Bioma, Juni 2018
Vol. 20, No. 1, Hal. 55-59

p ISSN: 1410-8801
e ISSN: 2598-2370

Phylogenetic Analysis Of Pigmented Marine Derived Yeast Associated With *Sargassum* sp. Based On Internal Transcribed Spacer (ITS)

Arina Tri Lunggani dan Endang Kusdiyantini
Departemen Biologi, Fakultas Sains dan Matematika, Universitas Diponegoro
Jl. Prof Soedharto, SH, Tembalang, Semarang 50275
rynatri7@gmail.com

Abstract

Karimunjawa Jepara is a region with high diversity, including the diversity of seaweed. Brown seaweed is potential marine organisms due to their ability to produce enzymes, pigments, and bioactive compounds. This ability makes brown seaweed one of the potential biological agents from the marine to be developed in the industrial field. However, most of these substances may not be produced by seaweed itself, but cooperation with microbes or even by bacteria or symbiotic fungi. Secondary metabolites which are pharmaceutical, enzyme, and cosmetic sources can be produced by microbial associan. The purpose of this study was to carry out phylogenetic analysis and morphological characterization of colonies from pigmented yeast associated with Sargassum sp. The results of the phylogenetic analysis indicate that isolates of KY 3 have 100% relative similarity with *Cystobasidium oligophagum*.

Keywords: *marine-derived yeast ; Sargassum sp. ;Karimunjawa ; phylogenetic analysis*

Abstrak

Karimunjawa Jepara merupakan daerah dengan diversitas yang cukup tinggi, termasuk diantaranya adalah diversitas rumput laut. Rumput laut coklat adalah organisme laut potensial sehubungan dengan kemampuannya memproduksi ensim, pigmen dan senyawa bioaktif. Kemampuan ini menjadikan rumput laut coklat sebagai salah satu agen biologi potensial dari laut yang akan dikembangkan di bidang industri. Namun, sebagian besar zat ini kemungkinan tidak diproduksi oleh rumput laut itu sendiri, melainkan kerja sama dengan mikroba assosian atau bahkan oleh bakteri atau jamur simbion. Mikroorganisme yang berasosiasi dengan organisme laut, biasanya memiliki metabolit sekunder yang dapat menjadi sumber farmasi, enzim, maupun kosmetik. Tujuan dari penelitian ini adalah untuk melakukan analisis filogenetik dan karakterisasi morfologi koloni dari khamir berpigmen yang berasosiasi dengan *Sargassum* sp. Hasil analisis filogenetik menunjukkan bahwa isolat khamir KY 3 mempunyai kesamaan relatif sebesar 100% dengan isolat *Cystobasidium oligophagum*.

Kata kunci : *khamir laut ; Sargassum sp ; Karimunjawa ; analisis filogenetik*.

INTRODUCTION

Marine seaweed habitats in the waters can be affected by exposure to ultraviolet light and the air leads to the formation of free radicals. The compounds formed are very reactive and is known as reactive oxygen species (ROS). In healthy seaweed, damage oxidative in the compounds they contain can be reduced by its ability to fight oxidation, which indicates the existence of an antioxidant defense system which protects cells (Vinayak *et al*, 2019). Over the past few years, brown seaweed and its extract have produced a large amount of interest in the pharmaceutical industry as a source of fresh bioactive compounds with enormous medicinal potential (Blanco-Pascual *et al* 2014; Pangestuti dan Kim, 2011; Banafa *et al*., 2013). One type of brown seaweed that can be utilized in the pharmaceutical field is the *Sargassum* sp. The Sargassum species is a member of tropical brown seaweed and is in shallow water. *Sargassum* sp. is a rich source of nutrients and bioactive compounds such as vitamins, carotenoids, dietary fiber, and most importantly is a source of medicine. This type of seaweed can produce pigments that have the potential to be anti-cancer, anti-inflammatory (Miyashita *et al*, 2011; Yende *et*

al., 2014). The main pigment of brown seaweed is fucoxanthin, which is one of the most abundant carotenoid pigments in nature, with an estimated 10% estimated total carotenoid production (Pangestuti, R. dan Kim, S.K. 2011).

