



The effect of grazing on the microbiome of two commercially important agarophytes, *Gracilaria firma* and *G. salicornia* (Gracilariaceae, Rhodophyta)

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Abstract

Grazing, which leads to losses in biomass and drastic declines in total crop production, is one of the main concerns in seaweed aquaculture. This is also thought to affect the composition of the associated bacterial communities which are believed to play a crucial role in determining the host's health and development. Apart from morphological impairment, studying changes in the prokaryotic microbiome composition and predicted functional responses to grazing will allow us to understand the underlying effects of grazing on the seaweed host. This study is the first report of the effect of grazing on the prokaryotic microbiome of two economically important agarophytes, *Gracilaria firma* and *Gracilaria salicornia*, by high-throughput sequencing targeting the V3-V4 variable region of the 16S rRNA gene. The results indicated that for *G. firma*, the microbiome composition of tissues grazed by marine herbivores had significantly more agarolytic bacteria *Marinagarivorans* sp. and *Algisphaera* sp. than in ungrazed tissues. The predictive functional metagenomics for this species revealed that grazing escalated the pathway activities related to nucleotide degradation, aromatic compound degradation and aerobic sugar metabolism, while pathways associated with cell wall synthesis, aerobic respiration, vitamin biosynthesis and amino acid biosynthesis were reduced. However, for *G. salicornia*, the bacterial communities were not significantly affected by grazing. Nevertheless, pathways relating to anaerobic respiration and amino acid, coenzyme and vitamin B-6 biosynthesis in this species were predicted to be more active in grazed tissues, whereas the microbiome of ungrazed tissues had higher activities in bacteriochlorophyll *a*, fatty acid, secondary metabolite and heme biosynthesis.

Keywords 16S metagenomics · Gracilarioid red algae · Grazers · Microbiota · Predicted functional pathways · Seaweed aquaculture

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Introduction

Species of *Gracilaria* are widely cultivated in Asian countries, including China, Indonesia, the Philippines and South Korea, as well as across the continent of South America in Chile, Peru and Argentina, as the principal resource for the agar industry (Kim et al. 2017). In Malaysia, small-scale farms of *Gracilaria manilaensis* Yamamoto & Trono (Yamamoto and Trono 1994) and *Gracilaria firma* C.F.Chang & B.-M.Xia (Chang and Xia 1976) have been established at the West Coast of Peninsular Malaysia since the mid-1990s (Phang 1998; Prud'homme van Reine and Trono 2001; Phang et al. 2019). *Gracilaria*, due to its high yields of good-quality agar with high gel strength, contributed nearly 80% of total agar production in the global market in the year 2009 (Santelices 2014). Turnover from *Gracilaria* aquaculture has also been maximized by utilizing the residue from agar extraction which