Advances in Engineering Research, volume 209
International Joint Conference on Science and Engineering 2021 (IJCSE 2021)

Diversity of Cellulolytic Fungi Isolated in Fermetodege: Fermented Feed Mixed Water Hyacinth, Rice Bran, and Corn Cob

Isnawati [1,*] Lisa Lisdiana[1], Mahanani Tri Asri[1], Guntur Trimulyono[1], Roni Afif Hidayat[1]

[1] *Department of Biology, Universitas Negeri Surabaya*, Surabaya, Indonesia
[*] *Corresponding author. Email:* isnawati@unesa.ac.id

ABSTRACT
Fermetodege is an innovation of fermented feed with a mixture of water hyacinth, corn cobs, and rice bran which was developed in the manufacture of ruminant feed by utilizing the role of microbes. Fungal communities in the feed fermentation process have a role as microbes that can degrade materials containing cellulose to increase the palatability of feed. This study aims to determine the diversity of cellulolytic fungi isolated from fermetodege, namely ruminant fermented feed mixed of water hyacinth, rice bran, and corn cobs that influence the fermentation process of feed ingredients. Fungi isolation was carried out using potato dextrose agar (PDA) media and screened by growing on carboxymethyl cellulose (CMC). Fungi isolates was analyzed based on Shannon-Wiener to get the diversity and evenness index, and the Simpson dominance index. There were nine cellulolytic fungi isolates with the Shannon-Wiener diversity index value in medium category 1.3423-1.8814 (1<H'<3), the evenness index 0.8340-0.9668, and the Simpson dominance index in the low category (close to 0) namely 0.1608-0.3105. The results showed that the community structure of cellulolytic fungi in the fermetodege had moderate diversity with an even distribution without the dominance of certain species of cellulolytic fungi during fermentation. The diversity of fungi in fermetodege: fermented feed mixture of water hyacinth, rice bran, and corn cobs has a diversity of fungi that have the potential to be used in the fermentation process.

Keywords *— Cellulolytic Fungi, Fermetodege, Diversity*

1. INTRODUCTION

Fermentation has been widely used in animal feed-making processes, especially ruminant cattle. Fermentation is used for the biodegradation of feed ingredients which generally consist of materials with high cellulose content. Feed ingredients with high cellulose content have low palatability values and are difficult to digest by livestock. Conventional feed ingredients, which are generally in the form of leaves and grasses, are also known to be uncertain in their availability for a certain period. Utilization of the fermentation method in the manufacture of animal feed aims to increase the palatability of feed, is also useful in helping farmers in providing feed for a longer period than conventional animal feed. The basic ingredients of fermented feed that can be used because of their abundance and high lignocellulose content include water hyacinth, rice bran, and corn cobs.

Water hyacinth (*Eichhornia crassipes*) is known as an aquatic plant that becomes an aquatic weed that grows rapidly.

Water hyacinth with uncontrolled growth in waters can have an impact on water utilization problems because it can cause silting and disruption of aquatic ecosystems [1]. Water hyacinth contains lignocelluloses that contain 16.4% cellulose, 42.8% hemicellulose, and 6.5% lignin [2]. Based on the proximate analyzed the water hyacinth content was 9.3% dry matter, 26.9% crude fiber, 12.4% ash content, and 10.5% crude protein [3]. High lignocellulose content and good other nutrient indicate that water hyacinth can be used as a base material for fermented animal feed. The use of water hyacinth as a raw material for fermented feed can also support environmental conservation efforts from the threat of pollution caused by the rapid growth of water hyacinth.

Rice bran and corn cobs are agricultural wastes that are used properly. Rice bran and corn cobs are materials with high lignocellulosic content, because of that, they have the potential to be used in the manufacture of fermented feed. Rice bran consists of 42.9% cellulose, 26% hemicellulose, and 22% lignin [4]. Based on the proximate analysis of rice bran, they contain 23.21% crude fiber, 12.35% ash, 16.72% protein, and 52.87% carbohydrate [5]. Corn cobs are known to have lignocellulose content consisting of cellulose 45.88%, hemicellulose 39.40%, and lignin 11.32% [6]. Proximate levels in corn cobs include 30.93% crude fiber, 2.26% ash, and 0.61% crude fat [7].

