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Isolation and Identification of Bacteria in the Rotifer Mass Culture Medium

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Abstract

The objectives of this study are to isolate, to identify and to determine the dominant bacteria in the rotifer (*brachionus rotundiformis*) culture medium. Stages of the method done were by isolating bacteria in the initial uptake condition and final condition of rotifer population, total bacterial analysis, biochemical tests and water quality. Availability of substrate (raw fish) collected at the base trigger an increase in the number of bacteria to 2.7 X 10^4 CFU / mL. It is identified that 97 isolates are Halococcus sp bacterial which are kemoheterotrof. The species that are able to survive during the cycle density on rotifer mass culture medium is H. saccharolyticus with a percentage of 54.6%.

Keywords: bacterial identification, rotten fish, halococcus sp., rotifers, water quality

1. Introduction

Rotifers is one of the microorganisms categorized to zooplankton associated with microorganisms. Rotifers lives cosmopolitan and found in waters with a high content of organic matter on the environmental where conditions are easy to change or unstable conditions, because rotifers are highly adaptable animals (Rumengan et al. 2007). Rotifer ability to adapt in a fluctuating environment is believed that it can be removed from its natural habitat into the container (medium) to be cultured, similar to other organisms can only live and grow well if the food supply are available both quantity and quality.

Rotifers as organisms that are filter feeders can consume the feed in the form of algae, yeast, bacteria, and detritus microencapsulation. Algae can be Nannochloropsis sp., Tetraselmis sp. (Purba 1995, Cheng et al., 2004, Sutomo et al., 2007), combined Nannochloropsis sp., Dunaliella sp., Isochrisys sp., And Pavlova sp., added with Bacillus sp., as probiotic bacteria (Erlania et al. 2010).

Organic matter such as oxygen, hydrogen, carbon and nitrogen is a constituent element of a compound of protein, carbohydrates, lipids (fats), vitamins, enzymes in fish flesh. Meat fish besides contain proteins, it also contain much water which are good medium for bacterial life. The presence of bacteria is very influential on the decomposition of these compounds. Therefore, fish will get a rapidly decaying process. The research objectives are to isolate, to identify and to determine the dominant bacteria in the rotifer mass culture medium

2. Material and Methods

2.1 Materials and Devices

A bath container used for place of rotifer culture; length of 125 cm, width 81 cm and height 75 cm. Other tools are sample bottles, cool box, DO meter, pH meter, saline meter, thermometers, analytical scales, hotplate, stirrer, spatula, measuring cups, Erlenmeyer, volume pipettors, pipette measure, pipette drop, Pasteur pipette, Bunsen, Ose needle, petri dishes, tubes Hach, Durham, autoclave, laminary water flow, incubators, ovens, microscopes, pans, stoves and water pumps.

Materials used include 0.2 kg of raw fish namely 'deho' (auxis thazard), Nutrient Agar (NA) media, Nutrient Broth (NB), distilled water, immersion oil, crystal violet solution, alcohol 70%, safranin solution, Lugol's solution, H_2O_2 solution, broth carbohydrates (glucose, fructose, lactose and sucrose), Motility Test Medium, Simmons Citrate Agar, MR-VP reagents, reagent Tetramethyl-p-fenilenediamine dihydrochloride and Kovac's reagent.

2.2 Scope of Research

Container used for rotifer mass culture is filled with seawater as high as 67 cm. The seawater is taken from the coastal waters of Poigar bay (± 200 meters from the location where sample of the research is taken) using a water pump. Then, the raw fish wrapped and put into the container as the source of feed for rotifer and inoculant seed of rotifer. The goal is to create a medium to grow rotifers using fish spoilage results or results of

decomposition by bacteria that occur in the decomposition process. Rotifers were inoculated as many as 282,000 individuals from the pool grounds located in the coastal region Poigar of South Minahasa. Rotifers were taken from the pool grounds from the former pond waters in Poigar, Bolaang Mongondow. Rotifer culture medium was not given food microalgae and not aerated.

Stages of bacterial sampling performed on the culture medium at the time of the initial conditions or after the seawater filled into the tub (sample A), the initial conditions of rotifer medium in the surface tub (sample B), the initial conditions of rotifer density medium at the base of bathtub (sample C), the final condition of the density of rotifers medium in the surface bath (sample D) and the final conditions of rotifer density medium at the base of bathtub (sample E). Sampling was taken using sterilized bottles. Then the bottles are put into the cool box so that the water temperature is under control at the time the sample was taken to the laboratory. Water quality sampling conducted in conjunction with bacterial sampling. Measurement of physico-chemical parameters of water quality include: temperature, salinity, pH, dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) presented in Table 1. After that, microbes are analyzed (Cappucino and Sherman 1992; Lay 1994) and identified (Holt et al 1994).

Table 1. Water quality parameters and methods used

Water quality parameter	Unit	Method
Temperature	ºC	In situ
Salinity	‰	In situ
pH	-	In situ
DO	mg/L	In situ
Amonia	mg/L	Elektroda selektif amonia
Nitrit	mg/L	Spektrofotometer
Nitrat	mg/L	Spektrofotometer

3. Results and Discussion

3.1 Medium Conditions

The values of pH in the rotifer mass culture medium is in the range of 7.07 to 8.07, while the ammonia in the initial conditions (seawater samples) of 0.02 mg / L, but levels vary after adding fish oil (presented in Table 2). Complex organic materials in fish flesh reformed into a more simple form of compounds such as glucose, glycerol, fatty acids and amino acids. Amino acids are oxidized to yield ammonia revamp protein and carboxyl compounds. Reducing nitrate to nitrite by bacteria, and then nitrite is reduced again and the result is ammonia. Low levels of DO due to the lack of aeration to increase dissolved oxygen content in the media and increased rotifer populations of organisms and bacteria that need oxygen. According Widayat et al (2010), when the DO below 1 mg / L the nitrification process is slow. Salmin (2005) states that the oxygen needed for the oxidation of organic materials and inorganic in aerobic process. Oxygen diffusion speed from the air depends on several factors such as temperature and salinity. The higher the temperature, the more increased activity of microorganisms that cause increased utilization of dissolved oxygen, but the bacteria will decrease (Arbain et al, 2008; Moertinah 2010). Heterotrophic bacteria play a role in the decomposition of organic loads such as Bacillus subtilis, Clostridium and Proteus sp., While the ammonia decomposition is Nitrosomonas and Nitrobacter (Widayat et al., 2010).

Table 2. The results of measurements of medium addition of raw fish

Sample	pH	NH_3 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	DO (mg/L)	Salinity (‰)	Temp. (ºC)
A	8,07	0,02	0,002	0,023	4,88	30	32,1
B	7,07	0,72	0,004	0,059	3,105	20,1	30
C	7,2	0,58	0,002	0,04	0,47	20	29
D	7,41	2	0,02	1,8	0,6	20,5	29
E	7,4	2	0,01	1,7	0,83	20,3	29

Description: A = samples of sea water; B and C = Cycle initial rotifer density; D and E = end of cycle rotifer

density.

3.2 Total Colonies of Bacteria (Total Plate Count)

TPC results of seawater samples (A) were taken from the bath of rotifer mass culture shows 3.5 X 10^2 CFU / mL, while the total value of bacterial colonies on the sample initial cycle in surface bath density rotifer medium (B) of 2.5 X 10^2 CFU / mL and at the base of bath medium (C), which is 7.5 X 10^2 CFU / mL (Table 3). The results of sampling B and C are performed while rotifer population rose to 472 ind / ml showed differences of TPC values, sample C is higher than sample B, allegedly influenced by the source of the bacteria substrate in the base of bath medium.

The difference of TPC value is also seen in samples D and E where samples taken done when the rotifer density reached 1383 ind / ml. The highest TPC number in the sample E are directly related to the activity of bacteria decompose the organic material in a medium (base medium bath). Bacteria use the decomposition of fish meat to fulfill its needs so that the population is growing in number. Jaya and Ramadan (2006) report that the more fish rotting, the greater the number of bacteria reached 5.00 X 10^8 colonies / g in 24 hours, while Badjoeri et al (2010) expressed the highest ammonium bacteria is 1.2 X10^{20} cells / g on the basis of depth 10-15 cm. The number of bacteria in fish are increasing along the time due to the optimal environment for the growth of bacteria that cause bacteria to grow to its full potential (Amin and Leksono 2001). The results of elevated levels of ammonia in the medium consolidate its decomposition by bacteria. Ammonia is toxic to some aquatic biota, but the ammonia levels are still good for the life of rotifers. Rotifers can breed with both ammonia levels ranging from 0.031 to 0.345 mg / L (Sumiarsa et al. 1996).

