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RESEARCH

Metabolomic profiling of a red alga, *Gracilaria changii*, under current ambient and elevated pCO_2 levels using an untargeted gas chromatography-mass spectrometry (GC–MS) approach

Pei-Tian Goh[1,2] · Sze-Wan Poong[1] · Xinqing Zheng[3] · Tao Liu[4] · Zizhong Qi[5] · John Beardall[4,6,7] · Phaik-Eem Lim[1]

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Abstract
Metabolomics offers valuable insights into the final stages of biological processes within organisms and holds promise for environmental monitoring. The escalating levels of anthropogenic CO_2 due to industrialization are projected to raise atmospheric pCO_2 to levels exceeding 1000 ppm by 2100. The ocean absorbs approximately 30% of this increase in CO_2, altering seawater chemistry and decreasing pH levels. In this study, untargeted gas chromatography-mass spectrometry (GC–MS) complemented by physio-biochemical analyses, was utilized to explore the impact of elevated pCO_2 on the growth, photosynthesis, agar yield and quality, and metabolite composition of the red alga *Gracilaria changii.* Although elevated pCO_2 did not increase the growth rate of *G. changii*, an increase in the photosynthetic electron transport rate suggests that photosynthetic carbon assimilation was enhanced. The extra photosynthate was used for other cellular processes including proton export to regulate cellular pH homeostasis given the excess H^+ in the environment, rather than being invested in new tissue growth. Thymine emerged as a key metabolite influenced by elevated pCO_2 in *G. changii*. Pathway analysis unveiled significant impacts on amino acid synthesis pathways in *G. changii* at high pCO_2. The concentration of compounds such as dopamine and glutamic acid, which are known to be triggered during stress response and provide antipathogenic bioactivity, increased in thalli cultured at higher pCO_2. Heatmap analysis indicates d-3 as the turning point for *G. changii* cultivated at higher pCO_2, where the macroalgae begin to regulate their metabolites to alleviate abiotic stresses from higher pCO_2 and to maintain essential metabolic functions.

Keywords Elevated pCO_2 · Metabolites · Algae · pH · Physiology · Rhodophyta

Introduction

Since the onset of industrialization, the atmospheric CO_2 concentration has increased from ~280 to ~420 ppm due to ongoing increases in human activities such as fossil fuel combustion and deforestation (NOAA 2023). About 30% of this CO_2 has been absorbed by the ocean, leading to acidification (Paine et al. 2023). While ocean acidification is primarily driven by external atmospheric CO_2, coastal ecosystems can also be acidified through local atmospheric

✉ Sze-Wan Poong
szewan@um.edu.my

✉ Phaik-Eem Lim
phaikeem@um.edu.my

[1] Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia

[2] Institute for Advanced Studies, University of Malaya, Kuala Lumpur, Malaysia

[3] Ministry of Natural Resources, Third Institute of Oceanography, Xiamen, China

[4] State Key Laboratory of Marine Environmental Science and College of Ocean and Earth Sciences, Xiamen University, Xiamen 361102, China

[5] College of Marine Life Sciences, Ocean University of China, Qingdao 2660003, China

[6] School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

[7] Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia