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Indigenous Bacteria Community Fluctuation During Fermentation of Water Hyacinth (*Eichhornia crassipes*) and Corncob (*Zea mays*)

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Abstract. Water hyacinth nowadays is a disturbing water-weeds. In fact, water hyacinth is potential for cattle feed if it is mixed with corncob, and thus increase the nutritional value. In cattle feed production, there are some indigenous fungi and bacteria to be used for fermentation. This research was aimed to know the fluctuation of bacteria and fungi community for 15 days of fermentation process. The bacteria and fungi were first inoculated and isolated in both NBA and PDA agar plate. Then, they were purified so as to make pure-culture that will be easily identified. Bacteria identification was conducted using *Microbact Identification Kits* (*Microbact™ GNB12A and 12B*) and identification book *Bergey's Manual of Determinative Bacteriology*. Fungi identification were conducted based on macroscopic and microscopic characteristics. Ecological parameters such as diversity, evenness, and dominance were analyzed on this research. There were eight bacteria species found, including *Bacillus laterosporus*, *B. badius*, *B. pantothenicus*, *B. brevis*, *Staphylococcus sciuri*, *B. stearothermophilus*, *Burkholderia pseudomallei*, and *Enterococcus duran*. The highest bacteria diversity were in the fourth day, the highest evenness occurred at the first, tenth, twelfth, and fifteenth day. All of the bacteria species were dominant during fermentation, except *Bacillus badius* at the eleventh day that was a subdominant bacteria.

1. Introduction

Up to now, the feed stock especially the cheap and nutritious one has become a main problem in breeding. The cheap and nutritious feed can be produced through fermentation process by utilizing farm-waste and weeds materials. The efforts in producing cheap and nutritious fermented feed can be made of farm-waste, such as rice-straw, corn-straw (its leaves and stems), corncob, *Leguminosae* waste, and other farm wastes. From all farm wastes, corncob has not been utilized optimally. Rice-straw is generally big-sized ruminants feed, such as cows so that in this research fermented feed is made from corncob as the main material.

Corncob is potential to be used as cattle feed because it contains 5,6% protein which is higher than the protein concentration in rice-straw (4,9%). The utilization of corncob is only for fuel (as substitutive component for wood), also for mushrooms growth media [1]. Some researchers had been utilized corncob as cattle feed, such as Sumaoang (corncob powder) [1]. Through fermentation process by utilizing microorganisms and molase addition, corncob not only becomes a nutritious feed but also becomes favourite food for pigs, goats, and poultries [1]. The corncob that has been blended also can be



used to replace cassava slices as buffalo feeds [2]. Then, Rostika and Safitri [3] used corncob that has been blended and fermented with various mushrooms as fish feed. Lardy and Anderson [4] stated that corncob can be utilized as ruminant feed alternative.

However, there are some weaknesses in using corncob as feed materials that up to now can not be overcome. The weaknesses including of the high natural lignocellulose concentration (45-55% cellulose, 25-35% hemicellulose, 20-30% lignin) and also high fiber concentration (930 g netral detergent fibers/kg dry materials; 573 g acid detergent fibers/kg dry materials) that are difficult to digest in cattle [5]. The corncob processing before it is used in fermentation, such as rarefaction, fermentation itself and addition other materials will increase the overall nutrient value and the quality.

The nutrient enhancement of fermented feed can be conducted by adding other materials, so that the fermented feed does not contains a single material only. Water weeds, water hyacinth (*Eichhornia crassipes*) is a very suitable added material. It is because the water hyacinth can grow very fast [6], contains high protein around 11.87% and 14.28 % percentage and contains high calcium and phosphorous concentration, and therefore stimulate the milk production if it is combined with suitable concentration [7]. The nutrient concentration did not change because of its growth geographical difference [8]. However, the use of water hyacinth as cattle feed has some weaknesses. Water hyacinth contains a very high rough fiber concentration, so that the carbon source is less good. The other weakness is that the water concentration of water hyacinth is relatively very high, the texture is smooth, and the protein is difficult to digest [9]. Based on Umsakul, Dissara, and Srimuang [10] research the mixing process of water hyacinth in feed production can increase the nutrient value of the feed.

