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Mini Review

Marine Bacteria from Eastern Indonesia Waters and Their Potential Use in Biotechnology

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ABSTRACT

Indonesian vast marine waters, which constitute 81% of the country's total area, have a great potential in terms of marine bacteria biodiversity. However, marine bacteria are still under-explored in Indonesia, especially in its eastern area. Known as one of the biodiversity hotspots worldwide, this area surely harbors various marine bacteria of particular interest. Despite the growing number of oceanic expeditions carried out in this area, only little attention has been attributed to marine bacteria. Limited literatures exist on the isolation of marine bacteria producing compounds with potential biotechnological applications from the aforementioned waters. There are two main causes of this problem, namely lack of infrastructures and limited competent human resources. In this paper, I will highlight the preliminary results of isolation and bioprospecting attempts on this group of bacteria during the last fifteen years. These results indicate that research activities on marine bacteria in this area need to be intensified, to uncover their potential applications in various biotechnological fields.

Keywords: marine bacteria, eastern Indonesian waters, biotechnological application

1. Introduction

Ocean proportion represents a major part of the biosphere, containing about 97% of the Earth's water. The majority of this biosphere is situated in the deep sea (depth greater than 1,000 meters), which accounts for 75% of the ocean's volume.

Indonesian eastern marine waters (EIW) constitute more than eighty per cent of this region's total area. The EIW were defined as waters extended from Makassar Strait to Banda and Arafura Seas horizontally, and from Pacific Ocean and Celebes Sea to Banda Sea vertically (Fig 1). Considering its vast coverage, EIW have a great potential in terms of marine bacteria biodiversity. Other than that, these waters are situated in the coral triangle area, one of the hotspot of world biodiversity.

However, marine bacteria are still under-explored in this area. Zobell and Morita (1959) were the first to report about deep-sea marine bacteria isolation attempts from Banda Sea during Galathea expedition in the 1950s, but not much were explored afterwards until early 2000 (Tapilatu, 2011). There were gaps in common knowledge on marine bacteria existence in EIW, in particular about their potential biotechnological applications.

This succinct article was expected to fill the information gap about how far the efforts have been carried out to identify the potential bacterial resources in biotechnological applications, in order to encourage the intensification of studies in this field. Information on marine bacteria research and their potential use in biotechnology are indispensable in positioning the current state of marine bacteria research in Indonesia to better orienting the future direction of these research activities in a sustainable humanosphere context.

Marine bacteria refer to bacteria group that require 35 ‰ NaCl (or the average salinity of seawater) to grow and multiply optimally. Their size range from 0.5-5.0 micrometres in length, and spherical bacteria are about 1 micrometer in diameter. This type of microorganisms can be found from coastal waters to the deep ocean. Prior reports confirm its existence even at the depth of 10 000 meters of Mariana Trench (cf. Kato et al., 1998; Deming et al., 1988). They can be found either as free-living, external or internal symbionts to macroorganisms with various level of relationship, from beneficial to pathogenic.

Figure 1. Indonesian map. The Eastern Indonesian Waters (EIW) were indicated with the blue line.

Marine bacteria constitute one portion of the most abundant microorganisms in the sea and have multiple functions in marine ecosystems. They influence the biogeochemical process and play important roles as food for larger predators, as well as decomposer in marine trophic web. They also mineralize toxic compounds which enter the sea naturally and/or through human activities. Most importantly, some of them produce natural compounds which are beneficial to humans particularly in biotechnological applications.

Biotechnology refers to technological applications using biological systems, living organisms, or derivatives thereof, to make or modify useful products or processes for human specific use. To date, marine microorganisms have been used as resources in various biotechnological applications worldwide (for a good review on the subject, please see Imhoff et al 2011). Albeit the rapid expansion of its exploration and consequent use in biotechnology worldwide, growing interest on the development of bacterial utilization in this particular field could only be noticed in the last fifteen years in Indonesia. Discoveries were made by various researchers, international and Indonesians alike, on diverse aspects of marine bacteria isolated from EIW. Regarding marine bacteria in Indonesia, the potential usages explored were mainly on bioremediation and pharmaceutical industries, as well as on mariculture, food and pulp/paper industries. Isolation attempts were not always carried out, and for the most part the efforts done concerning bioprospecting using the molecular biology approach or chemical fingerprints. Not much of the research carried out about marine bacteria in EIW compared to those carried out in the western part. During the last fifteen years, from a total of about eighteen papers published on marine bacterial isolation or exploration for biotechnology purposes, only four of them were using bacteria sampled from EIW.

