A review of studies on bacteria in the Banda Sea over the past seven decades (1950-2017)

Y Tapilatu¹

¹Center for Deep-Sea Research, Indonesian Institute of Sciences (PPLD LIPI), Jl. Y. Syaranamual Guru-guru Poka Ambon 97233, Indonesia

yosmina.tapilatu@lipi.go.id

Abstract. Studies on marine bacteriology in the Banda Sea were started from the 1950s. This paper is an attempt to review all works carried out in the last seven decades. Literature reviews indicated that there was not much research done on marine bacteria in the Banda Sea and adjacent waters such as Ambon Bay. Studies on bacterial biomass, indicator bacteria on water quality monitoring as well as bacteria potential as a producer of secondary metabolites were carried out. However, considering Banda Sea ecosystem that is mostly consisted of deep environments, it would be interesting to prioritize research activities on piezophiles or at least piezotolerant marine bacteria. There is thus an urgent need to determine the priorities in studying marine bacteria in this ecosystem, which should be focused on deep-sea ones.

Keywords: Banda Sea, marine bacteria, deep sea

1. Introduction

The Banda Sea (figure 1) is included in the Coral Reef Triangle Initiative (CTI) area. The CTI area is known to be the global centre of marine biodiversity [1]. The Government of the Republic of Indonesia has issued a Ministerial Decree No. 69/2009 stating the Banda Sea as one of the National Marine Conservation Area [2]. This is because the Banda Sea has natural resources that are yet to be utilized optimally. One of these under-explored resources from the Banda Sea is its marine bacteria.

Marine bacteria refer to the bacterial group that requires 35 ppm NaCl (or the average salinity of seawater) to grow and multiply optimally. Their size ranges from 0.5-5.0 micrometres in length. Spherical bacteria sizes are about 1 micrometre in diameter. This type of microorganisms can be found from coastal waters to the deep ocean. Prior reports [3,4] confirm its existence even at the depth of 10 000 meters of Mariana Trench. They can be found either as free-living, external or internal symbionts to macroorganisms with various level of relationship, from beneficial to pathogenic.

This bacterial group constitutes one portion of the most abundant microorganisms in the sea and has multiple functions in marine ecosystems. They influence the biogeochemical process and play important roles as food for larger predators, as well as the decomposer in the marine trophic web. They also mineralize toxic compounds which enter the sea naturally and/or through human activities. Some of them produce natural compounds which are beneficial to humans, particularly in biotechnological applications. Most importantly, marine bacteria are responsible in providing fifty percent of the oxygen that we breathe.

There are many reviews that have been published about marine bacteria in Indonesia [5] and more particularly about those from eastern Indonesian waters [6]. These reviews highlighted on studies...
about the biotechnology application potential of marine bacteria secondary metabolite from the area. However, to our knowledge none has been done especially about those carried out in the Banda Sea and adjacent waters under its influence, i.e Ambon Bay. This paper is an attempt to provide a comprehensive literature review, with the objective to provide the most updated review on marine bacteria studies carried out in the Banda Sea and Ambon Bay. It was expected to be a reference for future studies that will be carried out regarding marine bacteria in the aforementioned area.

Figure 1. Maps of Indonesia. The dashed line indicates the geographic area of the Banda Sea.

2. Methodology
Previously published literature contained information on marine bacteria studies in the Banda Sea from the last seven decades (1950-2016) were used as primary sources. Google search engine was used to initially track published literature available electronically in English and/or Indonesian languages, using combined keywords such as “marine bacteria banda sea” and “marine bacteria ambon bay”. This was because most of the published works were either written in Indonesian, or few of them were indexed by reputable scientific literature search engine. The results obtained served as starting point for further literature search, mainly by searching for reference lists in reviews and original research articles. Articles which were not available electronically were hand-searched in relevant journals available in our institution's library. There were several minimum criteria for inclusion. The first criterion included literature published in international, national and local journals/proceedings and graduate thesis. The full-text availability of literature obtained, and the relevance of the results presented with the context of this paper constituted the second and the third criteria.

