



Available online freely at [www.isisn.org](http://www.isisn.org)

# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2019 16(3):3085-3090.

OPEN ACCESS

## Diversity of indigenous bacteria during fermentation fermentoge: The ruminant feed made of water hyacinth (*Eichhornia crassipes*) and corn (*Zea mays*) cob

Isnawati\*, Guntur Trimulyono and Dwi Anggorowati Rahayu

Faculty of Mathematics and Natural Science, State University of Surabaya, Jalan Ketintang, 60231, Surabaya, Indonesia

\*Correspondence: [isnawati@unesa.ac.id](mailto:isnawati@unesa.ac.id) Accepted: 01 August 2019 Published online: 11 Sep 2019

This research aim to know the diversity of bacteria community for 15 days of fermentoge fermentation process. Fermentoge is the ruminant feed made of water hyacinth and corn cob. The procedure passed consisted of bacteria isolation, purification and identification. Bacteria identification based on the sequence of 16S rRNA gene. Parameters of community ecology such as diversity, evenness, and dominancy were analyzed on this research. There were 8 bacteria species, including *Staphylococcus sp.*, *Enterococcus sp.*, *Bacillus tequilensis*, *Bacillus brevis*, *Bacillus badius*, *Bacillus cereus*, *Bacillus aerius* and *Burkholderia sp.* The highest bacteria diversity were in the fourteenth day, the highest evenness occurred at the twelfth day fermentation process. Seven bacteria species were dominant during fermentation, except *Bacillus badius* that sub dominant.

**Keywords:** diversity, evenness, dominancy, fermentoge, fermentation

### INTRODUCTION

Water hyacinth grew very rapidly in organic materials water polluted (Ndimele and Jimoh, 2011). Up to now, water hyacinth has been assumed as disadvantageous water-weeds. The rapid growth of the plant in water-body can change the diversity of water organisms and endanger other organisms life (Gichuki, et al., 2012). Some kinds of efforts had been done to inhibit their growth. The efforts consisted of nutrient reduction in water-body (Gichuki, et al., 2012), removal of plants manually, giving 2,4-D or glyphosate (Labrada, et al., 1994) and paraquate (Jian-jun, et al., 2006).

Other efforts to reduce water hyacinth in water area was by functioning it as cattle-feed, because of the high concentration of protein around 11.87% to 14.28 % (Mako, et al., 2011), high

calcium and phosphor concentration, and could stimulate the milk production if it is combined with suitable feed concentration (Kumar, et al., 2011). Other analysis stated that water hyacinth contained dry material (8.7 to 9.3 g/100g), crude protein (10.1 to 11.2 g/100g), crude fiber (26.1 to 27.4 g/100g), nitrogen-free extract (47.2 to 50.2 g/100g), ether extract (1.1 to 1.8 g/100g), and total ash 12.3 to 12.4 g/100g, with metabolism energy 1999.7 to 2054.1 kcal/kg (Hossain, et al., 2015). Some researchers had been utilized water hyacinth as cattle feed like duck feed (Lu, et al., 2008; Mangisah, et al., 2009), and *Cyprinus carpio* feed (Mohapatra, et al., 2015).

Other waste materials that less utilized, but had potency to be functioned as cattle feed is corncob. Lignocellulose concentration of corncob consists of 45% to 55% cellulose, 25% to 35%

hemicellulose, and 20% to 30% lignin, which cannot be digested by digestive enzymes of pork (Kanengoni, et al., 2015). Corncob also contains 5.6% protein that is higher than rice straw (4.9%). Corncob had been used for pork, chicken, and goat feed (Sarian, 2016), buffalo feed (Wanapat, et al., 2012; Wachirapakorn, et al., 2016), fish feed (Rostika and Safitri, 2012), and various kinds of ruminant feed (Lardy and Anderson, 2009).