The basic ingredients of feed in the form of water hyacinth, rice bran, and corn cobs are fermented by utilizing the role of the microbial community, one of which is cellulolytic fungi. The microbial community under controlled conditions during the fermentation process plays a role in the biodegradation of organic materials such as lignocellulosic [8]. Cellulolytic fungi that have the potential to be used in the biodegradation process include the genera Aspergillus, Trichoderma, and Fusarium [9]. The use of fungi is known to increase the nutritional value of biomass materials with lignocellulosic content in ruminant feed [10].

2. METHODS

2.1. Materials and Methods Research Materials

The basic ingredients of fermented feed were used in this study consisted of water hyacinth, rice bran, corn cobs, and banana leaves. Materials for the isolation process and calculation of fungal diversity consisted of potato dextrose agar (PDA), carboxymethyl cellulose (CMC), distilled water, 70% alcohol, spiritus, peptone, $MgSO_4.H_2O$, K_2HPO_4, $(NH_4)_2SO_2$, $FeCl_3.6H_2O$, yeast extract, $MnSO_4$, and agar.

2.2. Fermentation Feed Making

Fermented feed made from water hyacinth, rice bran, corn cobs were made with the 1:1:1 composition of each ingredient. The water hyacinth used consists of stems and leaves. The feed ingredients must first be ensured in a clean condition. The feed ingredients are cut into small sizes (1-3 cm) and then was mashed. The refined feed material was dried to reduce the water content of the materials. The feed ingredients were steamed for 20-30 minutes. The feed ingredients after the steaming process were cooled and mixed evenly. The feed ingredients were then was put in a basket with banana leaves covering [11].

The fermentation process was carried out in this study was solid-state fermentation (SSF) and under natural conditions for 15 days. Fungi isolation was performed every day along the fermentation process [12]. Temperature and pH measurements were carried out during fermentation on the top, middle, and bottom of the feed. The temperature and pH of each measurement were recorded and averaged to obtain daily temperature and pH data.

2.3. Isolation Fungi Cellulolytic

Isolation of fungi was performed by the pour plate method using 10 g of feed samples taken at random about 10-15 cm from the top of the feed. The feed sample was suspended by adding sterile distilled water until the volume reached 100 ml. The sample was vortexed to homogenize the sample. Isolation of fungi was carried out by a series of 10^{-5} dilutions. Fungal isolation samples from the 10^{-5} dilution series were taken 1 ml and cultured on 10 ml of potato dextrose agar (PDA) media which had previously been added to the antibiotic chloramphenicol.

Isolates that grew on PDA media after three days of incubation were counted the number of colonies and recorded based on the number of each colony that had different morphology colonies. The fungal isolates were purified by the streak plate method based on the results of observations of the colony morphological differences of each fungal. The purified fungal isolates were grown on carboxymethyl cellulose (CMC) media to know fungi cellulolytic activity. The CMC media composition included 10 g carboxymethyl cellulose, 3 g yeast extract, 0.2 g $MgSO_4.H_2O$, 5 g K_2HPO_4, 0.5 g $(NH_4)_2SO_2$, 0.01 g $FeCl_3.6H_2O$, 0.001 g $MnSO_4$, 1000 ml distilled water, 5 g peptone, and 20 g agar [13].

