Identification of The Potential of Degrading Carrageenan in Red Algae *Kappaphycus alvarezii* Symbiotic Bacteria

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**Abstract**— *Kappaphycus alvarezii* is a red alga contained large amount of bioactive material, such as carrageenan. Carrageenan is useful as a raw material for several industries and can be degraded by marine bacteria through breaking the linkages in polysaccharide carrageenan into oligosaccharide carrageenan. The aim of this study is identification of degrading carrageenan in *K. alvarezii* symbiotic bacteria. The results showed there was 14 isolate bacteria, and all of the isolates have clear zone on congo red staining activity. The isolate bacteria were 7 genera as *K. alvarezii* symbiotic bacteria, such as *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Celeribacter* sp., *Pseudoalteromonas* sp., *Phaeobacter* sp. and *Cobetia* sp. *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Pseudoalteromonas* sp., *Phaeobacter* sp. were recognized to have strong interactions with carrageenan in red algae, while the other *Celeribacter* sp. and *Cobetia* sp. have strong interactions with alginate in brown algae.

**Keywords**— *Kappaphycus alvarezii*, carrageenan, degrading bacteria.

**I. INTRODUCTION**

*Kappaphycus alvarezii* is red algae that generally has cylindric thallus, smooth surface, cartilaginous and consists of several types based on the color, such as green, yellowish green, gray, brown and red (Parenrengi et al., 2010). *K. alvarezii* was lived in tidal habitats, coral reef flats and attached to hard substrates (Erlania, 2013). *K. alvarezii* was contained large amount of carrageenan and used as stabilizer and gelling agent in processed meat, ice cream, chocolate, pudding, pet food, shampoo, toothpaste and cleaning products industrial (Hotchkiss et al., 2016; Barret, 2018).

In the ecosystem, bacteria were played an important role because of its ability to degrade organic matters to inorganic matters (Ginting et al., 2019). The existence of symbiotic bacteria was to protect their host and produce secondary metabolites (Funty, 2015). The use of secondary metabolites in algae, for example as bioactive materials (Nurhaedar, 2008). Carrageenan as a bioactive material in algae can be degraded by marine bacteria, especially gram-negative bacteria and produced enzymes to degrade carrageenans and it was useful for several industries (Chauhan & Saxena, 2016). Carrageenans degradation was a process to break the linkages in polysaccharide carrageenans to be oligosaccharide carrageenan with low molecular weight (Ghanbarzadeh et al., 2018). Several studies showed that *Pseudoalteromonas* sp. (Li et al., 2013); *Tamlana* sp. was isolated from red algae *Hyalosiphonia caespitosa* (Sun et al., 2010) and *Cytophaga* sp. was isolated from red algae *Eucheuma gelatinue* (Mou et al., 2004) have degrading carrageenan ability. Based on previous several studies, there is no *K. alvarezii* symbiotic bacteria in degrading carrageenan. Therefore, it is necessary to conduct research on the identification of degrading carrageenans in *K. alvarezii* symbiotic bacteria.

**II. MATERIAL AND METHODS**

*Kappaphycus alvarezii* AND CARRAGEenan USED IN THIS STUDY

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Algae K. alvarezi were collected from USA Marine Biological Institute, Kochi University, Japan and floated in Uranouchi Bay, Tosa, Kochi Prefecture, Japan for one week to collect the bacteria. While several commercial carrageenan was used in this study, κ-carrageenan and λ-carrageenan purchased from Wako.

**ISOLATION OF K. alvarezi SYMBIOTIC-BACTERIA**

Artificial water agar medium was made with adding 0.5% carrageenan and incubated with red algae K. alvarezi for 3 days. After 3 days, the artificial seawater bacteria were dropped around 0.1 ml in marine broth agar. Moreover, bacteria were incubated at 25°C for 2 days to get the pure bacteria cultured.

**DEGRADING CARRAGEENAN SCREENING BY CONGO RED STAINING**

Bacteria in marine broth agar medium was inoculated to marine broth medium and incubated at 25°C for 2 days. Congo red agar medium was made from 4 gr gar medium, 25 ml 0.5% carrageenan and 1 ml congo red. Bacteria colonies from marine broth medium was dropped into congo red agar medium for 0.1 ml. The bacteria were incubated at 25°C for 2 days to identify the clear zone. Formed clear zone was an indicator of the carrageenan degrading activity existence. Bacteria with clear zone was inoculated and analyzed by 16S rDNA.

**16S rDNA ANALYSIS OF K. alvarezi SYMBIOTIC-BACTERIA**

A colony of 14 isolate bacteria were used as a template for PCR. Isolate bacteria were amplified by using universal primer pr0R2 (5' - AGAGTTTGATCMTGGCTCAG - 3') dan 534R (5' - ATTACCGCGGCTGCGTGG - 3'). PCR products were applied to agarose gel electrophoresis and purified using Wizard® SV Gel and PCR Clean-Up System (Promega). The purified DNA were sequenced by ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems Japan) using BigDye® Terminator v3.1 and analyzed with BLAST on NCBI.

