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Application of Microbe Consortium Bacillus maritimus and Virgibacillus Chiquensis from Sea Cucumber as Nitrate Booster in Semarang Wastewaters Potentially for Plants Bioactivator

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Abstract: This study aims to increase nitrate content in waste water after the introduction of consortium microbes from the species *Bacillus maritimus* and *Virgibacillus chiquensis* Waste water samples were collected from 3 different sites in Semarang city, namely Pekunden, Tembalang, and Kaligawe. Consortium microbe in the concentration of 10 mL was added to 100 mL sample from each site prior to incubation for 4 days. The results showed that there was a decrease in microbe density from 12×10 CFU. mL^{-1} to 3.0×10 CFU.mL^{-1}, meanwhile nitrate content increased almost six folds after the introduction of consortium microbes. Identification of pathogenic microbe in the samples found the species *Staphylococcus aureus* to be most prevalent. This study concluded that consortium microbe from the species of *Bacillus maritimus* and *Virgibacillus chiquensis* can be applied to increase nitrate content in waste water, which is usable as a component in bio activator for plants.

Key words: waste water, consortium microbes, nitrate, bio activator

1. Introduction

Indonesia has a high variety of marine and terrestrial biodiversity. This natural wealth has the potential to be developed to meet the needs of human life. As human population increases, the exploration and exploitation of marine biota is also increasing to be utilized mainly in the field of food through agricultural activities. One of the potential marine resources to exploit is sea cucumber. Sea cucumbers are marine biota that live on the seabed and are included in groups of animals that eat organic and suspended materials [1].

In digestive tract of sea cucumber *Holothuria atra* is known to live the type of symbiont bacteria *Bacillus maritimus a*nd *Virgibacillus chiquensis*. The bacteria are predicted to increase the nitrate content. The bacteria are known to synergize therefore they can be put together into a bacterial consortium tested for wastewater. Agents of organic material bioremediatory consortium can improve the quality of environment more efficiently and also have the ability to suppress the emergence of pathogenic bacteria. Synergistic interactions between bacterial consortia or their interactions with the environment cause degradation of organic contamination in waste [2]. In aquaculture, consortium bacteria are prepared as biocontrol and probiotics, has an antagonistic role for pathogens or can able to improve water quality as a bioremediant [3]

2. Material and Methods

The samples in this study were symbiotic microbes isolated from digestive track of sea cucumbers from Jepara waters, Indonesia. The results of identification

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of symbiotic bacteria by the 16S rDNA/PCR amplification method concluded that these types of bacteria were *Bacillus maritimus* and *Virgibacillus chiquensis.*

2.1 Source of Wastewater

Wastewater samples were taken from 3 different locations in Semarang city, namely Pekunden, Tembalang, and Kaligawe.

2.2 Isolation and Identification of Bacterial Colonies

Isolation of wastewater bacteria and pathogenic bacteria from the most odorous wastes, test the antibacterial activity of the bacterial sea cucumber consortium against bacteria that cause water pollution, measure bacterial density from each sample, test bacterial activity were carried out [4] The selected pathogenic bacteria samples collected were bacteria that had a very strong and dirty odor and high bacterial density. Identification of pathogenic bacteria by examining colony morphology, form of bacterial colonies, form of bacterial colonies, elevation of bacterial colonies, structure of bacterial colonies, colony shape, and color of bacterial colonies.

2.3 Measurement of Nitrate and Phosphate Content

Nitrate and phosphate content was determined by means of NitraVer Nitrate 5 Reagent Powder Pillow and PhosVer 3 Phosphate Reagent Powder Pillow for each wastewater sample and then measured spectrophotometrically. Measurement of pH and temperature of wastewater was done manually using pH paper and thermometer.

2.4 Treatment of Waste Water

100 mL of each wastewater sample from 3 different locations was poured into three different Erlenmeyer flasks (volume 250 mL) then 10 mL microbial consortium was added to each flask before incubation for 4 days.

2.5 Gram Staining of Bacteria

Gram staining of bacteria was determined by violet crystals and safranin procedure [4]

2.6 Test the Potential of Sea Cucumber Symbiotic Consortium As Anti-Bacterial

Test of the potential of sea cucumber symbiotic consortium as an anti-bacterial pathogen was carried out by dripping 10 µL of methanol extract from the bacterial consortium on a paper plate then affixed to media that had been inoculated with pathogenic bacteria obtained from 3 different wastewater sources in a petri dish. The inhibitory zones formed were given a positive sign and vice versa if there was no zone of inhibition then given a negative sign [5].

