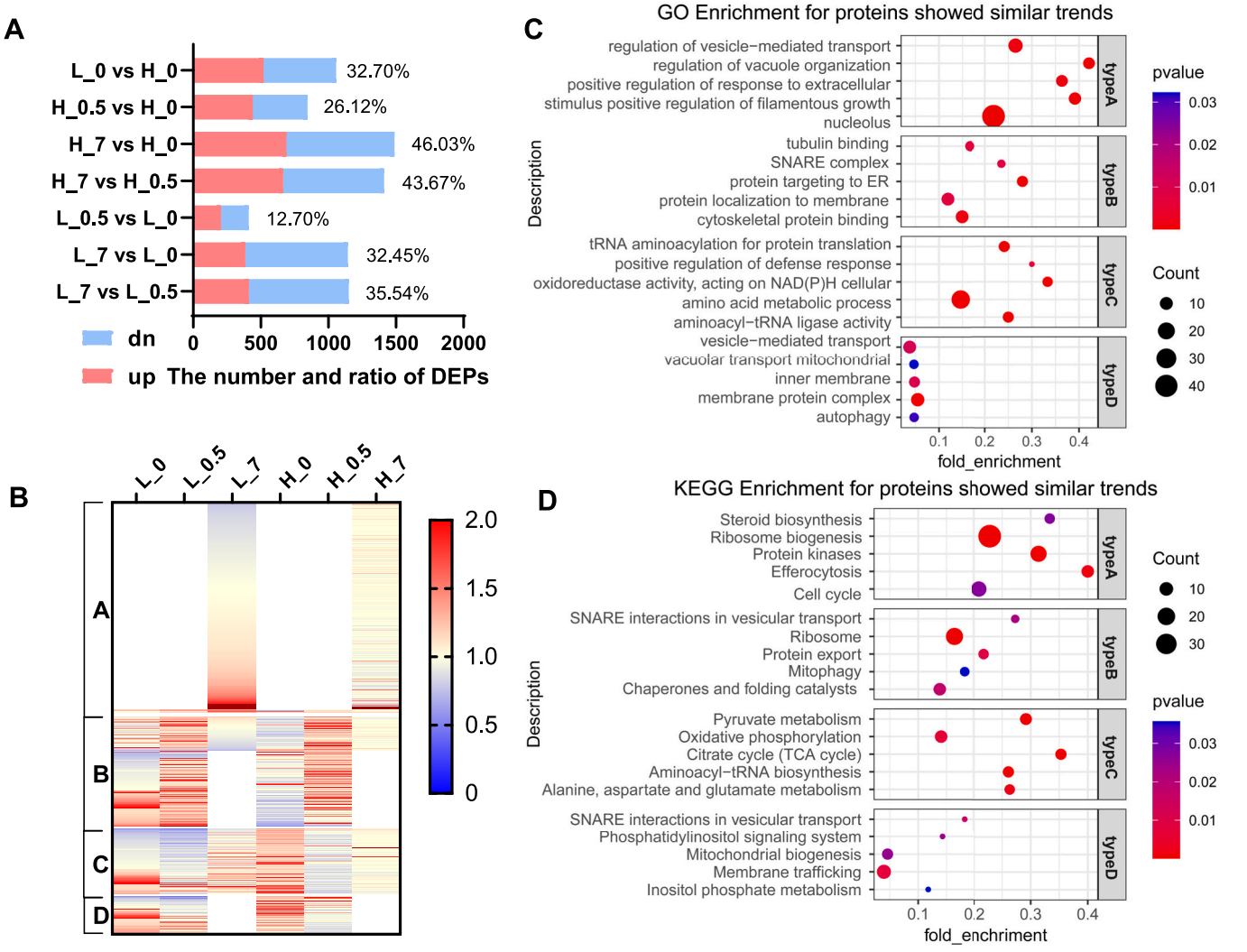

Fig. 3. (A) Quantification and proportion of differentially expressed proteins (DEPs) in Cd low-accumulation strain Le4606 (L) and Cd high-accumulation strain Le4625 (H) at different exposure times (0, 0.5 and 7 h). The y-axis represents the various contrast groups, while the x-axis indicates the number of DEPs. The terms "dn" and "up" refer to down-regulation and up-regulation of DEPs, respectively. All percentages are calculated relative to the total of 3235 proteins detected across all groups. (B) Visualization of 1318 proteins exhibiting similar expression trends. Each column represents a sample, and each row represents a protein. Blue colour indicates lower abundance, red indicates higher abundance, and blank signifies the absence of protein detection, as determined by TMT-based proteomic analysis. Type (A, ++) exhibited an increase at 0.5 and 7 h; type (B, +−) showed an increase at 0.5 h and a decrease at 7 h; type (C, −+) demonstrated a decrease at 0.5 h and an increase at 7 h; and type (D, −-) showed a decrease at both 0.5 and 7 h. (C) Dot plot of GO enrichment analysis of proteins showed similar trends. (D) Dot plot of KEGG enrichment analysis of proteins showed similar trends. The y-axis represents the fold enrichment, the dot size indicates the number of DEPs, and the dot colour represents the *p*-value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results indicate that 647 proteins are classified as type A, 370 as type B, 178 as type C, and 123 as type D. Notably, among type A proteins, nearly all were undetected until the 7-h mark, highlighting their significant role in the response to Cd stress in L. *edodes*. Additionally, many proteins associated with signaling pathways, such as SSK1, HOG1, NIK1, and TOR1, were identified.

GO enrichment analysis indicates that type A proteins are enriched in the positive regulation of filamentous growth and response to extracellular stimuli. Type B proteins are enriched in protein targeting to the endoplasmic reticulum (ER) and the SNARE complex. Type C proteins are enrichment in oxidoreductase activity and tRNA aminoacylation for protein translation. Type D proteins are enriched in membrane protein complexs and autophagy (Fig. 3C).

