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The Determination and Arrangement of a Combination of Enzyme Lactate Dehydrogenase of Bacteria Acinetobacter Sp. as a Device the Identity Important Bacteria Agent Composts

D. Sukmawati, R. Puspitaningrum, and Muzajjanah

Abstract. The number of garbage generated by the industry or society is a usual problem encountered by almost all urban centers, especially large cities such as Jakarta. Waste prevention strategy required quickly and accurately. One strategy for tackling the Junk was getting lactic acid-producing bacteria. It has been shown that lactic acid can increase the acceleration of organic matter such as an overhaul of lignin and cellulose as well as out causing toxic compounds arising from decay. This research will be conducted on the determination and characterization of the enzyme-producing compost bacteria LDH lactate dehydrogenase LDH - which in isolation from the garbage Landfill Rawasari. Methodology: Research carried out consists: isolation of lactic acid-producing bacteria; identification of microscopic, macroscopic and staining Gram; cellulose assay, and optimization of PCR conditions LDH enzymes producing bacteria. Isolation is performed by dilution method and the direct method. As many as 5-point sampling. Each stage is conducted from 10 grams of soil from the top surface of the compost. Isolation results obtained 100 isolate the bacteria. Base on the characteristic of macroscopic and microscopic observations retrieved 14 isolates of bacteria have shaped rods and brought forth a negative kind of Gram positive staining. Bacterial isolates with codes (BK1; BK3; BK4; BK5; BK6; BK7; BK8; BK9; BK10; BK11; BK12; BK 13). The potential bacteria with ability produce lactate dehydrogenase was BK1 and BK3. Base for analysis phylogenetic there was identification bacteria bak1 and bak3 where Acinetobacter sp.

INTRODUCTION

The schema of the application of compost is currently alleged to be the most common and effective in tackling food waste in urban areas. Basically, the process of making compost is assisted by microorganisms that degrade organic waste plays into high-quality compost [1]. Hence, be thought that needed new ways to take on the problem of barren. This study provides an important breakthrough in harnessing the waste problem with the approach to exploration and determination of bacterial fermentation process leading compost. In the procedure of making fermented compost, occurs the process of succession of microorganisms in each level and make a sort of different microorganisms [2].

Fermented product is acid-organic acids that are a result of hydrolysis of the fatty acid and also as a result of the natural process of the bacterial growth. One of the organic acids produced from that process is lactic acid. The lactic acid formed by the metabolism of anaerobic Glycolysis through the transition of private in enzymatic by the enzyme lactate dehydrogenase (LDH). It has been demonstrated that lactic acid can increase the acceleration of organic matter such as an overhaul of lignin and cellulose without causing toxic compounds arising from decaying organic material. These bacteria can inhibit the development of Fusarium. Thus, the swiftness of the organic waste decomposition depends on the lactic acid is created. On this research will be done testing the compost bacteria in LDH isolation from landfills of Rawasari.
Lactic acid fermentation process, not lose connection with the microorganism which became the activator in the formation of lactic acid. Microorganisms’ bacteria that can produce lactic acid known as lactic acid bacteria (BAL). Lactic acid bacteria can ferment carbohydrates to produce lactic acid. Lactic acid bacteria serve to elaborate on organic matter made by fermentation of lactic acid and glucose. Lactic acid will suppress the growth of harmful microorganisms as well as increase the degradation of organic materials [3, 4]. Lactic acid bacteria in anaerobe condition. The bacteria Bacillus in anaerobic conditions that have a very important role is an enzyme LDH because many Acetyl-CoA A converted into acetate to form ATP and produces NADH during glycolysis [5].

One case of bacteria lactate which isolated from compost is Bacillus sp. The enzyme from Bacillus sp. The bacteria are known mostly found in the temperature range 20-60 °C while above the range only strain Bacillus stearothermophilus can live [6]. Bacillus sp. have a metabolic enzyme that plays an important role in the degrade cellulose, lignin, and bioplastic [7]. Therefore, the role of the Bacillus sp. is very important in the initial process of producing compost [8].

The genus Bacillus originally proposed by Cohn in 1872 classified as bacteria that produce endospore (endospore-forming bacteria). This Genus has a number of species is very fantastic reached 146 types, including two types of the most popular namely B. anthracis and B subtilis [9]. Since Cohn, taxonomy Bacillus experienced some changes, until this time the genus Bacillus valid known only 88 types of bacteria [9] and around 18 types of found in compost [7].

