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Article

Biochemical and Metabolomic Responses of Antarctic Bacterium *Planococcus* sp. O5 Induced by Copper Ion

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Abstract: Copper toxicity has been a selective pressure on the sea-ice bacteria due to its widespread occurrence in Antarctica. Here, with a combined biochemical and metabolomic approach, the Cu^{2+} adaptation mechanisms of Antarctic bacteria were analyzed. Heavy metal resistance pattern of Pb^{2+} > Cu^{2+} > Cd^{2+} > Hg^{2+} > Zn^{2+} was observed. Copper treatment did increase the activity of antioxidants and enzymes, maintaining cellular redox state balance and normal cell division and growth. Metabolomics analysis demonstrated that fatty acids, amino acids, and carbohydrates played dominant roles in copper stress adaptation. The results indicated that the adaptation mechanisms of strain O5 to copper stress included protein synthesis and repair, accumulation of organic permeable substances, up-regulation of energy metabolism, and formation of fatty acids. This study increases the resistance mechanism understanding of Antarctic strains to heavy metals in extreme environments.

Keywords: Antarctic strain; copper stress; adaption responses; metabolomics

1. Introduction

Heavy metal pollution is unequivocal in that metal elements with biological toxicity cannot be degraded into non-toxic forms [1]. Unfortunately, concentrations of heavy metals can subsequently be biomagnified thousands of times through biomagnification in ecosystems [2]. Over the past few years, with the high level of industrial activity and the widespread use of pesticides and fertilizers, there has been a general and dramatic increase in the levels of heavy metals in the environment on a global scale, which has seriously threatened the stability of the ecological system and the health of human beings [3].

Bacteria are found almost everywhere on earth[4]. Although metals play an essential role, directly or indirectly, in key cellular processes such as aerobic metabolism and cellular respiration, heavy metal concentrations above the maximum threshold can alter the conformation of nucleic acids and peptides, disrupting cell wall integrity, enzyme specificity, oxidative phosphorylation and osmotic balance, thus becoming toxic to microorganisms [5, 6]. To counteract metal toxicity, microbes have initiated defense strategies and evolved several adaptive mechanisms [7], such as accumulation on a cell wall, transportation across the cell membrane, permeable membrane, intracellular sequestration, and enzymatic detoxification, which form the basis of different bioremediation strategies [6, 8]. The ability of an organism to regulate its metabolism is a central characteristic required for proliferation, hibernation, and survival of any assault [9]. To date, the metabolic mechanism of microbial heavy metal resistance has been elucidated in detail from different perspectives. Adjustment of the fatty acid composition of bacterial cell membranes is one of the vital mechanisms of adaptation to heavy metals [10]. Reconfiguring energy-generating processes are effective in resisting heavy metals. Under metal stress, partial microorganisms meet their energy requirement through substrate-level phosphorylation rather than oxidative phosphorylation [11]. The resistance of *Proteobacteria* to heavy metals maybe since the

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phylum can utilize a variety of organics as carbon and energy sources [12]. The presence of large amounts of long-chain fatty and phospholipid saturation contributes to low fluidity and rigidity of the membrane to prevent the bacterial cell from contaminants[13, 14]. Active efflux and precipitation are major factors in bacterial resistance toward metals [15]. The importance of phosphate efflux for heavy metal resistance was shown in the acidophilic bacterium *Acidithiobacillus ferrooxidans* and *Bacillus sphaericus* [16-18]. In *Escherichia coli*, the addition of copper (Cu) induced cellular intracellular degradation of polyphosphates, and phosphate exportation also supports this mechanism [19]. In addition, heavy metals are precipitated and eliminated by compounds produced by microorganisms in response to heavy metal stress also contributes to a degree of bacterial resistance to metals [15]. To circumvent aluminum (Al) toxicity, *Pseudomonas fluorescens* promotes the synthesis of citrate involved in the sequestration of Al [20]. *Desulfovibrio desulfuricans* can regulate the precipitation of metals by forming metal sulfide [21]. Metabolic levels of low molecular weight organic acids were upregulated to solubilize heavy metals by using them as final electron acceptors or by lowering pH [22, 23]. *Oxalobacter formigens* take up as much Pb as possible, converting it to oxalate for excretion from the intestine [24]. Bacteria remarkably increased metabolites associated with the stress response to rebalance the oxidative stress and osmotic stress caused by heavy metals. *Scenedesmus obliquus* increased lipid esters and Cys-GSH isomers for antioxidant defense mechanisms and prevention of reactive oxygen species under cadmium stress [25]. Ethanol tolerance involves an increase in glycine metabolism, which acts as protective osmolytes in *Escherichia coli* [26].