KEGG enrichment analysis indicates that type A proteins are enriched in ribosome biogenesis, protein kinases, and efferocytosis. Type B proteins are enriched in SNARE interactions in vesicular transport and ribosome. Type C proteins are enriched in the TCA cycle and oxidative phosphorylation. Type D proteins are enriched in membrane trafficking and mitochondrial biogenesis (Fig. 3D).

3.5. Different Cd responses between Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625

Previous data indicate that Le4625 has higher Cd enrichment capability, while Le4606 has lower Cd enrichment capability. Proteins showing similar trends not only reveal the general impact of Cd stress on L. *edodes*, but also help explain the potential causes of the differences in Cd enrichment capacity between Le4625 and Le4606. We focused on proteins showing similar trends but with significant differences in fold change values (FC1 / FC2 > 1.2 or < 0.833).

After 0.5 h of Cd exposure, 34 proteins exhibited a significant increase, while 20 proteins showed a significant decrease in Le4606. Notably, KAF8824890.1, annotated as a NADH-ubiquinone oxidoreductase subunit, showed a significant decrease in Le4606 after Cd exposure. In contrast, Le4625 exhibited 97 proteins with a significant increase and 93 proteins with a significant decrease. Among these, LCC3, annotated as a multicopper oxidase, showed a particularly pronounced increase in Le4625. LCC3 catalyzes the oxidation of a wide range of phenolic and non-phenolic substrates, while concurrently reducing molecular oxygen to water (Terrón et al., 2004; Zhang et al., 2023). Additionally, GDH2, annotated as glutamate dehydrogenase and crucial for maintaining glutamate homeostasis, exhibited a more pronounced decrease in Le4625.

After 7 h of Cd exposure, 143 proteins exhibited a significant increase in abundance, while 112 proteins showed a significant decrease in the Le4606. Among these, the most notable is long-chain base kinase LCB4, which exhibited a marked increase in Le4606. LCB4 triggers cellular responses such as Ca2+ mobilization and the inhibition of apoptosis

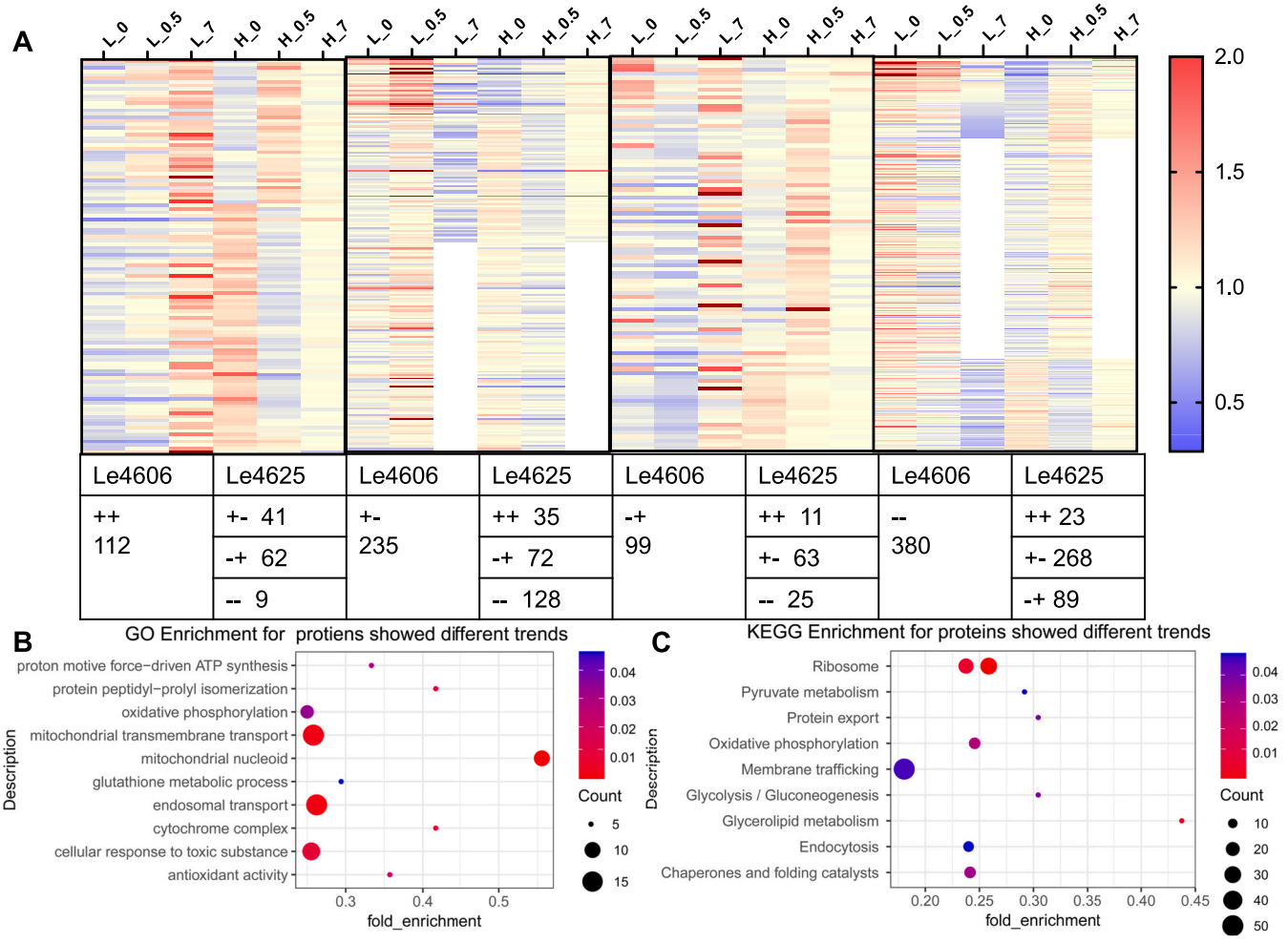

Fig. 4. (A) Heatmap for the proteins exhibiting different trends in Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625. The colour bar indicates the relative abundance of proteins, with blanks signifying undetected proteins. Symbols "+" and "-" denote increases and decreases in protein levels, respectively. The figure also provides the number of proteins exhibiting various alteration trends at 0.5 h and 7 h. (B) Dot plot representing GO enrichment for proteins showed different trends. (C) Dot plot depicting KEGG enrichment for proteins showed different trends. The Y-axis denotes fold enrichment, the dot size corresponds to the number of DEPs, and the dot colour indicates the *p*-value.

