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Characterization of Yellow Pigmented Bacteria Associated with *Gracilaria* sp.

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Abstract. Research on the kinship analysis of endophytic bacterial isolated from *Gracillaria* sp has been carried out. The presence of bacteria associated with *Gracilaria* sp. has enabled the use of these bacteria as a source of new bioactive compounds, such as biopigments. The research aims to isolated bacteria from *Gracilaria* sp., screened their symbiont bacteria that could potentially produce pigments. Sampling *Gracilaria* sp. conducted in the waters of the Island of Karimunjawa, Jepara. Furthermore, bacterial isolation was carried out, screening for pigment-producing bacteria and 16S rRNA sequence analysis. Research result showed that the symbiont bacteria isolate TK 373 produced consistent pigments after several regenerations, in several types of growth media incubated at room temperature. The results of 16S rDNA identification showed that the TK 373 isolate had the closest relationship with *Pseudoalteromonas* sp. with 98.72 % homology.

Keywords: *symbiont bacteria, pigment, Gracilaria* sp.

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1. Introduction

Indonesia gets a big advantage because it has a very large marine. These advantages include in the form of biodiversity, both in the form of community, species and genetic diversity. Indonesia's abundant natural wealth and biodiversity have prospects for this nation to develop bio-industry. One of these potential genetic resources is algae.

Various natural products from macro algae metabolites plays a very important role in the natural product discovery process [1,2,3]. Algae, has been widely used as a bioactive source metabolites such as proteins, lipids, mineral salts, polyphenols and polysaccharides which are very useful in various industries of food, pharmacy, cosmetics [4,5]. Red algae is a group of algae that has extraordinary potential, including the pigment content of red algae which has pharmaceutical, nutraceutical and cosmeceutical prospects [6, 7].

Marine microorganisms have a very large role in the life cycle at sea. One of them is as epiphytic microbes or microbes associated with marine organisms. Various scientific publications show that there are associations of microorganisms with marine organisms that also synthesize secondary metabolites such as their host organisms, including red algae symbionts [8, 9]. This is a consideration to explore

the red algae symbiont bacteria with the hope of providing a wider opportunity to get red algae bacterial associan that have biological abilities like their host.

2. Methods

2.1. Sampling site

Algae samples of *Gracilaria* sp. were collected from Karimunjawa island Indonesia. After collection, algae materials were placed onto sterile bottles containing autoclaved seawater and brought in chilled condition, to the Integrated Biotechnology Laboratory, Diponegoro University.

2.2. Isolation and purification of bacteria from algae

Fresh algae samples were washed with marine steriled water. Bacteria associated with *Gracilaria* sp. isolated according to the method of [10]. Using a sterile scalpel, seaweed tissue is cut in a size of about 0.1 cm3 and sprayed three times with sterilized sea water. These pieces are then rinsed for surface sterilization with sterile sea water. Zobell media are used to place *Gracilaria* sp tissue in an incubator at 28° C for 72 hours. Different colonies appear morphologically to be separated and purified.

2.3. Phylogenetic Analysis

Genomic DNA was extracted using the standard chelex protocol according to [11]. The 16S rRNA gene of the isolate was amplified with the bacterial universal 16S rRNA primers 27F and 1492R. The optimizations used were: 93° C for 30 seconds, 54.5° C for 30 seconds, and 72 ° C for 1.5 minutes. run 30 cycles. The presence of PCR products was confirmed by electrophoresis on 1 % agarose gels. Basic Local Alignment Search Tool (BLAST) is used to determine bacterial species that are closely related to potential bacterial isolates. Phylogenetic trees with bootstrap sampling were reconstructed by the neighbor-joining method, with 1,000 bootstrap replications was run in MEGA 7 [12, 13].

3. Results and Discussion

Efforts to obtain bacterial isolates pigmented brown algae symbionts were carried out using culture-dependent methods. This method is a physiological identification process of microbes by first isolating and purifying the bacteria from the host. Based on the results of isolation and purification, it was found that several isolates produced pigment. However, after several isolates regeneration processes were carried out, it turned out that only one isolate was able to show fertile growth and a consistent color, namely the TK 373 isolate.This isolate produced a bright yellow color when grown on Marine agar and Nutrient Agar media, incubated at room temperature (Figure 1)

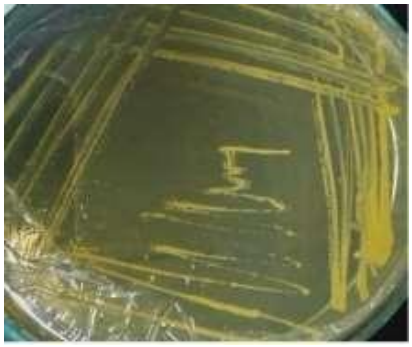

Figure 1. Colony characteristics of TK 373 isolates on ZMA media were incubated for 48 hours atroom temperature