Saputro [9] had conducted fermentation process in water hyacinth by using *Aspergillus niger* obtained the duration of fermentation in 8 days to obtain fermented feed with rough protein 18,84% percentage and 15,73% rough fiber. The other research related to the utilization of water hyacinth as fermented feed material has used tempe yeast as microorganism sources [11]. The feed produced can increase goat body weight. Research result conducted by Pioneer, Yurmiati, and Sinaga [12] on *New Zealand White* rabbit showed that the usage 20% water hyacinth provides body weight addition, but not influence the ratio usage efficiency.

There have been some efforts in increasing the feed quality by using fermentation technique. The fermented feed has some strengths such as high nutrient concentration, high digestibility, and high palatability [13].

Fermentation process always involves microorganisms, including bacteria and fungi. The rate of fermentation process depends on some factors, mainly the compatibility of microbes related to fermentation process of materials and physical-chemical parameters. It means that fermentation process using certain materials will have specific and selected microorganisms [8]. This has relation with the enzymes of the microbes, whether it is suitable to degrade the chemical compounds in the materials that will be fermented or not. The diversity of indigenous microorganisms in every fermentation phase need to be known to give scientific reasons that are important to make a fit formula. The indigenous microorganisms diversity that have been known will give information about microorganisms population that have dominant role in fermentation, so that the suitable starter formulation will be easier.

The other factor determining the fermentation rate is physical condition and chemical condition that support fermentation process. Physical condition consists of temperature, pH, and humidity [14]. The chemical condition that influences fermentation process include of the presence of carbon and nitrogen sources, also respiratory gases (in aerobic and anaerobic condition) and supplement molecules to support microorganisms population growth. Research result of Belal [15] showed that different carbon sources will cause different fermentation result in rice-straw to produce bioethanol.

In the research that have been conducted, the nutrient value of water hyacinth and corncob mixture was high with fermentation duration for 5 days [16]. The goats that have been given fermented feed of water hyacinth and corncob mixture show higher biomass compared to the biomass when using conventional feed [16, 17]. The other strength of water hyacinth and corncob mixture fermented feed, based on Saputro [9], is that it can improve the goat carcass, signing by increasing protein concentration

and lowering fat content. Those studies focus mainly on the use of tempe-yeast for fermenting agent which fulfill the standard needed 5 days.

The research about bacteria and fungi species involved in fermentation process, especially with water hyacinth and comcob mixture as materials has not been conducted. Knowledge about bacteria and fungi species related in fermentation process of certain materials is very essential to increase fermentation result quality and the rate of fermentation itself. This research will investigate the fluctuation of bacteria and fungi species involving in fermentation process from day to day during fermentation process, then the diversity, evenness, and dominancy of bacteria and fungi species will be analyzed.

2. Method

Basically in this research, there were some stages, each of stages will be detailed as follow:

2.1 Indigenous microbe sources preparation and isolation

Water hyacinth as microbes sources was taken from a river that the water had been ensured unpolluted by doing water quality test and heavy metal concentration test. The corncob was taken from the rice mill that had been ensured uncontaminated from dangerous substances. As initial indigenous microbes sources, firstly the production of fermented feed with water hyacinth and corncob mixture was conducted with ratio 1:1. This feed was made by some stages including materials cutting, materials drying, steaming, and materials incubation so that fermentation will occur naturally [17]. Everyday during fermentation process, the indigenous microbes isolation by taking materials randomly for 30 gr, then the materials suspended in sterile aquades, filtered, and cultured by pour plate method. Bacteria media culture used NA (Nutrient Agar for Microbiology Merck). Culture then incubated in 37°C temperature for 24-72 hours.

2.2 Purification, characterization, and identification of indigenous bacteria and fungi

The culture mixture was then purified. Characterization of pure bacteria isolates was conducted by macroscopic and microscopic observation. Identification of pure bacteria isolates was conducted using *Microbact Identification Kits (Microbact™ GNB12A and 12B)*.