(Iwaki et al., 2007). The most notable decrease in Le4606 was MDM34, which provides essential membrane resources for the formation of autophagosomes (Xu et al., 2019).

After 7 h of Cd exposure, 295 proteins exhibited a significant increase in abundance, while 110 proteins showed a significant decrease in Le4625. Among these, the most notable is CCH1, a protein annotated as the calcium and cadmium channel, which showed a marked increase in expression.

To elucidate the factors contributing to the differential Cd enrichment capabilities of Le4625 and Le4606, we focused on 826 proteins exhibiting distinct expression patterns (Supplementary Table A.2). As shown in Fig. 4A, there are 112 type A proteins in Le4606, while there are identified as 41 type B, 62 type C, and 9 type D proteins in Le4625. Le4606 contains 235 type B proteins, while there are identified as 35 type A, 72 type C, and 128 type D proteins in Le4625. Le4606 contains 99 type C proteins, while there are identified as 11 type B, 63 type C, and 25 type D proteins in Le4625. Le4606 contains 380 type D proteins, while there are identified as 23 type A, 268 type B, and 89 type C proteins in Le4625.

To analyze the distribution of functions across these 826 proteins, GO and KEGG enrichment analyses were performed. GO analysis indicated that proteins exhibiting different trends were significantly enriched in processes such as cellular response to toxic substances, glutathione metabolism, and antioxidant activity (Fig. 4B). KEGG analysis demonstrated that these proteins were enriched in pathways related to oxidative phosphorylation, membrane trafficking, and endocytosis (Fig. 4C).

3.6. Inherent differences of Cd low-accumulation strain Le4606 and Cd high-accumulation strain Le4625 without Cd stress

The observed differences may stem from the inherent characteristics of the strains themselves. Therefore, a comparative analysis was conducted between L_0 and H_0 to elucidate the differences between the two strains in the absence of Cd stress. As shown in Fig. 5A, Le4606 exhibited 538 up-regulated DEPs and 527 down-regulated DEPs. Notably, no proteins were exclusively expressed in either Le4606 or Le4625 under non-stress conditions. Gene Ontology (GO) enrichment analysis indicated that Le4606 possesses a higher abundance of proteins related to response to oxygen levels and vesicle-mediated transport, whereas Le4625 is enriched in proteins associated with response to hydrogen peroxide, peroxidase activity, oxidoreductase activity and glutathione metabolism (Fig. 5C). KEGG enrichment analysis indicated that Le4606 exhibits a higher abundance of proteins associated with the spliceosome, necroptosis, endocytosis, and lysosome pathways, while Le4625 demonstrates a greater prevalence of proteins linked to glutathione metabolism and proline metabolism (Fig. 5D).

Notably, proteins associated with antioxidant systems were identified in H_0 compared to L_0. Among these, AHP1 (fold change = 3.16), a typical 2-Cys peroxiredoxin, plays a crucial role in detoxifying various ROS to maintain cellular redox homeostasis (Brachmann et al., 2020).

To initially elucidate the differences between the two strains, serial biochemical indicators were assessed using kits from the Suzhou Keming Institute of Bioengineering. As shown in Fig. 5B, the DPPH assay revealed that Le4625 exhibited a higher total antioxidant capacity (T-

