J. Trop. Agric. and Fd. Sc. 43(2)(2015): 119 – 127

Identification of rice straw degrading microbial consortium

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Abstract
A microbial consortium consisting of 30 species of bacteria was developed to study the biodegradation of rice straw. Rice straw treated only with microorganisms showed better degradation results compared with rice straw pre-treated with urea, lime and palm oil mill effluent (POME). A reduction of 15.85, 24.22 and 10.25% in acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre (CF) of rice straw was observed after the treatment with microbial consortium. The study highlights the potential of microbial consortium to degrade rice straw efficiently.

Keywords: identification, rice straw, biodegradation, microbial consortium

Introduction

Rice straw consists of cellulose, hemicellulose and lignin that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross linkages (Sanchez 2009). It has been generally characterised to contain 28 – 36% of alpha cellulose, 12 – 16% of lignin, 15 – 20% of ash and 9 – 14% of silica, which is recalcitrant for degradation. Therefore, the hidden value of rice straw can be explored by biological conversion into value added products. Since bioconversion of lignocellulose material has become an emerging biotechnology trend in the modern world, rice straw may function as potential feedstock to produce biofuels (Ranjan and Moholkar 2013), bio-fertilizers (Srinivasan et al. 1983), animal feed (Prasad et al. 1998), bio-based industry chemicals (Horward et al. 2003; Parameswaran et al. 2010; Park et al. 2011; Zhu et al. 2012), cheap substrate for fermentation of enzymes (Hideno et al. 2011), biogases (Chen et al. 2012), mushroom cultivation (Zhang et al. 2002) and bio-compost (Zayed and Abdel-Motaal 2005; Yu et al. 2008).

In Malaysia, abundance of rice straw produced is disposed via open burning in rice fields during dry season. To mitigate open burning practice, which is hazardous for the environment, utilisation of rice straw for animal feed production, bioethanol production, lignocellulose enzyme production and bio-composting are seem to be potential. The microbial consortium can be used as a booster for bio-composting or *in-situ* biodegradation of rice straw. The aforementioned potential technologies and products generated from rice straw are highly dependent on effective lignocellulose degrading microorganisms. In nature, lignocellulose biomass can be degraded efficiently with the co-operation of many species of microorganisms. It has been reported that mixed culture of cellulolytic microorganism with non-cellulolytic

Article history
Received: 4.9.2014
Accepted: 12.12.2014

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microorganism is ideal for cellulose degradation (Kato et al. 2004). White-rot fungi (Tengerdy and Szakacs 2003; Huang et al. 2008), *Pleurotus eryngii* (Camerero et al. 1999), bacterial lignocellulose enzymes (Sarnklong et al. 2010; Zeng et al. 2013) and *Streptomyces badius* (Huang et al. 2008) were also regarded as efficient decomposer of natural lignocellulose. The biodegradation of cellulose biomass assisted by microbial communities has been proposed as a highly efficient approach for biotechnological applications (Wongwilaiwalin et al. 2010). Tuesorn et al. (2013) has highlighted the feasibility and eco-friendly strategy of lignocellulose waste biodegradation by microorganisms.

Thus, in this study, microorganisms were isolated and identified to screen for indigenous and potential rice straw degrading microbial consortium to decompose the complex structure of rice straw. The lignocellulose microbial consortium is expected to be advantageous for rice straw based agricultural and industrial applications in Malaysia.

Materials and methods

About 300 bacterial isolates collected from soil were subjected to pre-screening enzyme assays using agar plates. Carboxymethylcellulose (CMC) plate was used to identify cellulose-degrading bacteria (Sirisena and Manamendra 1995). Xylan agar consists of xylan from oat spelt was used to detect hemicellulose degrading bacteria (Padmavathi and Kavya 2011) and azure agar consists of azure B was used to isolate lignin degrading bacteria. Bacterial isolates were subjected to preliminary enzymatic assays based on the formation of haloes on agar plates.

Rice straw was obtained from Muda Agriculture Development Authority, Malaysia (MADA) and was cut into 10 – 15 cm long. Approximately 10 g of rice straw soaked in 100 ml of urea and lime with varying concentrations ranging from 2 – 10% for 24 h.

Haloes forming microorganisms were subjected for preparation of bacterial inoculum. All bacterial isolates were grown separately in nutrient broth. After 2 days of incubation at 30 °C with 150 rpm speed, each nutrient broth was transferred into a fresh enrichment medium consisting of malt extract 10.0 g/litre, yeast extract 4.0 g/litre and beef extract 6.0 g/litre. The mixed culture was incubated at 30 ºC in a rotary shaker at 150 rpm for 2 days. Sterilised glucose (10%) was used for microbial cell activation. Approximately 100 ml of microbial culture was added into each pretreated rice straw samples.

