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Interactive effects of warming and copper toxicity on a tropical freshwater green microalga *Chloromonas augustae* (Chlorophyceae)

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Abstract

Microalgae, the primary producers in aquatic ecosystems, are highly susceptible to heavy metal contamination. In this study, the interactive effects of warming and copper (Cu) toxicity on the physiology (cell density, photosynthetic efficiency, reactive oxygen species (ROS) production, and metal uptake in the biomass) and biochemistry (metabolite) of a freshwater green microalga, *Chloromonas augustae* (UMACC246), were elucidated. The microalgae were exposed to culture media supplemented with copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) at different concentrations (50, 150, 250 μM) at two temperatures, 25 °C (control) and 30 °C (sub-optimal), for 24 h. The results indicated that *C. augustae* exhibited a concentration- and temperature-dependent decrease in the cell density. Copper greatly affected the photosynthetic efficiency of *C. augustae* by reducing the maximum rate of relative electron transport ($rETR_m$), light harvesting efficiency (α), and saturation irradiance (E_k). Warming increased ROS production remarkably in the microalga. Untargeted metabolomics indicated that temperature contributed to the most significant variations in the cultures ($p < 0.05$) in comparison with Cu toxicity or both factors combined. Compounds such as amino acids and amines were significantly dysregulated in response to warming and Cu toxicity. Pathway analyses showed that the glutathione metabolism, sulfur metabolism, and mechanisms in the amino acid metabolism were regulated, suggesting that the microalga underwent primary metabolism restructuring for survival and adaptation. Overall, the data showed that warming enhanced Cu toxicity in the cultures. This implied that increasing water temperature and metal toxicity due to global warming and anthropogenic activities will probably exacerbate existing threats to the primary producers.

Keywords *Chloromonas* · Chlorophyceae · Copper toxicity · Metabolomics · Microalgae · Photosynthesis · Temperature stress

Introduction

Microalgae play a crucial role in photosynthetic carbon fixation and in driving the major primary productivity of food webs in the aquatic ecosystems (Häder and Gao 2015). The current shifts of global climate due to anthropogenic activities have been reported to pose numerous impacts on microalgae. For example, fluctuations in temperature, ultraviolet (UV) radiation, carbon dioxide (CO_2) levels, and the presence of excess pollutants in the environment can lead to changes in growth rate, photosynthetic efficacy, respiration rate, metabolism, oxidative damage, and trophic transfer in microalgae (Sampaio and Rosa 2019). Many reports have drawn inferences from a single environmental variable in understanding the response of microalgae to abiotic stresses. It has become increasingly evident that the natural environment is multivariate and thus the need to understand the response of

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