2.4. Cellulolytic Fungi Diversity

The diversity of isolates fungi was obtained by counting the number of colonies that grew on each day along of fermentation process. The diversity of fungi that have the dominant role in the fermentation process of feed making from water hyacinth, rice bran, and corn cobs was known by calculating the diversity index which was strengthened by the calculation of the evenness and dominance index. Diversity is determined based on the Shannon-Wiener diversity index, evenness index, and Simpson dominance index calculated by the formula followed below [14].

$$H = -\sum\left(\frac{n_i}{N}\right)\ln\left(\frac{n_i}{N}\right) \quad (1)$$

Note: H' = Shannon-Wiener diversity index, ni = the total number of individuals of the i-th species, and N = the total number of individuals in all species. The level of species diversity is classified as low if H'<1, moderate 1<H'<3, and high H'>3.

$$H = \sum\left(\frac{H'}{\ln S}\right) \quad (2)$$

Note: E = evenness index, H' = Shannon-Wiener diversity index, and S = number of species. Evenness is categorized as having an unstable species distribution if the index is close to 0 and classified as stable if the species distribution is close to 1.

$$H = \sum\left(\frac{n_i}{N}\right)^2 \quad (3)$$

Note: C = Simpson's dominance index, ni = the total number of individuals of the i-th species, and N = the total number of individuals in all species. The range of dominance index values ranges from 0 to 1 with the C value close to 0 indicating that there are no dominant species in the community, and if C is close to 1, there is a dominating species in the community

3. RESULTS AND DISCUSSION

3.1. Observation of Temperature and pH during Fermentation Process

Observation of temperature and pH parameters was carried out during the fermentation process of feed ingredients. Measurement of temperature and pH for 15 days in the feed fermentation process showed that there were differences in temperature and pH every day.

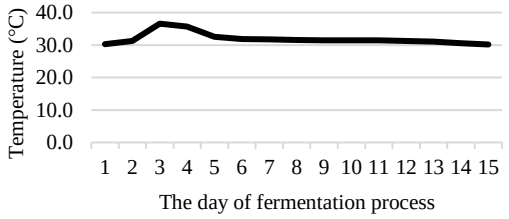

Figure 1. Graph of average temperature fluctuation for 15 days of the feed fermentation process

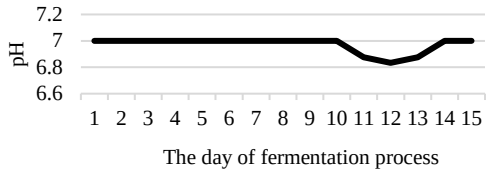

Figure 2. Graph of average pH fluctuation for 15 days of the feed fermentation process

The observation results of temperature and pH fluctuation during the fermentation process showed that there were no significant differences in temperature and pH changes, thus providing information that the isolation of fungi was correct for 15 days of fermentation because it was difficult to distinguish the rotation of each fermentation phase that took place.

Temperature and pH fluctuation during 15 days of the fermentation process indicated that the fermentation phase could not be identified. The highest temperature during fermentation was 36.6ºC, while the lowest temperature was 30.2ºC. Based on pH measurements, the highest pH was 7 and the lowest pH was 6.8. Temperature and pH fluctuation in the fermentation process indicated the cellulolytic fungi activity on the materials. Changes in temperature during fermentation indicate an effect of temperature on the enzymatic activity of the fungi during the fermentation process [15]. Changes in pH during the fermentation process indicated organic acids produced by fungi [16].

3.2. Fermentation of Feed Ingredients

Water hyacinth, rice bran, and corn cobs were chopped before the fermentation process to reduce the size of the material, to accelerate the drying and steaming process. Cutting the material into small sizes aims to make it easier for microorganisms to decompose the material. The drying process was used to reduce the water content of the feed ingredients. The low water content of the ingredients was needed to avoid the spoilage process of the material and to improve the fermentation process. The ideal water content of materials ranged from 30–40% [17].

The fermentation process was carried out in the natural condition with the solid-state fermentation (SSF) method. Solid-state fermentation is influenced by several factors including the kind of substrate used, temperature, water content, and microbial bioactivity [18]. The application of solid-state fermentation has a positive impact including reduced risk of contamination, needed low cost, easier to do, thereby increased productivity during the fermentation process [19].