The Antarctic, an isolated and usually considered pure land, is challenged by negative factors from human activities [27-29]. Heavy metals have been detected in abiotic samples, such as surface soil, atmosphere particulate, and snow in Antarctica [30]. What's more, the levels of metals in Southern Ocean organisms are significantly higher than those in other oceans [31].

As an essential micronutrient, Cu is employed by most organisms to perform different functions [32-34]. However, excessive amounts of copper can be toxic. Cu is one of the most common sources of heavy metals contributing to contamination in Antarctica [35]. Although adaptation strategies to copper have been relatively well described, the metabolic reprogramming that leads to these stress-induced changes in polar microbial lifestyles remains to be further elucidated.

The sensitivity of organisms to contamination is likely to vary latitudinally [36]. To adapt to the extreme environments of high latitudes, polar organisms have evolved unique characteristics, including lower metabolisms, living longer, growing to larger sizes, and having higher lipid content in tissues[37]. Most research has focused on low-temperature enzyme production and low-temperature adaptation mechanisms [38, 39]. In this study, cell growth, and physiological and biochemical variations of the Antarctic bacterium *Planococcus* sp. O5 after Cu^{2+} exposure was analyzed to explore the tolerance mechanism of the bacterium and reduce heavy metal stress. The results will help to elucidate the adaptive mechanism of polar microorganisms under heavy metal exposure.

2. Materials and Methods

2.1 Bacterial strains and culture

Antarctic strain *Planococcus* sp. O5 was isolated from Antarctic Sea Ice collected by the 23rd China Antarctic scientific expedition. To address the growth effect of Cu^{2+} exposure, strain O5 was cultured at 10℃ in 2216E liquid medium (5.0 g of peptone, 1.0g of yeast extract, and 0.015 g of iron phosphate tetrahydrate, in 1000 mL of purified and sterilized seawater) under the agitation of 120 rpm. Additional $CuSO_4$ (final concentration 0.5 mmol/L) was added as copper stress.

2.2 MIC determination of heavy metals

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The minimal inhibitory concentration (MIC) for the strain O5 was tested as described by Rajpert [40]. Log phase cultures of the strains were inoculated separately in 2216E liquid medium supplemented with Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, and Hg^{2+} at concentrations ranging from 0 mM to 1500 mM, and cell growth was monitored by performing the OD_{595} measurement with a UV spectrophotometer. The lowest concentration of the metal that could hamper microbial growth was regarded as the MIC of the test strain against the metal.

2.3 Measurements of electrical conductivity and biomass

The permeability of the membrane was measured using a conductivity meter, and the biomass was measured by OD_{595}[41].

2.4 Measurements of antioxidant system

Changes in antioxidant enzyme activity were measured to understand the impact of Antarctic bacteria. For this purpose, 100 mg fresh weight (FW) bacterial strain were homogenized in 20 mL of 50 mM phosphate buffer (pH 7.8) before centrifugation at 12,000 rpm for 30 min at 4°C. The collected supernatants were employed to determine the antioxidant enzymes activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase(APX) activities, and the content of glutathione (GSH) and carotenoid. SOD activity was assayed as described by Chowdhury and Choudhuri [42]. GR was measured following the method of Pinto, Mata, and Lopezbarea [43]. APX activity was measured by the method of Y, Nakano, and K Asada[44]. GSH content was performed as described in Anderson [45]. Whereas the multi-parameter flow cytometry method was recorded by Freitas [46] and was used to assay carotenoid content.

