ISECE 2010

International Seminar on Economic, Culture and Environment

The University of Mataram, Indonesia, 11-13 November 2010

Proceedings

ISBN 978-979-8911-67-5

PT Newmont
Nusa Tenggara

Proceeding of
International Seminar On Economic, Culture And Environment

November 11 – 3, 2010
Mataram, Indonesia

Reviewer :

Prof. Mahyuni, Ph.D.
Ir. Surya Hadi, M.Sc., Ph.D.
Dr. Muhamad Ali.
drh. Made Sriasih, M.Agr.Sc.,Ph.D.

Editor :

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Indonesian Ministry of Foreign Afairs,
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Preface

On behalf of the University of Mataram it is a pleasure and a great honor for me to welcome you to Lombok Island and to this international seminar on economic, culture and environment. Unram is one of the state universities in Indonesia which was founded in 1964. At the beginning of its establishment, it was only Law Faculty followed by Economic Faculty few years later. Now, Unram has eight faculties, i.e. law, economic, agriculture, animal husbandry, engineering, medical, and science faculties with about 1000 more lecturers and 450 supporting staffs and 17 000 students. Last month, October 2010, we celebrated our 48th anniversary. 48 years old if we make an analogy as the age of a human being is certainly a mature age. We realize that Unram is as it is now, because of supports from both government and stakeholders. To respond to this, Unram will continue to improve its maturity in all aspects. For instance, in recent years, our university has significantly improved the competency and qualification of its staff. This can be seen from the increase in number of our staff who gained their Ph.D from both domestic and overseas universities, including the increase in number of the professors. This is relevant as an approach to indicate progress. Now, we try to work even harder to reach our goal to be one of the World Class Universities. Because, we have huge potentials that need to be tackled professionally.

We have wide range of dry land area in both Sumbawa and Lombok island. In terms of facilities, we have standard laboratories to conduct collaborative research. So far, we have joint research with Nagoya University, Jichi Medical School in Japan, Arizona State University in United States, La Trobe University in Australia, Utrecht University in The Netherlands, SOAS in England. We expect to have more joint research in the future as this global world requires. This is also imperative that we need more colleagues to collaborate with in handling related issues for the betterment of the future life.

However, let us see what is going on in our region where malls, supermarkets, housing complex are built on very productive lands which sometimes are not based on comprehensive environmental studies. We expect that not in a very long time Unram would show its existence and scientific contribution on this global trend. This seminar is one of the efforts to show the world that we exist and ready to work together hand in hand to face the world's common concern. There is an urgent need of saving our planet from an even detrimental destruction. One of them is climate and environmental change, another is economic and cultural change.

In terms of economics, the discourse among Asean countries to unify their currency system is an interesting issue to be addressed proportionally. This seminar is expected to contribute some principle ideas and strategies on how Asean should tackle their internal problems to reach harmonious cooperation among its members. In relation to culture, there is a massive change in this global world. We are expected to refer to our local wisdoms as a filter of values which are destructive. Those issues are addressed proportionally in this seminar. Therefore, I expect that we gather in this place to contribute our best to tackle relevant issues based on our own discipline.

Last but not least, we need to give a special appreciation to the committee of this seminar because of their efforts this important event could be realized. Having said that, please excuse any inconvenience that we may cause. Finally, have a great seminar and please enjoy your stay in Lombok. Wassalamu'alaikum Wr Wb .Thank you

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PREFACE

Isolation of Soil Microbes to Improve Nutritional Value of Rice Straw As an Animal Feed

by
Made Sriasih*, Sulaiman N Depamede and Muhamad Ali

Laboratory of MicroBiotechnology, Faculty of Animal Sciences, Mataram University Jalan Majapahit No. 62, Mataram 83125, NTB- Indonesia