3.3. Cellulolytic Fungi Isolation

The results of the isolation and screening process of cellulolytic fungi obtained 9 fungal isolates based on the differences in colony morphology. Based on Tabel I, the number of all fungal isolates during 15 days of fermentation was 1192 colonies. Isolation and screening of cellulolytic fungi on fermented feed made from a mixture of water hyacinth, rice bran, and corn cobs obtained nine isolates including CF1, CF2, CF3, CF4, CF5, CF6, CF7, CF8, and CF9.

TABLE 1. Cellulolitic fungi colony of fermetodege

Isolate Code	Number of colonies each day of along fermentation process															Total colony
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
CF1	3	5	2			5	4	4	2	6	22	10	3	6	25	96
CF2	14	12	12	16	20	18	17	22	18	14	34	30	34	49	54	368
CF3	2	3	3	3	7	8	13	6	9	15	11	14	15	6	12	126
CF4				3	5	6	10	5	7	11	3	7	4	3	3	67
CF5	3	4	4	2	4											16
CF6			2	4												6
CF7	10	14	10	7	11	7	16	15	18	22	14	16				159
CF8								10	20	17	13	13	33	37	33	176
CF9						5	18	18	12	10	6	19	30	23	37	178
Total	32	38	33	35	47	49	78	80	86	95	103	109	119	124	164	1192

According to the number of colonies that grow on the media was influenced by differences in the absorption of nutrients in the substrate and ecological factors [20]. Substrate contained cellulose will be hydrolyzed into glucose by cellulase enzyme activity. The glucose resulting from this degradation process has functioned as a carbon source and energy for fungal growth [21].

3.4. Cellulolytic Fungi Diversity

The results of the analysis of fungal diversity stated that the diversity of fungi was classified as moderate with the results of evenness and dominance analysis showing that during fermentation the evenness of fungi was classified as the same without the presence of dominant species.

The diversity of cellulolytic fungi during the fermentation process of a mixture of water hyacinth, rice bran, and corn cobs as a whole based on the calculation of the diversity index belonging to the medium criteria with the highest diversity index value occurring on the 10th day of fermentation of 1.8814 and the lowest diversity index value of 1.3423 on the first day of fermentation. The value of the diversity index is classified as moderate indicating a balanced ecosystem condition with sufficient productivity and moderate influence of ecological factors [22].

TABLE 2. Index of diversity, evenness, and dominance of fungi during the fermentation process

Day of fermentation	Number of Fungi Colony	Index		
		Diversity	Evenness	Dominance
1	32	1.3423	0.8340	0.3105
2	38	1.4362	0.8923	0.2701
3	33	1.5432	0.8613	0.2544
4	35	1.5123	0.8440	0.2800
5	47	1.4351	0.8917	0.2766
6	49	1.6647	0.9291	0.2178
7	78	1.7097	0.9542	0.1897
8	80	1.7818	0.9157	0.1891
9	86	1.7966	0.9232	0.1793
10	95	1.8814	0.9668	0.1608
11	103	1.7356	0.8919	0.2046
12	109	1.8539	0.9527	0.1709
13	119	1.5289	0.8533	0.2397
14	124	1.4234	0.7944	0.2849
15	164	1.5756	0.8794	0.2287

The structure of the cellulolytic fungi community during the feed fermentation process had stable evenness and low dominance. The evenness index for 15 days of fermentation showed a stable community structure based on the evenness index value close to 1. The highest evenness index was on the 10th day of fermentation with a value of 0.9668 and the lowest evenness index was 0.8340 on the 1st day of fermentation. An evenness index close to 1 indicates that the distribution is even across species [23].

The dominance index obtained during 15 days of fermentation did not show a significant difference in index values. The highest dominance index value of 0.3105 occurred on the first day of fermentation, while the lowest dominance index value occurred on the 10th day of fermentation, which was 0.1608. A low dominance index indicates that there are no certain species that dominate in the community [23]. A low dominance index value has a relationship with evenness index results. According to the high evenness index value, the dominance index value tends to be low [24]. Based on the results of evenness and dominance indices, it showed that the community structure of cellulolytic fungi during fermentation was stable without a tendency for a type of cellulolytic fungi to dominate and each type of cellulolytic fungi had the same distribution of individuals.