2.5 GC-MS analysis of metabolites

2.5.1 Sample preparation

The bacteria supplemented with 0.5 mmol/L Cu^{2+} in the logarithmic and stable phases were acquired respectively by centrifugation at 12,000 rpm for 5 min at 4°C. Subsequently, 2 mL of 60% precooled methanol (-40°C) were added and placed on ice for 5 minutes to quench the cellular reaction. After centrifugation, the collected cell pellet was introduced to 0.5mL methanol (50%, -40°C), followed by rupture using the sonication method. The broken cells were centrifuged (4°C, 12000 rpm, 10 min), and 10 μl succinic-2,2,3,3-d4 acid (0.3 mg/mL) was added to the supernatant. Subsequently, the samples were dried, added to 100 μL of 20mg/ml pyridine amine hydrochloride, and oxidated for 1.5 h at 30 °C. Afterward, MSTFA (100mL) was used to derivate the samples by incubation at 37 °C for 0.5 h.

2.5.2 GC-MS analysis of metabolites

The metabolites were analyzed using GC-TOF-MS (Agilent 7890A). 1μL of the derivatized sample was injected into GC-MS, which was equipped with a DB-FFAP capillary column (60 m × 0.25 mm × 0.25μm). The elution program was set: Isothermal at 80 °C for 1 min, next to an increase of 2 °C min−1 up to 100°C, and ramped at 4 °C min−1 to 240 °C, and then hold for 15 min at 240 °C. The ion source temperature was maintained at 200°C. The mass spectrometer was set to scan a mass range of 50–800 m/z at 20 scans/s with an electron beam of 70 eV.

2.5.3 Data processing

The peak integration and peak alignment were conducted by applying the XCMS package of the R software, and components were identified and confirmed manually using the NIST library. Noise and low abundance components were eliminated from the data matrix based on a noise threshold (S/N>10). A ultimately generated a two-dimensional matrix consisting of retention time (RT) and mass-to-charge ratio (m/z) data pairs.

2.5.4 Statistical analysis

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The processed data matrix was submitted to the MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) for data pre-processing and multivariate statistical analysis. The data were normalized and then log-transformed. Principal component analysis (PCA) was performed to provide a general overview and to remove the irrelevant variable. Orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted to further investigate metabolic changes. Metabolites of interest were filtered using volcano plots with fold change (FC)≥1.2 and P-value <0.05. To explore the related metabolic pathways for differential metabolites, compounds of interest were imported into the MetaboAnalyst Pathway.

3. Results

3.1. Heavy metals resistance analysis

Typical bacterial growth was observed in the induced and normal groups (Figure.1a). Although the growth rate of Planococcus sp. O5 in the normal group was higher in the induced group during the first 72 hours. Subsequently, The growth of the copper exposure group presented an equal A_{600} value to the untreated group. The result indicated that the Cu stress significantly inhibited the growth of strain O5 and, on the contrary, the bacterium showed resistance to copper stress. The relative metal resistance of strain O5 was in order of $Pb^{2+} > Cu^{2+} > Cd^{2+} > Hg^{2+} > Zn^{2+}$(Figure.1b). The MIC reached 1.0 mmol/L, 0.8 mmol/L, 0.7 mmol/L, 0.6 mmol/L, 0.5 mmol/L, respectively. Planococcus sp. O5 exhibited a wide range of resistance to different metals. Meanwhile, the optimum concentration of copper stress was determined to be 0.5 mM.

3.2 Change in the membrane permeability

The determination of conductivity provides an indirect indication of membrane permeability. According to Figure. 1c, the conductivity of the control was stable, which was maintained between 200-230 mS/m. Correspondingly the conductivity of the bacteria-induced with Cu^{2+} increased slowly from days 1-7 and then rapidly, reaching 586 mS/m on the tenth day. These findings revealed changes in the integrity of the cell membrane after being challenged with *0.5* mM Cu.

