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RESEARCH ARTICLE

Abundance of Culturable Heterotrophic Marine Bacteria in *Ulva lactuca* Associated with Farmed Seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum*

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ABSTRACT

Ulva lactuca is one of the macro-epiphytes of farmed seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum* in Tawi-Tawi, southern Philippines, especially during the summer season, which affects the growth and health of farmed seaweeds. In this study, the abundance of culturable heterotrophic marine bacteria from *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum* was investigated in the seaweed farms of Tongsibalo, Sibutu, Tawi-Tawi, southern Philippines, using serial dilution procedure. Results revealed that the average bacterial counts obtained from *U. lactuca* associated with *Kappaphycus alvarezii*, *K. striatus*, and *E. denticulatum* were 2.48 x 10^{10} CFU g^{-1}, 1.14 x 10^{12} CFU g^{-1}, and 1.32 x 10^{11} CFU g^{-1}, respectively. In addition, agar-digesting bacteria were observed from *U. lactuca* samples associated with *K. alvarezii* and *K. striatus* manifested by the depression and liquefaction of the marine agar after 2-3 days which were suspected as pathogenic bacteria causing ice-ice disease. Therefore, *U. lactuca* may serve as a vector for these potential pathogens to farmed seaweeds.

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Introduction

In the marine environment, most colonizers on the surface of macroalgae are bacteria (Armstrong et al., 2000). Macroalgae can supply food (organic matter) and oxygen that are beneficial to associated bacteria. Seaweed-associated marine bacteria play an important role in the morphogenesis and growth of seaweeds, both direct and indirect ways (Singh & Reddy, 2014). The most abundant bacterial communities on the surface of seaweeds belong to Proteobacteria and Firmicutes (Albakosh et al., 2016). Marine bacterial communities associated with seaweeds provide a vast array of benefits such as producing plant growth-promoting substances, bioactive compounds, quorum sensing signaling molecules, and other effective molecules which are responsible for the seaweeds' normal development, morphology, and growth (Singh & Reddy, 2014).

However, some pathogenic bacteria in seaweeds can lead to the development of the disease (Largo et al., 1995a; Tahiluddin & Terzi, 2021a; Tahiluddin et al., 2021). *Cythophaga-Flavobacrterium* complex and *Vibrio-Aeromonas* complex are some of the identified aetiological agents of ice-ice disease in *Kappaphycus*, especially when the seaweeds are stressed due to low or high temperature, salinity, and light intensity which can lead to the whitening of the thalli (Largo et al., 1995b; Tahiluddin & Terzi, 2021a; Tahiluddin & Terzi, 2021b). *Vibrio* species entered the tissue of seaweeds by pumping the carraginase enzyme causing the thalli to become pale and white and resulted in making the tissue soft and easily

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broken, which is called medullary seam (Yulianto & Mira, 2009). Some bacteria have been associated with decaying microalgal blooms linking to degradation processes in microalgae (Teeling et al., 2012). Largo (2002) reviewed different seaweed diseases and indicated that seaweed diseases are generally caused by microorganisms coupled with unfavorable environmental conditions.

Green alga *Ulva lactuca* is more abundant in polluted sites due to high nutrient contents (López-Gappa et al., 1990). In Tawi-Tawi, southern Philippines, *U. lactuca* is one of the macro-epiphytes, which hinders seaweed production during "green tides" outbreak, especially in the summer season as a consequence of inorganic nutrient disposal after inorganic nutrient enrichment of *Kappaphycus* (Tahiluddin, 2018). Since heterotrophic marine bacteria are usually attached to seaweeds in general, epiphytic green alga *U. lactuca* attached to farmed seaweeds *Kappaphycus* and *Eucheuma* species may contain pathogenic bacteria. It might be possible that these bacteria may contaminate and infect the farmed seaweeds that could induce ice-ice disease, a disease that threatens seaweed industry in the Philippines. Thus, this study investigated the abundance of culturable heterotrophic marine bacteria in green alga *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum*.

Materials and Methods

Study Site

The study was carried out at the seaweed farms of Tongsibalo, Sibutu, Tawi-Tawi, southern Philippines (Figure 1). Sample analysis was done at the Microbiology Laboratory, College of Fisheries (COF), Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, southern Philippines (5.1042° N, 119.8121° E) from March 22, 2019 to April 1, 2019.

