ISOLATION AND IDENTIFICATION OF BACTERIA FROM BANANA STEM BIOPROCESS RESULT AS DIRECT FED MICROBIAL FOR RUMINANT

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Abstract

Direct Fed Microbial are beneficial microbes to improve microbial balance in the digestive tract. Isolation of bacteria aims to obtain the potential bacteria as Direct Fed Microbial for ruminant. This research used descriptive methods. Identification used API test (Analytical Profile Index) methods. The results showed that there are two bacteria has potential as Direct Fed Microbial, those are Bacillus licheniformis bacteria and Lactobacillus delbrueckii ssp delbrueckii bacteria, both have the same characteristics as the bacteria in the rumen.

Key words: Isolation, Identification, Bioprocess, Banana Stem, Direct Fed Microbial

INTRODUCTION

Direct Fed Microbial (DFM) is probiotic category often used in animal feed industry [4] [10]. Direct Fed Microbial is useful microbes that used to improve microbes balance in digestive tract. Direct Fed Microbial can be obtained from various media formed from a mixture of natural ingredients from available natural resources and preferred by microorganisms. Microorganisms that formed is called the indigenous microorganisms. Microorganisms that included in DFM is a group of bacteria and yeast (fungus) [1].

The working mechanism of DFM is balancing rumen ecosystem, suppresses lactic acid production, and helps in breaking down cellulose. This is known from the volatile fatty acids production, ammonia, and higher ability to digest fiber [8]. DFM ideal strain should not only survive the digestive tract but also have the ability to multiply in the digestive tract, resistant to gastric and bile fluid [3] [7].

Rumen ecosystem balance that occurs will stimulate livestock growth and productivity using body weight increase as growth indicators. Microorganisms growth media can be waste plants around the environment, for example the remains of plants such as banana weevil, rice straw, etc. [8].

Banana stem is the largest source of natural ingredients both in size and weight compared to rice straw or other materials for local microbial growth media. The banana plant is an evergreen crop and the growth is distributed to almost every regions in Indonesia.

Endophytic microbes both endophytic fungi and bacteria which is isolated from plants organs have the capability of producing secondary metabolites in accordance to the host plant even in larger quantities. In addition, these efforts are more efficient in producing bioactive compounds because it does not require plants in large numbers thus preserving the plants [5] [11].

Isolates obtained from banana stems bioprocess may require further identification thus it will facilitate the characterization and classification. It is important to facilitate its development. Based on this it is important to identify endophytic bacteria isolates derived from banana stems. Endophytic isolates that requires identification is isolates that have the

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ability to improve microbes balance in ruminants digestive tract.

MATERIALS AND METHODS

Research conducted at the Laboratory of Microbiology Department of Biology, Faculty of Science, Universitas Padjadjaran. The tools used are incubators, autoclaves, erlenmeyer, hotplate, aluminum foil, Bunsen lamp, petri dish, a digital scale, mortar porcelain, beakers, test tubes, microscope drop tube, glass objects, ose needle, digital cameras, and stationery.

The materials used in this study is a indigenous microbial liquid from banana stems bioprocess, MRS, NA, PDA media, methyl red indicator (methyl red), material for Gram stain test (crystal violet, lugol iodine, safranin, alcohol 95% and distilled water), NaCl, hydrogen peroxide (H_2O_2), cotton, API (Analytical Profile Index) Kit.

Research Procedure

1. Tools and Materials Sterilization.

All tools and materials to be used sterilized by autoclaving at temperatures of 121ºC and 15 lb steam pressure for 15 minutes [9].

2. Sampling.

Sampling was obtained from the local microbial fluid as a results of banana stems bioprocess. Samples were grown on agar medium. Once grown, bacteria were isolated to be identified.

