ISOLATION AND IDENTIFICATION OF BACTERIA IN THE COMPOSTING PROCESS OF RED ALGAE (*Glacilaria* sp) WITH THE ADDITION OF COW MANURE AND WASTE HOUSEHOLD

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Received : June 5, 2021 Accepted : October 16, 2021 Published : December 31, 2021

Abstract: Bacterial isolation research has been conducted from composting *Glacilaria* sp with the addition of cow dung and household waste. *Glacilaria* sp samples were taken from Gampoeng Neheun Pond, Mesjid Raya district, Aceh Besar. The process of composting *Glacilaria* sp with the addition of other materials is done with a ratio of 2:1, the composting time lasts for 20 days. Leachate sampling is carried out on days 0; 4; 8; 12; 16; and 20 as much as 10 ml. PH, color and smell measurements of the sample are performed on each leachate sampling. The observations obtained 39 Gram-negative isolates and 40 Gram-positive isolates. With morphological forms of cells namely Coccus, Bacill, Diplococcus, Streptoccus, Streptobacillus and Staphylococcus. The bacteria are most commonly found in composting household waste.
Keywords: Isolation; Identification; Composting.

Abstrak: Telah dilakukan penelitian isolasi bakteri dari pengomposan *Glacilaria* sp, dengan penambahan kotoran sapi dan limbah rumah tangga. Sampel *Glacilaria* sp berasal dari tambak Gampoeng Neheun, Kecamatan Masjid Raya Aceh Besar. Proses pengomposan *Glacilaria* sp dengan penambahan bahan lain dilakukan dengan perbandingan 2:1, waktu pengomposan berlangsung selama 20 hari. Pengambilan sampel lindi dilakukan pada hari ke 0, 4, 8, 12, 16, dan 20 sebanyak 10 ml. Dilakukan pengukuran pH, warna dan bau sampel pada setiap pengambilan sampel lindi. Hasil pengamatan diperoleh 39 isolat Gram negatif dan 40 isolat Gram positif. Dengan bentuk morfologi sel yaitu *Coccus*, *Bacill*, *Diplococcus*, *Streptoccus*, *Streptobacillus* dan *Staphylococcus.* Bakteri yang paling banyak ditemukan pada pengomposan limbah rumah tangga.
Kata kunci: Isolasi; Identifikasi; Pengomposan.

Recommended APA Citation :
Harahap, M. R., Yahya, H., Lubis, S. S. & Sugiati. (2024). Isolation And Identification of Bacteria in The Composting Process of Red Algae (*Glacilaria* sp) With The Addition of Cow Manure And Waste Household. *Elkawnie*, *7*(2), 408-417. https://doi.org/10.22373/ekw.v7i2.22536

Introduction

In the last five years, seaweed has become dominant in the industrial world and has become a trend in various fields such as health, food and agriculture. Every year there is an increase in seaweed growth, namely 11.8%. In 2017, seaweed production was recorded at 10.8 million tons, while in 2018 the government of the Director General of Aquaculture, Ministry of Maritime Affairs and Fisheries (KKP) targets seaweed production to increase by 20%, namely 13 million tons of total seaweed production in the year (Lina, 2019). *Glacilaria* sp is a marine resource that is easy to cultivate and has high economic value.

Every year the amount of seaweed production increases quite rapidly. The utilization of seaweed raw materials includes 18.56 tons of carrageenan and 6,000 tons of gelatin products per year, and the goods produced from these two products are 16,189 tons. Of this production amount, there are 8,371 tonnes of processed seaweed remaining in the form of solid waste, liquid waste and sorting waste. (Ministry of Industry of the Republic of Indonesia, 2015).

Use extracted liquid seaweeds in agriculture as plant growth in growth experiments (Kocira, 2016). Sources of producer waste from industry processing seaweed can form waste solid and waste liquid. Meanwhile, in the processing industry seaweeds into ATC (*Alkali Trade Cottonii*) or ATS (*Alkali Trade Spinosum*), waste originates from the remainder sorting and sized *thallus* mall amount that passes the filtering after the alkalization process.