### III. RESULT AND DISCUSSION

Bacteria isolation found 14 isolate bacteria with big size colonies. Congo red staining activity showed that there was clear zone in 14 isolate bacteria (Fig 1). Congo red was used for degrading carrageenans activity because congo red have strong interaction with polysaccharide which contained cellulose linked by β-1,4-glycosidic linkages (Teather & Wood, 1982). FAO (2003) was explained that red algae contain carrageenan and cellulose which insoluble in water and alkali. The clear zone was formed because of the reaction between congo red and β-1,4-glycosidic linkages in cellulose polymer (Missa et al., 2016).

Electrophoresis showed that the measurement of bacteria DNA fragment was about 500 bp and it was compared to the marker 1 kbp DNA (Fig 2). The purification of DNA was measured by absorbance 260 nm and 280 nm. It showed that the absorbance of purified DNA was about 1.73-2.07 (Table 1). Thermo Fisher Scientific (2010) explained that the ratio of absorbance 260 nm and 280 nm was about 1.8 and it was “pure” for DNA purification.

| Bacteria | A260/280 |
|----------|----------|
| KC1      | 1.73     |
| KC2      | 1.79     |
| KC3      | 1.82     |
| KC4      | 1.83     |
| KC5      | 1.78     |
| KC6      | 1.81     |
| KC7      | 1.92     |
| KC8      | 1.87     |
| KC9      | 1.94     |

**Fig 1: Congo red staining activity on 14 isolate bacteria**

**Fig 2: DNA fragment of K. alvarezi symbiotic bacteria**

**Table 1. Absorbance of K. alvarezi symbiotic bacteria DNA purification**
16S rDNA showed that there were 7 genera of symbiotic bacteria in *K. alvarezii*, such as *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Celeribacter* sp., *Pseudoalteromonas* sp., *Phaeobacter* sp. and *Cobetia* sp. (Table 2). Azizi *et al.* (2018) found some bacteria was associated with 4 types of *K. alvarezii* and classified by 11 genera, such as *Alteromonas* sp., *Aestuariibacter* sp., *Idiomarina* sp., *Jejuia* sp., *Halomonas* sp., *Primoskyibacter* sp., *Pseudoalteromonas* sp., *Ruegeria* sp., *Terasakiella* sp., *Thalassospira* sp. and *Vibrio* sp. All of bacteria in this study was gram-negative bacteria and have carrageenan degrading ability by congo red staining activity. Chauhan & Saxena (2016) explained that carrageenase enzyme was only produced extracellularly by gram-negative bacteria.

**Table 2. 16S rDNA of K. alvarezii symbiotic bacteria**

| Isolate  | Identity          | Reference                                      | Reference Name                      |
|----------|-------------------|------------------------------------------------|-------------------------------------|
| Isolate 1| Labrenzia sp.     | 99.77%                                         | *Labrenzia sp.* THAF35, Accession No. CP045380 |
| Isolate 2| *Alteromonas* sp. | 98.75%                                         | *Alteromonas tagae*, Accession No. NR_043977 |
| Isolate 3| *Alteromonas* sp. | 99.58%                                         | *Alteromonas tagae*, Accession No. NR_043977 |
| Isolate 4| *Vibrio* sp.      | 99.60%                                         | *Vibrio campbellii* MMRF1060, Accession No. |

The first *Labrenzia* sp. in red algae was *Labrenzia polysiphoniae* in red algae *Polysiphonia* sp. (Romanenko *et al.*, 2019). *Alteromonas* sp. was found as *Kappaphycus alvarezii* symbiotic bacteria and showed a pathogenetic. *Alteromonas* sp. was able to be pathogen agent that caused ice-ice symptoms (Syafitri *et al.*, 2017). On the other hand,
Alteromonas sp. showed a potential to degrade some polysaccharide, such as alginate (Neumann et al., 2015), ulvan (Koch et al., 2019); agar (Wang et al., 2005); t-carragenan (Barbeyron et al., 2019) and κ-carragenan (Barbeyron et al., 1994). Araki et al., (1999) and Zhu & Ning (2016) found high activity of κ-carrageenase enzyme through Vibrio sp. purification. Moreover, Pseudoalteromonas sp. had an ability to utilize κ-carrageenan and ƞ-carrageenan for their energy source (Hettle et al., 2019). Pseudoalteromonas sp. was also degraded κ-carrageenan (Liu et al., 2011) and ƞ-carrageenan (Guiabet et al., 2007). Furthermore, Phaeobacter inhibens was found in red algae Tichocarpus crinitus to degrade the carrageenan (Kalitnik et al., 2017). Based on the several studies, Labrenzia sp., Alteromonas sp., Vibrio sp., Pseudoalteromonas sp. and Phaeobacter sp. were recognized to have strong interactions with red algae through the utilization of red algae carrageenan. Whereas Celeribacter sp. and Cobetia sp. was found in brown algae through the utilization of brown algae alginate. Ihua et al. (2020) showed that Celeribacter sp. was found on brown algae thallus Laminaria digitata and Yagi et al. (2016) explained that Cobetia sp. was isolated from brown algae Padina arborescens with alginate degrading enzyme.

IV. CONCLUSION

In this study, we found 7 genera of Kappaphycus alvarezi symbiotic bacteria, such as Labrenzia sp., Alteromonas sp., Vibrio sp., Celeribacter sp., Pseudoalteromonas sp., Phaeobacter sp. and Cobetia sp. All of the bacteria showed an activity on congo red staining based on the formed clear zone. The clear zone was indicated the carrageenan degrading activity.

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