4. CONCLUSION

The diversity of cellulolytic fungi isolated from fermented feed mixed with water hyacinth, rice bran, and corn cobs during the fermentation process included moderate criteria based on the Shannon-Wiener diversity index with diversity index values ranging from 1.3423-1.8814 ($1<H'<3$). The evenness index ranged from 0.8340-0.9668 and Simpson's dominance index was categorized as low (close to 0) ranging from 0.1608-0.3105 indicating that the cellulolytic fungi community structure had an even distribution without the dominance of certain cellulolytic fungi during the fermentation process. The diversity of fungi in fermetodege: fermented feed mixture of water hyacinth, rice bran, and corn cobs has a diversity of fungi that have the potential to be used in the fermentation process. Fungal isolates that play a role in feed fermentation can then be selected to obtain candidate microbial starters in the fermentation process of cellulose materials.

REFERENCES

[1] B. A. Adanikin, G. A. Ogunwande, and O. O. Adesanwo. "Evaluation and kinetics of biogas yield from morning glory *(Ipomoea Aquatica*) co-digested with water hyacinth (*Eichhornia crassipes*)." Ecological Engineering, vol. 98, pp. 98-104, January 2017.

[2] S. Rezania, M. F. M. Din, S. M. Taib, S. E. Mohamad, F. A. Dahalan, H. Kamyab, N. Darajeh, and S. S. Ebrahimi. "Ethanol production from water hyacinth (*Eichhornia crassipes*) using various types of enhancers based on the consumable sugars." Waste and Biomass Valorization, vol. 9.6, pp. 939-946, March 2017.

[3] M. E. Hossain, H. Sikder, M. H. Kabir, and S. M. Sarma. "Nutritive value of water hyacinth (*Eichhornia crassipes*)." Online Journal of Animal and Feed Research, vol. 5.2, pp. 40-44, 2015.

[4] R. Sivaramakrishnan, B. Ramprakash, G. Ramadoss, S. Suresh, A. Pugazhendhi, and A. Incharoensakdi. "High potential of Rhizopus treated rice bran waste for the nutrient-free anaerobic fermentative biohydrogen production." Bioresource Technology, vol. 319, pp. 124193, January 2021.

[5] L. Liu, W. Wen, R. Zhang, Z. Wei., Y. Deng, J. Xiao, and M. Zhang. "Complex enzyme hydrolysis releases antioxidative phenolics from rice bran." Food Chemistry, vol. 214, pp. 1-8, January 2017.

[6] A. Shariff, N. S. Mohamad Aziz, N. L. Ismail, and N. Abdullah. "Corn Cob as a Potential Feedstock for Slow Pyrolysis of Biomass." Journal of Physical Science, vol. 27.2), pp. 123-137, 2016.

[7] K. A. Paynor, E. S. David, and M. J. G. Valentino. "Endophytic fungi associated with bamboo as possible sources of single-cell protein using corn cob as a substrate." Mycosphere, vol. 7.2, pp. 139-147, 2016.

[8] V. S. Varma, K. Ramu, and A. S. Kalamdhad. "Carbon decomposition by inoculating Phanerochaete chrysosporium during drum composting of agricultural waste." Environmental Science and Pollution Research, vol. 22.10, pp. 7851-7858, January 2015.

[9] A. L. Chafla, Z. Rodríguez, R. Boucourt, J. Espín, and L. Silva. "Isolation, selection and characterization of cellulolytic fungi from cocoa (*Theobroma cacao* L) hull." Revista Cubana de Ciencia Agrícola, vol. 50.3, pp. 411-420, 2016.

[10] S. J. A. Van Kuijk, A. S. M. Sonnenberg, J. J. P. Baars, W. H. Hendriks, and J. W. Cone. "Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review." Biotechnology advances, vol. 33.1, pp. 191-202, January–February 2015.

[11] H. Fitrihidajati, E. Ratnasari, Isnawati, and G. Soeparno. "Quality of fermentation result of ruminant feed production made of water hyacinth (*Eichornia crassipes*)." Journal of Biosantifika, vol. 7.1, pp. 62-67, 2015.