Table 3. TPC Results

Sample	Density of rotifer (ind/ml)	TPC value (CFU/mL)
A	0	$3,5x10^2$
B	472	$2,5x10^2$
C		$7,5x10^2$
D	1383	$5,2x10^3$
E		$2,7x10^4$

Description: A = samples of sea water; B and C = Cycle initial rotifer density;

D and E = end of cycle rotifer density; CFU = Colony Forming Units.

3.3 Morphology Characteristics Test and Biochemical Bacteria

Observations on colony morphology obtained in NA medium has flat surface and curved, rounded edges intact and forms a jagged and generally yellowish white (beige). Gram staining were performed on 97 isolates showed 87 isolates of negative Gram and the rest are positive with almost all forms of cocci. In motility showed 61 positive isolates and 36 negative isolates. Fermentation of carbohydrates gave a positive response. If the test results are negative then the bacteria use other nutrients as an energy source such as peptone. The catalases test results obtained 85 isolates are positive and the rest are negative, whereas oxidase of 88 isolates showed a positive reaction. The test of Indol and methyl red are vary in the result. while Voges Proskauer is positive indicating that this bacterium has the ability to catalyze organic acids resulting from the fermentation of carbohydrates to produce Acetylmethylcarbonil and ethanol. Citrate is derived almost all negative, the bacteria do not use citrate as the source of carbon but tends to use carbohydrates such as glucose, sucrose, lactose, maltose, mannitol and cellulose (Lay, 1994).

Halococcus consists of six species, namely H. morrhuae, H. saccharolyticus, H. hamelinensis, H. qingdaonensis, H. salifodine and H. dombrowskii (Legat et al., 2010). Goh et al. (2006) confirmed that the cells Halococcus shaped single or paired cocci and has negative Gram. The content contained on the form heteropolysaccharides cell wall binds to sulfate and N-acetylated sugars or amino acids (Steber and Schleifer 1975; Fendrihan et al. 2006). According to Holt et al. (1994), H. morrhuae be negative on glucose and lactose fermentation, whereas H. saccharolyticus gives positive results. These results are supported by research conducted Nada et al. (2011), among types Halococcus sp. a highly negative impact on the fermentation of carbohydrates (glucose and lactose

or) is H. morrhuae. Research conducted by Goh et al. (2006) reported the results of katalase test all strains positive. According to Oren (2006), Halococcus sp. oxidase positive, while Goh et al. (2006) stated that some species groups Halococcus the oxidase is negative. Halococcus sp. are positive indole. (Oren, 2006), but most isolates response negatively (Goh et al. 2006). Halococcus is an extreme Halofilik and it can grow within the pH 4.0 to 9.0 at 37 º C (Valera et al., 1979; Goh et al., 2006; Wang et al. 2007).

Halococcus sp. have roles as decomposers, organisms and are not detrimental to other organisms in this case the rotifer. Halococcus sp. identified as group of bacteria that use organic ingredients as energy so it is less likely for the microbes to metabolize the autotrophs. Holt et al. (1994), states that Halococcus sp. is kemoheterotrof (carbohydrates, alcohols, carboxylic acids, amino acids) as an energy source. Bacteria found cannot synthesize their own food, but the food is obtained by taking the form of a ready-made organic compounds or the rest of the dead organisms. Halococcus sp. plays an important role in the decomposition of organic material on the substrate raw fish meat put into the bath as a culture medium. The high content of protein or amino acids, carbohydrates and fatty acids in fish flesh is a good material for the needs of living bacteria. Conditions of medium with high organic matter content is also an environment for the rotifers. According Rumengan et al. (2007) the rotifers are also found in the environment that contains a lot of organic matter. The dominant presence of bacteria indicating that the bacteria are able to survive during the cycle density on rotifer mass culture medium (Table 4). When sampling, groups shaped motile negative Gram bacteria are single or in pairs in the rotifer culture medium carried along the water mass, so that it is dominant among other bacteria.

Table 4. The percentage of bacteria that were identified from the rotifer culture medium by Gram nature and motility

Nature	Form	Isolate	Percentage (%)
Gram-positive motile	Coccus	8	8,2
Gram-positive non motile	Coccus and Streptococcus	2	2,1
Gram-negative motile	Coccus and Diplococcus	53	54,6
Gram-negative non motile	Coccus and Streptococcus	34	35,1

4. Conclusion

Bacteria identified in rotifer mass culture medium with raw fish as the addition of medium is a bacteria that contribute to the decomposition of organic matter, so it does not inhibit rotifer populations. Bacteria which are dominant is motile Gram-negative bacteria identified as H. saccharolyticus with a percentage of 54.6%.

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