Agricultural solid wastes are available abundantly, and not yet fully utilized. Wastes, such as rice straw, could be used, for example, as an animal feed. However, its low digestibility due to high fiber content and low nutrition value has limited its application. In this research, isolation of soil microbes that is potential for degrading roughage had been initiated. The isolated microbes were then characterized and were used for treating rice straw. The quality of treated rice straw was analyzed using Van Soest analysis. Based on the appearance of microbial colonies, four types of fungi (designated as J1, J2, J3 and J4 respectively) and four types of bacteria (designated as B1, B2, B3 and B4 respectively) were identified. Van Soest analysis showed that treatment with fungi J3 gave the best results amongst soil microbes tested. Treatment with fungi J3 had decreased the content of neutral detergent fiber (13.45%), acid detergent fiber (9.25%) and lignin (9.65%) of treated rice straw. This finding suggests that the isolated microbes may be utilized as a good bio-activator agent that can increase the nutritional and economical values of rice straw, as an animal feed.

Keywords: *soil microbes, rice straw, Van Soest analysis, animals feed*

INTRODUCTION

Livestock industry in Indonesia is very prospective, however obstacles such as the cost of animal feed, which is relatively expensive, and unstable availability of animal feed have become major problems. One solution that can be offered to alleviate those problems is the utilization of resources that are cheap and are easily available.

In Indonesia, agricultural solid wastes are abundant, and not yet fully utilized. Rice straws, for example, is one of the common organic materials incorporated into a paddy field and is produced approximately 12 to 15 tons per every harvest time (Gumilar, 2010). Most of the rice straw is used as a bedding for animals or is burned. Gumilar (2010) reported that 70 – 80 percents of farmers in Indonesia usually burn the straws at their paddy field after harvesting. This may result in environmental pollution caused by global addition of carbon dioxide, a gas contribution to the green-house effect and likely high health cost through increase in the respiratory problems in the local population (Samar *et al*., 1999; Gumilar, 2010).

Rice straw is a by-product of rice production, and a great bio-resource as raw biomass materials for manufacturing value-adding protein for animal feedstock (Zheng *et al*., 2005). One of the key problems for the effective utilization of this resource as raw material for animal feed is the low ability of its lignocelluloses to hydrolysis, which is attributable to the crystalline structure of cellulose fibrils surrounded by the hemicellulose and the presence of the lignin which prevents the degrading enzymes to act on (Jahromi *et al*., 2010). Therefore, rice straw is low in nutritive value with low level of protein and low dry matter digestibility (Zheng *et al*., 2005; Wanapat *et al*., 2009; Nader & Robinson, 2010; Jahromi *et al*., 2010).

Various treatment methods have been used, with some success, to improve nutritive value of rice straw. The methods include physical, chemical and biological treatments (Ali *et al*., 2001; Meryandini *et al*., 2009; Wanapat *et al*., 2009; Khejonsart & Wanapat, 2010), but biological is preferred over chemical methods (Jahromi *et al*., 2010). Previous works have shown that high-protein animal feed has been successfully produced by microbial fermentation of cellulosic substrates using microorganisms such as *Candida arborea* (Zheng et *al*., 2005), *Trichoderma sp.* (Hong-Man *et al*., 2001) and *Aspergillus niger* K8 strain (Jahromi *et al*., 2010). Moreover, common practice of immersing rice straw under the ground has proven to be an effective way for the farmers to help degrading the straw. This may be attributed to the presence of soil microbes that are capable of decomposing lignin, cellulose and chitin (Malekzadeh *et al*., 1993; Jahromi *et al*., 2010). In addition, soil organisms are also known as one of the most sensitive biological markers, and the most useful agents for classifying disturbed or contaminated systems (Hong-Man *et al*., 2001).

The tiny microorganisms which can decompose cellulose (known as bioactivator) might be found living in several habitats in various places in Indonesia. The objective of the present study was therefore to isolate soil microbes of local isolate that are potential for improving the nutritional and economical values of rice straw as animal feed.