[12] Isnawati. "Cellulolytic Activity of Indigenous Fungi in Fermentation: Ruminant Fermented Feed Made of Water Hyacinth (*Eichhornia crassipes*) and Corn Cobs (*Zea mays*)." Journal of Biological Research and Its Applications, vol. 1.1, pp. 26-31, March 2019.

[13] I. N. Sumerta and A. Kanti. "Diversity of Yeasts Isolated from Natural Resources of Enggano Island, Bengkulu and Its Cellulolytic Potency." Berita Biologi, vol. 15.3, pp. 247-255, December 2016.

[14] M. A. Hanif, M. A. B. Siddik, M. R. Chaklader, A. Nahar, and S. Mahmud. "Fish diversity in the southern coastal waters of Bangladesh: present status, threats, and conservation perspectives." Croatian Journal of Fisheries: Ribarstvo, vol. 73.4, pp. 148-161, 2015.

[15] G. H. Hansen, M. Lübeck, J. C. Frisvad, P. S. Lübeck, and B. Andersen. "Production of cellulolytic enzymes from Ascomycetes: comparison of solid-state and submerged fermentation." Process Biochemistry, vol. 50.9, pp. 1327-1341, September 2015.

[16] A. P. G. Dezam, V. M. Vasconcellos, P. T. Lacava, and C. S. Farinas. "Microbial production of organic acids by endophytic fungi." Biocatalysis and Agricultural Biotechnology, vol. 11, pp. 282-287, July 2017.

[17] Y. Farida, H. Sasongko, and Sugiyarto. "Utilization of Local Plant as Fermented Feed and Supplements for Livestock in Sendang Village, Wonogiri District." Agrokreatif: Jurnal Ilmiah Pengabdian kepada Masyarakat, vol. 4.1, pp. 61-67, July 2018.

[18] P. K. Sadh, S. Duhan, and J. S. Duhan. "Agro-industrial wastes and their utilization using solid-state fermentation: a review. " Bioresources and Bioprocessing, vol. 5.1, pp 1-15, January 2018.

[19] P. Leite, D. Sousa, H. Fernandes, M. Ferreira, A. R. Costa, D. Filipe, M. Gonçalves, H. Peres, I. Belo, and J. M. Salgado. "Recent advances in the production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes." Current Opinion in Green and Sustainable Chemistry, 100407, 2020.

[20] M. Yu, J. Zhang, Y. Xu, H. Xiao, W. An, H. Xi, Z. Xue, H. Huang, X. Chen, and A. Shen, A. "Fungal community dynamics and driving factors during agricultural waste composting." Environmental Science and Pollution Research, vol/ 22.24, pp. 19879-19886, 2015

[21] J. Silva-Mendoza, A. Gómez-Treviño, U. Lopez-Chuken, E. A. Blanco-Gámez, L. ChávezGuerrero, and M. E. Cantú-Cárdenas, M. E. "Agave Leaves as a Substrate for the Production of Cellulases by *Penicillium* sp. and the Obtainment of Reducing Sugars." Journal of Chemistry, 2020.

[22] P. Binambuni, M. Langoy, and D. Y. Katili. "Diversity of Starfish Species on Bahowo Beach, Bunaken District, Manado City, North Sulawesi." Pharmacon, vol. 8.1, pp. 65-72, 2019.

[23] A. Munandar, M. S. Ali, and S. Karina. "Community Structure Macrozoobenthos Estuari Kuala Rigaih District District Of Setia Bakti Aceh Jaya." Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah, vol. 1.3, pp. 331-336, November 2016.

[24] S. Nurlinda, M. Kasim, and A. I. Nur. "Community Structure and Macrozoobenthos Condition in Reefrupted Coral Reef in the Village of Oyster Tanjung North, Moramo District, South Konawe District." Jurnal Manajemen Sumber Daya Perairan, vol. 4.2, pp. 123-133, 2019.