Only best degradation exhibiting treatment was selected for re-isolation of bacteria which was based on longevity and ability to withstand chemical pre-treatments that was observed phenotypically for a month of incubation period. Selected isolates were subjected to an antagonistic test to study the compatibility of microorganisms to live together in a consortium. Bacteria that are able to survive in a consortium were selected and identified.

The bacterial strains were identified using 16S rDNA amplification using universal 16S rDNA gene primer sets: 27f and 1492r.

Bacterial isolates were prepared as a mixed culture or in a consortium and inoculated on rice straw. Approximately 200 g of rice straw was subjected to this study. Four treatments with three replications were introduced as stated below:

a) 50 ml of 2% urea and 50 ml 3% lime + 100 ml of microbial consortium
b) 200 ml of diluted palm oil mill effluent (POME) only
c) 200 ml of microbial consortium only
d) 200 ml of water only

Water treatment of rice straw served as control treatment. The experiment was conducted on trays and in conical flasks. The submerged rice straw was monitored for about a month. Approximately 50 – 100 ml of fresh inoculum was added once a week.

Chemical fibre analysis and Scanning Electron Microscopy (SEM) analysis

Samples were sent for acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre (CF) analyses to evaluate the biodegradation of rice straw. ADF, NDF and CF content were determined according to AOAC official method using Fiber Tech System (AOAC 1997). Meanwhile the treated rice straw with microbial culture was also sent for electron microscopic scanning to study the morphological differences of treated and non-treated rice straw. ADF consists of cellulose and lignin, whereas NDF consists of cellulose, hemicellulose and lignin content. Thus, deduction of ADF value from NDF value gives an estimation of hemicellulose content in rice straw. Crude fibre is a structural and indigestible carbohydrate component such as cellulose, hemicellulose and lignin present in plant residue.

Statistical analysis

All data were subjected to statistical analysis of variance using one-way ANOVA. Differences of data at $p < 0.05$ level were considered significant.

Results

Out of 300 microorganisms, only 48 showed efficient degradation for all the three main components of rice straw, namely cellulose, hemicellulose and lignin. The efficiency was determined based on diameter of clear zone obtained in each agar plate (*Figure 1* and *Figure 2*). Clear zone is formed as a result of decomposition of the substrate by microorganism. A total of 30 re-isolated microorganisms that were tested for compatibility to live together were used to form a microbial consortium. The microorganisms in the consortium were generally identified as *Proteus mirabilis*, *Raoutella planticola*, *Serratia* sp., *Pseudomonas viridilivida*, *Klebsiella oxytoca*, *Bacillus fusiformis*, *Bacillus cereus*, *Klebsiella* sp., *Bacillus licheniformis*, *Corynebacterium urealyticum*,

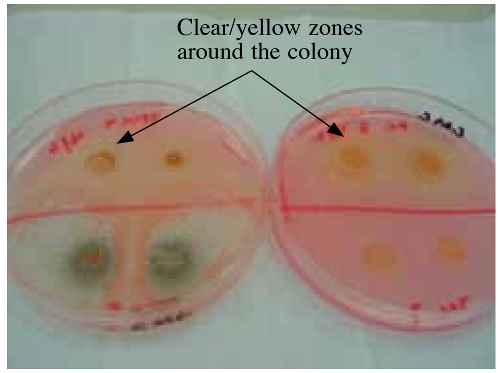

Figure 1. Cellulose degradation is indicated by clear/yellow zone around the colonies in carboxymethylcellulose agar

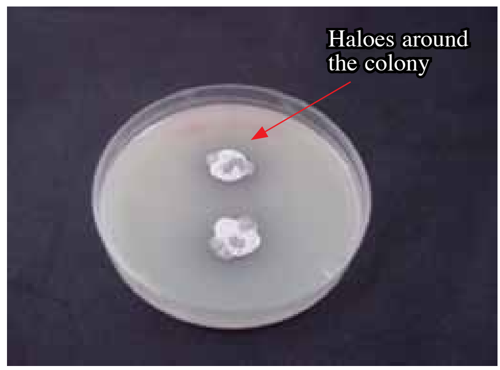

Figure 2. Haloes around the bacterial colonies in xylan agar

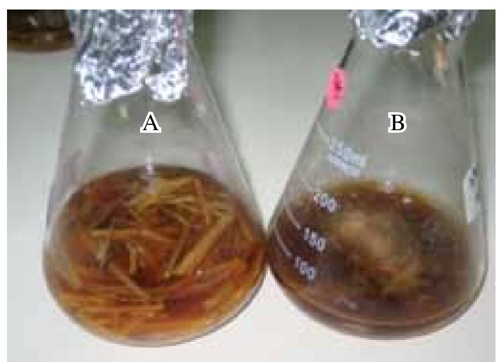

Figure 3. Rice straw submerged in liquid medium
A: Rice straw mixed with water
B: Rice straw treated with bacterial consortium

Cellulosimicrobium cellulans and *Bacillus subtilis* using 16s rDNA molecular identification technique. Among all these bacteria, *P. viridilivida*, *K. oxytoca*, *R. planticola* and *C. urealyticum* were reported for the first time as cellulose degrading microorganisms.