3. Results and discussion

3.1. Research history and challenges
There were only eight articles published about original research on marine bacteria from the Banda Sea and Ambon Bay during the last seven decades (1950-2017) (Table 1), which means that there was -in average- one article published every decade. This was very low in quantity compared to studies on marine bacteria in other parts of Indonesia. For example, literature search on line back in 2011 [5] and recently indicated that there were at least 27 original research articles published about marine bacteria studies in other parts of Indonesia in the period of 1970 to 2010.
Table 1. Summary of bacterial studies in the Banda Sea, 1950-2017.

| No | Sampling area  | Study type                                      | Methods used                          | Key findings/results                                                                 | Publication year | Reference |
|----|----------------|-----------------------------------------------|---------------------------------------|--------------------------------------------------------------------------------------|------------------|-----------|
| 1  | Banda Sea      | Exploration of deep-sea bacteria              | Conventional isolation using selective media | Isolation of deep-sea starch hydrolizers, nitrate reducers, sulphate reducers, ammonifiers and nitrifiers bacteria | 1959             | [7]       |
| 2  | Ambon Bay      | Indicator bacteria (coliform, faecal coli and faecal Streptococcus) | Most Probable Number (MPN) | Lower number in Ambon Bay compared to Jakarta Bay and Banten Bay                     | 1988             | [8]       |
| 3  | Banda Sea      | Marine bacterial ecology                      | Adenosine Tri Phosphate (ATP) measurement of two different periods | Bacterial numbers were not affected by the monsoon period                             | 1990             | [9]       |
| 4  | Banda Sea      | Marine bacterial ecology                      | Indirect measurement of cell numbers using Phycoerythrin absorbance assessment | Synechococcus-type species prefer deeper water column (twilight zone) and indicated diurnal pattern based on their low surface abundance | 1990             | [10]      |
| 5  | Ambon Bay      | Indicator bacteria                            | MPN                                   | Detection of coliform total, fecal coliform and E. coli at Lata village waters       | 1996             | [11]      |
| 6  | Ambon Bay      | Hydrocarbon-degrading bacteria (HCB) number assessment | MPN                                   | Higher density of HCB number at Ambon Inner Bay than at Ambon Outer Bay              | 1997             | [12]      |
| 7  | Ambon Bay      | Heterotrophic bacteria                        | TPC                                   | Higher density of heterotrophic bacteria at Ambon port waters, due to anthropogenic activity, with vertical distribution pattern in accordance with dissolved oxygen concentration in Ambon Bay | 1998             | [13]      |
| 8  | Ambon Bay      | Exploration of secondary-metabolite producer bacteria | Conventional isolation using selective media and initial assessment of the secondary metabolite produced profiles | Isolation of putative agarolytic bacteria, exopolysaccharide-producing bacteria and marine Actinomycetes | 2015             | [14]      |
This accounted for about six articles per decade in forty years. Five from eight published studies (Table 1) were carried out at Ambon Bay area. Three main subjects were treated, namely exploration of bacteria with potentially important functions (either ecologically or commercially) [7,14], marine bacterial ecology [9,10] and indicator bacteria as a part of Ambon Bay water quality monitoring [8,11-13]. These subjects were less varied compared to marine bacterial research carried out in other parts of Indonesian waters, with also varied methodologies (c.f [5] and references therein).

It is interesting to note, however, that the earliest study about marine bacteria in the Banda Sea was also the first marine bacteria study ever published that was carried out in Indonesian marine environment. The study was reported in the late 1950s, as part of the Danish world expedition, Galathea 2 that took place in 1951. Weber Deep, Banda Sea was one of the stations where ZoBell and Morita collected sediment samples from 7 250 m for subsequent incubations and attempt of isolations of putative in situ deep-sea bacteria [7]. They were the first to report the presence of starch hydrolyzers, nitrate reducers, sulphate reducers, ammonifiers and nitrifiers that were active in the differential media at 700 atm, and suggested that these were indigenous species which were probably limited by the nutrient content of sea water and sediment and not by high hydrostatic pressure or low temperature. Further literature research indicated that there was no record of continued studies regarding this sample afterward. Nevertheless, this was one of the seminal works on deep-sea bacteria globally afterward.

Research activities on marine bacteria in the Banda Sea have practically halted afterward and restarted in the 1990s. Whereas in Ambon Bay particularly, marine bacteria studies were started by Thayib and Razak in 1988, when they carried out observations on bacterial indicator on Ambon Bay water quality compared to Jakarta Bay and Bay of Banten [8]. The reason for this long gap of activities could possibly due to lack of proper infrastructures to carry out the research in this field at that period. The Snellius II Expedition in 1984-1985 resulted in two original research articles on marine bacteria. The first one by Vosjan et al. [9] investigated on the microbial biomass, respiratory activity and bacterial numbers in the Banda Sea as a function of the two monsoon periods, northwest and southeast. The ATP measurement as relative biomass of less than 50 µm microorganism at the eastern part of Banda Sea indicated that it was not affected by the monsoon period. A similar trend was observed also when the respiratory activity was measured. They also found out that the bacterial numbers tended to increase during the night.

