

# New Tridecapeptides of the Theonellapeptolide Family from the Indonesia Sponge *Theonella swinhoei*

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**Submission date:** 13-Mar-2019 03:39PM (UTC+0700)

**Submission ID:** 1092547672

**File name:** 7\_Ecotourism.pdf (303.52K)

**Word count:** 2454

**Character count:** 12737

## 3 Ecotourism challenges occurrence of enteropathogenic in Manado bay and Bunaken National Park (BNP)

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(Received 20 August, 2012; accepted 20 September, 2012)

### 1 ABSTRACT

Development activities in coastal areas have to be concerned with the environmental carrying capacity, sustainability of natural resources, and have to be integrated with various activities related to the improving the welfare of local communities. Potential of BNP is so high, especially in relation to tourism, which is attractive to many international and domestic tourists. Therefore, as a part of sustainable management, the environmental condition especially water quality should be well known. Previous researches have shown that enteropathogenic bacterium was positive in Manado bay, all strains grown well at extreme conditions (pH, temperatures, and in NaCl). Also have been found in scad at "Bersehati" fish market, which could survive in the environmental conditions during selling. The aim of this study is to assess the water quality of Manado Bay and BNP, especially enteropathogenic bacteria. Samples were taken monthly from March to August 2011, at 5 locations, using GPS to ensure the sites. All samples were taken from surface layer (0-1 m layer), and filled into sterilized bottles, kept in an cool box, and than immediately transported to laboratory in less than an hour after collected. *E.coli*, Total *Vibrio sp.*, and *Vibrio cholerae* were assessed using MPN method. Samples of fish from the traditional market, and seaweed cultivated in BNP areas, were also assessed with the same method. Results shown that *Vibrio sp.* grown well in all locations, more than WHO standard. Although less than WHO standard, *Vibrio cholerae* and *E. coli* were found in BNP. In Manado bay both pathogenics were found more than WHO standard. Assessment on fish and seaweed also shown positive results.

**Key words :** Enteropathogen, *Vibrio sp.*, *Vibrio cholerae*, Water quality.

### Introduction

Biodiversity of "Bunaken National Park" (BNP) is one of the Indonesian sustainable investment, not only attractive for tourism, but also as a centre of world coral biodiversity. The park contain about 53% of the world reef biodiversity. The figures encouraged the United Nations (UN) to support

World Ocean Conference (WOC) and Coral Triangle Initiatif (CTI) summit 2009 in Manado, Indonesia, to discuss the ways to save the valuable coral reef from destruction by human as well as global warming.

Destruction of coral reef can increase intensity of UV-B, which can destroy phytoplankton as a primary resource in marine food chain. Reducing phytoplankton means reducing consumption of

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CO<sub>2</sub> in sea water, and that has been identified as one of many factors contribute to global warming. Many researches have shown that global warming contributed in occurrence and biological changes of marine pathogenic bacterium.

Manado Bay is a semi-closed waters, but is directly connected to Sulawesi sea, caused the bay very dynamics with strong tidal currents along the coast. Geographically, BNP is integrated with Manado bay, in where many of small and big rivers are terminated. Most of the domestic wastes are dumped into those rivers and will accumulate in Manado bay, and finally will affect BNP. Dien (1999) and Ijong and Dien (2001) found that some of big rivers terminating in Manado bay were already contaminated by *E. Coli* and *Vibrio sp.* with concentration higher than WHO standards.

Indonesian Ministry of population and Environment on the Letter of Decree No. Kep-02/MENKLH/1998, already stated that for tourism and recreation activities, water quality standard should be Coliform <1000, and pathogenic bacteria have to be nil. Distribution of *Vibrio sp.* in marine environment especially in tropical areas are very wide, and commonly found as contaminant (Kiiyukia *et al.* 1991). Cholera caused by *V. cholerae* is a global killer.