Rice straw treated with microbial consortium in liquid culture exhibited good decomposition compared with rice straw treated with water that served as control in this experiment (*Figure 3*). Rice straw degradation did not happen in control conical flasks although it was soaked in water for about a month. To quantitate the degree of decomposition, fibre content of rice straw treated with various chemical and biological treatments on trays was determined (*Table 1*). ADF and NDF analysis can give a rough estimation on the lignocellulose degradation though they are commonly used for feedstock. ADF, NDF and CF contents were reduced by 15.85, 24.22 and 10.25% respectively in rice straw treated with microbial consortium as compared with rice straw treated with water. Microbial treatment was not significant as compared with chemical and POME treatments (*Table 2*). Thus, the efficiency of microbial consortium is as good as chemical treatments using urea and lime or biological treatment using agricultural waste such as POME. It is also noticeable that microbial consortium can be used for animal feed treatment using rice straw as it is economically feasible than the urea and lime treatment that has been widely used in animal feed preparation.

The data also clearly showed that microbial consortium was able to reduce the hemicellulose of rice straw better than other treatments. The hemicellulose in rice straw treated with bacteria was only 5.8%, whereas rice straw treated with chemicals and POME retained 17% and 11.6% of hemicellulose respectively. Rice straw treated with microbial consortium showed the lowest value of crude fibre compared with rice straw treated with chemicals and POME.

Figure 4a shows that rice straw inoculated with bacteria had penetrated the outer structure of rice straw which is consist of lignin. On the other hand, *Figure 4b* shows the intact structure of rice straw that was not disrupted. The physical structural disruption of rice straw inoculated with microbial consortium matches the efficiency of the microorganisms in enhancing degradation.

Discussion

In this study, a different approach was taken to isolate the rice straw degrading microorganisms from a consortium. The microorganisms were encouraged to live harmonically in a consortium at the very beginning stage itself to allow adaptation to chemicals and competitive indigenous microorganisms. Urea and lime pre-treatments were introduced as these chemicals have been used widely

Table 1. Chemical analyses of lignocellulose content of rice straw experimented on trays (solid state)

	ADF (%)	NDF (%)	CF (%)
Rice straw + 2% urea + 2% lime + bacterial inoculum	43.8 ± 0.58*	60.8 ± 0.29*	27.7 ± 0.18*
Rice straw + POME	36.9 ± 0.72*	48.5 ± 0.37*	22.4 ± 0.36*
Rice straw + bacterial inoculum	39.9 ± 0.10*	45.7 ± 0.22*	19.5 ± 0.40*
Rice straw + water	55.7 ± 0.41*	70.0 ± 0.26*	29.6 ± 0.42*

Each value represents a mean ± standard deviation (SD) of three replications
*Significant at $p < 0.01$

Table 2. Statistical analysis for fibre analysis of rice straw treated with chemical, POME and microbial consortium

Acid detergent fibre

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance		
Column 1	3	120.43	40.14333	9.397733		
Column 2	3	120.02	40.00667	15.13213		
Column 3	3	121.6	40.53333	11.96333		
ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.448156	2	0.224078	0.018421	0.981803	5.143253
Within groups	72.9864	6	12.1644			
Total	73.43456	8				

Neutral detergent fibre

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance		
Column 1	3	154.8	51.6	68.2171		
Column 2	3	155.6	51.86667	65.12333		
Column 3	3	154.6	51.53333	61.62333		
ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.186667	2	0.093333	0.001436	0.998565	5.143253
Within groups	389.9275	6	64.98792			
Total	390.1142	8				

Crude fibre

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance		
Column 1	3	68.83	22.94333	17.76063		
Column 2	3	69.3	23.1	17.56		
Column 3	3	70.5	23.5	15.97		
ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.494422	2	0.247211	0.014459	0.985679	5.143253
Within groups	102.5813	6	17.09688			
Total	103.0757	8				