As a solution that is offered at the end of a series of multi years is this research with find bacteria compost is superior to get the identity of LDH. On the man known that LDH is an enzyme with a different isoform combination order in the organ of the heart and muscle tissue. Therefore, LDH becomes key enzymes of anaerobic respiration metabolism lactate producers. In this research, we have to get the new strain isolate mesophilic bacteria from the compost in Rawasari (Indonesia), In order to determine the taxonomic position and phylogenetic from this organism, then the first year of this research is planned to make a determination of the tree phylogenetic through the full analysis 16 rRNA from this strain.

**MATERIALS AND METHODS**

**Isolation of Bacillus sp.**

Isolation of bacteria was done using dilution plating method [10]. A total of 1 g sample was in a test tube containing 9 ml of sterile distilled water (dilution 10-1) after test tube was vortexed for ± 1 minute. Furthermore, doing the same to the dilution 10-6. On dilution of 10-4, 10-5 and 10-6 sample was inserted into 2 petri dishes that have already contained NA (Nutrient Agar). Samples were incubated 7 days at a room temperature (25-30 °C).

**Identification of Bacteria**

Identification of bacteria was based on the observation of morphological characters, both macroscopically and microscopically and Gram staining bacteria by Allison et al. [11].

**RESULTS AND DISCUSSION**

**The Isolation of the Bacteria Isolates from East Jakarta Rawasari**

The isolation of the bacteria isolates done from the ground in the area of waste disposal Rawasari East Jakarta. The isolation of the done in January 2016. Sampling the land obtained from the sampling point 5. Each point taken as much as 10 grams’ land. As much as 1 grams done technique of dilution is an incubation temperature of 30 °C for 2-3 days. Sampling method using electrolyte deficits plating method with the medium of insulation in the form of nutrients. The isolation of the results obtained 110 isolates.

The results of the isolation and then done liquefaction and observed characters macroscopic and microscopic. Based on the characters macroscopic and microscopic obtained as much as 13 isolates the bacteria in the form of bars. The bacteria in the form of bars or known Bacillus sp. found in the samples of the land. Yabe et al. [10] reported the results of the isolation from samples of the land in the roots of plants found bacteria genus Bacillus sp.
In the amount of money. The land is a source of nutrient for the growth and development of all organisms. The results of the test testing catalase all isolate shows positive results, this shows all the bacteria that have acquired the ability to produce an enzyme catalase. Macroscopic testing and microscopic bacteria Bacillus sp. and testing catalase and motility.

Testing characteristic bacteria isolation from TPS Rawasari results done the motility test, catalase and dye test Gram. Motility test results show bacteria bak 1 until bak 14 test results positive, catalase, motile, have the form of morphology bacilli and observations grams is positive gram (Fig. 1).

The Condition of the Optimization PCR LDH Genes in the Bacteria Bacillus sp.

LDH genes (therefore for this study of the primary genes LDH on bacteria Bacillus sp. used primarily: Forward: 5'CAA AAA CCT GGT patient GAG ACA CGC CTT 3' (24 bases). Reverse: 3' ACAGAGC TTCCCTGTTTGGAGCCAC 5' (24 bases), because the reverse so writing for the order of the primary behind so: CAC CGA GGT TCC TTC GAG ACA. The length of the nucleotides which will be obtained from the amplification process is over 312 nucleotides. Do optimization of bacteria with the code sink 1 and sink 3 LDH gene optimization results obtained PCR conditions with the condition: Amplification region 16S rDNA using forward primary and primary reverse the results of the primary creation LDH genes in Bacillus.

PCR reaction using the Go Green Master mix Taq (Promega) with the volume of the end of the 25 µl consisting of 7.5 µl nuclease free water (NFW), 12.5 µl Go TaqGreen (Promega Master mix), 1 µl each primary (ITS5 and primary ITS4) and 3 µl DNA template. The term of the PCR (Thermo Scientific™ Arctic™ Thermal Cycler) as follows: 95 °C for 2 minutes, followed by 35 for the stage denatures cycle at a temperature 95 °C for 15 seconds, annealing at a temperature 58 °C for 30 seconds and elongation at a temperature 72 °C for 1 minutes. The final Elongation at a temperature 72 °C for ten minutes and soaking at a temperature 4 °C. The optimal PCR conditions produce a band with the length of the base balance between 500-600 bp. The condition is the optimum acquired genes LDH uses PCR method (Fig. 2).