Glacilaria sp has some element important things contained therein that is element macro such as N, P, K, Ca, Mg and C. Meanwhile for micronutrients there are such as Fe, Cu, Zn, Boron, Na, Cl and Mn. Meanwhile, regarding the composition of chemicals contained within *Gracilaria* sp and the calculated from heavy dry namely: nutrient K 5791.8 mg/100g; Cl 1980.3 mg/100g; Mg 580.5 mg/100g; P 311.4, mg/100g; Na 291.8 mg/100g; Ca 218.5 mg/100g; Zn 4.5 mg/100g and Cu 0.60 mg/100g. *Thallus Gracilaria* sp contains agaropectin, minerals, cellulose, hemicellulose and lignin (Basmal, 2016).

Fertilizer liquids made from *Glacilaria* sp are superior among them that is, rich in macronutrients, micro and substances regulator growth nutrients. Therefore, that's seaweed can used as fertilizer liquid organic, because it is rich in macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Bo, Cu, Cl, Zn and Mn) needed for the growth of the plant.

Research Methods

Preparation Sample

Sample research used red seaweed (*Glacilaria* sp) obtained from Gampoeng Neheun pond, Mesjid Raya district, Aceh Besar, cows' dung obtained from Lamteh village, Meuraxa subdistrict, Aceh Besar and waste house stairs obtained from restaurant and markets in the Aceh Besar district. The research was carried out at the microbiology laboratory at the State Islamic University of Ar-Raniry.

Liquid Fertilizer

The composting process is carried out using a modified compost drum. Then the ratio used in making liquid fertilizer is 2:1 (Adityawarman, 2015) in the form of organic material and water. Seaweed waste is first rinsed with clean water. After washing it thoroughly, the seaweed solid waste is cut into 0.5 – 1 cm pieces (Loppies & Yunas, 2017) using a knife. The length of composting time is around 20 days. In this study, the composition of *Glacilaria sp* seaweed waste, cow dung waste and household waste were made in a ratio of 60: 40 (60% for *Glacilaria* sp seaweed waste, and 40% for cow dung waste or household waste). Meanwhile, sampling for microbial analysis is carried out from the beginning, middle and end of the fermentation process. Observation time intervals are every 4 days starting from 0, 4, 8, 12, 16, 20 (Vishan et al., 2017). The process of stirring waste biomass and aeration is carried out every 2 days (Michalak et al., 2016), for 20 minutes. At the time of sampling, the pH and temperature of the liquid fertilizer were also measured.

Isolation of Bacteria from Leachate

A leachate sample of 1 ml was taken and put into a test tube containing 9 ml of 0.9% NaCl. The same procedure was carried out until the dilution was 10^{-4} (Vishan et al., 2017). A total of 0.1 ml was taken with a micropipette and then spread on NA media (Pal et al, 2017). Incubation is carried out at room temperature for 24 - 48 hours. Morphological observations (shape, elevation, edges and color of colonies) were carried out to see differences in colonies to obtain different isolates. Then the isolate was purified to obtain a pure culture using the scratch plate method.

Characterization of Bacteria

Identification of bacteria is carried out by observing the morphology of bacterial colonies and Gram staining (Pal et al., 2017). The isolate or pure culture that has been obtained is then viewed morphological characteristics by observing the colony bacteria including shape, edges, elevation, colony color and Gram stain (Rahmadian, 2018).

Biochemical Test

The biochemical test namely the test used to identify the ability of bacteria and to know bacterial metabolic activity. The metabolites produced by bacteria from biochemical tests use reagents chemistry as well as the ability of bacteria to utilise compounds certain as source carbon and sources of energy (Septian, 2018). Several biochemical tests were used in the research namely the TSIA test, SIM test, SCA test, Urease test and Catalase test.