Not significant at $p < 0.01$

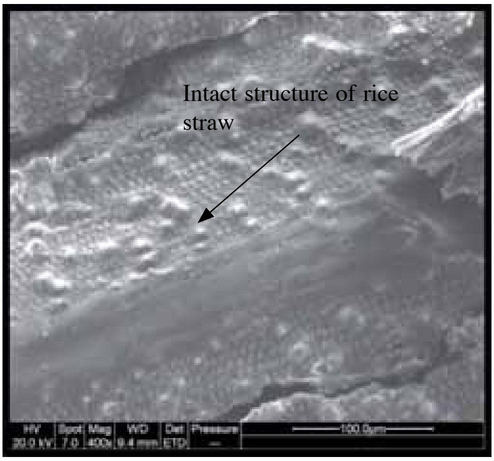

Figure 4a. Surface of rice straw not treated with bacteria under X500 magnification using scanning electron microscope

Figure 4b. Surface of rice straw treated with bacteria under X500 magnification using scanning electron microscope

in treating rice straw for ruminant feed. The general idea of pre-treatment is to remove or alter the hemicellulose or lignin, decrease the crystallinity of cellulose and increase the surface area (Van Soest 2006). Urea treatment is known for effective biodegradation of hemicellulose in rice straw. However, this usage of urea for large-scale operation is not viable due to economic constraints. On the other hand, lime treatment is more effective in delignification of rice straw (Sarnklong et al. 2010).

Combination of both urea and lime was reported as a better pre-treatment for rice straw. In this study, urea and lime treatment was introduced before the re-isolation of microorganisms to make the microorganisms compatible with various environmental conditions involved especially during ruminant feed preparation. Since the selected microorganisms were compatible with urea and lime, the microbial consortium can also be used together with chemical treatments if necessary. Glucose was added to microbial inoculum not only to activate the cells and increase the metabolic activity of microorganisms, but also to supply readily utilisable source of carbon in order to pave the way for lignin degradation.

The microbial consortium was able to degrade lignocellulose content of rice straw without chemical pre-treatment. Rice straw treated with bacteria has shown the same efficiency of biodegradation as chemical (urea and lime) and POME treatment. Therefore, microbial consortium can be used as a substitute for urea and lime pre-treatment in ruminant feed preparation. It can also be used together with urea and lime as the chemicals will not hamper the survival of microorganisms in the consortium. Besides ruminant feed preparation, microbial consortium is also capable of hastening and enhancing degradation of rice straw in composting process.

Microorganisms in a group or consortium work synergistically where growth, biological processes and enzymatic activities are conducted more effectively and efficiently than microorganisms on an individual population basis (Kato et al. 2005). Similarly, Tuerson et al. (2013) has reported that the symbiotic relationship among the microorganisms in consortium provides the basis for its stability and efficiency. Microorganisms in a consortium also maintain metabolic and ecological compatibility and stability.

Wongwilaiwalin et al. (2010) reported that the biomass degrading capability is based on the functional and structural stability of a microbial consortium. Some microorganisms are superior to others in degrading different component of rice straw namely cellulose, hemicellulose and lignin. Thus, the most efficient bacteria in cellulose, hemicellulose and lignin degradation were mixed together to function as a strong and competent microbial consortium. Yang et al. (2004) has indicated that microbial consortium has advantage over single culture where it helps to grow in agriculture residues that are low in nutrients and protects the culture from contamination. Thus, a lignocellulose microbial consortium was developed in this study for an efficient biodegradation of rice straw.

Conclusion

A microbial consortium consisting of 30 species of bacteria with the ability of degrading cellulose, hemicellulose and lignin was formed in this study. The consortium was found to degrade rice straw effectively without chemical pre-treatment. The microbial consortium developed in this study has the potential to be used as an alternative economical approach for urea and lime pre-treatment in ruminant feed preparation. It is also suitable to be used in composting of rice straw especially to improve the nutrient contents and quality of compost and to hasten the composting process as well.

Acknowledgement

The authors wish to acknowledge ScienceFund, Ministry of Agriculture, Malaysia for providing financial assistance to conduct this project (RS 05-03-08-SF0012).

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Abstrak
Konsortium mikrob yang mengandungi 30 spesies bakteria telah dihasilkan untuk mengkaji degradasi jerami padi. Jerami padi yang dirawat dengan mikrob sahaja telah menunjukkan degradasi yang lebih baik apabila dibandingkan dengan jerami padi yang dirawat dengan urea, kapur dan efluen kilang minyak sawit (POME). Pengurangan sebanyak 15.85, 24.22 dan 10.25% telah diperhatikan dalam ADF (*acid detergent fibre*), NDF (*neutral detergent fibre*) dan CF (*crude fibre*) selepas dirawat dengan konsortium mikroorganisma. Kajian ini telah menonjolkan potensi konsortium mikrob untuk mendegradasi jerami padi dengan berkesan.