

Abundance of heterotrophic marine bacteria, *Vibrio*, and marine fungi in green seaweed *Caulerpa racemosa* in Sibutu, Tawi-Tawi, Philippines

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Abstract

Seaweeds have been used in the human diet for centuries, particularly in Asia. Green seaweed from the genus of *Caulerpa* is consumed throughout the Pacific and Southeast Asia, with high economic value. In the Philippines, *Caulerpa* spp. are one of the seaweed delicacies among locals eaten as salads. In Tawi-Tawi, southern Philippines, *Caulerpa racemosa* var. *occidentalis* is one of the edible seaweeds which is sold in the market center in Bongao, Tawi-Tawi and Tongehat, Sibutu, Tawi-Tawi is one of the sources of this seaweed. However, the microbial load of this green seaweed has not yet been explored and has remained unstudied. Hence, in this study, we determined the microbial load (colony-forming unit, CFU) of heterotrophic marine bacteria, *Vibrio*, and marine fungi from *C. racemosa* var. *occidentalis* in coastal waters of Tongehat, Sibutu, Tawi-Tawi, Philippines, following the serial dilution procedure. Results revealed that the heterotrophic marine bacteria recorded the highest abundance of 5.9×10^{10} CFU g⁻¹. *Vibrio* abundance was 1.2×10^2 CFU g⁻¹, and marine fungi load was 4.1×10^3 CFU g⁻¹. The low presence of *Vibrio* in this study indicates that the *C. racemosa* var. *occidentalis* contains low potential pathogens and may be safe for human consumption.

Introduction

Seaweeds are important coastal products that are commonly used as a food source (Delan et al., 2015), and they have been utilized in human diet for decades in Asia, and now also spreading rapidly in western countries (Løvdaal et al., 2021). *Caulerpa* genus is a

green seaweed, commonly called as “sea grapes,” popularly consumed in the Pacific and Southeast Asian countries (Paul et al., 2014). *Caulerpa racemosa*, one of the species of sea grapes with high economic value, contains many nutritional components and can be used as a functional food item for humans (Magdugo et al., 2020). Compared to

other seaweeds, it contains a greater amount of protein, crude lipids, and fiber (Bhuiyan et al., 2016). Hence, some local people of the northern Philippines utilize *C. racemosa* as a traditional medicine to relieve cough or asthma (Dumilag and Javier, 2022).

Seaweed diversity in the Philippines is high, with a total of 1 291 seaweed species (Ang et al., 2013). Among these, the most important genera are *Kappaphycus*, *Eucheuma*, *Gracilaria*, *Caulerpa* (Trono and Largo, 2019), and *Sargassum* (Yangson et al., 2022); all these seaweeds are commercially cultivated throughout the country (Trono and Largo, 2019; Tahiluddin et al., 2021a; Tahiluddin et al., 2021b; Tahiluddin et al., 2021c; Estrada et al., 2021; Sarri et al., 2022; Dumilag et al., 2022; Tahiluddin et al., 2022a; Tahiluddin et al., 2022b), except for *Sargassum*, which is still in its infancy (Yangson et al., 2022). In Tawi-Tawi, Philippines, *C. racemosa* is one of the edible seaweeds that is abundant in the local market, normally prepared by the consumers as a salad mixed with vinegar, tomatoes, and onions, and is considered as one of the essential traditional diets of the local people (Dumilag, 2019). This green seaweed is usually collected from its natural habitat. One of the important sources of *Caulerpa* species in the local market of Tawi-Tawi, is the Sibutu Island, particularly in the coastal water of Tongehat.

Microbial communities thriving on the surface of the seaweeds are very complex, dynamic, and composed of a consortium of microorganisms, including fungi, bacteria, diatoms, spores, protozoa, and marine invertebrate larvae (Singh and Reddy, 2014). The abundance of microorganisms can be used as an indicator of the food quality and safety of edible seaweeds. For instance, the seaweed's high bacterial load may indicate the health and age of the plant and may negatively affect the sensory quality and shelf-life of the product, but it does not imply

that it is unsafe to eat. On the other hand, low bacterial counts do not necessarily imply that it is safe since some pathogens, such as *Vibrio*, even a small numbers, are sufficient to cause serious human health problems (Løvdal et al., 2021). Moreover, *Vibrio* and marine fungi are normally associated with seaweeds in the natural marine environment (Tahiluddin and Terzi, 2021a; Tahiluddin and Terzi, 2021b; Tahiluddin et al., 2021c; Bermil et al., 2022). The presence of opportunistic *Vibrio* and marine fungi can cause ice-ice disease in red seaweeds (Zainuddin et al., 2019; Bermil et al., 2022). In green seaweeds, like *Caulerpa* species, *Vibrio* and marine fungi are naturally occurring microorganisms (Rizzo et al., 2013; Rizzo et al., 2016; Kataržytė et al., 2017). In this study, we investigated the abundance of microorganisms, such as heterotrophic marine bacteria, *Vibrio*, and marine fungi in green seaweed *C. racemosa* var. *occidentalis* in Sibutu, Tawi-Tawi, Philippines. This would serve as part of a baseline information when it comes to microbial loads of *C. racemosa* var. *occidentalis* since this green seaweed is consumed by the local people in the area and nearby places.