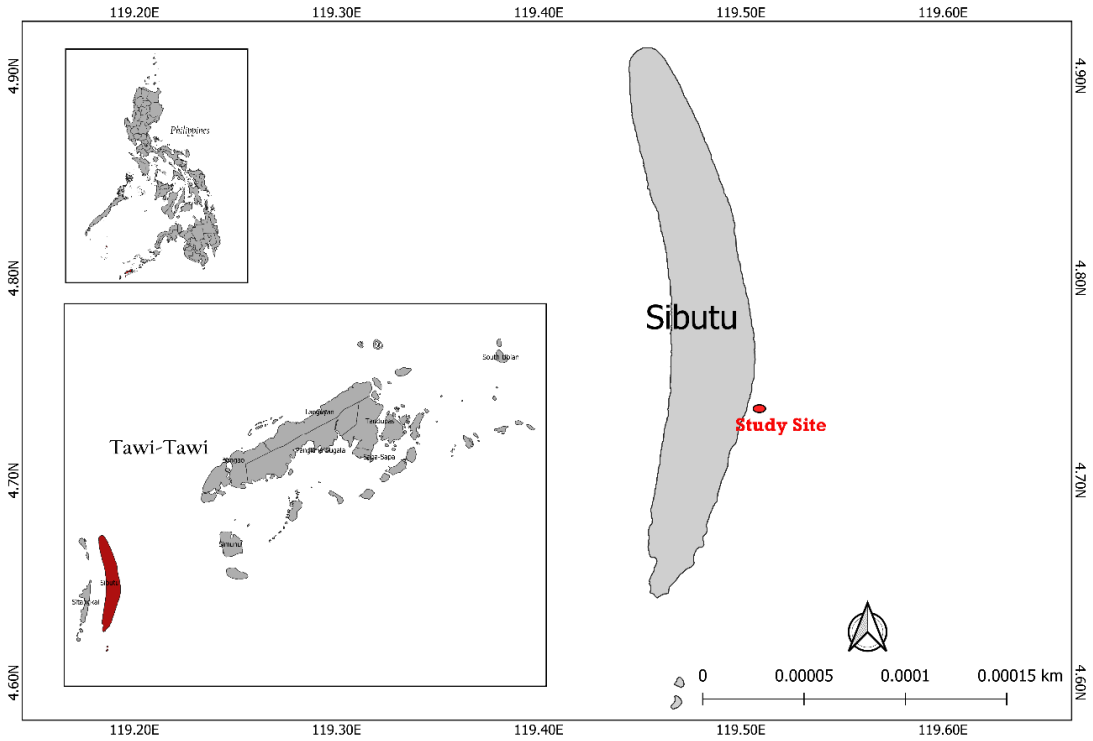

Figure 1. Map of the study area showing the study site (Source: QGIS version 3.18)

Sample Collection

Ulva lactuca samples were collected individually in three different farmed seaweeds such as *E. denticulatum*, *K. alvarezii*, and *K. striatus*. Nearly 5 g of samples were placed in different sterile containers with filtered 20 ml seawater. All samples were collected in triplicate. These were chilled inside the styrofoam using seawater ice and were immediately stored in the freezer in the study site.

Transporting of Samples

Frozen samples inside the styrofoam were transported via motor launch to Chinese Pier, Bongao, then from Chinese Pier to Microbiology Laboratory, COF, MSU-TCTO, Sanga-Sanga, Bongo, Tawi-Tawi, and was immediately stored in the freezer until analysis.

Microbiological Analysis

The microbiological analysis of seaweed samples was performed under sterile conditions. One (1) g of sample was mashed into small pieces using a sterile razor blade and placed in 9 ml diluents. Saline peptone water (0.1% peptone with 0.85% sodium chloride) was used as diluents. The microbiological cultivation was performed by taking 1 ml from the appropriate dilutions (up to eleventh dilution), consisting of 1 g of sample homogenized in 9 ml of saline peptone water, using the spread plate technique in triplicate. From each dilution, an aliquot of 0.1 ml was spread-plated on the marine agar and incubated at room temperature for 2 days (Chellaram et al., 2013). Colonies were counted manually. Colony-forming units (CFU g^{-1}) of culturable heterotrophic marine bacteria were calculated using the formula below (FDA, 2001):

$$N = \frac{\Sigma c}{[(1 \times n_1) + (0.1 \times n_2) + (0.01 \times n_3) \times (d)]} \quad (1)$$

Where, N= number of colonies for ml or g of sample,

Σc=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.

Data Analysis

The data expressed in colony-forming units (CFU g^{-1}) were analyze using one-way analysis of variance using SPSS version 20.