The BLAST results showed that the TK 373 isolate had the highest similarity with Pseudoalteromonas with 98.72% similarity (Table 1). 97-99% similarity for 16S rRNA sequences for bacteria showed similarity at the genus level, whereas> 99% similarity in gene sequences was the criterion used to

identify isolates at the species level [14]. The results of phylogenetic reconstruction of bacterial isolates based on partial gene sequences of 16S rRNA showed the closeness of TK 373 to *Pseudoalteromonas* sp. (Figure 2).

Table 1. Homology of partial gene sequences of 16S rRNA isolate TK 373 with GenBank Database

Isolate code	Homology	Query cover (%)	Score	E-value	Per Ident	Ascension number
TK 373	*Pseudoalteromonas* sp. strain S14	91%	2084	0,0	98,72 %	KX989351.1

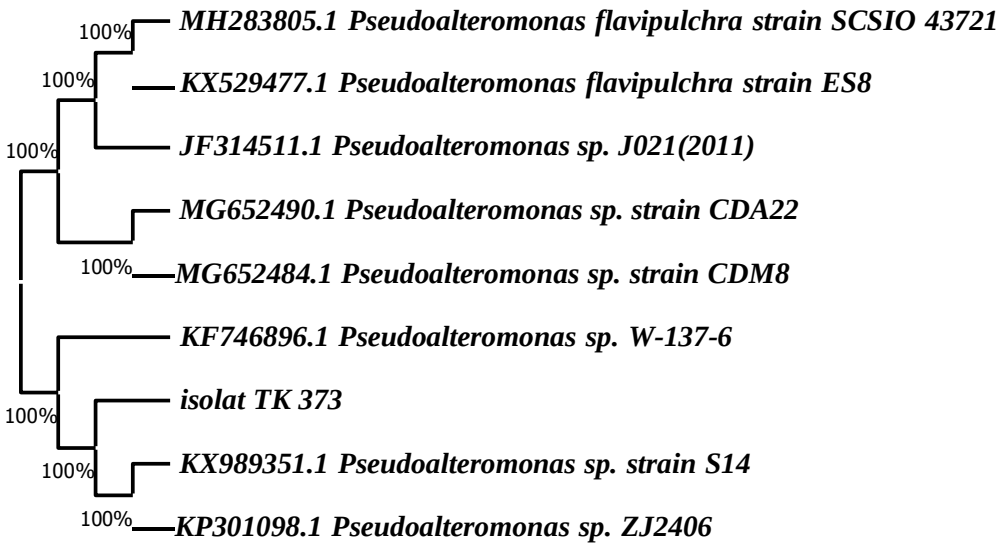

Figure 2. Reconstruction of the TK 373 phylogenetic tree with reference strains obtained from NCBI. A neighbor joining tree was calculated using partial 16S rRNA gene sequences.

Many researchers have reviewed the Pseudoalteromonas. [15] stated that pseudoalteromonas are true marine bacteria, this is because the Pseudoalteromonas require a sea water base for growth. Pseudoalteromonas are gram-negative bacteria, motile and have polar flagella, are heterotrophic, and have GC content between 38 - 50% [16]

The genus Pseudoalteromonas is an interesting topic of study. This is due to its potential and uniqueness. This potential is the productive capacity of its metabolite production, while its uniqueness is seen in terms of its association with other organisms [17]. Based on these two points of view, it can be said that their ability to associate with other organisms is the key to the abundance of potential metabolites from the Genus Pseudoalteromonas [15]. The results of this study also support this argument. Isolate TK 373 was related to Genus Pseudoalteromonas, isolated from Gracilaria sp. on sampling sites in marine waters.

The metabolite production capacity in Pseudoalteromonas is usually associated with pigmentation, for example, P. tunicata CCUG 26757 and P. rubra DSM 6842 strains produce various pigments that are involved in antibacterial and antifungal activity [18,19,20,21]

Isolate TK 373 produces a bright yellow color. This of course gives hope that these isolates are potential. Screening for bioactive potential needs to be done as part of the bioprospection of potential local isolates that can be applied in the industrial sector.

4. Conclusion

Research on molecular characterization and morphotypes of local bacterial isolates with symbionts in Gracilaria sp. has provided findings that TK 373 isolate has the closest similarity to Pseudoalteromonas, one of the potential genera where most of its members are able to produce pigments that can be explored further for its potential.

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