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Characterization of Microorganism Isolated From “Fermege”: The Ruminant Fermented Feed from Water Hyacinth (*Eichhornia crassipes*)

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Abstract— The aim of this study was to determine the number of bacteria and fungi in the fermentation process and characterization of these microorganisms. There were several steps in this research including production of fermented feed, isolation of indigenous microorganisms, purification, characterization of microorganisms, species identification and test of cellulolytic activity. Results showed that eight bacteria and four fungi indigenous from the fermented feed were isolated. The species were *Bacillus badius*, *B. subtilis*, *B. brevis*, *B. pumilus*, *B. cereus*, *Xenorhabdus luminescent*, *Pseudomonas stutzeri* and *P. diminuta*. In addition, the species of fungi were *Aspergillus niger*, *A. flavus*, *Mucor sp.* and *Cunninghamella sp.* All of isolates shown the excellent cellulolytic activity. *Aspergillus niger*, *Cunninghamella sp.*, *B. brevis*, *B. cereus*, and *P. diminuta* showed the best cellulolytic activity. We recommend the use of microorganisms in the manufacture of cellulose fermentation starter component of the formula for producing ruminant feeds.

Keywords— water hyacinth; cellulotic degradation; cellulolytic bacteria; cellulolytic fungi; fermented feed production

I. INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is included in perennial aquatic herb or the aquatic weed. The water hyacinth (*Eichhornia crassipes*) is the most successful colonizers in the plant world. This free-floating freshwater plant has spread from tropical South Africa [9]. Water hyacinth has high rate multiplication. Water hyacinth has a long roots which are commonly suspended in water. The abilities of water hyacinth are higher growth rate, pollutant absorption efficiency, and renewability shows that using this plant can be considered as a suitable technology for the treatment of wastewater [8]. The structure of aquatic plants root in particular water hyacinth can present suitable environment for the aerobic microorganisms to function in the sewage system [6]. Water hyacinth can grow very rapid and float on the water surface. The growth is very difficult to control. There are many problems that occur due to the uncontrolled growth of water hyacinth such as covering the surface of water affecting on decreasing biodiversity, evaporating water rapidly and microhabitat for disease vectors [1].

Consequently, some efforts should be made to prevent environmental damage that caused the plant. The water hyacinth has been used as a phytoremediation agent [5]. Water

hyacinth is possible to become feed fermented because of the high nutrient content. Water Hyacinth dry material contains crude protein (10,1-12 g/100g), crude fibre (26,1-27,4 g/100g), ash (12,3 -12,4 g/100g), and energy 1999,7-2054,1 Kcal/kg. According to [5] water hyacinth has more advantages impact in environment and human health such as biogas, production of animal feed and compost. Also, feeded goat by fermented water hyacinth showed an increased level of body weight [4]. Moreover, the lamb analysis showed that the lamb of the goat feed with fermented water hyacinth had high protein and low-fat [7].

The fermentation process is essential to be applied in the feed production in order to the feed produced that contains high nutrient. The feed had a good level of digestibility and palatability. The report found that fermentation period as long as six weeks using *Aspergillus niger* shown the best result. In this process, *Aspergillus niger* is a probiotic. The addition of probiotics accelerates and enhance the fermentation process [3] [4]. Probiotics are living microorganisms that can improve the health and physiological benefits when consumed. The mixture of the microorganism as probiotic more advantageous than the using of a single microorganism.

Fermentation process always involves microorganisms like bacteria and fungi. The speed of fermentation process depends on several parameters such as the suitability of the type of microbes that work in the fermentation process, and chemical and physical conditions. Therefore, it needs to isolate specific inoculum [2]. Fermentation process associated to enzymes that belong to the microbes.

Water Hyacinth contains high amount of cellulose. Water Hyacinth has a high percentage of cellulose and hemicellulose (44% to 66,9% of dry weight basis) and low component of lignin from 3.5% to 9.5%. The content of water hyacinth is sufficient to extract fermentable sugars. The presence of lignin can make it resistant to degradation due to compact structure between cellulose and hemicelluloses [6]. These microorganisms are unique in terms of their diversity and properties. Based on the facts, each substance naturally has different indigenous microorganisms from other ingredients. The isolates were used as starter formulation that are expected to accelerate the fermentation process. This study carried out

the isolation, characterization and cellulolytic activity of bacteria and fungi that were contained in the fermented feed made from water hyacinth.