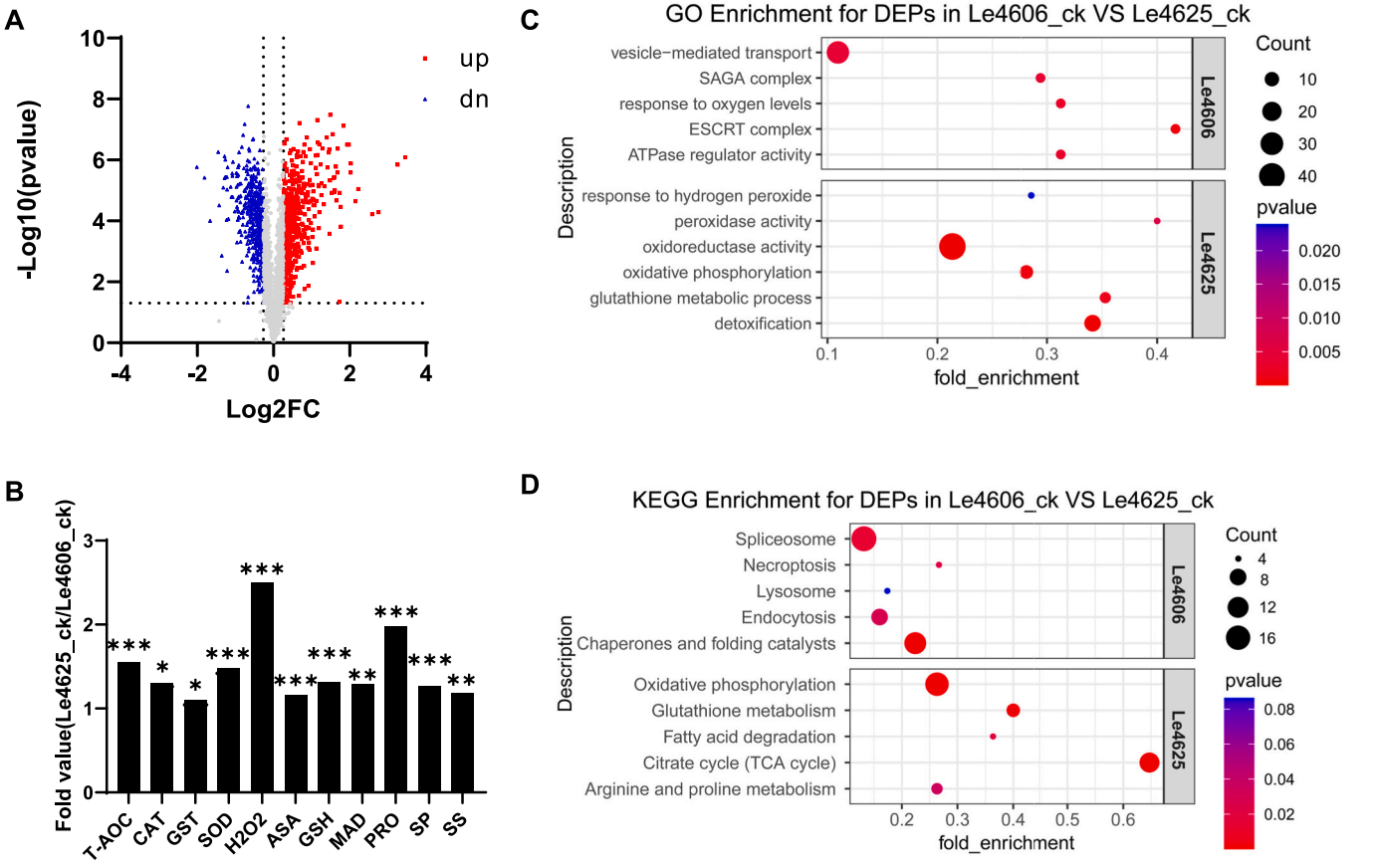

Fig. 5. (A) Volcano Plot of DEPs between H_0 and L_0. (B) Comparative analysis of biochemical indicators between the two strains at the mycelial stage under non-Cd stress conditions. The y-axis represents the fold change (H_0/L_0). Abbreviations: T-AOC, total antioxidant capacity; CAT, catalase; GST, glutathione S-transferase; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; ASA, ascorbic acid; GSH, L-glutathione; MDA, malonic dialdehyde; PRO, proline; SP, soluble protein; SS, soluble sugar. Statistical significance is denoted as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (C) GO enrichment analysis for DEPs between H_0 and L_0. (D) KEGG enrichment analysis for DEPs between H_0 and L_0. The y-axis represents the fold enrichment, dot size represents the number of DEGs, and dot colour represents the *p*-value.

AOC). Compared to Le4606, Le4625 demonstrated higher activities of antioxidant enzymes, including catalase (CAT), glutathione S-transferases (GST), and superoxide dismutase (SOD). Additionally, Le4625 showed higher levels of non-enzymatic antioxidants, such as ascorbic acid (ASA), L-glutathione (GSH), and proline (Pro). Soluble protein (SP) and soluble sugar (SS) are critical osmotic adjustment substances, and their levels are notably higher in Le4625. Additionally, higher concentrations of metabolites such as hydrogen peroxide (H_2O_2) and malonic dialdehyde (MDA) were observed in Le4625. These findings suggest an enhanced antioxidant capacity in the Cd high-accumulation strain Le4625, which aligns with the GO and KEGG enrichment analyses for DEPs.

4. Discussion

4.1. Effect of Cd stress on L. edodes

Cadmium can accumulate in L. *edodes*, subsequently entering the food chain. Previous research has shown that the fruiting bodies of fungi can accumulate substantial quantities of heavy metals, posing a significant threat to human health (Pei et al., 2015). In this study, we observed that Cd stress inhibits mycelial growth, affects yield, and induces ROS-mediated oxidative damage, consistent with previous studies (Dong et al., 2023; Yu et al., 2020). The yield of Le4625, characterized by higher Cd accumulation, is reduced, while the yield of Le4606, with lower Cd accumulation, increases. Our previous research also revealed that, aside from an increase in single rod yield, no significant differences were observed in Le4606. Conversely, although the yield of Le4625 decreased, the size of individual mushrooms increased under Cd stress (Zhai et al., 2023). These results suggest that Cd stress influences agronomic traits in L. *edodes*, particularly in Le4625. This phenomenon may because that Cd stress triggers mycelial resource reallocation from biomass production to stress defense, limiting primordia initiation (with yield decreased) but enhancing carbon allocation to existing fruiting bodies (with size increased) (Durand et al., 2018). Or modifications in fruiting body cell wall plasticity allow size expansion despite yield loss (Meyer et al., 2015; Pérez et al., 2021). This phenomenon may also stem from the heightened sensitivity and responsiveness of Le4625, in contrast to the greater stability of Le4606 at the protein level under Cd stress. The pronounced phenotypic differences suggest that L. *edodes* may employ distinct strategies to cope with Cd stress, just as in plants (Song et al., 2025). By integrating findings from KEGG and GO enrichment analyses, our primary focus is on proteins and metabolites involved in detoxification, signaling pathways, antioxidant systems and autophagy.

4.2. Cadmium accumulation and detoxification

The voltage-gated high-affinity calcium channel, comprising the CCH1 and MID1 subunits, is essential for Cd uptake in yeast (Xiong et al., 2015). CCH1 gene deletion leads to heightened sensitivity to Cd and increased accumulation of cadmium ions (Gardarin et al., 2010; Ruta et al., 2014). Elevated CCH1 levels in Le4625 suggest enhanced cadmium uptake capacity in this strain.

