Bacteria Attached on Cultured Seaweed *Gracilaria changii* at Mengabang Telipot, Terengganu

*Najiah Musa and Lee Seong Wei*

Department of Fisheries Science and Aquaculture, Faculty of Agrotechnology and Food Sciences, Universiti Malaysia Terengganu (UMT) 21030 Mengabang Telipot, Terengganu, Malaysia

**Abstract:** Massive disease outbreak in cultured seaweed *Gracilaria changii* was reported in Mengabang Telipot marine hatchery of Universiti Malaysia Terengganu, Malaysia in December 2007. These seaweeds were previously collected from Morib beach, Selangor and cultured at marine hatchery at UMT. After two weeks at the hatchery, 50% of seaweeds were found to have pale stalks which withered its production and led to death. Quantitative count for total bacteria count, *Vibrio* count and *E.coli* count were carried out on both healthy and diseased seaweeds. In addition, qualitative test for the bacteria present on the seaweed samples was carried out. Six types of different bacterial species were identified using a commercial identification kit (BBL Crystal, USA). They were *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Pasturella haemolytica*, *Vibrio alginolyticus* and *Vibrio cholerae*. In terms of quantitative count (CFU g⁻¹), there was no significant differences between the healthy and diseased seaweed on total bacteria count, *Vibrio* count and *E. coli* count. Antibiogram of the selected bacterial isolates were also determined in the present study and revealed that all isolates were sensitive to oxolinic acid, nalidixic acid, chloramphenicol and flumequine but all were resistant to lincomycin.

**Key words:** seaweed • *Gracilaria changii* • *Vibrio* • *E. coli* • antibiogram

**INTRODUCTION**

People from northern Peninsular Malaysia incorporated agrophytic seaweed *Gracilaria changii*, locally known as “Kerabu Sarah” as side-dish during meals. It can be found abundantly in mangrove areas in Malaysia and Thailand [1]. At present, many studies were focusing on bioactive compound isolation from seaweed for medicinal purposes due to its high mineral content. However, very few studies were conducted on the presence bacteria in seaweed. [2] reported that seaweed containing *V. parahaemolyticus* could cause gastroenteritis to seaweed consumers. In the present study, healthy seaweed was previously collected from Morib beach, Selangor and brought back and cultured at Mengabang Telipot UMT’s marine hatchery. After two weeks at the hatchery, 50% of seaweeds were found to suffer from pale stalks which withered its production and resulted in death. Therefore, qualitative and quantitative studies were carried out as well as determining antibiogram of the isolated bacteria from healthy and diseased seaweeds.

**MATERIALS AND METHODS**

**Isolation and Identification of the Bacteria:** 30 gram of each healthy and diseased seaweed was sampled from Mengabang Telipot marine hatchery at Universiti Malaysia Terengganu, Malaysia. All samples were run in triplicates. They were washed with sterile saline water following by homogenization in physiological saline. Ten fold serial dilutions viz. 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ were performed on the samples Aliquots of 0.1 ml from each dilution were inoculated onto Tryptic Soy agar (TSA) incorporated with 2% NaCl for total bacteria count, Thiosulphate Citrate Bile salt (TCBS) for *Vibrio* count and Eosin Methylene Blue (EMB) agar for *E.coli* count (Merek, Germany) by spread plate method. They were incubated for 24 to 48 hour at room temperature. After that, colony forming unit (CFU g⁻¹) of bacteria on the inoculated agar plate was recorded. Single and pure of bacteria colonies were selected randomly and stocked in deep tube tryptic soy agar (TSA) (Merek, Germany) with 2.0% NaCl for the further identification using commercial bacterial identification kit (BBL, USA). Water parameters

**Corresponding Author:** Dr. Najiah Musa, Department of Fisheries Science and Aquaculture, Faculty of Agrotechnology and Food Sciences, Universiti Malaysia Terengganu (UMT) 21030 Mengabang Telipot, Terengganu, Malaysia
such as pH and temperature were also recorded at the culture sites.

**Antibiotic Susceptibility Test:** The identified bacterial isolates were cultured in tryptic soy broth (TSB) (Oxoid, England) for 24 h at room temperature. The bacterial cells were then collected by centrifuged at 14,500 rpm for 5 min by using minispin (Ependoff, Germany). The concentration of the bacterial cells was adjusted to $10^8$ colony forming unit (CFU) by using physiological saline and monitored with Biophotometer (Ependoff, Germany) before swabbing onto the prepared Mueller Hinton agar (Oxoid, England). After 10 min, the tested antimicrobial disks were placed on the agar with a sterile forceps. The plates were then place inverting and incubated for 24 h at room temperature. Twenty antimicrobial agents were applied in the present study. They were erythromycin (30 μg/disk), spiramycin (100 μg/disk), oxytetracycline (30 μg/disk), amoxicillin (25 μg/disk), colistin sulphate (25 μg/disk), doxycycline (30 μg/disk), florfenicol (30 μg/disk), flumequine (30 μg/disk), fosfomycin (50 μg/disk), lincomycin (15 μg/disk), nitrofurantoin (50 μg/disk), novobiocin (30 μg/disk), oleandomycin (15 μg/disk), oxolinic acid (2 μg/disk), chloramphenicol (30 μg/disk), ampicillin (10 μg/disk), kanamycin (30 μg/disk), nalidixic acid (30 μg/disk), furazolidone (15 μg/disk) and sulphamethoxazole (25 μg/disk) (Oxoid, England). Finally, antimicrobial susceptibility result of the present isolates was determined according to National Committee for Clinical Laboratory Standards (NCCLS).

