Morphotypes and molecular characterisation of pink pigmented bacterial symbiont of Turbinaria sp.

by Endang Kusdiyantini

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A T Lunggani[1], E Kusdiyantini[1], F D Imtiyaz[1]

[1]Biology Departement, Faculty of Science and MathematicsDiponegoroUniversity, Jl. Prof H. Soedharto, SH, Tembalang, Semarang 50275, Indonesia

Corresponding author: arinalunggani@live.undip.ac.id

1. Introduction

Brown algae have played an important role in the marine ecosystem and provided many biologically active compounds for industry such as antibacterial activities and anti-cancer agents [1][2][3]. However, the exploration was restricted by a conservational issue. To overcome the problem, symbiotic bacteria harboring in some organisms by forming mutualistic association is becoming a hot issue since they could produce similar compounds as produced by the host organisms, in this case, marine organisms, especially brown algae [4][5]. The compounds produced by the bacteria could protect the host from pathogen and extreme environment [6]. Thus, the exploration of symbiotic bacterium associating with brown algae has increased over time and should be developed extensively.

*Turbinaria*sp. is a genus of brown algae that grows on rocky substrates of the marine environment, interestingly, it yields many compounds that are used as antihistaminic agents and antiviral activities [7] antibacterial compound [8]. Moreover, the bacteria residing beneath their tissues are diverse and exhibiting a unique ability [9][10][11]. Also, other studies revealed that the antibacterial activity of bacteria isolated from brown algae effectively inhibits many bacterial pathogens. However, many symbiotic bacteria, especially those isolated from the marine environment, are not properly identified and characterized. By identifying and characterizing the marine pigmented bacteria with better methodology, there would be the fundamental information that values for industrial development.

Therefore, this study aimed to isolate, characterize and identify the pigmented bacteria associated with *Turbinaria* sp.

2. Material and methods

2.1. Microorganism

KRT-7 isolate was successfully isolated from *Turbinaria* sp. that obtained from Karimunjawa Island, Jepara, Central Java.

2.2. Morphological characteristic and physiology

The isolate was cultivated in Zobell Marine Agar (ZMA) and Potato Dextrose Agar (PDA) for about 24 hours. The cells were transferred to 10 mL of physiological saline solution and homogenized the solution using vortex before the bacterial cell solution was examined using VITEK. The motility of bacterium was assessed using a microscope and morphological assessment was done using gram staining.

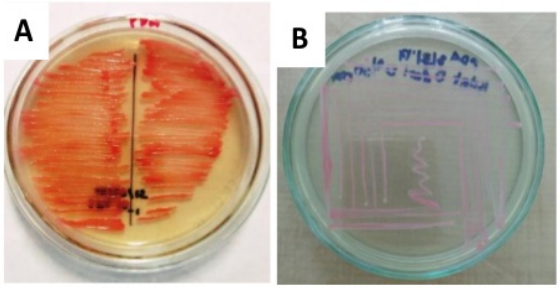

Figure 1. Pigment of KRT 7 isolatein (A) Potato Dextrose Agar (PDA) and (B) Zobell Marine Agar (ZMA) after 96 hours of incubation on 27°C.

2.3. DNA extraction

The DNA extraction was done using Chelexmethod [12]

2.4. Amplification of 16S rRNA and sequencing

5 μL of DNA template was mixed with 25 µL MyTaq™ Mix according to the company protocols. 1.5 μL of forwarding primer 27F (5'- AGAGTTTGATCMTGGCTCAG 3'-) and 1.5 μL of reverse primer 1429 R (5'- TACGGYTACCTTGTTACGACTT-3') were added to the mix solution and sterilized Aquabidest was transferred to the solution to reach the total volume of 50 μL. Amplification was done using polymerase chain reaction (PCR) machine with 2 minutes of pre-denaturation in 94°C before the sample was brought to 30 cycles of denaturation (94°C for 1 minute), annealing (55°C for 1 minute) and elongation (72°C for 2 minutes). After that, the PCR product was visualized using the electrophoresis machine before it was sequenced by 1st Base Laboratories SdnBhd, Malaysia.