Results and Discussion

The seaweed from red algae (*Glacilaria* sp) is obtained from Gampoeng Neheun pond, Mesjid Raya district, Aceh Besar. Whereas cows' dung is obtained from Lamteh village, subdistrict Meuraxa Aceh Besar and waste house stairs are obtained from restaurants and markets in Aceh Besar. At stage isolation a total of 79 isolates.

Table 1. Physical data on fertilizer liquid *Glacilaria* sp

Treatment	Observation (day)	pH	Temperature (°C)	Color	Smell	Amount isolate
Glacilaria	0	6.10	24	Pink	A little fishy	
	4	6.15	25	Pink	Fishy	8
	8	6.20	27	Pink	Fishy and slightly smell rotten	8
	12	6.50	30.1	Pink and slightly white ivory	Rotten	5
	16	5.79	34.6	White ivory	Rotten stings	4
	20	5.30	36.2	Cream yellowish	Rotten stings	4
Glacilaria + Waste House Ladder	0	6.12	25	White ivory	Rotten	
	4	6.16	25	White ivory	Rotten	7
	8	6.22	28.1	White ivory	Rotten	6
	12	5.80	32.3	White ivory/cream	Rotten	6
	16	5.10	34	White ivory brownish	Rotten A little sour	4
	20	4.50	37.8	Cream tanned	Rotten pungent and slightly sour	2
Glacilaria + Cow Manure	0	6.15	25	Yellow greenish	Rotten	
	4	6.15	25	Yellow greenish	Rotten	8
	8	6.66	28.8	Yellow greenish	Rotten	6
	12	6.15	33.4	Yellow greenish	Rotten stings	4
	16	6.12	37.2	Yellow and a few chocolates	Rotten stings	4
	20	5.79	39.2	Yellow and brown	Rotten stings	3

Based on research that has been carried out, it is known that after an incubation period of 20 days, the compost has a different aroma and texture starting on the first day of composting. On the first day, the compost had not yet released an odor and was still rough. However, after 20 days the compost emits an unpleasant aroma (rotten smell) and rot occurs in the materials contained in the compost. A very strong odor is emitted by *Glacilaria* sp compost with the addition of cow dung, while compost with the addition of household waste emits an unpleasant and sour odor. For *Glacilaria sp* compost, adding one litre of distilled water does not emit a strong, rotten aroma like compost with the addition of household waste and cow dung. Meanwhile, the leachate produced by these three compost fertilizers is also different.

There are two important processes in the composting process, namely microbial activity and changes in organic material. In the first stage, microbes start the composting process by increasing the temperature through oxidation of organic material, then decomposing most of the organic material that can be decomposed and increasing the stability of organic residues. In this process, microorganisms degrade organic materials through chemical synthesis to convert the materials into humic. The by-products of the composting process are leachate, carbon dioxide and heat. In the second stage, the remaining organic material in humic substances is changed through humification to improve the quality of the compost. However, decomposition continues until the organic material is converted into stable humic substances. When microbial activity decreases, temperature decreases (Palaniveloo, 2020).

Glacilaria sp compost produces pink-colored leachate, *Glacilaria* sp compost with the addition of household waste produces ivory white/cream colored leachate, while *Glacilaria* sp compost with the addition of cow dung produces leachate which is greenish yellow to brownish yellow. Compost maturity can also be marked if there has been a change in texture, color and a more pungent aroma (Agus, 2014).

An increase in temperature and pH occurs as a result of microbial activity in degrading organic material in compost. Increasing the temperature in the compost ripening process, there are three types of microbes, namely at temperatures below 20 ^{0}C there are only Psychrotrophic types of microbes. Usually, these microbes appear at the beginning of composting. Furthermore, in the temperature range of 25 - 30 ^{0}C, the type of microbes that grow at this temperature are mesophilic which appear on the 10th day of the composting period. The last one is a type of thermophilic bacteria that lives at a temperature range of 60 ^{0} C. This increase in temperature occurs due to microbial activity in degrading organic material in compost. (Chinakwe, 2019).