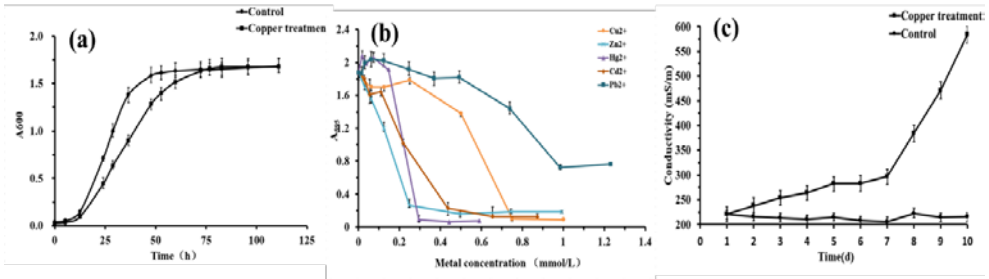

Figure 1. (a) Growth curves of the fungus *Planococcus* sp.O5 in the absence and presence of copper. (b) Heavy metals tolerances of *Planococcus* sp.O5 of Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and Hg^{2+}, separately. (c) Effect of Cu^{2+} on the conductivity of *Planococcus* sp.O5.

3.3 Response of the antioxidant system

To further understand the biochemical mechanisms of strain O5 induced by Cu^{2+}, it was also measured the content of antioxidant substances and the activities of antioxidant enzymes, as shown in Figure 2. Throughout the experiment, the enzymatic activity of SOD, GR, and APX remained almost constant in the absence of Cu^{2+} induction. After exposure to 0.5 mmol/L Cu^{2+}, SOD and GR activities rapidly increased, reaching a maximum on the second and third days. In contrast, APX activity decreased and was

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significantly lower than the control at all times. The changes in enzymatic activity were also clarified by isoenzyme analysis. Under 0.5 mM Cu^{2+} stress, carotenoid and GSH contents accumulated rapidly and remained significantly higher than the untreated group.

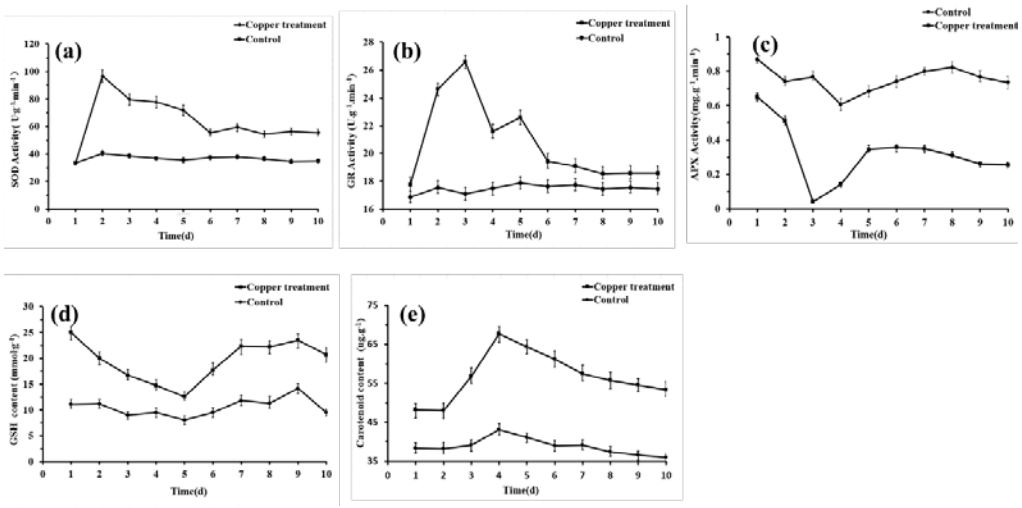

Figure 2. Effects of 0.5 mmol/L Cu^{2+} on the activities of SOD (**a**), GR (**b**), APX (**c**), GSH (**d**), and Carotenoid (**e**) of *Planococcus* sp.O5.

3.4 Metabolic response of strain O5 to Cu induction

3.4.1 Metabolic profile analysis

In the PCA score plot (Figure.3a), all 24 samples data were all within the 95% confidence ellipse, indicating that no sample contained outliers. Although there was a noticeable separation between the Cu exposure and the control groups in the PCA, the treatment and control groups were intermixed. To further investigate the metabolic variation induced by copper stress, an OPLS-DA was performed. As demonstrated in (Figure.3b,3c), the treatment and control groups demonstrated a more apparent separation. As shown in Figuers S1 and S2, the R2 values for the OPLS-DA model were satisfactory (>0.945, >0.99), thereby accounting for most of the variation between the samples. The Q2 values were significantly higher (>0.728, 0.7), so the vast majority of variation could be predicted.