2.3 Indigenous bacteria and fungi diversity observation during fermentation process

During fermentation process (for 15 days), observation and documentation including numbers and types of isolates that grow were conducted everyday. Then, counting of diversity parameter was conducted index of heterogeneity or index of diversity, index of evenness, and index of species richness. Index of diversity was counted using Shannon-Wiener formula as follow: $H' = - \sum (p_i \log p_i)$ (H' is index of diversity, p_i adalah ratio of individual number in a species and total individual numbers). Index of evenness is counted by using formula $E = H' / \ln(S)$ (E is index of evenness of a species, H' adalah Shannon-Wiener index of diversity, S is numbers of species found, \ln is natural logarithm). Index of species richness is counted using formula $D_i = n_i/N$ (D_i is species dominancy, n_i adalah number of individuals in a species, N adalah number of individuals of all species).

3. Result and Discussions

The result obtained from daily observation from day to day in 15 observation days. The data obtained then was analyzed the community diversity, evenness, and daily species dominancy was conducted during fermentation process. The result obtained then presented in Table 1 as follow.

Table 1. Diversity, evenness, daily species dominance during water hyacinth and corn cob mixture fermentation process

Day of fermentation	Total Bacteria Numbers	Diversity	Evenness	Bacteria Dominancy
1	134,5	0,2981	0,4301	<i>Bacillus laterosporus</i> (56,13%) <i>Bacillus stearothermophilus</i> (43,87%)
2	242	0,4708	0,4286	<i>Bacillus laterosporus</i> (39,67%) <i>Bacillus stearothermophilus</i> (26,03%) <i>Enterococcus durans</i> (34,30%)
3	304,5	0,5346	0,3856	<i>Bacillus laterosporus</i> (33,66%) <i>Bacillus badius</i> (5,42%) <i>Bacillus stearothermophilus</i> (23,15%) <i>Enterococcus durans</i> (37,77%)
4	308	0,7146	0,3988	<i>Bacillus laterosporus</i> (33,77%) <i>Bacillus badius</i> (7,63%) <i>Bacillus pantothenicus</i> (10,55%) <i>Bacillus brevis</i> (8,60%) <i>Bacillus stearothermophilus</i> (21,1%) <i>Burkholderia pseudomallei</i> (18,34%)
5	307,5	0,6216	0,3862	<i>Bacillus laterosporus</i> (41,63%) <i>Bacillus badius</i> (18,21%) <i>Bacillus pantothenicus</i> (5,69%) <i>Bacillus brevis</i> (12,52%) <i>Bacillus stearothermophilus</i> (21,99%)
6	284	0,4591	0,4179	<i>Bacillus laterosporus</i> (45,78%) <i>Bacillus badius</i> (22,36%) <i>Bacillus stearothermophilus</i> (31,87%)
7	280	0,4818	0,3475	<i>Bacillus laterosporus</i> (49,46%) <i>Bacillus badius</i> (8,39%) <i>Bacillus pantothenicus</i> (6,96%) <i>Bacillus stearothermophilus</i> (35,18%)
8	278	0,3872	0,3525	<i>Bacillus laterosporus</i> (52,16%) <i>Staphylococcus sciuri</i> (7,01%) <i>Bacillus stearothermophilus</i> (40,83%)
9	298	0,4687	0,4268	<i>Bacillus laterosporus</i> (47,82%) <i>Bacillus badius</i> (5,20%) <i>Bacillus brevis</i> (8,22%) <i>Bacillus stearothermophilus</i> (38,76%)
10	210	0,3004	0,4334	<i>Bacillus laterosporus</i> (47,51%) <i>Bacillus stearothermophilus</i> (52,49%)
11	391	0,6525	0,4054	<i>Bacillus laterosporus</i> (24,78%) <i>Bacillus badius</i> (4,47%) <i>Bacillus stearothermophilus</i> (25,54%) <i>Burkholderia pseudomallei</i> (20,05%) <i>Enterococcus durans</i> (25,16%)
12	289	0,4764	0,4336	<i>Bacillus laterosporus</i> (30,62%) <i>Bacillus stearothermophilus</i> (34,08%) <i>Enterococcus durans</i> (35,29%)
13	342	0,7502	0,3855	<i>Bacillus laterosporus</i> (23,39%)