METHOD

1. Soil microbes isolation

The media used for isolating soil microbes is potato dextrose agar (PDA) which composed of yeast extract (0.1%), peptone (0.6%), glucose (1%), NH_4NO_3 (0.5%), KCl (0.05%), $FeSO_4.7H_2O$ (0.001%), $CuSO_4.2H_2O$ (0.0001%), $MgSO_4.7H_2O$ (0.05%) and agar (2%).The microbia were isolated from decayed rice straw, which was taken from farming area located at Kekalik, Ampenan – Mataram, by using two different methods. Firstly, decayed rice straws were cultured directly onto PDA, followed by incubation at 37^{o}C for 48 hours. Secondly, decayed rice straws were mixed with double distilled water, vortexed and diluted to 10^4 dilution. One milliliter of the mixtures was then inoculated onto PDA using pour plate method. The plates were incubated as described above.

2. Characterization of soil microbes

The microbes that grow on the agar plate were first grouped based on the color of colony. Every group was then re-streaked on to PDA plate and incubated, in order to get separate colonies of bacteria and of fungi. The processes were repeated several times until pure colonies of bacteria and fungi were obtained. Characteristics of bacterium were then confirmed by Gram staining while methylene blue staining was applied for confirmation of fungi colonies.

For Gram staining, bacterial cells that have already been fixed on to glass slide were first stained with Gram A solution (crystal violet) followed by Gram B solution. The slide was washed with water and subsequently stained with Gram C solution. After the addition of Gram D solution (saffranin), the slide was washed again, air-dried and observed under the microscope. Hyphae of the fungi obtained were determined using methylene blue stain. Briefly, approximately 10 µl of stain were added on to glass slide and were mixed with the hyphae. Slide was covered with cover glass before being examined using microscope.

3. Soil microbial growth measurement

To determine the growth of soil microbes isolated, they were all inoculated on to LBA plates (Tryptone [1%], yeast extract [0.5%], NaCl [1%] and agar [2%],). The bacteria were cultured by streaking on to the plates whilst the fungi were inoculated using pour plate method. The number of colonies appeared on the plates were counted with colony counter. In addition to culturing on solid media, bacteria were also cultured on liquid media (LB broth) and

were incubated with shaking at 37°C. The degree of turbidity was then measured using spectrophotometer.

4. Application of soil microbe inoculants on to rice straws

4.1. Preparation of bacterial and fungal inoculants

Bacteria and fungi colonies were picked and inoculated separately on to 5 ml of potato dextrosa broth (PDB) consisting of yeast extract (0.1%), peptone (0.6%), glucose (1%), NH_4NO_3 (0.5%), KCl (0.05%), $FeSO_4.7H_2O$ (0.001%), $CuSO_4.2H_2O$ (0.0001%) and $MgSO_4.7H_2O$ (0.05%). Following overnight incubation with vigorous shaking (140 rpm) at 37°C, 3 ml of freshly grown overnight cultures were mixed with 300 ml of fresh PDB. The culture was incubated in a rotary shaker at 37°C for 16 to 24 hours or until the optical density of the microbial suspension corresponded to an absorption value of approximately 0.6 at a wavelength of 450 nm.

4.3. Preparation of rice straw

Five hundred grams of fresh rice straw were chopped nicely and packed using paper bag before autoclaving set up at 121°C, 1.5kgf/cm^2 for 15 minutes. The straw was then treated with either 100 ml of bacterial or fungal inoculants.

5. Experimental design

Rice straw prepared was treated with either bacterial (B1, B2, B3 and B4) or fungal inoculants (J1, J2, J3 and J4) with three replications. The straw which is treated with PDB only was included as a control treatment. All treatments were then incubated for 7 days at 28°C. After 7 days, fermentation processes was stopped, and 30 grams of sample were taken from all treatments. Samples were put in to the oven for 24 hours at 60°C and dry matter of each sample was recorded. All samples were then blended separately and were used for analysis of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose and lignin contents using Van Soest's method (Van Soest *et al*., 1991).

RESULTS AND DISCUSSION

1. Isolation and characterization of soil microbes

Based upon the color of colonies appeared, there were four group of bacteria and four group of fungi isolated. Bacterial isolates were designated as B1, B2, B3 and B4 whilst fungal isolates were marked as J1, J2, J3 and J4. All groups were re-streaked separately in order to obtain pure isolates.