Contamination of pathogenic bacteria in human can happen through two ways, i.e. direct and indirect contacts. Direct contact for example contact through wound, skin, ear, nose and mouth. Indirect contact is by accidentally drinking sea water, or consumed a contaminated seafood (US-EPA, 2005). Some of infected bacteria such as *Vibrio Sp*, Coliform group (*E. coli*, *Salmonella*, *Shigella*) were originated from sea water, faeces, waste, and raw food (Walters, 2005).

Infection diseases are already global health problems. In Indonesia, the diseases especially occur in coastal regions. A report from Bengkulu Health Office 2003, stated that dominant diseases in coastal regions are: skin diseases, upper tract respiratory infection, malaria, diarrhoea, ear infection, tuberculosis, and dengue fever. Manado Public Health Centre (2002) reported that dominant diseases in coastal regions are: diarrhoea, skin diseases, typhoid and cholera.

The aims of this study are to assess enteropathogenic bacteria in Manado bay and BNP.

## Research Methodology

### Sampling Methods

Water sample were taken at early morning (6 to 7am), from five locations, i.e. in front of Liang, Bunaken (Negeri), Kuala Jengki (Tondano river), Sario, and Bahu (Fig. 1), with a distance of 10-50 meters from beach line. Samples were taken using sampling bottles and immediately filled into sterilized sample bottles, kept in cool box, and then immediately transported to the laboratory.

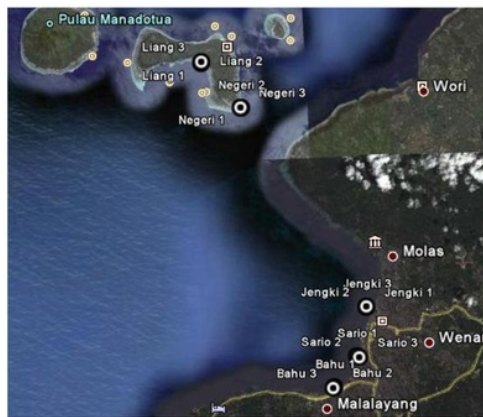


Fig. 1. Map of sampling locations

As supporting data, samples of fish from Manado's fish market and seaweeds from cultivated areas in BNP, were also taken and analyzed for the same assessments as water samples.

### Microbiological Assessments

Microbiological assessment consists of total bacterial count and identification of pathogenic bacteria. Total bacteria count have been done for coliform (TC) and *E. coli* (TEC). Sample was inoculated into enriched BGLB media, and incubated at 37°C. All positive samples were streaked to Eosin Methylene Blue (EMB) agar, and then incubated at 37°C. After one day, the growth of typical *E. coli* were observed, where pink color for TC and metallic for TEC.

Total Vibrio (TV) had been counted by inoculated sample into enriched Alkaline Pepton Water (APW) media, and incubated at 37°C. All positive sample were streaked to Thiosulphate Citrate Bile Salt

Succrose (TCBS) agar, and then incubated at 37°C. After one day, the growth of typical *Vibrio cholerae* were observed, and were counted using MPN Table (Bloggett, 2006)

4 Isolation and identification based on fisiological and biochemical assessments using Bargey's Method (Holt *et al.* 1994).

## Results and Discussion

### Enteropathogenic in Water

#### Total coliform

Distribution of coliform in Manado bay (Malalayang, Sario, and Tondano river) ranged between 74-1100 cfu/100 mL, while in BNP (Liang, Bunaken-Negeri) between 9 – 760 cfu/100 mL (Fig. 2). The figure shows that total coliform in Manado bay already exceeded Indonesian Government Standard, while in BNP the highest (> 200 cfu/100 mL) only found in Liang during June to August and in Bunaken during August.