3.4.2 Identification and analysis of differential metabolites

To discover the significantly modified metabolites induced by copper exposure, it was assessed for changes in metabolite abundance using the filtering function of the volcano plot. 13 different metabolites were filtered based on *FC* and the p-value. (Table *S1*, Table *S2*,). Among them, 4 metabolites were significantly reduced and 9 metabolites were significantly increased. The differential metabolites included energy, amino acids and lipids.

3.4.3 Perturbed biological pathway responded to Cu stress

Pathway analysis was carried out to explore the pathways associated with the copper response. The result was as shown in Figure. 3. The shades of color and size of each circle were based on p-values and pathway impact values. The redder and larger pathway circles indicate that the pathway was significantly perturbed. By pathway enrichment analysis, the MetaboAnalyst Pathway identified 6 and 7 major metabolic pathways in the logarithmic and stable growth phases, with pyruvate metabolism, butyrate metabolism, glycine serine and threonine metabolism showing the most significant changes. Similarly,

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the most significant changes in the metabolic pathway also had the most critical impact during the stable growth phase. P values and impact factors for the significant pathways are shown in Tables S2 and S3.

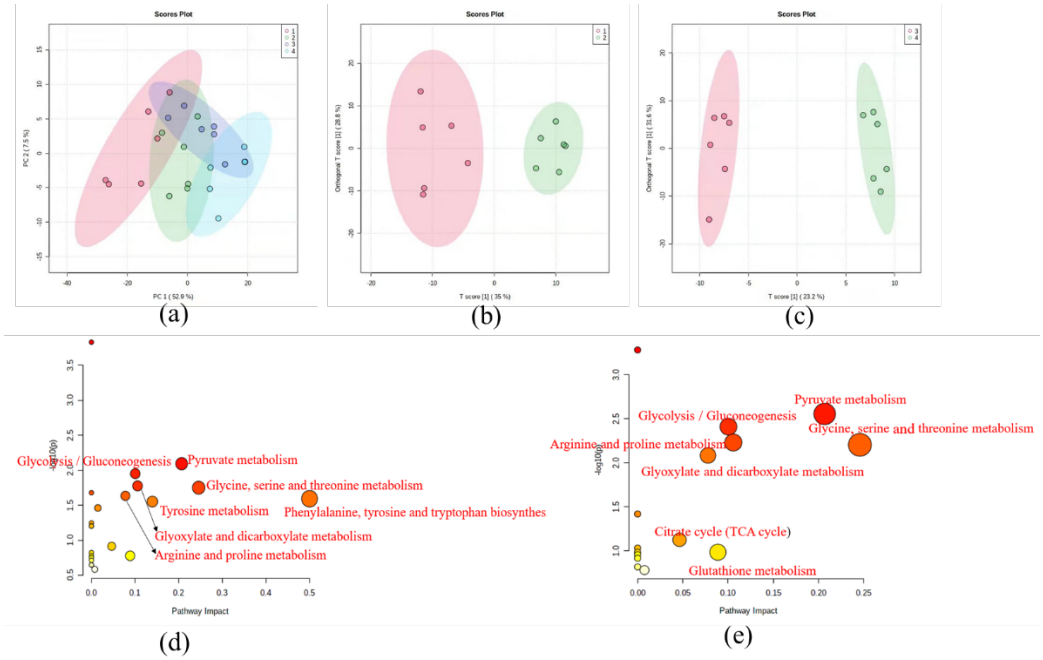

Figure 3. (a) shows the PCA models for a total of 24 samples. The numbers 1,2 represent the control group and copper exposure in the logarithmic phase, and the number 3,4 represents the control group and copper exposure in the stable growth phase. (b) (c) Separation of control (red) and copper exposure (green) samples in logarithmic phase and stable growth phase by using OPLS-DA, respectively. (d) (e) Changes of metabolites mapped to the metabolic pathways exposed to 0.5 mmol/L Cu2+ of Planococcussp.O5 in logarithmic and stable growth phases, respectively.