The phylogenetic analysis describes the taxonomic classification of organisms based on evolutionary history. The order that can be used in this study is the Internal Transcription Spacer (ITS). ITS is flanked by encoding areas of 18S, 5.8S and 26S rDNA in each unit on a series of chromosomes. The purpose of this study was to carry out a phylogenetic analysis of pigmented yeast associated with *Sargassum* sp. originating from Indonesia based on the sequence of ITS genes. Karimunjawa waters are directed as sampling locations for exploration of brown seaweed as host of pigmented yeast so that it can make a positive contribution to pharmaceutical and industrial development of industries

MATERIALS AND METHODS

Sample preparation, isolation and yeast purification.

A sample of brown seaweed was taken in the waters of Karimunjawa, Jepara, Indonesia. The yeast was isolated from seaweed that has been cleaned with sterile sea water, then thallus of small seaweed is cut into the flask tube. The sample was then placed in a shaker at a speed of 100 rpm, and incubated at room temperature for 24 hours. After the planned incubation time was achieved, 100 µL was taken from 1/10, 1/100 dilution and inoculated on Marine agar medium and Potato Dextrose Agar then incubated for 72 hours at room temperature. Microbial growth was observed, then yeast isolation and purification was based on the shape, size, morphology and pigmentation of the colonies.

Pigmented yeast screening

Pigmented yeast screening was carried out by growing pigmented yeast on potato agar medium and incubating at room temperature. Observed growth and pigmentation of colonies formed. Yeasts that are able to grow well and show relatively stable colony colors were selected as potential isolates.

Molecular identification.

DNA amplification in molecular phylogenetic analysis based on ribosomal DNA sequences (rDNA) in the ITS (Internal Transcribed Spacer region) region using primary ITS 1 (5′-TCC GTA GGT GAA CCT GCG-3′) and ITS 4 (5′-TCC TCC GCT TAT TGA TAT GC-3). The condition applied when the PCR amplification was pre denaturation: 95 ºC for 5 minutes, denaturation; 95 ºC for 1 minute, annealing; 57 ºC for 1 minute, for 34 cycles, extension for 72 ºC for 1 minute, final extension 72 for 7 minutes. Visualization rRNA PCR products is done through electrophoresis. Furthermore, the gel electrophoresis to see the DNA bands formed and visualized using Geldoc and the size of the fragment is determined by comparing it to the standard 1 KB Ladder. The PCR products were then sequenced and analyzed using MEGA 7

RESULT AND DISCUSSION

This research begins with the isolation of yeast associated with *Sargassum* sp. from Karimunjawa Jepara waters. *Sargassum* sp. samples were taken at a depth of 1 - 1.5 meters with snorkeling techniques. Isolation of yeast associated with Sargassum sp. is done by maceration, a technique that is carried out by destroying a solid sample by mashing it using mortar, so that microbes on the surface or in the sample can be released, then continued with multilevel dilution using sterile sea water, and grown on PDA for 72 hours of incubation

Isolation yeast isolate from *Sargassum* sp. obtained 5 pure isolates. Pigmented yeast isolates obtained from *Sargassum* sp. were relatively less compared to other types of microbes, namely pigmented bacteria which were also obtained from the host *Sargassum* sp. from the same waters. In the subsequent screening, only one yeast isolate was obtained potential in terms of the stability of the color it produced, namely KY3 isolates. *Sargassum* sp. samples obtained and morphology of yeast KY3 isolates are presented in Figures 1 and 2.

Fig. 1. *Sargassum* sp. as a host sample

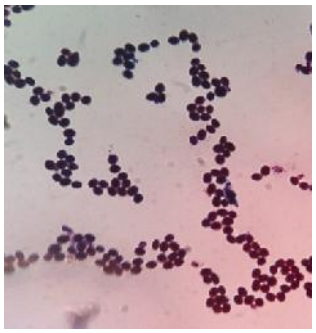

Fig 2. Morphology of KY 3 isolates

Color products or natural pigments in food ingredients are in great demand among the public given the dangers of using synthetic dyes. Natural dyes currently available are only used traditionally and are generally made from high-level plants, such as pandan leaves and suji leaves. However, natural dyes are still unable to compete with synthetic dyes sold on the market (Venil *et al*, 2009)