Fermetoge is a fermented feed made of water hyacinth and corn cob that has some advantageous, including increasing digestibility, nutrient absorbability level by cattle, and balancing the rumen microflora and decreasing pathogen-microorganism population (Missotten, et al., 2015). Other strength of fermented feed is the ability of reducing pathogen- microbe growth in digestive tract, helping stomach to reach low pH so that can kill the microbes that is carried by food (Missotten, et al., 2015). The using of fermented feed for chicken had increased its body weight, caused more aggressive behavior, strengthen egg-shell without reducing the egg production (Engberg, et al., 2009). In fact, cellulase enzymes produced by some bacterial in fermetoge can be beneficial for degrading cellulose available in the starter culture from cellulosic substrate such as other plant materials or agricultural waste, which may contribute to the fermented feed quality.

The fermentation process involves bacteria and fungi. The rate of fermentation process depends on some factors, mainly the compatibility of microbes with materials that will be fermented. It means that fermentation process using certain materials will have specific and selected microorganisms (Boboescu, et al., 2014). The specificity of microbes enzyme were play role in fermentation of specific materials. The diversity of indigenous microorganisms in every fermentation phase or day to day along fermentation process should be determined, in order to create a suitable starter consortium. Fungi were involved in fermentation process with water hyacinth and corncob mixture as feed materials is known in the previous research (Isnawati, et al., 2018). In this research displayed the bacteria were involved in the fermentation process to fermetoge production.

The knowledge about bacteria species related in fermentation process of certain materials are very essential to increase the rate and quality of fermentation result. This research investigate the fluctuation of bacteria in fermentation process from day to day in the fermetoge production. The diversity, evenness, and dominancy of bacteria

species will be analyzed.

## MATERIALS AND METHODS

The fermetoge was made by some stages: Water hyacinth and corncob were cut then dried. Dry materials were steamed, then mixed with ratio 1:1. After that, the mixture were incubated in order to naturally fermentation process (Fitrihidajati, et al., 2015). Every day during fermentation process, the indigenous bacteria was isolated by taking 30g materials randomly, then was suspended in sterile aquades, filtered, and cultured by pour plate method. Nutrient Agar (NA) for Microbiology (Merck) was used as the media culture. Then, the culture was incubated in 37°C for 24-48 hours. After 24-48 hours incubation, the culture was purified by streak plate method, then incubated for 24-48 hours again to get the pure culture. The pure culture as the DNA source for identification needs were grown in the Nutrient Broth (liquid) medium. DNA extraction was performed by QIAMP DNA Mini Kit (Qiagen). The DNA sample (DNA template) amplified by PCR (BioNer PCR Cycler) with the Primer Forward 341 and Primer Reverse 907 for 35 cycles. DNA result of amplification process (DNA amplicon) of each isolate was sequenced and the bacteria were identified to species or genus level using BLAST.

## RESULTS

The result presented in Table 1 as follow. Based on Table 1, from day to day there were different diversity, evenness, and species dominancy. By using culture method in nutrient agar, eight bacteria species was successfully isolated, consisted of *Staphylococcus sp.*, *Enterococcus sp.*, *Bacillus tequilensis*, *Bacillus brevis*, *Bacillus badius*, *Bacillus cereus*, *Bacillus aerius* and *Burkholderia sp.* The highest bacteria diversity were in the fourteenth day, the highest evenness occurred at the twelfth day. Seven of the bacteria species were dominant during fermentation, except *Bacillus badius* that was sub dominant.

Feed materials consist of mixture water hyacinth and corncob that contain high cellulose. Because of that, the indigenous bacteria had been isolated from this material were dominated by bacteria which have cellulolytic activity. The number of species increased day to day, and then decreases in the middle of the fermentation process. But, at the end days of fermentation process there were seven species of bacteria.

**Tabel 1; Diversity, evenness, and dominance index daily of bacteria species during water hyacinth and corncob mixture fermentation process in the fermentoge production**