The second study was done by Zevenboom, who carried out research on the relative abundance of red-pigmented picocyanobacteria during August 1984 and February/March 1985 [10]. It was observed in this study that Synechococcus-type species showed a preference for deeper layers where light intensities were low, and indicated diurnal pattern based on their low surface abundance. Unfortunately, no further studies were carried out to follow up the results obtained. Yet these were few of the rare studies on bacterial ecology in the Banda Sea.

As adjacent marine environment that is heavily influenced by the Banda Sea, Ambon Bay became one of the locations where research activities were carried out intensively in the 1990s. This included the few studies on water quality indicator [11], hydrocarbon-degrading [12] and heterotrophic [13] bacteria, carried out from 1996 to 1998 in Ambon Bay coastal waters. Unfortunately, all research activities were suspended due to civil unrest from 1999 to 2004.

Studies on marine bacteria in Ambon Bay were restarted in around 2010, on water quality indicators and continued until 2016. In parallel, preliminary studies about marine bacteria from this area and their potential as producers of bioactive compounds were conducted (cf. [14]). Nevertheless, no studies were carried out on marine bacteria in the Banda Sea after 2004 to our knowledge. This was due to several factors, i.e. lack of adequate funding, infrastructure, and competent human resources. Sampling effort requires substantial fund, as expedition in the Banda Sea is costly, in terms of logistic and technical details to accommodate all researchers involved. With its vast area, sampling in the Banda Sea cannot be accomplished in a single expedition of ten cruising days, as were carried out in the last five years by Center for Deep-Sea Research, Indonesian Institute of Sciences (LIPI). As they can only be seen using microscopes and takes hours of bench works and
expensive culture media ingredients, marine bacteria are not one of the priorities for the research to be funded, in contrast with other marine organisms that are easily visible, colourful and highly publicized.

Other than that, lack of proper and basic infrastructures in marine bacteria hinder studies in this field on Banda Sea ecosystem. For instance, as per author’s experience, it is difficult to obtain adequate storage facilities for samples, let alone isolates, such as -80°C freezer or liquid nitrogen container in Maluku Province’s capital, Ambon. This is not only due to the limited funding in government institutes research equipments purchase but also in the absence of seller or dealer of substantial provision such as liquid nitrogen.

The limited funding and infrastructures combined with limited competent human resources in the field worsen the situation. Informal discussions carried out with the local undergraduate students indicated that they feel discouraged to pursue interest in marine bacteria studies because of the long hours spent in laboratory works and the expensive culture media or molecular biology kits and disposable items.

3.2. Strategies for future research

Applied bacterial studies are important aspects that should be put forward in the future orientation of research in the Banda Sea. As previously mentioned in the introduction, the development of marine and fisheries sector in Banda Sea ecosystem is important for Indonesia, in particular for the people of Maluku Province. Limited infrastructures, research funds and competent human resources require the following strategies: 1) Prioritization of the understudied deep-sea bacteria research, notably the exploration of those with potential application in biotechnology; 2) Provision of the existing laboratories with adequate equipment for sampling, analysis, and preservation of deep-sea bacteria; 3) Provision of multi-years funding for the community structure analysis and bioprospecting works; 4) Preparation of the critical mass of researchers in the field of marine bacteria.

The first strategy is needed to optimize the utilization of the limited available resources. Marine microbes in the Banda Sea have yet to receive the attention they deserve as one of, if not the most, important actors in this environment. However, considering the challenges faced as detailed in the previous section (3.1), prioritization is necessary to ensure that the available resources are put to good use. Other than what has been done by Schwartz and Morita in the 1950-s, not much if any is known about the role of deep-sea bacteria in general in Banda Sea ecosystem. Specific studies such as bacterial community structure need to be carried out to gain better understanding of these roles. In parallel, considering the great potential of deep-sea bacteria of Banda Sea as producers of beneficial compounds, bioprospecting works could be also carried out. An unpublished report of preliminary attempts in isolating putative exopolysaccharide-producing bacteria from Ambon Bay sediment (400 m) [15] yielded one isolate capable of producing such compound. Bacterial exopolysaccharide has wide range application in biotechnology, including pharmacy [16].

Adequate equipments for sampling, analysis, and preservation of deep-sea bacteria in relation with the first strategy will thus be indispensable. This could be done in multiple years and could be started with basic equipments such as -80°C freezer, liquid nitrogen container, pressurized microbial incubation chamber or adaptable shakers. The first two are needed for isolate storage. The pressurized microbial incubation chamber will be useful to mimic the living condition in the deep sea. The adaptable shaker will be necessary to ensure the provision of oxygen inside the pressurized chamber.

The third strategy (continual funding) is essential in ensuring that the work carried out would not be done only in initial phases and had to be stopped due to the lack of funding, but would be accomplished thoroughly. This could be secured not only from internal funding but also through collaborations with other institutes or universities, in national and international levels.