3. Results and Discussion

3.1 Odor Test Results

Water aroma test results from 3 different waste sources are presented in Table 1. As seen in Table 1, Pekunden and Tembalang wastewater were smelled of bad odor compared to control. While, Kaligawe wastewater was remained smelling bad after being incubated for 4 days.

3.2 Identification of Wastewater Pathogen Bacteria

The results of identification of the morphological character of bacterial colonies showed that the bacteria were filamentous, irregular and circular. The edge of the colony varied, namely in the form of filamentous, serrated, lobate, and undulate. While colony elevation was low convex, the inner structure varied from wavy

Table 1 Organoleptic observations of odors from water samples from 3 different locations after 4 days period of incubation.

No	Sample of waste water	Day 1	Day 4
1.	Control	No smelled	No smelled
2.	Pekunden	No smelled	Smelled bad
3.	Tembalang	No smelled	Smelled bad
4.	Kaligawe	Smelled bad	Smelled bad

interlace, blur opaque, finely granular, and translucent. The surface of colony was rough, smooth and glistening. The color of all bacterial isolates was white (Table 2).

3.3 Gram Bacterial Staining

The observation of gram staining showed that all sample bacterial isolates became purple. The results showed that bacterial pathogenic isolates were gram positive bacteria with staining to purple, so it was concluded that bacteria were a type of *Staphylococcus aureus* bacteria.

3.4 Test the Potential of the Sea Cucumber Symbiosis Consortium as Anti-Bacterial

The study of bacterial sensitivity from the sea cucumber symbiosis consortium showed that it had the ability to inhibit the growth of *Staphylococcus aureus* tested bacteria in wastewater samples at Pekunden but did not have activities at Kaligawe and Tembalang wastewater locations as shown in Table 3.

3.5 Water Quality Measurement of pH and Temperature in Wastewater

The results of measurements of water quality on pH at the time before treatment and after the bacterial treatment of sea cucumber symbiont consortium showed that the pH value of wastewater decreased from 7.59 to 6.65 after being treated with bacterial sea cucumber consortium type *Bacillus maritimus* and *Virgibacillus chiquensis*. Meanwhile, the temperature of wastewater before and after the bacterial treatment showed a decrease from 25℃ to 23℃ as shown in Table 4.

Table 2 Characteristics of pathogenic bacterial isolates from wastewater.

No	Code	Form	Margin	Elevation	Inner Structure	Surface of Colony	Pigmentation
1	Sample 1	Filamentous	Filamentous	Low convex	Wavy Enterlace	Rough	White
2	Sample 2	Irregular	Serreted	Low Convex	Blur Opaque	Rough	White
3	Sample 3	Irregular	Lobate	Low Convex	Finely Granular	Smooth	White
4	Sample 4	Circular	Undulate	Low Convex	Finely Granular	Smooth	White
5	Sample 5	Irregular	Undulate	Low Convex	Translucent	Glistening	White

Table 3 Qualitative test results of sea cucumber extract samples against test bacteria.

	Location of wateswater		
	Pekunden	**Tembalang**	**Kaligawe**
Consorsium bacteria	+	-	-

Table 4 pH and temperature of wastewater before and after incubated with symbiont consortium bacteria for 4 days.

Source of wastewater	pH		Temperature (°C)	
	Before	After	before	After
Pekunden	7.47±0.14	6.66±0.02	25.30±0.26	23.24±0.08
Tembalang	7.50±0.01	6.70±0.09	25.33±0.06	23.07±0.12
Kaligawe	7.11±0.08	6.70±0.12	25.37±0.06	23.53±0.40

Table 5 Nitrate (ppm) and phosphate (ppm) content of wastewater after incubated with symbiont consortium bacteria for 4 days.

Sources	Nitrate		Phosphate	
	Before	After	before	After
Pekunden	0.71±0.13	4.69±0.98	0.96±0.16	4.30±0.56
Tembalang	0.62±0.10	4.00±0.31	0.71±0.07	4.55±0.07
Kaligawe	0.48±0.01	2.13±0.78	3.46±0.05	5.16±0.55

3.6 Nitrate and Phosphate Content in Wastewater

Nitrate and phosphate content was determined by means of NitraVer Nitrate 5 Reagent Powder Pillow and PhosVer 3 Phosphate Reagent Powder Pillow for each wastewater sample and then measured spectrophotometrically (table: 5). Measurement of pH and temperature of wastewater was carried out manually by means of pH paper and thermometer.