Day of fermentation	Total number of Bacterials	Diversity index	Evenness index	Dominancy
1	134.5	0.2981	0.4301	<i>Staphylococcus sp.</i> (56.13%) <i>Enterococcus sp.</i> (43.87%)
2	242	0.4708	0.4286	<i>Staphylococcus sp.</i> (39.67%) <i>Enterococcus sp.</i> (26.03%) <i>Bacillus tequilensis</i> (34.30%)
3	304.5	0.5346	0.3856	<i>Staphylococcus sp.</i> (33.66%) <i>Bacillus brevis</i> (5.42%) <i>Enterococcus sp.</i> (23.15%) <i>Bacillus tequilensis</i> (37.77%)
4	308	0.7146	0.3988	<i>Staphylococcus sp.</i> (33.77%) <i>Bacillus brevis</i> (7.63%) <i>Bacillus badius</i> (10.55%) <i>Bacillus cereus</i> (8.60%) <i>Enterococcus sp.</i> (21.1%) <i>Bacillus aerius</i> (18.34%)
5	307.5	0.6216	0.3862	<i>Staphylococcus sp.</i> (41.63%) <i>Bacillus brevis</i> (18.21%) <i>Bacillus badius</i> (5.69%) <i>Bacillus cereus</i> (12.52%) <i>Enterococcus sp.</i> (21.99%)
6	284	0.4591	0.4179	<i>Staphylococcus sp.</i> (45.78%) <i>Bacillus brevis</i> (22.36%) <i>Enterococcus sp.</i> (31.87%)
7	280	0.4818	0.3475	<i>Staphylococcus sp.</i> (49.46%) <i>Bacillus brevis</i> (8.39%) <i>Bacillus badius</i> (6.96%) <i>Enterococcus sp.</i> (35.18%)
8	278	0.3872	0.3525	<i>Staphylococcus sp.</i> (52.16%) <i>Burkholderia sp.</i> (7.01%) <i>Enterococcus sp.</i> (40.83%)
9	298	0.4687	0.4268	<i>Staphylococcus sp.</i> (47.82%) <i>Bacillus brevis</i> (5.20%) <i>Bacillus cereus</i> (8.22%) <i>Enterococcus sp.</i> (38.76%)
10	210	0.3004	0.4334	<i>Staphylococcus sp.</i> (47.51%) <i>Enterococcus sp.</i> (52.49%)
11	391	0.6525	0.4054	<i>Staphylococcus sp.</i> (24.78%) <i>Bacillus brevis</i> (4.47%) <i>Enterococcus sp.</i> (25.54%) <i>Bacillus aerius</i> (20.05%) <i>Bacillus tequilensis</i> (25.16%)
12	289	0.4764	0.4336	<i>Staphylococcus sp.</i> (30.62%) <i>Enterococcus sp.</i> (34.08%) <i>Bacillus tequilensis</i> (35.29%)
13	342	0.7502	0.3855	<i>Staphylococcus sp.</i> (23.39%) <i>Bacillus brevis</i> (6.58%) <i>Bacillus badius</i> (5.70%) <i>Bacillus cereus</i> (5.99%) <i>Burkholderia sp.</i> (6.43%) <i>Enterococcus sp.</i> (25.59%) <i>Bacillus tequilensis</i> (26.32%)
14	298	0.7888	0.4054	<i>Staphylococcus sp.</i> (25.67%) <i>Bacillus brevis</i> (10.91%) <i>Bacillus badius</i> (7.72%) <i>Bacillus cereus</i> (10.74%)

				<i>Burkholderia sp.</i> (8.56%) <i>Enterococcus sp.</i> (26.85%) <i>Bacillus aerius</i> (9.56%)
15	306.5	0.6010	0.4335	<i>Staphylococcus sp.</i> (22.35%) <i>Bacillus brevis</i> (25.78%) <i>Bacillus badius</i> (27.24%) <i>Enterococcus sp.</i> (24.63%)

Based on bacteria diversity data during fermentation process as in Table 1, *Staphylococcus sp.* and *Enterococcus sp.* have been detected the presence in all of day of fermentation process. The presence of that bacteria indicate signal that the two species could utilize the cellulose degradation as the energy and carbon sources (Alruman, 2016). It was different from the members of *Bacillus* group which presence at the end of fermentation process. This indicated that the members of *Bacillus* group need compounds from bacterial metabolism that work at the beginning of the fermentation process.