Gram staining of pure bacterial isolates presented in Figure 1 shows that bacterial isolates obtained were different in their shape but were similar in their sensitivity to Gram's dye solution. The B1 group is mono-bacilli and shows sensitivity to crystal violet (stained purple), and therefore considered as Gram positive bacteria. Another group, B2, is also Gram positive but has a chain of bacilli-shaped (streptobacilli). The last second groups, B3 and B4, are sensitive to crystal violet with short monobacilli-shaped. Similarity in their sensitivity to crystal violet suggests that the cell wall of all isolates may compose of similar structures. Gram positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50 – 90 percents) whilst Gram negative have a thinner layer (10 percents of cell wall) (Prescott *et al*., 1993). The multilayered nature of its peptidoglycan will trap the crystal violet-iodine complexes, and thus stains the cell purple. Methylene blue staining of the fungi indicates that the four groups of fungi isolated have different characteristic of hyphae (data not shown).

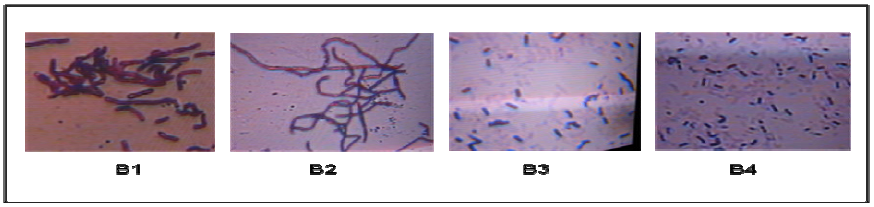

Figure 1. Gram staining of the bacterial isolates

2. Growth of soil microbe isolates

2.1. Fungal isolates

To monitor the growth of the fungal isolates, they were cultured on LB Agar (LBA) and were incubated for 72 hours. The results are presented in Figure 2. The figure presents that only one (J4) out of four isolates that has not grown after 24 hours incubation. After two days of incubation period, the growth of the J2 group is better than the J1 and J4. The isolate showing the fastest growing as shown by the number and the size of colonies at 48 hours was the J3 group. Extended period of incubation up to 72 hours again confirms that the J3 isolate grew the fastest with thick and wide colonies shown on LBA plate.

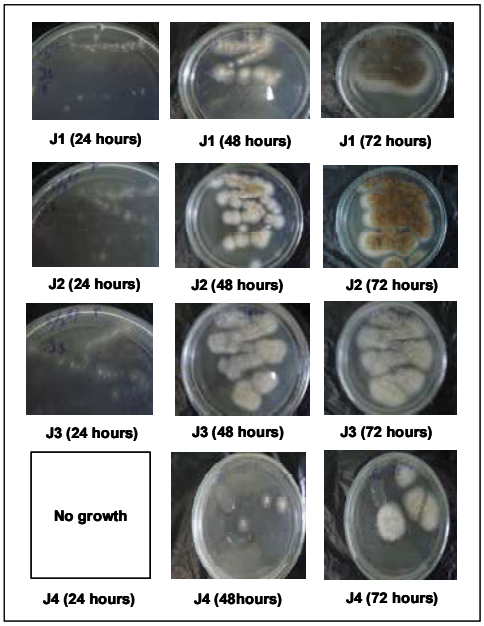

Figure 2. The growth of the four fungal isolates on LBA plates over period times of 24, 48 and 72 hours of incubation at 37^{o}C.

2.2. Bacterial isolates

Figure 3 depicts the growth of bacterial isolates on LBA plates. The results suggest that bacterial isolates had different growth rate. The growth of the B3 and the B4 groups is faster than the other two groups (B1 and B2). However, the B1 group showed more colonies on the plate suggesting that its' growth rate is better than that of the B2 group. To confirm these data, all isolates were cultured on liquid-type media (LB broth). The degree of turbidity (cloudiness) exhibited by a broth culture gives an indication of the number of organisms present, and correlates positively to the value of OD reading measured by spectrophotometer The results presented in Table 1 show that the B2 group has the highest $OD_{450\ nm}$ reading indicating that this group grew well in the liquid type media. The difference of growth rate obtained when culturing microorganisms on agar and liquid-type media may be attributed to a variety of factors including pH, degree of aeration and the state of medium itself (Prescott *et al*., 1993). It is

well known that solid media contains agar which is derived from seaweed. Agar itself contains nutrients that may allow some microbial growth.