In regard with the fourth strategy, human resource with skills in marine bacteria is lacking in Maluku Province. As a consequence, efforts should be done to raise young and/or potential researchers’ awareness on the importance of advancing studies on bacteria from Banda deeper waters.
There is a need of representing the marine bacteria subject as not an intimidating one, and this would take further socialization and communication works to the young generation early on.

4. Conclusion
There is still a lot of work to be done about marine bacteria in the Banda Sea. Bacteria from Banda deeper waters are still under explored. Challenges in marine bacteria study in the Banda Sea require strategies that should be carried out to ensure accomplishment of necessary research works. These studies would not only contribute to the basic knowledge about their presence and potential roles in Banda Sea ecosystem but also could lead to the discoveries of potential producers of beneficial compounds. These future studies could only be possible within regional and international collaborative research scheme, as large numbers of infrastructures, as well as equipment and human resources, will be needed to accomplish them.

References
[1] Burke L, Reytar K, Spalding M and Perry A 2011 Reefs at risk. Revisited (Washington DC: World Resources Institute) p 124
[2] Indonesian Minister of Marine Affairs and Fisheries 2009 Ministerial Decree on the Marine Protected Area Establishment in Maluku Province No 69/2009 [Internet] accessed on Apr 12th, 2017 from http://kkji.kp3k.kkp.go.id/index.php/dokumen/regulasi-hukum/keputusan-menteri/finish/14-ke-putusan-menteri/187-kepmen-kp-no-kep-69-men-2009-tentang-penetapan-kawasan-konservasi-perairan-nasional-laut-banda-di-provinsi-maluku
[3] Kato C, Li L, Nogi Y, Nakamura Y, Tamaoka J and Horikoshi K 1998 Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters Appl. Environ. Microbiol. 64 1510–13
[4] Deming J W, Somers L K, Straube W L, Swartz D G and MacDonald M T 1988 Isolation of an obligately barophilic bacterium and description of a new genus, Colwellia gen. nov Syst. Appl. Microbiol. 10 152–60
[5] Tapilatu Y 2011 Marine prokaryotes in Indonesia: research history and potential use in biotechnology Proc. 1st Int. Symp. for Sustainable Humanosphere Oct 3, 2011 (Ambon: R & D Unit for Biomaterials-LIPI) pp 21–9 doi: 10.13140/2.1.3345.8563
[6] Tapilatu Y 2016 Marine bacteria from eastern Indonesia waters and their potential use in biotechnology Omni Akuatika 12(1) 81-5 doi: http://dx.doi.org/10.20884/1.oa.2016.12.1. 32
[7] Zobell C E and Morita R Y 1959 Deep-Sea Bacteria Galathea Report 1 139–54
[8] Thayib S S and Razak H 1988 Observation on the bacteriological indicator, heavy metal and pesticide at Ambon Bay, Banten Bay and Jakarta Bay, Perairan Indonesia: Biologi, Budidaya, Kualitas Perairan dan Oceanografi, pp 124–31
[9] Vosjan J H, Nieuwland G and Ruyitno 1990 Monsoon differences in microbial biomass, respiratory activity and bacterial numbers in the Banda Sea Neth. J. Sea Res. 25(4) 501–11
[10] Zevenboom W 1990 Picocyanobacteria in the Banda Sea during two different monsoons Neth. J. Sea Res. 25(4) 513–21
[11] Indah L and Chasnah E 1996 Kualitas Mikrobiologi Perairan Pantai Teluk Ambon Bagian Dalam Ditinjau Dari Segi Kesehatan Lingkungan Prosiding Seminar dan Lokakarya Pengelolaan Teluk Ambon (Ambon: LON LIPI) pp 56–62
[12] Sutiknowati L I 1997 Sebaran Kepadatan Bakteri Pemecah Minyak di Teluk Ambon Seminar Kelautan LIPI–UNHAS (Makassar: LON LIPI–UNHAS) pp 260–64
[13] Sutiknowati L I 1998 Abundance and distribution of heterotrophic bacteria in Ambon Bay (a preliminary study) Perairan Maluku dan Sekitarnya 12 17–24
[14] Tapilatu Y 2015 Isolation of marine bacteria in Ambon Bay with potential biotechnological features Taprobanica 7(3) 143-50
[15] Tapilatu Y 2012 In search of exopolysaccharide–producing bacteria from Maluku deep–sea area Technical Report L’Oreal–UNESCO Indonesian National Fellowship for Women in Science 2011 p 4

[16] Tapilatu Y 2015 Status of Drug Discovery Research Based on Marine Organisms from Eastern Indonesia Proc. Chem. 14 484–92