The indigenous bacteria diversity that involved in fermentation process of water hyacinth and corncob mixture were different from day to day. The diversity of indigenous bacteria consist of species variation, and the numbers of bacteria that grow in those materials. The overall bacteria diversity during fermentation process were classified low (Shannon-Wiener index of diversity value = 0,7065). This indicated that during fermentation process of water hyacinth and corncob mixture there was not a big species diversity of bacteria, only a few bacteria species involved in the fermentation process. The index of evenness each bacteria about 0,3398 was categorized low. This indicated that the bacteria species diversity were not distributed, only certain bacteria species involved in the fermentation process. Seven of the bacteria species are dominant species during fermentation process, except the *Bacillus badius* that was sub dominant. Ramos, et al., (2011) state that each different phase fermentation (mesophylic, termophylic, cooling and maturing) has different microorganisms and involve some kinds of bacteria and fungi.

## CONCLUSION

In summary, fermentation process of the mixture of water hyacinth and corncob involved eight bacterial species including *Staphylococcus sp.*, *Enterococcus sp.*, *Bacillus tequilensis*, *Bacillus brevis*, *Bacillus badius*, *Bacillus cereus*, *Bacillus aerius* and *Burkholderia sp.* The diversity, evenness, and dominance of related bacterial in

those fermentation materials from day to day are various. The highest diversity was on the fourteenth day, while the highest evenness occurred at the twelfth day. Seven of bacterial species were dominant during fermentation process, except *Bacillus badius* that was sub dominant species.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEDGEMENT

We would like to thank staff from Biology Department of Mathematics and Natural Science Faculty, State University of Surabaya and Biologi Department of Science and Technology faculty, Airlangga University for their help in this project.

## AUTHOR CONTRIBUTIONS

ISN designed and performed the research and also wrote the manuscript. GTM and DAR performed bacteria identification, counted diversity and evenness value, identification of bacteria dominance and reviewed the manuscript. All authors read and approved the final version.

## Copyrights: © 2019@ author (s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

## REFERENCES

- Alrumman, S. A. 2016. Enzymatic Saccharification and Fermentation of Cellulosic Date Palm Waste to Glucose and Lactic Acid. Brazilian Journal of Microbiology, 47: 110-119.
- Boboescu, I. Z., Ilie, M., Gherman, V. D., Mirel, I., Pap, B., Negra, A., Kondorosi, E., Biro, T., & Maroti, G. 2014. Revealing the Factors