Upon entering the cell, Cd binds to GSH, synthesized by GSH1 and GSH2, forming a $GSH(Cd)_2$ complex (Iddrisu et al., 2024). Due to its high affinity for Cd and its role as a Cd chelator, GSH levels significantly decrease in response to Cd exposure (Xu et al., 2014). Glutathione S-transferase enzyme GST2 conjugates Cd to GSH to facilitate detoxification (Zheng et al., 2018). Previous studies show that the introduction of extracellular metals results in a rapid decrease in YAP1 protein levels, consistent with our proteomic data observed at 7 h (Oya et al., 2022). YAP1 regulates genes associated with reducing Cd uptake, vacuolar sequestration, and excretion, thereby contributing to metal homeostasis (Grant et al., 1996). The more pronounced reduction in GSH and GST2 levels in Le4606 suggests that this strain exhibits enhanced Cd detoxification capabilities via the GSH pathway.

Major Facilitator Superfamily (MFS) transporters are integral membrane proteins that facilitate the translocation of a wide range of substrates by harnessing the energy stored in electrochemical gradients. These transporters are crucial in the extrusion of deleterious compounds, including heavy metals (Drew et al., 2021). In this study, three MFS general substrate transporters, KAF8823702.1, KAF8826854.1, and KAF8832711.1, were identified. Notably, the expression of KAF8823702.1 was significantly elevated in Le4606 at 7 h. These findings suggest an enhanced capacity for cadmium extrusion in Le4606.

The tricarboxylic acid (TCA) cycle is a crucial bioenergetic pathway that facilitates ATP synthesis through oxidative phosphorylation. It also plays a crucial role in modulating NADH/NADPH homeostasis, scavenging ROS, producing ATP via substrate-level phosphorylation, and signal pathways, while also supplying essential metabolites to mitigate various cellular disruptions (MacLean et al., 2023). In Le4606, numerous proteins associated with the TCA cycle are up-regulated. Notably, succinate dehydrogenase subunit 4 (SDH4), a key protein involved in succinate degradation, is significantly induced under Cd stress, as reported in previous studies, and similarly induced in Le4606 (Ji et al., 2020). Increased levels of isocitrate dehydrogenase (IDH2) enhance NADPH production, essential for the maintaining reduced GSH and peroxiredoxin systems (Smolková & Ježek, 2012). Consequently, the TCA cycle is enhanced in Le4606, contributing to cellular homeostasis in this strain.

Previous studies show that the vacuole plays a crucial role in Cd homeostasis by storing Cd for future use under deficient conditions, sequestering excess Cd for detoxification, and buffering rapid fluctuations in intracellular Cd levels (Sharma et al., 2016). The ABC transporter YCF1 facilitates the transport of the $GSH(Cd)_2$ complex into vacuoles, enhancing Cd tolerance and accumulation (Guo et al., 2012; Paumi et al., 2009). Proteomic analyses reveal that ZRC1 is detected exclusively in Le4625 at 7 h. ZRC1 is a metal transporter that translocates Zn/Cd into vacuoles (MacDiarmid et al., 2002). Previous studies show that the absence of ZRC1 increases Cd sensitivity, suggesting its crucial role in conferring Cd resistance (Pan et al., 2017). The absence of ZRC1 detection in the low-Cd-accumulating strain Le4606 under all tested conditions suggests a defective vacuolar Cd^{2+} sequestration pathway in this strain. In contrast, the high-Cd-accumulating strain Le4625 exhibited transient ZRC1 expression at 7 h post-exposure, indicating its capacity to pump Cd^{2+} into vacuoles via proton gradient-dependent transport. This functional dichotomy provides a mechanistic explanation for their divergent Cd accumulation phenotypes: Le4625 efficiently isolates Cd into vacuoles, reducing cytosolic toxicity and enabling sustained uptake, whereas Le4606—lacking this transient sequestration—relies on slower, less efficient cytoplasmic chelation, ultimately limiting total Cd accumulation.

Proline, a key osmoticum, plays a significant role in enhancing tolerance to environmental stress. Previous research shows that proline mitigates Cd toxicity by reducing accumulation and re-establishing redox homeostasis (Wang et al., 2022). Further studies show that proline protects cells from metal toxicity by chelating metal ions in the cytoplasm and detoxifying ROS (Tripathi et al., 2013). Our data reveal that proline hyperaccumulation occurs in Le4625, suggesting it is an adaptive strategy in response to Cd stress.

Overall, once Cd enters the cytoplasm, it binds to GSH or proline and is sequestered into vacuoles or effluxed to mitigate toxicity. Le4625 exhibits higher Cd absorption capacity, greater proline hyperaccumulation, and enhanced sequestration into vacuoles. In contrast, Le4606 demonstrates lower Cd absorption capacity but a stronger detoxification ability via GSH conjugation mediated by GST2, an enhanced TCA cycle, and more robust Cd extrusion.

4.3. Cadmium -induced oxidative stress

Our previous transcriptomic study showed that Cd stress activates a

range of antioxidant stress response pathways (Yu et al., 2020). To further understand Cd-induced oxidative stress, the biochemical and proteomic results were integrated. Upon entering cells, Cd disrupts metabolic processes, leading to oxidative stress and generating substantial amounts of ROS, including O_2^-, H_2O_2, HO^-, ROOH, and ROO· (Jovanović et al., 2022; Singh et al., 2016). Mitochondria, a key site for ROS production, generate O_2^- through single electron reductions of O_2, which SOD converts to H_2O_2 (Hillmann et al., 2015). The downregulation of succinate dehydrogenase complex subunit A (SDH1) suggests a blockade in the electron transport chain of the oxidative respiratory pathway, leading to increased ROS levels (Chen et al., 2024). Conversely, upregulation of cytochrome *c*1 (CYC1), an electron carrier protein, indicates enhanced ROS production, which is also implicated in apoptosis and the regulation of hyphal morphogenesis (Sellem et al., 2007; Zeng et al., 2023). Proteomic and physiological analyses reveal that Cd stress induces ROS production within 0.5 h, especially in Le4606.