4. Discussion

The consortium microbe found in sea cucumber intestines are identified as *Bacillus maritimus* and *Virgibacillus chiquensis.* The existence of these two species of bacteria is tied to the diet of sea cucumbers. Sea cucumbers play an important role in the nutrient cycle of marine ecosystems by consuming sediment and moving sand, thus occupying niches similar to earthworms in terrestrial ecosystems [20]. Sea cucumbers, marine biota that live on the seabed, are filter feeders, which means they eat all the particles found on the sea floor [1]. Bacteria are often found in marine sediments, marine animals and various other environments and have a high diversity of microbes from the genera Bacillus and Vibrio [20]. Genera of bacteria found in the intestines of sea cucumbers are different, depending on where they live in the intestine [13]. His finding showed that the anterior intestine is dominated by Proteobacteria (61%) and Bacteroidetes (22%), the medium intestine is similar but with lower Bacteroidetes (4%), and the posterior intestine was remarkably different, dominated by Firmicutes (48%) and Bacteroidetes (35%). Bacteria that found in Stichopus japonicas intestine are: Legionella sp., Brachybacterium sp., Streptomyces sp., Propionigenium sp. and Psychrobacter sp, although there is no information about the position of bacterial isolates [17].

Bacillus is a Gram positive, rod-shaped, with various species being obligate aerobic or facultative anaerobic, and have endospore as a structure that ensures survival when the environmental conditions are inhospitable [6]. The shape of *Bacillus* spores (endospores) varies depending on the species [7]. Bacillus has advantages over other microorganisms because it can survive for a long time, in environmental conditions that are not favorable for its growth [8]. Bacillus bacteria are found on land or in the ocean. Bacillus found on land have potential as Plant growth-promoting rhizobacteria (PGPR) [16]. *Bacillus* isolates found in the sea, such as those in the intestines of sea cucumbers, play an important role in individual health and growth [18].

Microbial consortium from Bacillus bacteria has also been proven to be the best antagonist bacteria for controlling plant diseases [21]. *Bacillus maritimus* is a bacterium that is often found in sediments [9], while sea cucumbers are filter feeders and live on the sea floor. Therefore, it is very feasible if the type of *Bacillus maritimus* bacteria is a symbiont within the digestion system of sea cucumbers.

Virgibacillus chiquensis is a bacterial species found on compost media [10]. This means that the bacteria can function as a decomposer. This is evident from the results of studies that show that the bacterium consortium *Bacillus maritimus* and *Virgibacillus chiquensis* can increase the value of nitrate and phosphate content in wastewater up to four times the concentration. Thus, the microbial consortium is useful as a bioactivator which can increase the value of nitrate and phosphate in plants. The research products can be used as plant growth-promoting bacteria (PGPB) since they can promote root growth. The introduction of PGPB had an impact on root growth and absorption of nitrates [14]. It is generally known that increasing plant growth is mediated by the binding of Nitrate in compost husk [12]. However, the results of the study show that the use of *Bacillus maritimus* and *Virgibacillus chiquensis* consortium directly impacts in significant value in increasing Nitrate and Phosphate levels.

Changes in the odor of wastewater from foul-smelling to odorless are thought to be because the microbial consortium functions as an antibacterial agent that suppresses pathogenic bacteria in wastewater [11]. The treatment of samples from three research locations, namely wastewater in Pekunden location, Undip reservoir and Kaligawe found an increase in water odor after treatment in all samples, even though the microbial consortium was a bacterium that functions as an antibacterial agent. Several Bacillus groups are bacteria that produce secondary metabolites to suppress pathogen growth [6]. The odor that arises after the treatment of each sample is suspected because the microbial consortium conducts nitrification, which is a characteristic of a group of bacteria capable of compiling nitrate compounds from ammonia compounds which generally take place aerobically. Therefore, the odor in each sample is due to the content of ammonia in the sample which is converted to nitrate by the microbial consortium. In agriculture, nitrification is very beneficial because it produces nitrate compounds which are vital for plant growth.

Bacillus maritimus and *Virgibacillus chiquensis* as microbial consortium can enhance phosphate content. This is because the microbial consortium functions as a phosphate solvent, increasing the concentration of phosphate. The treatment of wastewater from all sampling locations shows changes in water pH from base to normal. The water temperature during treatment also decreases, from 25°C to 23°C. This means that the microbial consortium has a positive role as a decomposer in waste water samples, by increasing the content of Nitrate and Phosphate, and supported by other environmental parameters, namely water pH and temperature. There are already studies proving that bacteria from the sea have the potential for plant growth, which means that endophytic bacteria are not the only ones that have the potential to be useful in plant growth [15].

5. Conclusions

The study concluded that the bacterium consortium *Bacillus maritimus* and *Virgibacillus chiquensi* are decomposter bacteria which can increase the concentration of nitrate and phosphate in wastewater.

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