- Influencing A Fermentative Biohydrogen Production Process Using Industrial Wastewater as Fermentation Substrate. *Biotechnology for Biofuels*, 7: 139-154.
- Engberg, R. M., Hammersh, M., Johansen, N. F., Abousekken, M. S., Steinfeldt, S., & Jensen, B. B. 2009. Fermented Feed for Laying Hens: Effects on Egg Production, Egg Quality, Plumage Condition and Composition and Activity of The Intestinal Microflora. *Journal British Poultry Science*, 50(2): 228-239.
- Fitrihidajati, H., Ratnasari, E., Isnawati, & Soeparno, G. 2015. Quality of Fermentation Result of Ruminant Feed from Water Hyacinth (*Eichhornia crassipes*), *Journal of Biosaintifika*, 7 (1): 62-67.
- Gichuki J, Omondi R, Boera P, Okorut T, Matano A S, Jembe T & Ofulla A. 2012. Water Hyacinth *Eichhornia crassipes* (Mart.) Solms-Laubach Dynamics and Succession in The Nyanza Gulf of Lake Victoria (East Africa): Implications for Water Quality and Biodiversity Conservation. *The Scientific World Journal* 10: 11-20.
- Hossain, Md. E., Sikder, H., Kabir, Md. H., & Sarma, S. M. 2015. Nutritive Value of Water Hyacinth (*Eichhornia crassipes*). *Journal of Animal and Feed Research*, 5(2): 40-44.
- Isnawati, Ni'matuzahroh, & Surtiningsih, T. 2018. Diversity of Indigenous Fungi During Ruminant Feed Fermentation Made of Water Hyacinth (*Eichhornia crassipes*) and Corn (*Zea mays*) cob. *Bioscience Research*, 15(3):2797-2801.
- Jian-jun, C., Yi, D., & Qi-jia, Z. 2006. Invasion and Control of Water Hyacinth (*Eichhornia crassipes*) in China. *J Zhejiang Univ SCIENCE B*, 7(8):623-626.
- Kanengoni, A. T., Chimonyo, M., Ndimba, B. K., & Dzama, K. 2015. Potential of Using Maize Cobs in Pig Diets — A Review. *Asian Australas. J. Anim. Sci.*, 28(12): 1669-1679.
- Kumar, A., Sharma, P.C., Kumar, A., & Negi, V. 2011. A Study on Phenotypic Traits of *Candida* Species Isolated from Blood Stream Infections and Their In Vitro Susceptibility to Fluconazole. *Al Am een J Med Sci*, 7(1):83-91.
- Labrada R, Caseley J C and Parker C. (1994). *Weed Management for Developing Countries*. FAO. New York. USA.
- Lardy, G., & Anderson, V. 2009. Alternative Feeds for Ruminant. NDSU. Dakota.
- Lu, J., Fu, Z., & Yin, Z. 2008. Performance of Water Hyacinth (*Eichhornia crassipes*) System in The Treatment of Wastewater from A Duck Farm and The Effects of Using Water Hyacinth as Duck Feed. *Journal of Environmental Sciences*, 20(5):513-519.
- Mako, A. A., Babayemi, O. J., & Akinsoyinu, A. O. 2011. An Evaluation of Nutritive Value of Water Hyacinth (*Eichhornia crassipes* mart. solms-laubach) Harvested from Different Water Sources as Animal Feed. *Livestock Research for Rural Development*, 23:106-110.
- Mangisah, I., Sukamto, B., & Nasution, M. H. 2009. Implementation of Fermented Eceng Gondok in Duck Ration. *Journal of The Indonesian Tropical Animal Agriculture*, 34 (2): 127-133.
- Missotten, A. M., Michiels, J., Degroote, J., & De Sme, S. 2015. Fermented Liquid Feed for Pigs: An Ancient Technique for The Future. *Journal of Animal Science and Biotechnology*, 6: 4-13.
- Mohapatra, S. B. 2015. Utilization of Water Hyacinth (*Eichhornia crassipes*) Meal as Partial Fish Protein Replacement in The Diet of *Cyprinus carpio* Fry. *European Journal of Experimental Biology*, 5(5): 31-36.
- Ndimele, P. & Jimoh, A. 2011. Water Hyacinth (*Eichhornia crassipes* [Mart.] Solms.) in Phytoremediation of Heavy Metal Polluted Water of Ologe Lagoon, Lagos, Nigeria. *Research Journal of Environmental Sciences*, 5(5): 424-433.
- Ramos, C. L., de Almeida, E. G., Freire, A. L., & Schwan, R. F. 2011. Diversity of Bacteria and Yeast in The Naturally Fermented Cotton Seed and Rice Beverage Produced. *Food Microbiology*, 28(7): 1380-1386.
- Rostika, R. & Safitri, R. 2012. Influence of Fish Feed Corn-Cob Was Fermented by *Trichoderma sp.*, *Aspergillus sp.*, *Rhizopus oligosporus* To The Rate of Growth of Java Barb (*Puntius gonionitus*). *APCBEE Procedia*, 2:148-152.
- Sarian, Z. B. 2016. 'Corn Cobs Converted into Nutritious Animal Feed' Version: 30 October 2016. <http://www.zacsarian.com/category/agri-ideas>.
- Wachirapakorn, C., Pilachai, K., Wanapat, M., Pakdee, P. & Cherdthong, A. 2016. Effect of Ground Corn Cobs as A Fiber Source in Total Mixed Ration on Feed Intake, Milk Yield and Milk Composition in Tropical



Lactating Crossbred Holstein Cows. *Animal Nutrition* 2: 334-338.

Wanapat, M., Pilajun, R., Kang, S., Setyaningsih, K. & Setyawan, A. R. 2012. Effect of Ground Corn Cob Replacement for Cassava Chip on Fermentation and Urinary Derivatives in Swamp Buffaloes. *Asian-Aust. J. Anim. Sci.*, 25(8): 1124-1131.