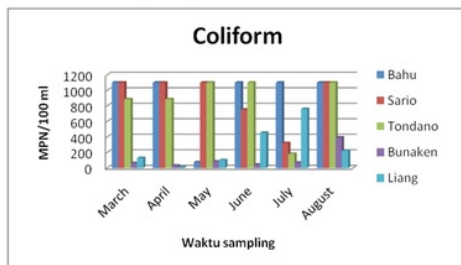


Fig. 2. Distribution of coliform

The high amounts of coliform in Manado bay, caused by domestic waste came from Manado city by the rivers. In BNP total coliforms are usually low (<200 cfu/100 mL), except during June to August because the rainy season, where the water from the rivers of Manado can reach BNP.

#### *E. coli*

Distribution of *E. coli* ranged between 0 – 570 cfu/100 mL, where in Manado bay ranged between 13 – 570 cfu/100 mL, while in BNP it ranged between 0 – 38 cfu/100 mL.

According to WHO (1968) and APHA, the maximum allowed for drinking water is 1 cfu/100 mL for swimming pool 200 cfu/100 mL, and for recre-

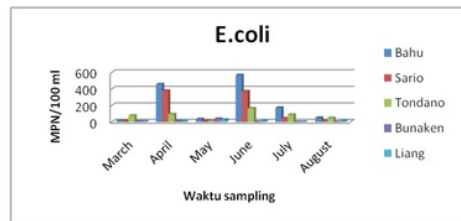


Fig. 3. Distribution of *E. coli*

ation 1000 cfu/100 mL. Disease associated to *E. coli* is diarrhoea.

#### Total *Vibrio sp*

Distribution of Total *Vibrio* ranged between  $1.6 \times 10^1$  –  $1.1 \times 10^3$  cfu/100 mL, where in Manado bay ranged between  $6.0 \times 10^1$  –  $1.1 \times 10^3$  cfu/100 mL, while in BNP ranged between  $1.6 \times 10^1$  –  $3.9 \times 10^2$  cfu/100 mL (Fig. 4).

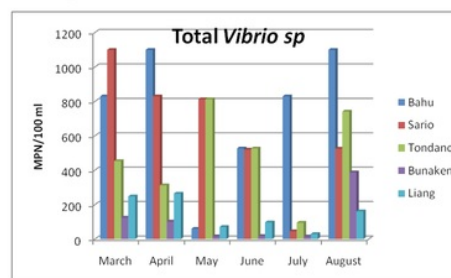


Fig. 4. Distribution of total *Vibrio sp*

Like coliforms, total vibrio in Manado bay was higher than in BNP. This is also because of domestic waste disposal. *Vibrio* could pose serious problems if found in swimming or diving area, because it can cause many problems to public health. The case of infection by *Vibrio sp.* in recreation area in USA reported by Fewtrell and Jones (1992) are leg gangren, endotox shock, ear infection, and atitis externa. Dien (1999)b also found *Vibrio cholera* and *V. parahaemolyticus* in Manado Bay.

*Vibrio sp.* are halophile and can grow in sea water with salinity range of 20-40‰ (Anonymous, 2011). Salinity of Manado Bay ranged between 27.0-34.0‰. The same as salinity, pH value during observation also in the range of optimum pH for *Vibrio sp.* to grow, i.e. pH 6.5-8.5.

#### *Vibrio cholerae*

Distribution of Total *V. cholerae* ranged between 0.4

$\times 10^1 - 1.1 \times 10^3$  cfu/100 mL, while in Manado bay ranged between  $0.8 \times 10^1 - 1.1 \times 10^3$  cfu/100 mL, while in BNP ranged between  $1.6 \times 10^1 - 1.9 \times 10^2$  cfu/100 mL (Fig. 5). Fig. 5, shows that *V. cholerae* at Manado bay were higher than in BNP, especially during March-May, while in BNP the highest were found during March-April, and also August, but still less than 200 cfu/100 mL.