FIGURE 1. Macroscopic observation and coloring its gram bacteria (bacteria codes: bak 1-8) origin TPS Rawasari, incubation 48 hours nutrient medium so that the temperature of the incubation period 30 °C.
Testing the Activity of Cellulose Bacteria

Testing the ability of bacteria to produce an enzyme cellulose done in 13 isolates bacteria with isolated code (bak 1; bak 3; bak 4; bak 5; bak 6; bak 7; bak 8; bak 9; bak 10; bak 11; bak 12; bak 13; and bak 14). It produces the enzymes cellulose testing based on the method. The tests are performed using the medium Czapex Dextrose so that (CDA), Carboxyl Methyl Cellulose (CMC), Congo red 1 %. The test results obtained 7 (50 %) isolates produce clear zone and 7 isolates (50 %) negative produce clear zone. The largest average zone of nodes obtained from the bacterial isolates with the code bak 4 (11.39 cm); bak 5 (12.64 cm); bak 6 (12.76 cm); bak 8 (8.73 cm); bak 10 (10.95 cm); bak 12 (8.56 cm); and bak 14 (10.26 cm). The bacteria with the code bak 5 and bak 6 have a greater potential average zone of nodes that produced by 12 cm (Fig. 3).

Bacteria have the ability to produce an enzyme cellulose. The genus bacteria enzyme cellulose producer among other Lactobacillus [12]. Cellulolytic organisms such as owning the leavened cellulose extracellular complex consisting of at least 3 enzymes, namely: endotracheal intubation β-1,4 gluconate, β-1,4 gluconate and β-glucosidase. This enzyme complex ties hydrolysis β-1,4 glycosides on the cellulose and derivatives thereof be cellobiosa and glucose [12]. Cellulose can hydrolysis Endoglucanase will randomly produce glucose.

Phylogenetic analysis of bacterial bak1 and bak 3 comes from the same genus, namely Acinetobacter sp. but still have a low homology values that are not within a single clade with other species (Fig. 4).

Based on phylogenetic analysis obtained bacteria bak 1 and bak 3 identified into the bacteria genus Acinetobacter sp. Allison et al. [11] found the bacteria Acinetobacter calcoaceticus was the bacteria that produce L-lactate and L-mandelate. The bacteria A. calcoaceticus using lactate and mandelate as sole sources of carbon and energy for growth. The present study also shows that bacteria Pseudomonas putida, Acinetobacter calcoaceticus and Escherichia coli have lactate dehydrogenase [13]. Acinetobacter baumannii is an opportunistic human pathogen causing a wide range of infections including wound, urinary tract infections. Henein et al. [14] reported Acinetobacter baumannii can produce L- lactate dehydrogenase for Exhibits no Cytotoxicity in 3T3 Mouse Fibroblast Cells. Lactate dehydrogenase (LDH) is an intracellular enzyme that is present in almost all cells. The activity of LDH reaction will be increased at almost all the organs or tissues when in a State of destruction, damaged cells or in conditions of lack of oxygen. The enzymes LDH pyruvate into lactic convert was instrumental in the cytosol [15].
CONCLUSIONS

The isolation of the bacteria from the TPA Rawasari obtained 100 isolates. As many as 14 isolates showed discernible morphological features in the form of bars with positive gram coloring results. Bacterial isolates the potential for producing an enzyme LDH motility test done, and cellulose. The test results obtained 7 (50 %) isolates produce clear zone and 7 isolates (50 %) negative produce clear zone. The largest average zone of nodes obtained from the bacterial isolates with the code BK 4 (11.39 cm); Bk 5 (12.64 cm); BK6 (12.76 cm); BK8 (8.73 cm); Bk 10 (10.95 cm); Bk 12 (8.56 cm); and BK 14 (10.26 cm). The bacteria with the code BK 5 and BK 6 have a greater potential average zone of nodes that produced by 12 cm. It produces optimization testing LDH gene showing the isolate with the code BK1 and BK3 shows positive results with the optimization condition 95 °C for 2 minutes, followed by 35 for stage denaturize cycle at a temperature 95 °C for 15 seconds, annealing at a temperature 58 °C for 30 seconds and elongation at a temperature 72 °C for 1 minutes. The final Elongation at a temperature 72 °C for ten minutes and soaking at a temperature 4 °C.
REFERENCES