II. MATERIALS AND METHODS

Isolation procedures of indigenous microorganisms in the water hyacinth compost that capable to degrade of cellulose was performed using the following steps. Composting of water hyacinth was taken 10 g and was suspended in 90 mL of sterile water. This suspension was shaken at 150 rpm for 30 min at 30°C. Then the technique of dilution rank minus ten to seven series (10^{-7}) dilution was performed. The suspension of the 10^{-7} dilution was taken as 100µL and was locked in the salt medium (NaNO_3 0,5g L^{-1} ; K_2HPO_4 1g L^{-1} ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0,5g L^{-1} ; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0,02g L^{-1} ; KCl 0,2g L^{-1} and that 20 g L^{-1} was set at pH 7.5, the media was enriched with 0.05% (w / v) AZCL HE-Cellulose (Megazyme, Bray, Ireland) as the single carbon source. Furthermore, the incubation was carried out at 35 ° C for 7 days. The colonies of bacteria that can degrade cellulose will be surrounded by a blue zone. This indicated of their cellulase enzyme activity in the medium in a petri dish. Each colony that was surrounded blue zone was isolated and was subcultured to medium Trypto-Soya Agar (TSA; Nissui Pharmaceutical, Tokyo, Japan), as pure culture.

Similarly, the isolation of fungi with the cellulase enzyme activity was done using bacterial isolation, except its media composition. Isolation of fungi in order to get pure culture was performed in the three different kinds of media, MEA (malt extract agar), PDA (potato dextrose agar) and CA (Czapek's agar). The fungi with cellulase activities was determined by plate method screening on medium (PSM) using a solution that contain Mendel's mineral salt like following composition, urea 0.3 g L^{-1} ; $(\text{NH}_4)_2(\text{SO}_4)$ 1.4 g L^{-1} ; KH_2PO_4 0.2 g L^{-1} ; CaCl_2 0.3 g L^{-1} ; MgSO_4 0.3 g L^{-1} ; yeast extract 0.25 g L^{-1} ; and protease peptone 0.75 g L^{-1} with CMC (carboxymethyl cellulose) 10 g L^{-1} and to be 17.5 g L^{-1} . After few weeks, the fungi that fungi were incubated at a temperature of 25°C ($\pm 2^\circ\text{C}$) for 7 days. Fungi with cellulolytic activity will be characterized by the hydrolysis zone around it. For a more visible and easier to observe, the medium was colored with 1% Congo Red dyes for 30 minutes, and was washed with 1M NaCl for 20 minutes.

The pure isolates of bacteria were characterized in terms some kinds of the character of the colony like Gram stain, motility observation, catalase enzyme activity. The Microbact Identification Kits (Microbact™ GNB 12A dan 12B) were used in this research. The pure isolates of fungi were characterized by morphological observation using microscope of the colony, hypha, color and shape of the spore.

III. RESULTS

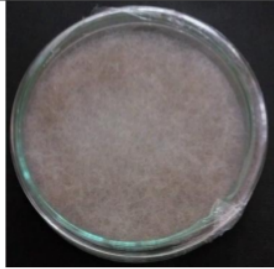
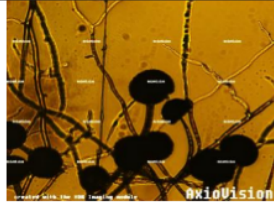
The number of bacteria that was obtained in this study is shown in Table 1. Based on the result, it can be stated that bacteria in a fermentation process of water hyacinth that contained eight isolates. Regarding the character of optical view, it was dominated by translucent colonies (4 colonies), the colony with opaque optical character (3 colonies), and the transparent colony (1 colony). Regarding the character of


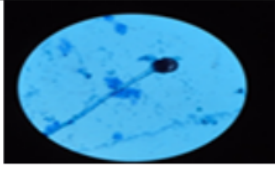
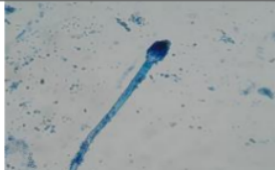
surface view, there are 4 colonies with a smooth shiny surface, not shiny smooth (3 colonies) and the colony with a rough surface (1 colony). Most colonies of white (7 colonies) and only one colony that is not pigmented (transparent). There were four kinds of colonies have the irregular shape, rhizoid shape 2 colonies, a circular shape one colony and filamentous one colony also. There are 3 kinds of elevation colonies were raised as much as 5 colonies, convex 2 colonies, and flat 1 colony. There are 5 different forms edges of colonies were filamentous as many as 2 colonies, lobat 2 colonies, undulate one colony, two entire colonies and erode one colony.