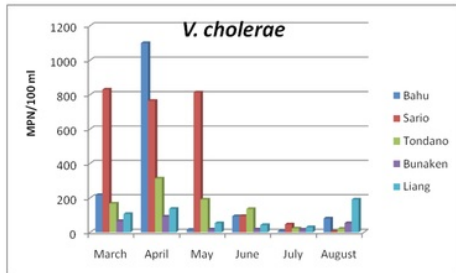


Fig. 5. Distribution of total *Vibrio cholerae*

### Enteropathogenic in Fish and Seaweeds

#### In Fish

The occurrence of *V. cholerae* in fish was assessed in Yellowtail (*Caranx leptolepis*) ranged between  $1.5 \times 10^1 - 1.1 \times 10^3$ . Figure 6 shows that 3 bacteria, i.e. Coliform, *E. coli*, and total vibrio were dominant, while *V. cholerae* even though low but was still found, especially during August.

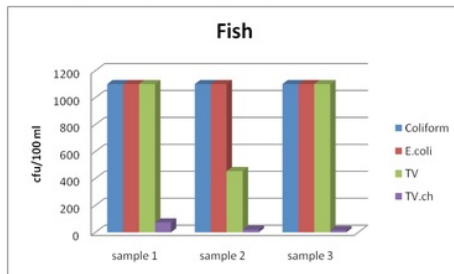


Fig. 6. Distribution of enteropathogenic in fish

Naturally, fish is not sterile. In certain part of fish i.e. in skin contained bacteria  $10^2 - 10^6$ /cm<sup>2</sup>, gills  $10^3 - 10^5$ /cm<sup>2</sup>, and intestine  $10^3 - 10^7$ /cm<sup>2</sup>. However, the flesh of healthy fish is sterile. Contamination of *V. cholerae* in fish and other seafoods, can cause foodborne diseases. Food poisoning caused by *V.*

*parahaemolyticus* occurs very often (40-70%) in Japan, because they eat fresh seafood (shasimi). The Case of contamination *V. vulnificus* in oyster, also occurred in the coast of California, Oregon, Washington, and British Columbia. According to Anonymous (1999) about 20 million Americans consumed fresh oyster every year. In fact, *Vibrio* not only caused problem to human, but also caused diseases to other biota, such as coral reef and seaweeds.

#### In Seaweed

The occurrence of *Vibrio cholerae* in seaweeds ranged between  $0.7 \times 10^1 - 1.1 \times 10^3$ /cm<sup>2</sup>, where dominated by Coliform, *E. coli*, and total *Vibrio*. Figure 7 shown that coliform still high during July and August, while *E. coli* dominated in July and then decreased sharply in August. On the other hand, total *vibrio* which was low in July increased sharply in August. *Vibrio cholerae* although low, but still exist in seaweeds. Cultivated area of seaweeds in BNP areas are usually close to villages, so poor sanitation and hygiene in villages can affect the occurrence of pathogenic in fish and seaweeds.

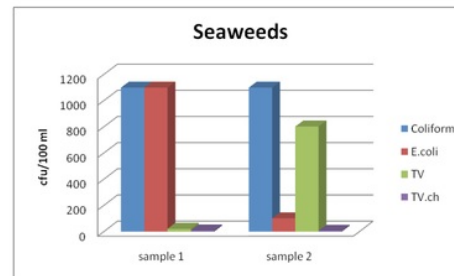


Fig. 7. Distribution of enteropathogenic in seaweeds

### Conclusion

Total coliforms were very high in Manado bay during March to August, and in BNP during June to August in Liang and August in Bunaken village. *Vibrio sp.* grew well in all locations more than WHO standard except in Bunaken village. Although less than WHO standard, *Vibrio cholerae* and *E. coli* were found in BNP. In Manado bay both pathogenics were found to be exceeding than WHO standards. Assessment of fish and seaweed also shows positive results.

To reduce domestic waste of Manado's rivers is

very important, in order to keep the Manado bay especially BNP suitable for international tourism destination in the future.

### Acknowledgments

This research was supported by BPPS scholarship, Directorate General Higher Education, Indonesian Ministry of Education and Cultural.

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