Marine bacteria with potential biotechnological features from EIW

Radjasa et al. (2007) begun the exploration of this type of bacteria by trying to isolate psychrophilic bacteria from sea water sampled at about 400 to 800 m at Makassar Strait. They succeeded in isolating 27 Gram negative isolates, having similar 16S rRNA sequences with bacteria from *Psychrobacter, Pseudomonas* and *Vibrio* genuses. *Psychrobacter* is the group of bacteria known to have adapted to cold temperature. The latter two genuses are known to be commonly found in marine environment. The isolation was not followed by further identification of metabolites or enzymes produced, but existing literatures on *Psychrobacter* indicate the potential of this group to produce lipolytic enzymes that can be applicable in versatile industry fields, notably those with systems operating at low temperature (see Elleuche et al., 2016).

Murniasih and Rasyid (2010) carried out bacterial symbionts isolation attempts from sponges growing at Barang Lompo coast, South Sulawesi. From seventy five pure isolates obtained, sixty indicated the capacity to inhibit the growth of *Bacillus subtillis, Staphylococcus aureus* and *Vibrio eltor.* However, further identification of these isolates as well as type of active substances produced is yet to be done.

At the same year, three mollusks' (*Conus miles*) symbionts with 16S rRNA sequences identical with *Pseudomonas* and *Vibrio* were isolated from Bastiong Coast in Ternate, North Maluku (Pringgenis, 2010). Antibacterial activity tests indicated that these isolates could inhibit multiple drugs resistant pathogenic bacteria such as *Klebsiella, Pseudomonas, Staphylococcus, E. colli* and *Enterobacter*. Bioactive compounds analysis using GC-MS revealed that these three symbionts produced antibacterial agent from acid group that could prove useful in pharmaceutical application, in

particular in disinfectant industry.

In 2012, Yulianti et al. reported the isolation of L-glutaminase producing bacteria from Sangihe Talaud Sea water, taken at the depth of 400 m. This bacteria 16S rRNA sequence was 96% similar with *Pseudomonas aeruginosa* strain CG-T8. L-glutaminase is an enzyme known to be used as flavor enhancer and antileukemic agent.

If previously mentioned studies were conducted mainly on the Sulawesi and North Maluku waters, in 2015 a study was published about antimicrobial compound producing bacteria from Papua waters. Mogea et al. (2015) reported the identification of antimicrobial compound produced by *Pseudomonas aeruginosa* NS3. This bacteria was a symbiont isolated from an ascidian (*Phallusia julinea*) found in Doreri Gulf, Manokwari, West Papua. The antibacterial compounds produced contain phenazine, and it showed inhibition activity against Methicillin-resistant *Staphylococcus aureus*, Entero-pathogenic *E. coli*, *Candida albicans.*

Marine bacteria from Maluku waters were only recently explored for their potential use in biotechnological applications. This was due to lack of infrastructures, as well as competent human resources with particular interest on this field. Preliminary studies conducted indicated that these types of bacteria can be isolated from Maluku waters. For example, a marine bacteria producing exopolysaccharide (EPS), a chemical compound known to have potential use in diverse areas of biotechnology industries (pharmaceutical, food and medical) was isolated from sediment collected at 400 m from Ambon Outer Bay (Tapilatu, 2012a). Analysis of its 16S rRNA sequence indicated its similarity with *Bacillus subtilis*, known as a producer of EPS (cf. Ivanova et al., 1999). Another attempt carried out in 2012 led to the isolation of pigment producing bacteria from seawater collected at the surface of Ambon Bay (Tapilatu, 2012b).

In 2013, a further attempt in Ambon Bay waters led to the isolation of two pure actinomycetes isolates with the capacity to produce antibacterial compounds (1-octadecene and methylisoborneol) as their secondary metabolites (Tapilatu, 2015).