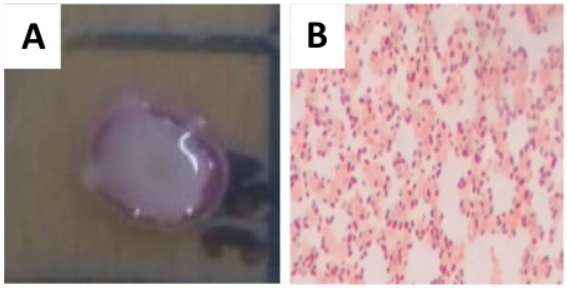

Figure 2. (A) Macroscopic and (B) microscopic Characteristic of KRT 7 isolate in Zobell Marine Agar (ZMA).

2.5. Construction of phylogenetic tree

The amplified PCR product was trimmed, edited and aligned using Bioedit software, also, it is analyzed its similarity with the sequences that were available in BLASTn from the GenBank database(https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using MEGA 7 [13].

3. Result and discussion

Through random purposive sampling, 10 isolates were successfully isolated from *Turbinaria* sp. collected from Menjangan Kecil, Karimunjawa island, Indonesia. However, there was only one isolate that survived after culturing all isolated bacteria in PDA and ZMA (figure 1) medium for 24 hours named KRT 7 isolate. The reason behind this survival is that growth media could affect the growth pattern of microorganisms as well as the content of compound produced. Several studies concluded that the growth of microorganisms and secondary metabolites secreted is affected by the availability of nutrients within the medium [14][15].

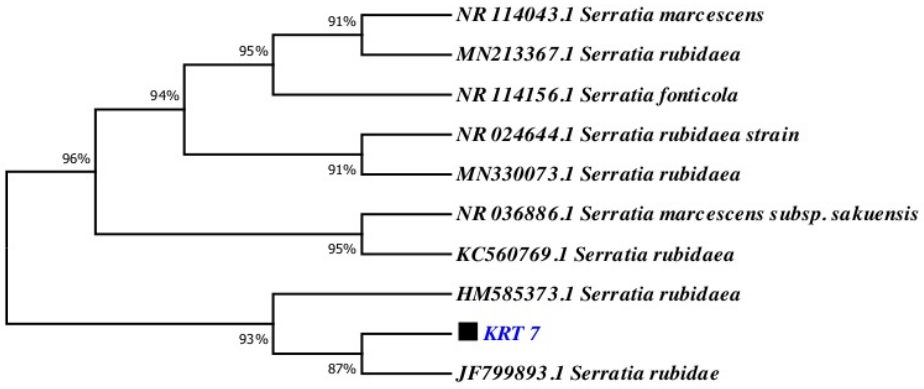

Figure 3. Phylogenetic tree constructed using the partial 16S rRNA gene sequences of KRT 7 isolate.

16S rRNA sequence of the isolate was amplified using both 27F and 1492R primers as forward and reverse primers, respectively. When the DNA's band was visualized, 1.5 kb of the band appeared to be which was confirmed as the DNA of KRT 7 isolate because the ranges of bacterial DNA are commonly about 1522 to 1534 bp [16]. Based on the results of molecular identification, which then made phylogenetic tree reconstruction, KRT 7 isolates were similar to *Serratiarubidaea* CIFRI P-TSB-51-ZMA strains deposited on NCBI with accession code JF799893.1 with 97% similarity. *Serratia rubidaea* strain CIFRI P-TSB-51-ZMA is a salt-tolerant bacterial isolate obtained by Behera B.K *et al* from the East Coast of India [17]. Furthermore, the morphological characteristics of KRT 7 isolate are rods and short, have a pale red pigment, have a circular and smooth texture, and appear to be a gram-negative bacterium.

S. rubidaea is a bacterium which commonly found in soil, water and food. The bacteria could cause opportunistic infection because it is identified as a pathogenic bacterium causing many diseases in humans such as in the respiratory tract, skin, digestive tract and liver. However, the species also shows many biotechnological applications. For instance, *S. rubidaea* isolated from a waste sample of a slaughterhouse in India exhibits protease activity that could be used as vaccine and enzyme peptide synthesis [18]. Eight rhamnolipids, a compound that showed biocontrol activity [19] and increased plant immunity [20], have been produced by *S. rubidaea*SNAU02 obtained from hydrocarbon-contaminated soil [21]Nevertheless, the in-depth study regarding other potencies remains unclear and could be explored in the future studies.

4. Acknowledgment

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References

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