Pratiwi (2013), stated that the increase in temperature in each compost treatment was not the same, this occurred because many factors influenced it. The temperature changes that occur during the composting period show that there is mesophilic and thermophilic microbial life which play alternating roles. The gradual decrease in temperature is due to organic material which can be broken down by microbes and is a factor indicating the maturity of the compost. When the temperature decreases, mesophilic microbes develop, whereas when the temperature increases, thermophilic microbes begin to develop. Therefore, temperature is an important factor determining compost maturity and the types of microbes that live in the media.

Apart from the temperature factor, pH is also an important factor in the compost ripening process. Acidic pH is formed due to the presence of simple organic acids. pH experiences changes in acidity levels over time the incubation period results in the decomposition of proteins and the release of ammonia. Increasing and decreasing pH is a sign that microbial activity is occurring in degrading organic material (Ismayana et al. 2012).

The low pH at the end of the composting process is the result of the high activity of lactic acid bacteria in degrading fermentable sugars which are broken down into lactic acid and other organic acids, plus carbon dioxide and ethanol in limited oxygen conditions. In addition, *Bacillus species* are known to secrete catabolic enzymes, such as proteases, which through proteolysis can increase pH. High temperature and high pH environments are active stages of composting where *Actinobacteria* and *Bacillus* spp. at high concentrations, it begins to decompose into hemicellulose, cellulose and lignin. The number of Gram-positive bacteria negativity decreases sharply in both units when the temperature reaches the thermophilic phase (Partanen, 2010).

The physical quality of the compost (including color, odor and texture) shows that the physical quality of the compost meets the requirements of SNI 19-7030-2004 criteria. According to Suwatanti (2017), compost has an earthy smell and a blackish color is formed as a result of this. Meanwhile, the texture of the compost changes to become smooth as a result of microbial activity degrading organic material. These three physical parameters can indicate the characteristics of good physical quality of compost. According to Ismayan a, 2012, the texture of compost is good if the final form does not resemble the shape of the material because it has been destroyed due to natural decomposition by microorganisms that live in the compost.

Based on observations on the morphology of isolate colonies, it was found that 79 isolates were successfully isolated from *Glcilaria* sp liquid fertilizer with the addition of household waste and cow dung. Each bacterial isolate has different morphological characteristics. The characteristics of bacterial colonies observed include shape, color, elevation and edges. As seen in Table 2, there are dominant colony shapes, namely round and irregular. Meanwhile, the color of the colonies that appeared after isolation were white, pink, yellow, cream and greenish-white. Identification of microbial cell morphology using gram staining, namely by looking at the ability of the bacterial cell wall to absorb purple crystal dye and/or safranin after the bacterial cells are fixed on a glass object (Ginting, 2019). The number of

Gram-positive isolates was 40 isolates, while the number of Gram-negative isolates was 39 isolates. Based on Table 1, the longer the composting process, the smaller the number of isolates obtained. The decrease in the number of microorganism isolates occurred in line with changes in physical factors in the compost. In research, Chinakwe (2019), reported that temperature is important in the quantity and type of microorganisms in the composting process. Temperature 20 and 40 °C is alternation of mesophilic bacteria, but thermophilic microorganisms are only active at temperatures between 40 and 70 °C. In addition, the density of bacteria is always higher than the density of fungi regardless of the length of the composting period. Increasing temperature causes a decrease and increase in the number of microbes from day 20 to day 25 of composting.

In general, the number of bacteria was slightly higher in the *Gracilaria* sp composting treatment with the addition of household waste. This matters because the composition of household waste is more complex. Household waste consisting of food scraps can consist of carbohydrates, proteins, lipids and small amounts of inorganic compounds. The composition varies according to the type of food waste and its constituents. The bacterial density is quite high when compost is mixed with leftover food waste because it contains a lot of organic material that microbes need to reproduce (Palaniveloo, 2020).