4. Discussion

Heavy metal ions pose serious ecological risks as they are highly toxic, non-degradable, bioaccumulate and biomagnify as a result of the food chain. Some metal ions are essential for the biological functioning of organisms. However, excess copper ions in cells are detrimental through various induced physiological, biochemical, and genotoxic effects [47]. The presence of copper ions forces the microorganisms to adopt different survival strategies. Thus, only copper-resistant microorganisms could resist, utilizing trace amounts of metals for metabolic functions while resisting or detoxifying its excess. To discover the adaptation strategies of Antarctic microorganisms to heavy metal stress, a combination of biochemical and metabolomic approaches was used to analyze the response to copper stress *Planococcus* sp. O5 bacteria isolated from Antarctic sea ice samples.

4.1 Heavy metals resistance

In this study, the Antarctic bacterium *Planococcus* sp. O5 exhibited relatively extensive tolerance to Cu^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}, especially Pb^{2+} and Cu^{2+}. The MIC of the heavy metals ranged from 0.5 to 1.0 mM (Figure.1b). Similar bacterial resistance to multiple heavy metals was reported in other Antarctic strains, such as Antarctic *Rhodotorula mucilaginosa* toxicity pattern of $Cd^{2+} > Pb^{2+} = Mn^{2+} > Cu^{2+} > Cr^{3+} > Hg^{2+}$ [48, 49]. Antarctic bacteria isolated from rock lichen located on the biogeographic polygon of Ukrainian Antarctic Station toxicity pattern of $Cr^{3+} > Ni^{3+} > Cu^{2+} > Co^{2+} > Hg^{2+}$ [48, 49]. The multiple heavy metals

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resistance is attributed to some heavy metals with similar toxic mechanisms and detoxification processes[50]. In the presence of 0.5 mmol/L Cu^{2+}, *Planococcus* sp. O5 experienced a significant decline in growth rate, indicating that 0.5 mmol/L Cu^{2+} in solution had a toxic effect on the growth (Figure. 1a). The emergence of a lag phase may be due to the consumption of glutathione (GSH) as an adaptation to oxidative stress caused by copper which requires additional energy for its synthesis. A similar adaptation mechanism has been observed in *Aspergillus niger* [51]. The stationary growth phase in the Cu-exposed cultures was attained at 70 h, which was slower than the control group. However, the biomass observed at the end of the stationary phase was similar for both groups. Thus, copper-challenged Antarctic sea ice bacteria appeared to have adapted to the presence of this toxic metal.

4.2 Effect on redox status

Reactive oxygen species (ROS), including superoxide anion (O^{2-}), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2), are among the important toxicities exerted by heavy metals on most organisms by altering the reducing environment [52 53]. In the Antarctic bacterium *Planococcus* sp. O5, the cell membrane was damaged by lipid peroxidation (Figure. 1c), and similar responses occurred in other microorganisms [54]. However, strain O5 could take advantage of various antioxidative defense systems to rebalance the redox status (Figure. 1c).In the study, the activity of SOD reached a rapid maximum after the first day of Cu exposure. The reason for this may be that SOD was the first line of defense against ROS and can dismutase $\bullet O^{2-}$ to H_2O_2 [55], and such a rapid oxidative stress response has been reported in *E. coli* strains [56]. Glutathione (GSH), an essential indicator of the redox environment, plays a major role in cellular defense response against oxidative stress [57]. In our study, copper resistance could also be explained by the activation of thiol-rich groups of glutathione, which would be associated with a metal complex mechanism of Sulphur-rich bonds. This detoxification mechanism has been demonstrated in yeast [58]. The higher activity of GR catalyzes the reduction of GSSG into GSH in the presence of coenzyme β-nicotinamide adenine dinucleotide 2′-phosphate hydrate (NADPH) [59], which has been thought to be related to resistance to oxidative stress in a microorganism [60]. The increased GR activity boosted the regeneration efficiency of GSH to keep the intracellular redox balance. Carotenoids were mostly located in the cell membrane, acting as antioxidant protectants for the cell membrane integrity [61]. The increase in carotenoids enhanced the rigidity of the plasma cell membrane, suggesting that it is important to regulate different antioxidant mechanisms to protect cells from endogenous damage by copper-induced oxygen radicals.