The most recent attempt was the isolation of bacteria producing antibacterial activity from sediment samples retrieved at the depths of 100, 115 and 1 457 m in Aru Sea (Tapilatu, 2016, Fig 2). This attempt led to the isolation of ten bacterial isolates. The 16S rRNA sequence analysis of two isolates, namely isolates PPLD3 and PPLD5, indicated their potential as producer of beneficial metabolites.

Isolate PPLD3 16S rRNA sequence was 98% similar with *Paracoccus zeaxanthinifaciens* strain ATCC 21588. This bacteria produce zeaxanthin, a yellow pigment from carotenoid group that is used in the poultry feed industries to enhance the pigmentation of the end product. Other than that it is also used in the prevention of age-related macular degeneration in humans (Berry et al., 2003).

The second isolate, PPLD5 was identified as having 98% similarity of its 16S rRNA sequence with *Bacillus mojavensis* B0621A. The latter bacterium was isolated from *Pinctada martensii*, a pearl oyster found in the South China Sea and known to produce three lipopeptides that indicated, among others, cytotoxic activities against the human leukemia (HL-60) cell line (Ma et al., 2012). However, further identification efforts remain to be carried out on the isolates PPLD3 and PPLD5, to confirm their capacity in producing beneficial metabolites compounds.

These works, albeit very preliminary, confirmed again the high biodiversity of marine bacteria isolated from Indonesian waters, as well as their multiple potential uses in biotechnological applications. They indicated the vast resources of marine bacteria yet to be explored for their potential use in biotechnology.

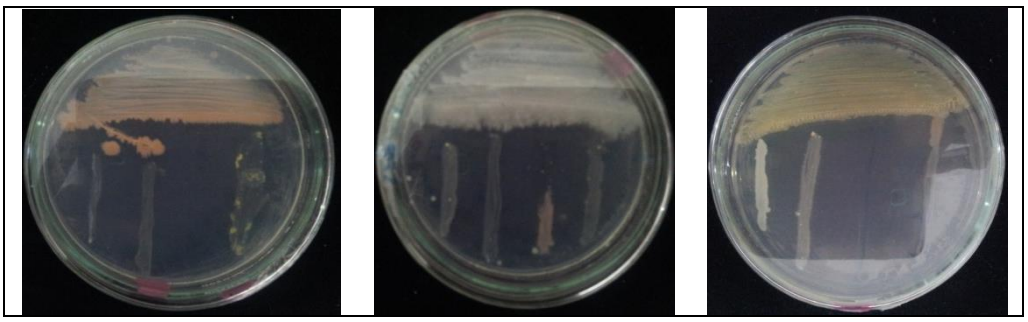

Figure 2. Three of ten pure isolates obtained in an ongoing antibacterial activity test. All were isolated from sediment sample retrieved at 1000 m depth in Arafura Sea.

2. Conclusion and perspective

There are still a lot of works left to be done in the future especially in EIW. A part of these waters is included in the Wallacea, the virtual biogeography line which indicated one of the biodiversity hotspots worldwide. This area surely harbors various marine bacteria of biotechnological interest.

Considering the great potential of marine bacteria studies in Indonesia, and particularly at the EIW, the future works could include the isolation and characterization of other group of marine microbes (archaea, fungi) with useful properties for biotechnological applications, such as in optical data storage and food industries production. Research activities on biotechnologically potential bacteria should be intensified, giving the fact that this group of marine procaryotes is relatively well characterized and identified. Beside the aforementioned biotechnological applications, the research could also explore the potential use of marine bacteria in producing various enzymes for molecular biology and diagnostic kits, as well as in nutraceuticals and cosmetics industries. Other potential uses include biopolymer production for numerous applications ranging from biodegradable plastics to pharmaceutical and medical polymers such as dental biomaterials and bio-adhesives (Imhoff et al., 2011).

Studies on marine extremophilic microbes constitute another aspect of future works to be intensified because of their potential in biotechnological applications. For this purpose, explorations could be carried out on EIW with depth more than 1000 m, such as in Banda Sea (average depth 4500 m) or Sulawesi Sea (average depth 5000 m). These future studies could only be possible within regional and international collaborative research scheme, as large numbers of infrastructures, as well as equipments and human resources will be needed to accomplish them.

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