Day of fermentation	Total Bacteria Numbers	Diversity	Evenness	Bacteria Dominancy
				<i>Bacillus badius</i> (6,58%) <i>Bacillus pantothenicus</i> (5,70%) <i>Bacillus brevis</i> (5,99%) <i>Staphylococcus sciuri</i> (6,43%) <i>Bacillus stearothermophilus</i> (25,59%) <i>Enterococcus durans</i> (26,32%)
14	298	0,7888	0,4054	<i>Bacillus laterosporus</i> (25,67%) <i>Bacillus badius</i> (10,91%) <i>Bacillus pantothenicus</i> (7,72%) <i>Bacillus brevis</i> (10,74%) <i>Staphylococcus sciuri</i> (8,56%) <i>Bacillus stearothermophilus</i> (26,85%) <i>Burkholderia pseudomallei</i> (9,56%)
15	306,5	0,6010	0,4335	<i>Bacillus laterosporus</i> (22,35%) <i>Bacillus badius</i> (25,78%) <i>Bacillus pantothenicus</i> (27,24%) <i>Bacillus stearothermophilus</i> (24,63%)

Based on Table 1, the diversity, evenness, and species dominancy were different in day-to-day observation. By using culture method in Nutrient Broth, eight bacteria species were successfully isolated, consisted of *Bacillus laterosporus*, *B. badius*, *B. pantothenicus*, *B. brevis*, *Staphylococcus sciuri*, *B. stearothermophilus*, *Burkholderia pseudomallei*, and *Enterococcus duran*. The highest bacteria diversity was in the fourth day, the highest evenness occurred at the first, tenth, twelfth, and fifteenth day. All of the bacteria species were dominant during fermentation, except *Bacillus badius* at the eleventh day that was a subdominant bacteria.

Feed materials consisting of water hyacinth and corncob contain high cellulose. Because of that, the indigenous bacteria that previously have been isolated from the material are dominated by bacteria that have cellulolytic activity. From those, there are 2 bacteria that do not show cellulolytic activity, namely *Bacillus brevis* and *Burkholderia pseudomallei*. Even though the two bacteria cannot degrade cellulose, this two species can inhibit water hyacinth and corncob mixture because they can use cellulose degradation result by other bacteria as carbon and energy sources. Based on bacteria diversity data during fermentation process as in Table 1, the presence of *Bacillus brevis* and *Burkholderia pseudomallei* have been just detected in the fourth day of fermentation process. This gives signal that the two bacteria can utilize the cellulose degradation result by other bacteria to fulfill their needs. This is very different to *Bacillus laterosporus* which is present in the feed since the first day until fifteenth day, when the isolates taking stopped.

The indigenous bacteria diversity involved in fermentation process of water hyacinth and corncob mixture are different from day to day. The diversity of indigenous bacteria consisted of species variation and the numbers of bacteria that inhibit those materials. The overall bacteria diversity during fermentation process are classified low (Shannon-Wiener index of diversity value = 0,7065), which means that the diversity is low. This indicates that during fermentation process of water hyacinth and corncob mixture, there was not a big species diversity, only a few species involved in the fermentation process. In every species, individual numbers are not even with index of evenness 0,3398 which means that the evenness is low. This indicates that the species diversity are not distributed, only certain species involved in the fermentation process of water hyacinth and corncob mixture. The dominant species during fermentation process are *Bacillus laterosporus*, *B. badius*, and *Enterococcus durans*. Each

different fermentation (mesophylic, termophylic, cooling and maturing) has different microorganisms variation. Fermentation process of a material involve some kinds of bacteria and fungi [18].

4. Conclusion

In fermentation process, the mixture of water hyacinth and corncob involves eight bacteria species including *Bacillus laterosporus*, *B. badius*, *B. pantothenicus*, *B. brevis*, *Staphylococcus sciuri*, *B. stearothermophilus*, *Burkholderia pseudomallei*, and *Enterococcus duran*. The diversity, evenness, and dominance of related bacteria in those fermentation materials from day to day are various. The highest diversity is at the fourth day, while the highest evenness occurred at the first, tenth, twelfth, and fifteenth days. All of bacteria species are dominant during fermentation process, except *Bacillus badius* at the eleventh day that was a subdominant bacteria.

Based on the research result obtained, there are bacteria diversity, evenness, and dominance fluctuation from day to day during fermentation. Therefore, in the utilization of fermented feed as indigenous bacteria consortium starter, all the caught isolates have to be sponsored everyday.

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