4.3 Metabolic reprogramming

A metabonomic analysis was performed to understand the metabolic response of bacterium O5 to copper ions. Pathway enrichment analysis showed that copper stress can produce metabolic reprogramming which leads to alterations in many metabolites, particularly energy, amino acid and lipid metabolism, forming a metabolic network in response to copper stress.

4.3.1 Energy metabolism

Carbohydrate and energy metabolism served as key attributions of adaptive responses to heavy metals [49]. In organisms, lactic acid, a by-product of anaerobic metabolism, and pyruvic, an end product of glycolysis by Embden Meyerhof pathway, were conversed to lactate acid by oxidation of nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase [62]. Under copper stress, bacteria activated the catabolism of intracellular sugars to produce more energy and amino acids as a method of defense. As a result, pyruvate acid and NADPH accumulated abundantly in the matrix and

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led to an aggregation of lactic acid in the intracellular. Furthermore, this energy pattern probably alleviates the cytotoxicity induced by excess Cu by decreasing intracellular ROS levels [63].

4.3.2 Amino acid metabolism

Amino acids are commonly used for osmoregulation, energy sources, protein synthesis, metabolite precursors, and signaling molecules in all living cells [64]. In this study, Cu stress decreased proline levels but promoted tyrosine, glycine, and lysine content. Glycine, an organic osmolyte [65], has been reported to be closely relevant to the Cu^{2+} tolerance of *Pseudomonas* [66]. In addition, glycine is a precursor of glutathione [67], which mitigates oxidative damage from reactive oxygen species. A previous study demonstrated that lysine altered NADPH flux to produce glutathione, thereby enhancing tolerance to oxidative stress [68]. Hence, in this study, it was expected that lysine-overproducing would exhibit higher tolerance to copper stress. Surprisingly, proline levels were decreased, which has not been observed in other organisms in response to environmental stress [69-72]. Proline metabolism is highly relevant to redox homeostasis, protein and nucleotide synthesis, and ATP production, especially closely associated with the progression of oxidative stress [73]. Consequently, in this study, proline levels were down-regulated, suggesting that under copper stress oxidative homeostasis is disrupted, leading to lipid peroxidation [74]. Tyr is susceptible to modification under conditions of cellular redox imbalance. Hence, the oxidation of phenylalanine, the precursor of Tyr, is a marker of oxidative stress [75]. Under conditions of ROS overaccumulation, tyrosine can be formed by either phenylalanine hydroxylation or oxidation [76]. Up-regulated tyrosine might be a strategy for oxidative stress. Increased concentrations of Tyr were observed in plants as a response to biological stress [77]. Moreover, upregulation of glycine, lysine, and tyrosine was considered to repair damaged proteins and activate renewal proteins synthesis [78]. The mentioned studies implicated the protein biosynthesis mechanisms underlying enhancement stress-induced.

4.3.3 Lipid metabolism

Stearic acid and palmitic acid have been identified as quantitative markers of cell stress, with which overproduction and accumulation of ROS were frequently associated [79]. This research showed that Cu stress increased cell membrane permeability in Antarctic bacterium strain O5 (Fig.1c), which implied that membrane integrity was disrupted by lipid peroxidation. This damage mechanism induced by copper has also been previously reported in filamentous fungus *Paecilomyces marquandii* [80]. Cell membranes consist mainly of lipid bilayers and embedded proteins, which allow selective transport of solutes across the membrane to facilitate physiological processes such as respiration and signal transduction [81]. The integrity and fluidity of cell membranes influenced by the lipid composition and unsaturation of fatty acids were critical for the survival of organisms in response to external stresses [82]. Stressors typically lead to a reconfiguration of lipid metabolism, which reduces membrane fluidity, sequestering metals outside of the cytoplasm [83]. It was found that upregulation of fatty acids (dodecanoic acid, stearic acid, and palmitic acid) was associated with copper ion-induced stress, indicating a low fluidity of the cell membrane to effectively prevent copper ions from entering the cytoplasm [84, 85]. There is a correlation between stress resistance and longevity in nematodes[86, 87]. In brief, the regulation of fatty acids significantly enhanced resistance to copper and oxidative.