In addition to the characteristics of the colony in this study, isolates were observed the cell characteristics. The first as the form of the cell. By simple staining it was got several forms of cells namely streptobacilli, streptococci, monococcus, staphylococcus, and monobacillus. Based on gram staining, there were 6 isolates was gram-positive and gram -negative was 2 isolates. On the examination of the motility of isolates were obtained 5 isolates motile and non-motile 3 isolates. All isolates showed positive catalase activity.

Bacteria species in this research was shown in Table 2. Based on Table 2, there were eight bacteria species that was found in the fermented feed that was made from water hyacinth. These bacteria species were *Bacillus badius*, *B. subtilis*, *B. brevis*, *B. pumilus*, *B. cereus*, *Xenorhabdus luminescens*, *Pseudomonas stutzeri* and *P. diminuta*. In addition, the fungi that are found in this research is displayed in the following Table 3. Based on Table 3 there were four kinds of the fungi species in the fermented feed. They are *Aspergillus niger*, *A. flavus*, *Mucor sp.* and *Cunninghamella sp.*

TABLE III. IDENTIFICATION FUNGI INDIGENOUS FROM WATER HYACINTH

Morphology	The species of fungi
	<i>Aspergillus niger</i>
	

Morphology	The species of fungi
	<i>Aspergillus flavus</i>
	<i>Mucor</i> sp.
	<i>Cunninghamella</i> sp.

All of indigenous bacteria in this fermented feed have cellulolytic activity. Based on the Figure 1, there were three species of bacteria that had high cellulolytic activity, namely *B. brevis*, *B. cereus*, and *P. diminuta*. Based on the Figure 2 there were two species of fungi that had high cellulolytic activity, namely *Aspergillus niger* and *Cunninghamella* sp.

IV. DISCUSSION

Cellulase was a complex enzyme that contains at least three kinds of enzymes so that the cellulose was contained in the raw material can be perfectly digested into glucose. Glucose is a simple organic compounds or nutrients that can be absorbed by the digestive system of ruminants. The third group of enzymes found in the cellulase enzyme complex were exoglucanase, endoglucanase, and beta-glucosidase [6]. Exoglucanase and endoglucanase digest chain of glucose, whereas beta-glucosidase was cut to completely digestion residues into short glucose chain, as a result of cellulose digestion perfectly.

The more variations cellulolytic bacterial species involved in fermentation process, the faster of fermentation process takes place [3][4]. The more variations of bacteria, the greater number of enzymes involved in the fermentation process or by way between these bacteria work synergistically. Some investigators known as the species of *Bacillus* can degrade cellulose, based on the its capacity to use carboxymethylcellulose (CMC) as single carbon source in laboratory culture. Water hyacinth is cellulosic materials, because of its cellulose. Besides that, there is a *Bacillus* species as indigenous microorganisms in this plant. *Xenorhabdus poinari* is the cellulolytic bacteria in the gut of three different phytophagous insects, *Oxyvelox* belonging to order orthoptera and *Propylea quatuordecimpunctata* and *Aspidomor phamiliaris* both belonging

to order coleopteran. Indigenous bacteria in water hyacinth in this research were identified as *Xenorhabdus luminescent*. Indigenous bacteria *Pseudomonas* spp. found in Mahanadi River Delta were identified as cellulolytic bacteria. Cellulolytic activity of this bacteria like *Pseudomonas stutzeri* and *P. diminuta* were found in this research.