3. Bacteria isolation.

After samples obtained, then serial dilution performed. Serial dilution method that performed by taking 1 ml sample is inserted into a test tube containing 9 ml of distilled water thus 10^{-1}dilution obtained, 10^{-2} dilution obtained by taking 1 ml of dilution 10^{-1} and inserted into a test tube containing 9 ml of distilled water, and serial dilution maintained up to 10^{-9}. As Much 1 ml of 10^{-8} and 10^{-9} dilutions were taken and then placed in a petri dish containing agar medium. The mixture was leveled and incubated with the cup in inverted position for 24-48 hours at a temperature of 30ºC [2].

4. Identification of Bacteria.

After incubation for 48 hours, bacteria isolated with quadrant scratches method several stages to obtain 1pure isolates. Isolates were then identified using API Kit. Cells morphological observation includes gram stain test, cell lines, as well as the physiological properties test with catalase test and oxidase test.

Cell Morphology

1. Gram Stain.

The slide is cleaned with alcohol and passed several times on a bunsen flame, then bacterial isolates taken aseptically withose needle and smeared on a glass slide. Bacterial isolates then colored with drops of purple violet and abandoned for 1 minute, isolates then washed with running water and wind dried. Bacterial isolates then spilled with iodine drops and abandoned for 1 minute and washed with running water and wind dried. Furthermore, bacterial isolates is given drops of 95% alcohol for 30 seconds, then drained with water and wind dried. Bacterial isolates are then etched with safranin for 30 seconds and washed with water, dried with absorbent paper and wind dried, then microscope observation was performed. Gram-positive bacteria marked with purple indicates that the bacteria are able to bind crystal violet color, while gram-negative bacteria are marked with pink color indicates that bacteria are not able to bind crystal violet color and only stained by safranin (counter dye) [6].

2. The Cell Shape.

Bacteria that grows later observed microscopically for cell shape on glass slide thus the shape is known (cocci or rods).

The Physiological Traits

1. Catalase Test.

Two drops of H_2O_2 is placed on a clean glass slide. Bacterial isolates were taken using oseneedle, then transferred to a glass slide and mixed. Positive test characterized by the formation of oxygen bubbles that indicates the organisms in question produces catalase enzyme that converts hydrogen peroxide into water and oxygen.

2. Oxidase Test

In a sterile glass object oxidase paper strip was storedthen take bacteria from the slant NA culture using sterile ose and rub on the oxidase paper strip on object glass and Physiological NaCl was etched. Wait for color change on oxidase paper strip, when its changed into blue it indicates oxidative property.

Identification of Bacteria

Microbial identification using API Kit. Bacterial isolates was known at earlier test, The nused on further tests to determine the species name. Two API Test kit used, CHB medium for genus Bacillus and CHL medium for genus Lactobacillus.

API 50 CH - CHB Medium

Preparation stages is adding sterile distilled water to a strip. The first stage is manufacturing pure isolates of Bacillus embedded in MRS or NA, then incubated for 24 hours. The second stage is suspension manufacturing by adding 1 mL of NaCl 85% on tilted agar thus bacterial suspension formed. The next stage is making Mac Farlan II inoculum, prepare 5 mL of NaCl 85% on a test tube, and then bacterial suspension is etched to obtain MacFarlan II, the next stage is to plant suspension at CHB medium. Then as much as 2n suspension is added into 5 ml of CHB medium, then it is homogenized. Next step is planting on strip, as much as 120-150 mikromili on each strip. Then incubated for 48 hours at a temperature of 37ºC. After the results obtained by matching the API Test in installation on OSXP (Operating System Windows XP).

API 50 CH - CHL Medium

Stages of preparation is adding sterile distilled water in a strip. The first stage is manufacturing pure isolation of Lactobacillus which is planted on on MRS, then its incubated for 24 hours. Second stage is making suspension by adding 2 ml of NaCl 85% in to tilted agar to form bacterial suspension. The next stage of making inoculum MacFarlan II, prepare 5 mL of 85% NaCl on test tube, then bacterial suspension is dropped into it to obtain MacFarlan II.