Brown seaweed is a type of potentially high economic seaweed, containing vitamins, minerals, fiber, sodium, potassium, and bioactive compounds in the form of secondary metabolites, and the most important nutrients are pigments. *Sargassum* sp. is a seaweed with wide application in medicine, food industry, cosmetics and pharmacy. The potential pigment possessed by *Sargassum sp*. can be utilized as a potential source of pigment (Heo, S.J. and Jeon, Y.J., 2009; Lunggani *et al*., 2018). On the other hand it is known that abundance, diversity, and distribution, and marine microbes and their functions, both planktonic microbes that are free living and those associated with eukaryotes are unlimited genetic resources (Egan *et al*., 2008; Steele *et al*., 2011; Eckert and Pernthaler, 2014). Epiphytic yeast and endophytes live on the surface and in tissues inside or even in their host cells. The current study shows that there is a complex interaction between the host and yeast associations, for example the host provides nutrition, while the asosian microbes produce chemicals for environmental defense and biological opponents. This phenomenon encourages researchers to explore more broadly the benefits of the *Sargassum* sp. pigment as a host of microbes capable of producing natural pigments.

One of the common stages in understanding microbes that is not yet known to be potential is one of them is phylogenetic analysis of these microbes. Search through morphological identification is sometimes hampered by the scarcity of certain taxonomic characters. This is due to the possible morphological transition of many species in response to changes in environmental conditions and part of their life cycle (Yarza *et al*., 2017). The combination of morphological and phylogenetic information can help in understanding the characteristics of isolates. Added by Jeewon, *et al*., 2013**,** understanding phylogenetic relationships is a prerequisite for understanding the microbial metabolism and ecological lifestyle. The next stage is screening biological potential of these microbes in accordance with the description of each research objective

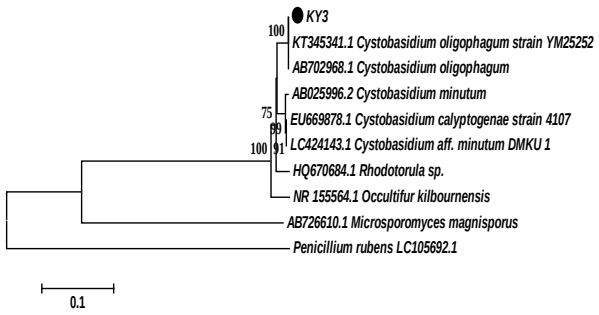

Fig 3. Phylogeny Tree of KY3 Isolate based on ITS sequence using Neighbor Joining Analysis.

The amplification of the ITS gene region was successfully performed. This is evidenced by the presence of single bands in the gel wells of each amplicon (repeated sample twice) that are parallel to the 600 pb marker . The nucleotide base sequence

of KY 3 isolates was then used as the basis for phylogenetic tree construction to determine the kinship relationship. The nucleotide sequences obtained from the sequencing process were then compared with the data on Genebank using the BLASTn program on the website of the National Center of Biotechnology Information (NCBI). The construction of phylogenetic trees was carried out using the Mega 7 program. The results of phylogenetic tree construction as shown in Figure 3. As mentioned by Jeewon, *et al*., 2013, identification of potential isolates is very important, related to the development of these isolates at a further research stage. Based on BLAST analysis, yeast KY3 has 100% similarity with *Cystobasidium oligophagum***.**

Obtaining pigmented yeast isolates from the sea is an interesting subject from biotechnology perspective. in general, this yeast has a broad spectrum capable of utilizing extensive carbon sources source and grow well at high temperatures, and are also capable of forming large biomass. Moreover, the yeast isolates obtained were endophytic microbes from the host *Sargassum* sp. which are organisms with broad spectrum secondary metabolites as well. KY 3 is a yeast that is capable of producing pigments. as part of the pigment, Q10 is also an attractive product from biotechnology. This Q10 component is widely used as a treatment and cosmetics. The use of KY 3 isolates gives hope that these isolates can be developed as agents for pigment production. Added by Vyas, S. and Chhabra, M., 2017, *Cystobasidium oligophagum* is included as oleaginous yeast, so this KY 3 isolate is an promossing candidate isolate, candidate agents were developed as subjects for biodiesel production

CONCLUSION

Isolate KY 3 as a marine-derived yeast pigmented contributes scientific information about pigmented yeast associated with *Sargassum* sp. as a potential genetic resource of Karimunjawa Island, Indonesia. This isolate has 100% relative similarity with *Cystobasidium oligophagu*/

ACKNOWLEGMENT

The authors are thankful to Siu Semar Saratu Langden for her assistance in this research

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