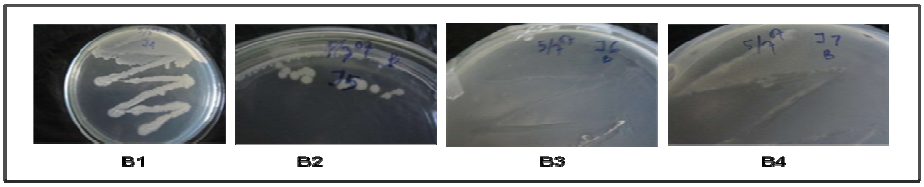

Figure 3. The growth of bacterial isolates on LBA plates

Table 1. The growth rate of the four bacterial isolates on LB broth

Group	OD $_{450\ nm}$ reading		
	0 min	60 mins	90 mins
B1	0.08	0.10	0.21
B2	0.08	0.40	1.22
B3	0.08	0.20	0.83
B4	0.08	0.10	0.61

3.3. Application of soil microbe inoculants on to rice straw

Five hundred grams of straws were treated with isolated soil microbial inoculants. Van Soest analysis to determine the NDF, ADF, cellulose and lignin content of treated rice straw were carried out and the results were presented in Table 2.

Table 2. Mean values of dry matter, NDF, ADF, cellulose, hemicellulose and lignin contents of treated rice straw

	Treatment								
	Control	**J1**	**J2**	**J3**	**J4**	**B1**	**B2**	**B3**	**B4**
Dry matter	88.76	88.56	89.87	88.82	89.14	90.84	90.08	90.27	90.51
NDF	67.39	67.59	64.09	58.32	65.22	66.36	68.88	67.46	74.50
ADF	47.61	46.58	48.90	43.20	54.29	52.30	51.11	55.62	52.60
Hemicellulose	19.78	21.01	15.19	15.12	10.94	16.56	16.77	11.34	21.90
Cellulose	32.18	30.93	30.66	29.47	34.30	30.20	17.88	36.16	31.22
Lignin	5.29	5.93	4.77	4.78	5.58	6.71	5.29	5.44	8.17

Table 2 shows that application of the J3 inoculant on rice straw generally has improved the nutritional values of the straw. This was shown by a decrease in NDF (13.45%), ADF (9.25%) and lignin (9.65%) of rice straw after treatment. The reduction of the NDF content of rice straw, particularly after treatment with the J3 inoculants, was due to the reduction of hemicellulose and cellulosa contents. Extensive research on the effects of *Aspergilus niger* on nutritive value of rice straw has indicated that reduction of lignocelluloses content in the rice straw after treatment is assumed to be the activities of cellulose, beta glucosidase and xylanase enzymes of the inoculants used (Pothiraj *et al*., 2006; Jahromi *et al*., 2010). Therefore, the finding in this study indicates that the J3 fungal group may possess such activities. The lowest ADF value of rice straw after the J3 treatment as shown in Table 2 also indicates that the treated rice straw can be considered to be feed quality for livestock. Although treatment with the B3 bacterial inoculants has shown a significant decrease in cellulose (44.4%) of the treated straw, the NDF, ADF and lignin content are still higher than treatment with the J3 fungal group. Fungi have been known to play an important synergistic role on the ruminal digestion of fiber by physical and chemical breakdown the lignified stem tissue which high potent fibrolytic enzymes for fiber degradation (Theodorou *et al*., 1992; Trinci *et al*., 1994)

Conclusion

Several soil microbes were isolated from decayed rice straw in this study. Amongst bacterial and fungal isolates tested, one fungal local isolate (designated as J3) has shown potential for improvement of low quality roughage such as rice straw *in vitro*. Further experiments in a large scale concept are required to support this finding.

Acknowledgement

The authors would like to express their most sincere thanks to all who have assisted the experiments in this study. This work was financially supported by the Regional Planning Bureau of West Nusa Tenggara through Research on Regional Development Capacity.

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