Results and Discussion

Bacterial counts (CFU g^{-1}) from *U. lactuca* associated with farmed seaweeds are shown in Table 1. Bacterial counts from *U. lactuca* associated with farmed seaweed *K. striatus* were found to be higher in colony-forming units (1.14×10^{12} CFU g^{-1}) than that associated with *K. alvarezii* (2.48×10^{10} CFU g^{-1}) and *E. denticulatum* (1.32×10^{11} CFU g^{-1}). However, statistically, there were no significant differences among the samples from three farmed seaweeds.

Table 1. Bacterial counts (colony forming units) from *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum*

Farm	Bacterial Count (CFU g^{-1})
K. alvarezii	2.48×10^{10}
K. striatus	1.14×10^{12}
E. denticulatum	1.32×10^{11}

The average number of bacterial counts in *U. lactuca* obtained in healthy branches of *Kappaphycus* spp. and *E. denticulatum* ranged from 10^{10} to 10^{12} CFU g^{-1}. Our results were three-fold lower than the previous study of Tahiluddin et al., (2021), where the thalli of nutrient-enriched *K. striatus* had heterotrophic marine bacterial abundance of 10^{16} CFU g^{-1}. The average number of colony-forming units of heterotrophic marine bacteria from *K. alvarezii* in healthy and ice-ice disease thalli were determined as 10^4 to 10^6 CFU g^{-1} (Largo et al., 1995a). Bacterial counts in healthy and ice-ice disease branches of *E. denticulatum* were 10^3 to 10^5 and 10^6 to 10^7, respectively. *U. lactuca* attached from *K. alvarezii* recorded the lowest bacterial counts, and this may be due to the antibacterial properties in *K. alvarezii* (Largo et al., 1995a). *K. striatus* had the highest bacterial counts, perhaps due to the slow water movement (0.06 m s^{-1}) in the farm compared with *K. alvarezii* (0.11 m s^{-1}) and *E. denticulatum* (0.14 m s^{-1}) farms. It is possible that the motile bacteria were able to colonize the *U. lactuca* and attached the farmed seaweeds when the water movement was slow (Largo et al., 1999).

Agar-digesting bacteria are reported to be associated with ice-ice disease in *Kappaphycus* (Largo et al. 1999; Tahiluddin et al., 2021) and in diseased *Gracilariopsis heteroclada* (Martinez & Padilla, 2016). In this study, agar-digesting bacteria were observed in the marine agar and created depression and liquefied after 2-3 days. These were present in both *U. lactuca* associated with *K. alvarezii* and *K. striatus* that could be one of the promoters to the development of ice-ice disease in the farmed seaweeds. Agar-digesting bacteria were not present in *U. lactuca* associated with *E. denticulatum* compared to *Kappaphycus* spp. This is maybe due to the chemical defense mechanism of *E. denticulatum,* which excretes volatile hydrocarbons that serve as a defense mechanism against epiphyte infestation and ice-ice disease (Pang et al. 2015).

Other studies identified these agar-digesting bacteria as the *Vibrio* group isolated from *K. alvarezii* (Largo et al. 1995a) and *Gilvimarinus chinensis* isolated from *K. striatus* (Tahiluddin et al., 2021). During the attack of pathogenic bacteria in farm seaweeds, after 48-72 hours, agar-digesting *Vibrio* species entered the tissue of *K. alvarezii* and *K. striatus* until the medullary seam by pumping the carraginase enzyme causing the thalli to become pale and white, and the soft tissue was easily disintegrated leading to ice-ice disease (Yulianto & Mira, 2009). This may indicate that agar-digesting bacteria from *U. lactuca* may contaminate the farmed *Kappaphycus* spp. that can cause ice-ice disease once they dominate, especially when the farmed *Kappaphycus* spp. are stress due to unfavorable environmental conditions.

Conclusion

The abundance of culturable heterotrophic marine bacteria in *Ulva lactuca* associated with farmed seaweeds *K. alvarezii, K. striatus*, and *E. denticulatum* were 2.48×10^{10} CFU g^{-1}, 1.14×10^{12} CFU g^{-1}, and 1.32×10^{11} CFU g^{-1}, respectively. The presence of agar-digesting bacteria in *U. lactuca* from healthy *K. alvarezii* and *K. striatus* farms may be an indicator of pathogenic bacteria that cause ice-ice disease. Therefore, *U. lactuca* may serve as a vector for these potential pathogens to farmed *Kappaphycus* spp. that can contribute to ice-ice disease development but still need to be validated and further studied.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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