The number of droplets to obtain MacFarlan II called n after obtained MacFarlan II, the next stage is planting on strip at CHL medium. A total of 2n of the suspension inserted on 5 ml CHL medium, then homogenized. The next stage is planting on strip, as much as 120-150 mikromili on each strip. Put strips on anaerobic container which has been given aerogen. Then incubated for 48 hours at a temperature of 37ºC. Having obtained the results by matching API Test program that installed on OSXP (Operating System Windows XP).

Research Parameter

The parameters of this study include (1) isolates number; (2) cell morphology, which is Gram staining properties and forms of bacterial isolates; and (3) the physiological properties and bacteria isolates with direct fed microbial potential.

Table 1 API KIT Identification Results : *Bacillus licheniformis*

Strip Profile Note	API 50 CHB V4.1 - + - - + + + - + - + + + + + + + + + - + + + + + + + + + + + + + - - + + + - + + - + - - - - - - -				
Significant taxa	%ID	T	Tests against		
Bacillus licheniformis	99.9	0.41	ADO 1%	SBE 8%	DUL 1%
Next taxon	%ID	T	Tests against		
Bacillus circulans	0.1	0.03	ADO 1% SOR 20%	SBE 1% TAG 20%	DUL 1% INO 1%

Table 2 API KIT Identification Results : *Lactobacillus delbrueckii ssp delbrueckii*

Strip Profile Note	API 50 CHL V5.2 - - - - - - - - - - - + + + - - - - - - - - + - - + + - + - - + + - - - - - - - - - - - - - - - - -			
Significant taxa	%ID	T	Tests against	
Lactobacillus delbrueckii ssp delbrueckii	51.2	0.8 ESC 24%	SAL 10%	TRE 20%
Lactobacillus acidopilus3	47.5	0.82		
Next taxon	%ID	T	Tests against	
Lactococcus lactis ssp hordniae	0.1	0.8	ARB 100%	MAL 0%