Cells aim to control or neutralize ROS to maintain functionality. Cellular detoxification systems include antioxidant enzymes and redox molecules, such as CAT, GSH, glutathione peroxidases (GPX2), glutathione reductase (GLR1), thioredoxin (Trx), peroxiredoxins (PRX1), and thioredoxin reductase (TRX3). A significant increase in the levels of most of these antioxidant enzymes and redox molecules was observed at 0.5 h. The Trx system plays a key role in detoxification by regulating the redox environment. Among these enzymes, PRX1 is the most effective at neutralizing H_2O_2. Trx reduces PRX1, while TRX3 reduces oxidized Trx. Additionally, protein disulfide isomerase PDI1, a thiol-disulfide oxidoreductase, has been identified as involved in biotic stress (Zhang et al., 2018). Higher protein expression and reduction of Trx system protein PRX1, TRX3 and PDI1 are observed in Le4606, indicating a stronger Trx antioxidant system in this strain.

In Le4606, an increase in protein expression accompanies the reduction in Trx system proteins PRX1, TRX3, and PDI1. An alternative mechanism for neutralizing H_2O_2 involves the ASA-GSH cycle. To counterbalance the reduction in GSH, GPX1 converts GSH into its oxidized form (GSSH), a process reversed by GLR1. Notably, GLR1 expression is elevated to compensate for the greater reduction in GSH content in Le4606 at 7 h.

Additionally, cytochrome *c* peroxidase (CCP1) enhances tolerance to oxidative stress by preventing ROS accumulation and acts as a negative regulator of mycelial growth (Charizanis et al., 1999). CCP1 modulates enzymes involved in the ASA-GSH cycle, including GPX2 and GST. The B-ZIP transcription factor YAP1 regulates proteins induced by oxidative stress, including CCP1, TRX3, TRR1, and GPX2 (Mulford & Fassler, 2011).

Collectively, CAT in Le4625 plays a significantly more critical role in regulating H_2O_2 concentration. Elevated protein expression and reduction of Trx system proteins PRX1, TRX3, and PDI1 contribute to the enhanced oxidative stress tolerance observed in Le4606. Increased reduction of ASA and GSH in Le4606 aids in neutralizing H_2O_2, thereby maintaining redox balance.

4.4. Signal pathways

MAPK cascades are activated in response to intracellular stress signals. The HOG pathway is the most well-characterized stress-activated MAPK pathway in filamentous fungi. It is activated by various stressors, including osmotic stress, oxidative stress, and heavy metals in filamentous fungi (de Nadal & Posas, 2022). The activation of the HOG pathway involves YPD1, phosphorylated YPD1, HOG1 in both the cytoplasm and nucleus, and the SSK2-PBS2-HOG1 complex (Cheetham et al., 2007; Tomar et al., 2013). Upon Cd stress exposure, MAPKKK SSK2, MAPK HOG1, and the response regulator SSK1 are upregulated. However, no PBS2 proteins were detected in the proteomic analysis. The fungal histidine kinase receptors NIK1 and CHK1 are crucial for osmotolerance and oxidative stress response; mutants deficient in these receptors exhibit increased sensitivity to peroxide (Chapeland-Leclerc et al., 2007; Yoshimi et al., 2005). Additionally, SSK1 and HOG1 either directly or indirectly regulate CHK1, contributing to mycelium development (Feng et al., 2022; Li et al., 2004). Additionally, SIT4 is involved in the dephosphorylation of SNF1 and plays significant roles in mycelium development by regulating cell wall biogenesis, osmosensing, and protein translation (Lee et al., 2004). SNF1 represses the activation of the HOG signaling pathway and is involved in filamentous growth (Shirra et al., 2005; Wang et al., 2023). Moreover, SNF1 can inhibit the TOR pathway (Hardie, 2007).

The RAM pathway, including the kinase CBK1 and the zinc finger transcription factor ACE2, contributes to filamentation and the response to hypoxia or oxidative (Saputo et al., 2016; Wakade et al., 2020) stress. Proteomic analyses revealed that the RAM pathway is activated following Cd stress exposure.

The target of rapamycin (TOR) pathway regulates cellular growth in response to diverse stressors, including nitrogen deprivation, elevated osmolarity, and increased temperature (Kawai et al., 2001). When TOR is active, SCH9 directly phosphorylates RPS6, activating targets involved in ribosome biogenesis, amino acid biosynthesis, and osmotic stress response (Alves de Castro et al., 2016). Proteomic analyses indicate that TOR1 kinase is activated by Cd stress after 7 h. This observation aligns with previous reports suggesting that *tor1* is a non-essential gene, required primarily under starvation and other stressors (Weisman & Choder, 2001).

Overall, the HOG, RAM, and TOR pathways are activated in response to various stresses induced by Cd exposure, including oxidative and osmotic stress. These pathways regulate filamentous growth, consistent with experimental observations indicating a reduction in growth rate and biomass of mycelium under Cd stress.

4.5. Autophagy and apoptosis

Autophagy is activated to ensure cellular survival in response to various stress conditions. In fungi, autophagy occurs in two forms: macroautophagy and microautophagy. Macroautophagy progresses through several stages: induction, nucleation, expansion and completion, docking and fusion, and degradation and efflux (Dargemont et al., 2012).

Proteomic analysis indicates that GCN2 plays a significant role in the cellular response to Cd stress, particularly at the 7 h. Activation of GCN2 inhibits the translation of non-essential proteins and promotes the translation of essential ones (Grallert & Boye, 2013). The GCN2 signaling pathway regulates stress-induced autophagy gene expression, with SIT4 enhancing GCN2 activity (Zhao et al., 2023). TOR1 functions as a nutrient-sensing element that modulates autophagy via phosphoprotein (Khalfan & Klionsky, 2002). Previous studies show that HOG1, involved in the hyperosmotic stress response, is not essential for bulk autophagy and the cytoplasm-to-vacuole targeting pathway (Liu et al., 2014).