**Result analysis:** The results of the total bacteria count, *Vibrio* count and *E. coli* count between healthy and diseased *G. changii* were analysed with t-Test using SPSS version 13.0 software.

**RESULTS**

In the present study, total bacteria count, *Vibrio* count and *E. coli* count between healthy and diseased *Gracilaria changii* showed no significant difference ($p < 0.05$). Healthy and diseased *G. changii* result for total bacteria count were $2.9 	imes 10^6$ ± 6.31 and $3.5 	imes 10^6$ ± 3.42 CFU g$^{-1}$, *Vibrio* count were $1.6 	imes 10^5$ ± 3.24 and $1.7 	imes 10^5$ ± 2.90 CFU g$^{-1}$ and *E. coli* count were $2.2 	imes 10^7$ ± 8.54 and $1.8 	imes 10^7$ ± 4.29 CFU g$^{-1}$, respectively. A total of six different bacterial species were successfully identified in the present study. They were *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Pasteurella haemolytica*, *Vibrio alginolyticus* and *Vibrio cholera*. Water parameter showed that pH and temperature fluctuated from 6.8 to 8.0 and 26 to 28°C, respectively.

In the present study, antibiotic test revealed that percentage of antibiotic resistance incidences was 24.3% whereas the percentage of antibiotic susceptibility and intermediary sensitive incidences among the present isolates to the tested antibiotic was 63.2% and 12.5%, respectively. All isolates were found to be resistant to lincomycin whereas only one out of twenty two isolate was sensitive to sulphamethoxazole. In the present study, all the isolates were sensitive to oxolinic acid, nalidixic acid, chloramphenicol and flumequine.

**DISCUSSION**

In the present study, majority of the isolates from seaweed were pathogenic to humans namely *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Pasteurella haemolytica*, *Vibrio alginolyticus* and *Vibrio cholera*. Although the seaweeds were washed with normal saline, huge number of bacterial pathogens found in seaweed samples suggests that its intimate association via the bacterium’s invasive capability to penetrate epithelial membrane [3]. This is supported by reported study that many bacteria species are dwelling in the agar and carrageenans derived from seaweeds [4]. [2] observed that *V. parahaemolyticus* could be isolated at a higher rate from seaweed as compared to water which indicated that seaweed could act as a bacteria reservoir since it can provide better conditions during adverse environmental conditions.

In the present study it showed that although bacteria were found in heavy load from seaweed samples, these bacteria most probably did not contribute to the death of seaweed as they were also present in healthy seaweed samples. These findings could imply that the seaweed from our studies, without significant signs except for the pale stalks but bearing various types of bacteria, might also have developed advanced stages of disease under unfavourable conditions. According to [5], successful seaweed development depends on several factors such as turbidity, nutrient levels, phytoplankton blooms, temperature and salinity fluctuations. In this study, fluctuations of temperature and pH could cause stress to seaweed that triggers the development of “ice-ice disease”. Ice-ice” disease in seaweeds is marked by the presence of whitening and pale stalks and consequently resulted in withering its production [6]. It has been reported that when the seaweed in under stress, it emits a moist organic substance that attracts bacteria in the water and induces the “whitening” and hardening of the seaweed branches [7].
In another experiment, different scenario of bacterial counts found on damaged tissue sites and undamaged sites of seaweed. [8] found that bacteria were consistently more abundant on intact seaweed surfaces as they were embedded in a surface mucilage layer while on damaged seaweed a greater percentage of bacteria would be loosely-attached bacteria. Moreover, deteriorating water quality, heavy metals and organic pollutants can cause problems to seaweed culture. [9] reported that seaweed are good in absorbing heavy metals. It is therefore advisable to culture seaweed away from areas with heavy pollutants loads. So far, there appears to be little information on pollutant residues in seaweed cultured in Asia.

The presence of various types of bacteria in the present study is not a serious threat to the seaweed culture at UMT although the bacteria were recovered from both healthy and diseased seaweed, they did not cause disease in seaweed. However, considering the susceptibility of seaweed to various potential environmental stressors, it would be advisable to conduct regular health studies on the seaweed, especially on its water source.

The antibiotic patterns showed alarming result which warrant further investigation particularly the source of water used to culture seaweed. In the present study it showed that seaweed contained high level of pathogenic bacteria which are hazardous to human. This indicated that these cultured seaweed is deem not suitable to be eaten raw. Our data suggested that bacteria found in seaweed might originate from an environment that contains faecal and other human clinical pathogens. Hence it is necessary to estimate the potential risks and associated risks in future studies. Therefore, it is important to examine both safety and microbiological aspects related to this cultured seaweed. According to [10], the absence of filtering process in macro algal indicated that it does not have the ability to concentrate faecal pathogens in its tissues. In short, contamination with faecal organisms may be direct results of growth in polluted waters containing human or animal faecal material.

In the future more studies should be carried out to ensure the safety of cultured seaweed focusing on several aspects such as environmental factor effects, quality of marine water and effect of drying on faecal pathogens found in seaweed.

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