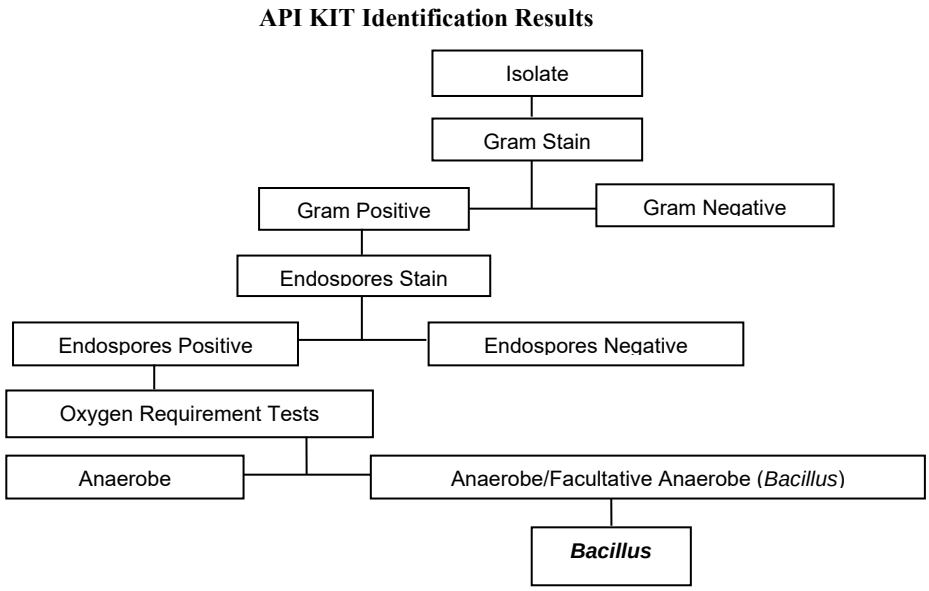

Fig 1. Identification Flow with gram stain test

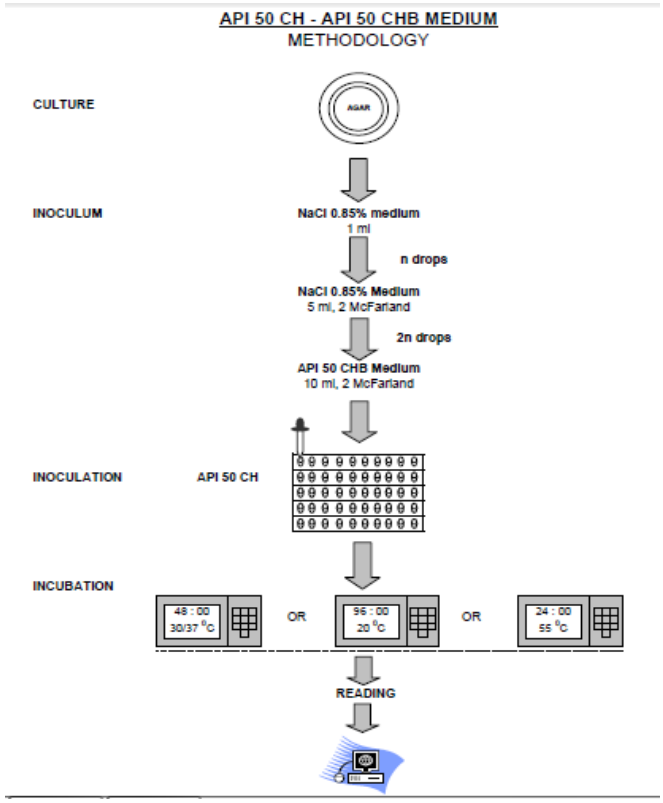

Fig. 2 Identification Flow with API Kit

RESULT AND DISCUSSION

Physiological test an advanced stage necessary to identify a bacterium. Physiological test performed in this study include catalase and oxidase test. Catalase test is used to determine the presence of catalase enzyme in bacterial isolates. Catalase is an enzyme that can catalyze the decomposition of hydrogen peroxide (H_2O_2) into water and O_2. Hydrogen peroxide is toxic to the bacterial cells because this material is able to deactivate enzymes in cells and is very harmful to the bacterial cell itself. This test is very important to know the nature of a bacterium to the need for oxygen.

In this research, the isolates potential as probiotics are gram positive as Bacillus and Lactobacillus. Based on the test using API kit, the results showed that there are two bacteria has potential as Direct Fed Microbial, namely *Bacillus licheniformis* and *Lactobacillus delbrueckii ssp delbrueckii* which has characteristics similar to the bacteria in the rumen.

Bacillus licheniformis and *Lactobacillus delbrueckii ssp delbrueckii* has characteristics similar to bacteria in rumen that can be used as DFM on ruminants. DFM good characteristics according has a good probiotic requirements [4]:

1) Strain capable of exerting a beneficial effect on the host animal, such as increased growth or resistance to disease.

2) Strain should be non-pathogenic and non-toxic.

3) The strain should be present as viable cells, preferably in large quantities.

4) The strain should be able to survive and metabolize in the digestive environment, for example, should be resistant to low pH and organic acids.

5) The strain should be stable and able to stay for an extended period of time under storage conditions.

In Table 1, are presented the results of identification using API test that shows the accuracy of the results *Basillus licheniformis* 99.9% and 0.1% *Basillus circulans*. Bakteri *Basillus licheniformis* has the characteristics of fiber and protein degradation due to produce cellulase and protease enzymes.

In Table 2, are presented the results of identification using API test showed 51.2% accuracy of the results *Lactobacillus delbrueckii ssp delbrueckii* and *Lactobacillus acidopilus* 47.5% and 0.1% *Lactococcus lactis ssp hordniae.* Bakteri *Lactobacillus delbrueckii ssp delbrueckii* has characteristics similar to the group *Laktobacillus casei* which has a beneficial effect on the digestive tract.