Autophagosome formation requires the activity of ATG1, which is critical for recruiting core ATG proteins to the pre-autophagosomal structure (PAS) (Nakatogawa et al., 2009). Various cellular compartments, including the endoplasmic reticulum (ER), the Golgi apparatus, and the plasma membrane, may contribute to phagophore nucleation and expansion (Reggiori & Klionsky, 2013). YPT1 is involved in membrane trafficking from the ER to the phagophore and facilitates recruitment of additional ATG1 molecules to the PAS. The TRAPP I complex activates YPT1 at the early Golgi apparatus, while TRAPP II complex activates the YPT31/32 functional pair at the late Golgi (Lipatova & Segev, 2019). The SEC23-SEC24 complex initiates COPII vesicle formation, which can increase autophagosome numbers under stress conditions (Wang et al., 2017). The activities of ATG5, ATG12, and ATG7 are essential for phagophore expansion (Arakawa et al., 2017; Xu et al., 2019). Specifically, ATG12 is activated by ATG7 in an ATP-dependent manner, and ATG5 is the sole target of ATG12 modification

(Ohsumi & Mizushima, 2004). The ESCRT-III subunit SNF7 interacts with the scaffold protein ATG17 in a VPS21-dependent manner, facilitating ESCRT-III recruitment. Subsequently, ESCRT-III and VPS4 catalyze the closure of the isolation membrane, forming a sealed autophagosome (Jiang et al., 2021). The greater magnitude of changes in ATGs and other autophagy-associated proteins in Le4606 indicates higher autophagy activity under Cd stress.

The fusion of autophagosomes with vesicles from the cytoplasm-to-vacuole targeting pathway requires SNARE machinery (Khalfan & Klionsky, 2002). YKT6 is a novel autophagosomal SNARE protein critical for vesicular fusion pathways and cycles between the cytosol and membrane-bound compartments through reversible lipidation (Matsui et al., 2018; McGrath et al., 2021). Phosphorylation of YKT6 by the ATG1 kinase on autophagosomes inhibits its interaction with vacuolar SNARE proteins. Upon dephosphorylation, YKT6 forms a SNARE complex with VAM3, VAM7, and VTI1, facilitating fusion of autophagosomes with the vacuole (Barz et al., 2020).

Micro-autophagy removes cytoplasmic components directly at the vacuole through tonoplast invagination. CDC48 and its cofactor SHP1 are novel components involved in the micro-autophagy of the nucleus, particularly in autophagosome biogenesis and fusion with lysosomes (Dargemont et al., 2012).

Autophagy and apoptosis often co-occur within the same cell, with apoptosis frequently following autophagy (Chatterjee et al., 2014). GSH depletion and calcium influx into the cytoplasm are hallmarks of apoptosis (Chandel et al., 2016). Cadmium stress induces the release of vacuolar calcium into the cytosol via the YVC1 channel (Gardarin et al., 2010; Ruta et al., 2014). KAF8831479.1 is a magnesium-dependent enzyme that catalyzes ATP hydrolysis, coupled with calcium transport. The greater reduction in GSH content, along with the corresponding increase in YVC1 and KAF8831479.1 level in Le4606 at 0.5 h, suggest a higher incidence of apoptosis in Le4606.

Overall, our findings suggest that Cd stress induces autophagy and apoptosis as mechanisms to remove damaged components and restore cellular homeostasis. Notably, Le4606 exhibits enhanced autophagy and apoptosis to maintain cellular homeostasis. This may explain the lack of significant differences in the primary agronomic traits of Le4606 following exposure to Cd stress.

4.6. Schematic of response to Cd stress in L. edodes

To elucidate the molecular mechanisms by which L. *edodes* regulates and adapts to Cd stress, a comprehensive schematic diagram was constructed, highlighting key proteins and metabolites involved in this process(Fig. 6). Detailed quantitative data are presented in Supplementary Table A.3. The specific relationships and potential mechanisms are elaborated upon in the previous sections. In general, Le4625 exhibits enhanced Cd absorption, proline accumulation, and vacuolar sequestration for detoxification, contributing to high Cd accumulation. Conversely, Le4606 excels in glutathione-mediated detoxification, thioredoxin antioxidant activity, tricarboxylic acid cycle activity, autophagy, and Cd extrusion, contributing to low Cd accumulation.

Our comparative proteo-physiological analysis reveals that natural L. *edodes* populations have evolved two distinct Cd response strategies. Specifically, we demonstrate that divergent detoxification strategies drive Cd accumulation phenotypes.

Cd high-accumulation strain Le4625 employ a 'Vacuolar Priority Strategy' to sequester Cd^{2+} into vacuoles via proton-coupled antiport characterized. This mechanism minimizes cytoplasmic toxicity while enabling sustained uptake, as vacuoles act as non-toxic sinks. Cd low-accumulation strain Le4606 adopt a 'Cytosolic Chelation Strategy' by ASA-GSH cycle to bind Cd^{2+} in the cytoplasm.