Materials and Methods

Study Site

Sea grape *C. racemosa* var. *occidentalis* samples were collected from coastal waters of Tongehat, Sibutu, Tawi-Tawi, Philippines (Figure 1) and analyzed at Microbiology Laboratory, College of Fisheries (COF), Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi from March 31 to April 23, 2019.

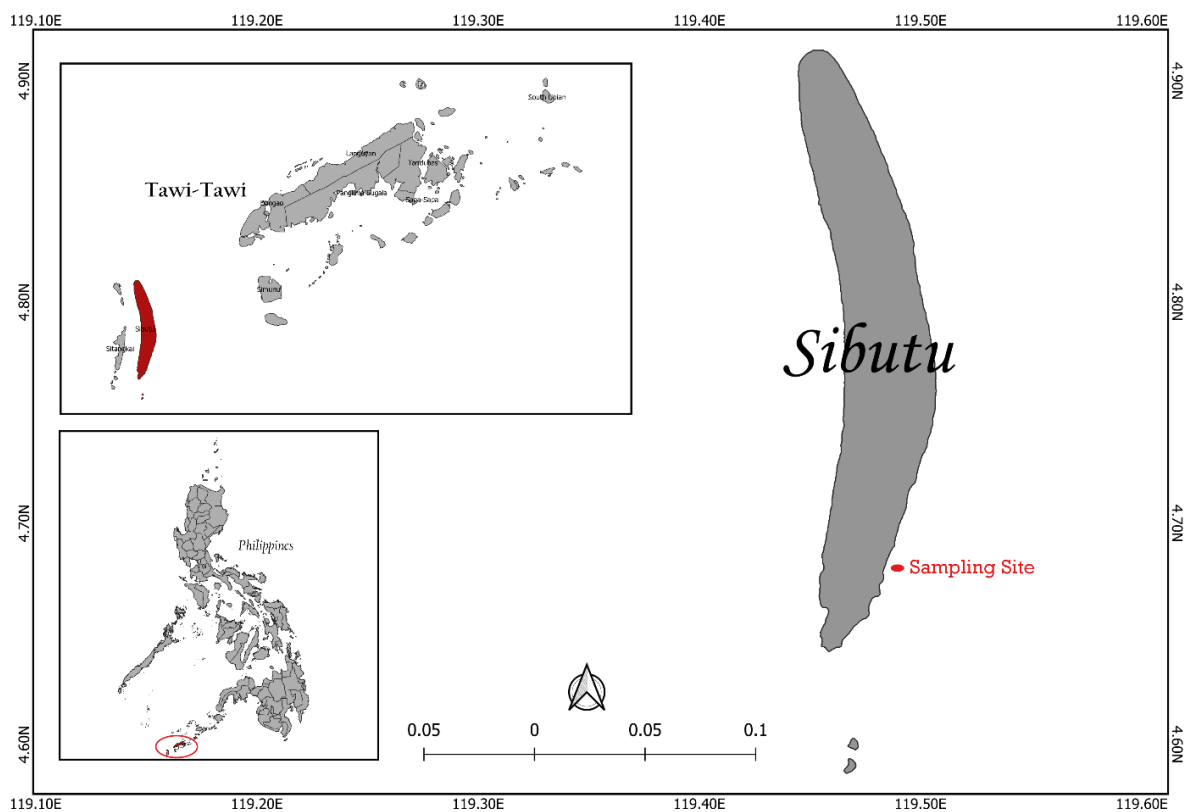


Figure 1. Map of the sampling site

Collection of Samples

About 10 grams of a whole part of healthy *C. racemosa* var. *occidentalis* were collected and placed in a sealed sterile glass jar containing 200 mL of sterile seawater. These samples were placed in disinfected styrofoam with sterile seawater ice in order to chill the samples and were immediately transferred to a freezer in Tongehat, Sibutu.

Transporting of Samples

The frozen samples in a sealed sterile glass jar were placed in clean and disinfected styrofoam together with seawater ice. These were transported from the sampling site to Microbiology Laboratory, COF, MSU-TCTO, Sanga-Sanga, Bongao, Tawi-Tawi for 3.5 hours. The samples were immediately frozen again for 24 hours before analysis.