Lactobacillus is a type of lactic acid producing bacteria, including gram-positive bacteria, facultative anaerobic and microaerophilic. The existence of lactobacillus bacteria is an indication of a healthy environment, because this bacterium is a normal microflora in the gastrointestinal tract environment and living beings both on land and in water. Lactobacillus metabolic capability to produce lactic acid and peroxidase is an effective way of inhibiting these bacteria in a variety of microbial pathogens that cause disease. So that lactobacillus bacteria are used as probiotics to apply in directly on the environment as well as on the feed mixture.

Bacillus is a bacteria that has a rod-shaped cells, has a type of gram-positive cells, grow well can aerobic conditions, and in some species can grow in semi-anaerobic conditions. These bacteria can survive temperatures mostly hot environment, and produces an enzyme that is able to decompose organic matter types of carbohydrates, protein, and fat [4].

The bacteria Bacillus and Lactobacillus is a bacterium that includes DFM groups. Bacillus and Lactobacillus is a genus of gram-positive, facultative anaerobic or microaerophilic. Bacillus and Lactobacillus are bacteria that can break down proteins, carbohydrates, fats in food, to help the absorption of essential elements such as minerals, amino acids, and vitamins that are needed for survival. This bacterium measuring 0.7 to 1.1 x 2.0 to 4.0 µm. The bacteria Bacillus and Lactobacillus acid tolerant. These bacteria form colonies and is part of heterofermentatif facultative species. The bacteria are also able to survive in the digestive environment, for example, resistant to low pH and organic acids [4].

CONCLUSION

Based on the research results, we can conclude there are two bacterial isolates potentially as Direct Fed Microbial from the resultsof banana stems bioprocess, namely *Bacillus licheniformis* and *Lactobacillus delbrueckii ssp delbrueckii.*

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REFERENCES

[1] Association of American Feed Control Officials (AAFCO), 1999: *AAFCO Official Publication.* Atlanta: Georgia Dept. of Agric., USA. pp. 307-308.

[2] Darmayasa, 2008: Isolasi dan Identifikasi Bakteri Pendegradasi Lipid (Lemak) pada Beberapa Tempat Pembuangan Limbah dan Estuari DAM Denpasar. *Jurnal Bumi Lestari* 8:122-127.

[3] FAO/WHO, 2001: *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.* Córdoba, Argentina. 1-33

[4] Fuller, R., 1989: Probiotics in man and animals.*J. Appl. Bacteriol.*

[5] Guo B., Y. Wang, X. Sun, & K. Tang, 2008: Bioctive Natural Products from Endophytes: *A Review. Applied Biochemistry and Microbiology 44(2): 136-142.*

[6] Hadioetomo RS., 1993: *Mikrobiologi Dasar dalam Praktek Teknik dan Prosedur Dasar Laboratorium.* Gramedia, Jakarta.

[7] Kailasapathy, Kaila & Chin, James, 2000: Survival and therapeutic potential of probiotic organisms with reference to Lactobaccillus acidophilus and Bifidobacterium spp. *J. Immunology and cell biology*.78: 80-88.

[8] Mamilianti, Wenny, 2012: *Pengaruh Mol (Mikroorganisme Lokal) terhadap PenggemukanSapi Potong sebagai Upaya Peningkatan Pendapatan Peternak.* FakultasAgribisnis Universitas YudhartaPasuruan.

[9] Marlina, 2008: Identifikasi Bakteri Vibrio parahaemolitycus dengan Metode BIOLOG dan Deteksi Gen ToxRnya Secara PCR. *Jurnal Sains dan Teknologi Farmasi.* 13(1):11-17.

[10] Schrezenmeir, J. & de Vrese M., 2001: Probiotics, Prebiotics and Synbiotics.Approaching a Definition U.3. *Am.J.of Clin.Nutr*., Vol. 73, No.2: 361-364.

[11] Strobel G. A. & B. Daisy, 2003: Bioprospecting for Microbioal Endophytes and Their Natural Products. *Microbiol.Mol. Biol*. Rev. 67: 491-502.