Table 2. Biochemical test results

Test	**Results**								
	XA3	**XA2**	**XB1**	**XB4**	**XD4**	**YB1**	**YD1**	**YD4**	**ZC1**
Grams	-	-	-	-	+	-	-	-	-
SCA	-	+	-	+	-	+	-	+	+
Urea	-	-	+	+	-	+	+	+	-
TSIA	+	-	+	-	+	+	-	+	+
Catalase	+	+	+	+	+	+	+	+	+
SIM	+	-	+	-	+	-	-	-	-
Genus	**Escherichia sp**	**Pseudomonas sp**	**Citrobacter sp**	**Alkaligenes sp**	**Bacillus sp**	**Proteus sp**	**Brucella sp**	**Klebsiella sp**	**Salmonella sp**

Information: X = isolate from composting *Gracilaria* sp., Y = isolate from composting *Gracilaria* sp. and cow dung, and Z= isolates from composting *Gracilaria* sp and household waste

Based on the results of biochemical tests on *Simon Citrate Agar* (SCA) media for bacteria of the genus *Pseudomonas* sp, *Alkaligenes* sp, *Proteus* sp, *Klebsiella* sp and *Salmonella* sp showing reaction positive, this signifies bacteria the capable use citric as source carbon and energy. Whereas bacteria of the genus *Escherichia* sp, *Citrobacter* sp, *Bacillus* sp, and *Brucella* sp show reaction negative, which means bacteria the not capable use citric as the only source of carbon and energy (Gultom, 2019). The results of the urea test on bacteria XA3, XA2, XD4, and ZC1 showed a reaction negative. Meanwhile, bacteria XB1, XB4, YB1, YD1, and YD4 show a reaction positive, this is marked by happen change in the color of the media from red become pink. Color change happens because the moment the urease enzyme dissolves bonds carbon and nitrogen make ammonia. There is ammonia causing the medium to become alkaline/base so that indicator *Phenol red* will be changed become pink in the media. This matter causes happen reaction and produces urease (Wahyuni, 2018).

Test results on bacterial *Triple Sugar Iron Agar* (TSIA) media *Pseudomonas* sp, *Alkaligenes* sp, and *Brucella* sp show a reaction negative, this signifies that bacteria the not capable of fermenting glucose, lactose and sucrose. Whereas bacteria *Escherichia* sp, *Citrobacter* sp, *Bacillus* sp, *Proteus* sp, *Klebsiella* sp, and *Salmonella* sp show reaction positive, this signifies bacteria the capable ferment lactose and sucrose (Meze, 2020). Catalase test results show positive for all samples that are marked with formation gas bubbles because bacteria the capable produce the enzyme catalase. Enzyme catalase is used to break down the hydrogen peroxide formed from the respiratory process aerobic and toxic to bacteria, into dihydrogen oxide (H_2O) and oxygen (O_2) (Wahyuni, 2018). The biochemical test results on bacterial *Sulphite Indole Motility* (SIM) media *Escherichia* sp. and *Citrobacter* sp. show reaction positive and characteristic motile. Meanwhile, bacteria of the genus *Pseudomonas* sp, *Alkaligenes* sp, *Proteus* sp, *Brucella* sp, *Klebsiella* sp. and *Salmonella* sp. show negative reactions and are not motile.

Conclusion

Based on the results of research during the 20-day composting period of *Glacilaria* sp. liquid fertilizer with the addition of household waste and cow dung, then isolated and 79 isolates were obtained, with 40 isolates being Gram-positive and 39 Gram-negative. Biochemical test results done as many as 5 tests, namely, TSIA, SIM, SCA, UREA and Catalase, and 9 types were obtained bacteria among them namely the genus *Escherichia* sp, *Pseudomonas* sp, *Citrobacter* sp, *Alkaligenes* sp, *Bacillus* sp, *Proteus* sp, *Brucella* sp, *Klebsiella* sp. and *Salmonella* sp.

Acknowledgement

The authors express gratitude to Universitas Islam Negeri Ar-Raniry Banda Aceh through PUSLITPEN LP2M for supporting this research. This research was funded by DIPA UIN Ar-Raniry Banda Aceh with the contract number of the grant agreement: 490/PPK-UIN/PUSLIT/I/2020. And the students who helped were Cut Ristina and Tuti Marlina.

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