Metabolic profile analyses demonstrated that the copper stress resulted in metabolic reprogramming. Among them, bacteria activated the breakdown of intracellular sugars to generate more energy in response to copper adaptation. Amino acids supported diverse functions, including maintaining appropriate cell status by an increase of osmotic substances, producing new stress proteins, and repairing damaged or misfolded proteins. While the accumulation of fatty acids contributes to reducing the fluidity of the cell membrane and preventing copper ions from entering the cytoplasm.

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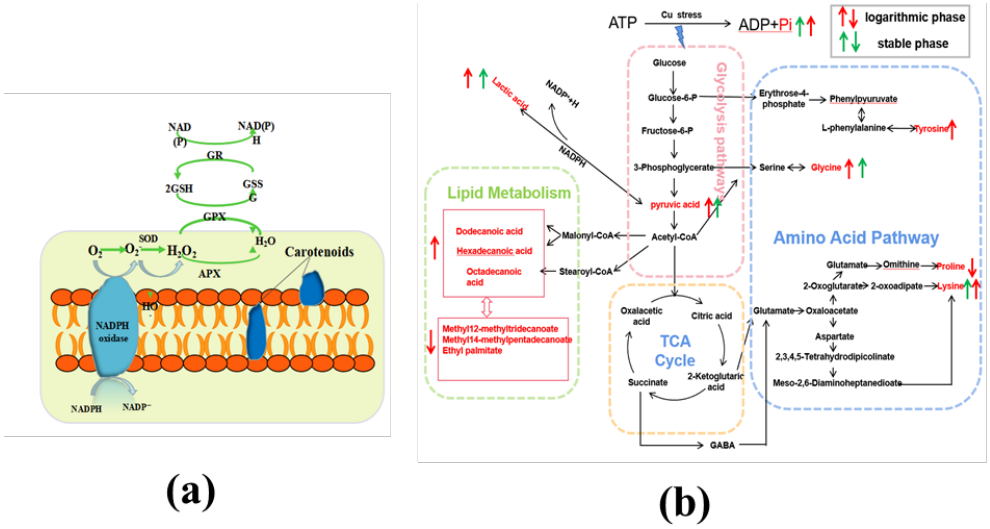

Figure 4. *Planococcus* sp. O5 presentation of systematic tolerance mechanisms. The identified significantly different metabolites were integrated into pathways(b).

5. Conclusions

In this study, biochemical and comparative metabolomic analyses were performed to understand the response of the Antarctic sea ice bacterium *Planococcus* sp. O5 to Cu^{2+}. The results indicated that strain O5 exhibited a broad range of resistance to heavy metals such as Pb, Cu, Cd, Hg, and Zn. Under copper stress, strain O5 maintained intracellular redox homeostasis through a secondary increase in antioxidant enzymes and antioxidant substances. *Planococcus* sp. Strain O5 in the presence of Cu stress acquired an inherently different metabolite profile, including amidic acid, organic acid and fatty acid. Adaption mechanism of strain O5 was included in protein synthesis and repair, organic osmolytes accumulation, energy metabolism up-regulation, and fatty acids formation. This study laid a theoretical foundation for revealing the biochemical and metabolomic response mechanisms of the polar bacterium to heavy metals.

Author Contributions: Ziyi Cheng., writing—original draft preparation and formal analysis, Cuijuan Shi., Xiujun Gao., methodology, validation, and formal analysis, Xiaofei Wang., investigation, Guangfeng Kan.; writing—review, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Shandong Provincial Natural Science Foundation, China [ZR2021MC180].

Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by Shandong Provincial Natural Science Foundation, China.

Conflicts of Interest: The authors declare no conflict of interest.

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