Microbial Analysis

The microbial analysis of the sea grape samples was carried out under sterile

conditions following the method of Tahiluddin et al. (2021c). One (1) gram of sample was chopped using a sterile razor blade and forceps and placed in a test tube with 9 mL diluent (0.1% peptone with 0.85% NaCl). This was mixed for 2-3 minutes using a vortex mixer. One (1) mL was diluted to the next test tube with 9 mL diluent and serially diluted up to 10^{11} dilutions for the heterotrophic marine bacteria, 10^3 dilutions for the *Vibrio*, and 10^4 dilutions for the marine fungi. An aliquot of 0.1 mL of each dilution was spread-plated into marine agar (Scharlau), thiosulfate-citrate-bile salts-sucrose agar (TCBS; TM MEDIA), and malt extract agar (TM MEDIA) and incubated at room temperature for 2 days (heterotrophic marine bacteria and *Vibrio*) and 14 days (marine fungi). Grown colonies were counted manually. The colony-forming units (CFU g^{-1}) were calculated using the formula (Maturin and Peeler, 2001).

$$N = \frac{\sum C}{[(1 \times n_1) + (1 \times n_2) + (1 \times n_3) \times (d)]}$$

Where:

N= Number of colonies per g of sample

$\sum C$ = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d= Dilution from which the first counts were obtained.

Results and Discussion

The abundance of marine microorganisms from *C. racemosa* var. *occidentalis* is shown in Table 1. The heterotrophic marine bacterial load was found to be higher, reported at 5.9×10^{10} CFU g⁻¹. *Vibrio* count was low at 1.2×10^2 CFU g⁻¹, while the number of marine fungi was 4.1×10^3 CFU g⁻¹.

Table 1. Abundance of marine microorganisms (CFU g⁻¹) from *Caulerpa racemosa* var. *occidentalis*

Microorganism	Microorganism count (CFU g ⁻¹)
Heterotrophic marine bacteria	5.9×10^{10}
<i>Vibrio</i>	1.2×10^2
Marine fungi	4.1×10^3

The total heterotrophic marine bacterial count (5.9×10^{10} CFU g⁻¹) in *C. racemosa* var. *occidentalis* determined in this study was relatively greater than reported in *C. lentillifera* with an abundance of 10^6 CFU g⁻¹ sampled from the water of Bohol, central Philippines (Delan et al., 2015) and 10^7 CFU g⁻¹ obtained from the tank culture in Japan (Kudaka et al., 2008). In the Mediterranean Sea, the bacterial load of *C. cylindracea* was found to be 10^7 CFU mL⁻¹. When compared with other green seaweed, our result was parallel to *Ulva lactuca* (10^{10} to 10^{12} CFU g⁻¹) collected from the adjacent coastal water of our sampling site (Tahiluddin et al., 2021a).

Vibrio abundance in *C. racemosa* var. *occidentalis* in this study was relatively low at 1.2×10^2 CFU g⁻¹, 10 fold lower than reported by Rizzo et al. (2013) sampled from Brindisi, Italy. In the Mediterranean Sea, the abundance of *Vibrio* from *C. cylindracea* was found to be 10^3 to 10^4 CFU mL⁻¹ (Rizzo et al., 2016). In red seaweed *Kappaphycus striatus*, the number of *Vibrio* was 10^4 CFU g⁻¹ sampled in a similar site in this study (Tahiluddin et al., 2021c).

The marine fungal load in chlorophytes (green seaweed) ranged from 0.02×10^4 to 1.2×10^4 CFU g⁻¹ (Kataržytė et al., 2017). In this study, marine fungi associated with *C. racemosa* var. *occidentalis* had an count of 4.1×10^3 CFU g⁻¹. In red seaweeds *K. alvarezii* and *K. striatus*, the loads of marine fungi were 10^2 to 10^3 and 10^3 to 10^4 , respectively, investigated in the same sampling site of this study (Bermil et al., 2022). In mangrove-associated sponges, different marine fungi had a count of 10^2 CFU g⁻¹ (Calabon et al., 2019).

Microorganisms are naturally associated with seaweeds, which play a huge role in both the growth and morphogenesis of macroalgae in direct and/or indirect ways (Singh and Reddy, 2014). Hence, their abundance in *C. racemosa* determined in this study may reflect its natural microbiome and help in these roles. However, the final microbial load may be higher than in the natural habitat since edible seaweeds may also get contaminated or re-contaminated while handling and processing until reaching the consumers' tables (Løvdal et al., 2021).

Conclusion

The collected *C. racemosa* var. *occidentalis* had a high load of heterotrophic marine bacteria, although it was in a good state. A low count of marine fungi was recorded, and the total of potentially pathogenic microorganisms such as *Vibrio* was also low. This study suggests that *C. racemosa* var. *occidentalis* harbors naturally occurring microorganisms, which may play important roles in the green seaweed. However, sea grapes that are marketed and displayed for sale, such as in the local public markets, need to be investigated as the final microbial load may be associated with poor handling and may lead to contamination of edible sea grapes.

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Ethical approval

The author declares that this study complies with research and publication ethics.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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