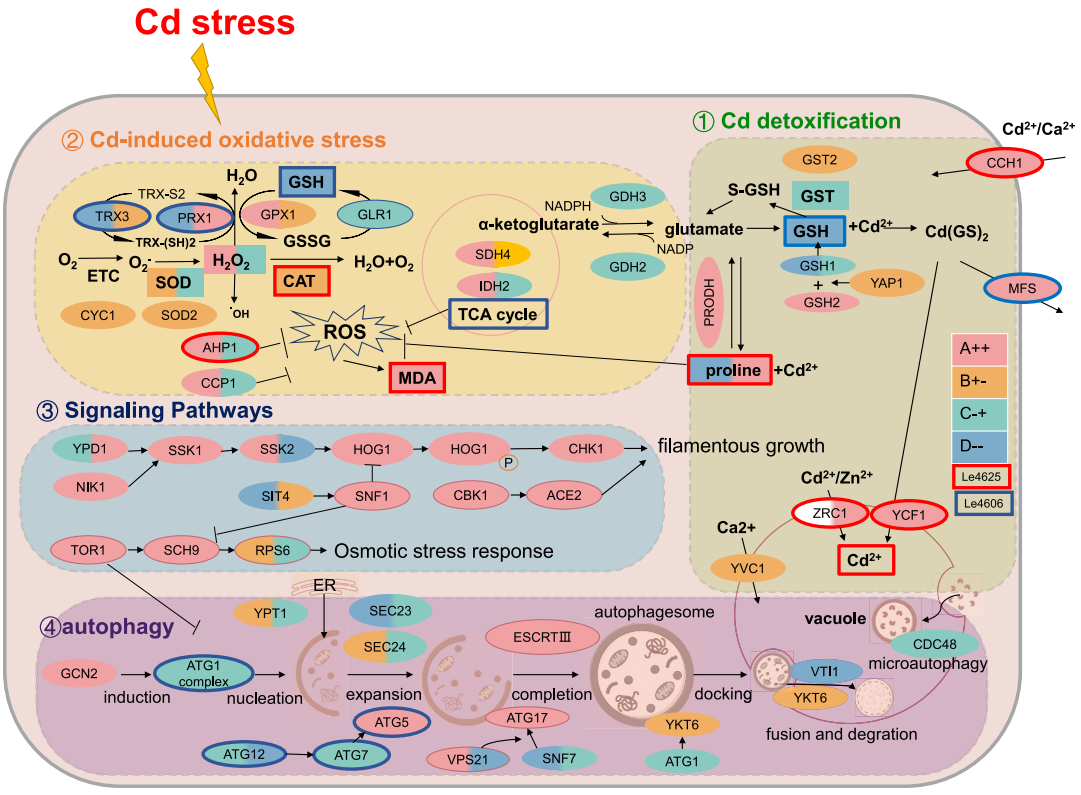

Fig. 6. Schematic of response to Cd stress in *L. edodes*. The different colours represent distinct response trends: Type A (++: increased at 0.5 and 7 h, pink); Type B (+−: increased at 0.5 h and decreased at 7 h, yellow); Type C (−+: decreased at 0.5 h and increased at 7 h, cyan); and Type D (−-: decreased at 0.5 and 7 h, blue). The colour on the left side indicates the response in Cd low-accumulation strain Le4606, while the colour on the right side indicates the response in Cd high-accumulation strain Le4625. Red-coloured boxes highlight a greater contribution in Le4625, while blue-coloured boxes indicate a greater contribution in Le4606. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

These findings provide actionable molecular targets for precision breeding. For example, replace the native ZRC1 promoter with a fruiting body-specific repressor to block vacuolar Cd storage in fruiting bodies.

5. Conclusion

In this study, we investigated the physiological and proteomic differences between the Cd low-accumulation strain Le4606 and the Cd high-accumulation strain Le4625 after exposure to Cd stress for 0.5 and 7 h. By integrating experimental results with existing literature, we propose a preliminary mechanistic hypothesis for the response of L. *edodes* to Cd stress at protein level. Upon Cd entry into the cytoplasm, it binds to GSH or proline and is subsequently sequestered into vacuoles or expelled from the cell to mitigate toxicity. To counteract oxidative damage, the antioxidant system is activated. Moreover, the HOG, RAM, and TOR pathways are triggered in response to the various stresses induced by Cd exposure, including oxidative stress and osmotic stress. These pathways can influence filamentous growth, aligning with experimental observations that show a reduction in the growth rate and biomass of mycelium following Cd exposure. Furthermore, Cd stress induces autophagy and apoptosis as mechanisms to maintain cellular homeostasis.

Our findings indicate that L. *edodes* employs distinct strategies to cope with Cd stress for survival. Specifically, the Cd high-accumulation strain Le4625 demonstrates a greater capacity for Cd absorption, increased proline hyperaccumulation, enhanced sequestration of Cd into vacuoles for detoxification, and maintenance of redox balance through CAT and peroxiredoxins. In contrast, the Cd low-accumulation strain Le4606 exhibits a weaker capacity for Cd absorption but possesses a more robust GSH detoxification mechanism, a stronger TRX and GSH-ASA cycle antioxidant system, an intensified TCA cycle, increased autophagy and apoptosis, and a more effective Cd extrusion capability. This study provides novel insights into the response of L. *edodes* to Cd stress at the proteomic level, revealing adaptive mechanisms for resistance.

The selection of low-cadmium varieties is a critical strategy to prevent Cd poisoning in the food chain. Our findings provide a theoretical foundation for breeding varieties with low Cd accumulation while retaining desirable agronomic traits. Based on our results, future breeding efforts should prioritize varieties with enhanced GSH detoxification capabilities over those relying on vacuolar sequestration for detoxification.

However, our current work establishes foundational mechanisms of Cd response only in L. *edodes* mycelia, stage-specific analyses from primordia initiation to mature fruiting bodies are essential for a holistic understanding. And most of the proteins discussed have only been reported in other species, and there are significant challenges remain before practical applications can be realized. Further validation of the protein's function in L. *edodes* is required, particularly regarding vacuolar sequestration and glutathione-mediated detoxification mechanisms. The protein ZRC1 associated with vacuolar sequestration will be the focus of our subsequent studies.

CRediT authorship contribution statement

Yansha Wu: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation. **Dandan Zhai:** Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation. **Ning Jiang:** Validation, Methodology, Investigation. **Qiaozhen Li:** Validation, Methodology, Investigation. **Meiyan Zhang:** Methodology, Investigation. **Chunyan Song:** Resources, Funding acquisition. **Haoran Dong:** Methodology, Investigation. **Xiaodong Shang:** Resources, Funding acquisition. **Hao Yu:** Writing – review & editing, Project administration, Conceptualization. **Hailong Yu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144739.

Data availability

Data will be made available on request.

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