Aspergillus niger is a kind of fungi that has role as the cellulase source. Cellulase production by *Aspergillus niger* under submerged and solid state fermentation was done with kinds of substrate like coir waste and agricultural residue like finger millet hulls, sorghum hulls, soybean hulls, groundnut husk, banana peels, corn stalk, cassava peels, sugarcane bagasse, saw dust, rice straw and sheanut cake. The substrates used by *Aspergillus niger* to produce cellulase contain cellulose and the fact indicate that *Aspergillus niger* have cellulolytic activity. In the water hyacinth, *Aspergillus niger* and *A. flavus* found as indigenous microorganism because of its cellulolytic activity.

V. CONCLUSION

In this study water hyacinth has a high percentage of cellulose and hemicellulose and low component of lignin. It were eight isolates of bacteria and four isolates of fungi with the variation of cell and colonies characteristics have been found. The bacteria species were *Bacillus badius*, *B. subtilis*, *B. brevis*, *B. pumilus*, *B. cereus*, *Xenorhabdus luminescens*, *Pseudomonas stutzeri* and *P. diminuta* while the fungi species were *Aspergillus niger*, *A. flavus*, *Mucor* sp. And *Cunninghamella* sp. Among bacteria species that found in this research, *B. brevis*, *B. cereus*, and *P. diminuta* show high cellulolytic activity. *Aspergillus niger* and *Cunninghamella* sp. were fungi that had high cellulolytic activity.

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APPENDIX

TABLE I. INDIGENOUS BACTERIALS CHARACTERS FROM WATER HYACINTH

Characters	Isolate sample							
	¹³ <i>P1</i> <i>Bacillus badius</i>	<i>P2</i> <i>Bacillus subtilis</i>	<i>P3</i> <i>Bacillus brevis</i>	<i>P4</i> <i>Bacillus pumilus</i>	<i>P5</i> <i>Xenorhaptus luminescens</i>	<i>P6</i> <i>Bacillus cereus</i>	<i>P7</i> <i>Pseudomonas stutzeri</i>	<i>P8</i> <i>Pseudomonas diminuta</i>
Oxidase	+	+	+	+	-	-	+	+
Motilitas	+	+	+	+	+	+	+	+
4 rate	+	+	+	+	-	+	+	+
Lysine	+	+	+	+	-	-	-	-
Ornithine	-	+	-	+	-	-	-	-
H ₂ S	-	-	-	-	-	-	-	-
Glucose	-	+	-	+	-	-	-	-
Mannitol	-	+	-	+	-	-	-	-
Xylose	+	+	-	+	-	-	-	-
ONPG	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-
Urease	-	-	-	-	+	-	+	-
VP	+	-	-	+	+	+	-	-
Citrate	-	+	+	+	-	-	-	-
TDA	-	-	-	+	-	-	-	-
Gelatin	+	+	+	-	+	-	+	+
Malonate	-	-	-	+	+	-	-	-
Inositol	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-
Arginine	+	-	-	-	-	-	-	-
Gram's stain	+	-	-	-	-	-	-	-
Form	+	+	+	+	+	+	+	-

TABLE II. BACTERIALS CHARACTERIZATION AND IDENTIFICATION

Isolate Code	Morphology of Colony						Morphology of Cell			
	Optical View	Surface View	Pigmentation	Form	Elevation	Edges colony	Simple stain	Gram's stain	Motility	Catalase Activity
P1	Transparent	Rough	No pigmentation	Filamentous	Raised	Filamentous	Streptobacillus	Positive	Non motil	Positive
P2	Translucent	Smooth dull	White	Irregular	Raised	Lobate	Streptococcus	Positive	Motil	Positive
P3	Opaque	Smooth dull	White	Irregular	Flat	Lobate	Streptobacillus	Positive	Non motil	Positive
P4	Opaque	Smooth shiny	White	Irregular	Convex	Undulate	Mono-coccus	Negatip	Non motil	Positive
P5	Translucent	Smooth dull	White	Irregular	Raised	Entire	Mono-coccus	Negatip	Motil	Positive
P6	Opaque	Smooth shiny	White	Circular	Convex	Entire	Staphylococcus	Positive	Motil	Positive
P7	Translucent	Smooth shiny	White	Rhizoid	Raised	Filamentous	Mono-coccus	Positive	Non motil	Positive
P8	Translucent	Smooth shiny	White	Rhizoid	Raised	Erote	Mono-bacillus	Positive	Non motil	Positive

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