ORGANIC ACIDS PRODUCTION OF RICE STRAW FERMENTED WITH SEVERAL TYPES OF MICROORGANISM AT DIFFERENT TEMPERATURES

Y. Yanti[1], Surahmanto[2], A. Purnomoadi[2] and Y. Kawamoto[3]

[1]Faculty of Agriculture, Sebelas Maret University,
Jl. Ir. Sutami No. 36A, Kentingan, Surakarta 57126 - Indonesia
[2]Faculty of Animal Science and Agriculture, Diponegoro University,
Tembalang Campus, Semarang 50275 – Indonesia
[3]Faculty of Agriculture, University of the Ryukyus,
1 Senbaru,Nishihara, Okinawa 903-0213 - Japan
Corresponding E-mail: yuliyanti@uns.ac.id

Received July 26, 2012; Accepted August 28, 2012

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui produksi asam organik dari fermentasi jerami padi dengan berbagai tipe mikroorganisme pada berbagai suhu. Rancangan penelitian yang digunakan adalah split-splot Rancangan Acak Lengkap. Petak utama adalah perlakuan suhu (25, 35, 45°C) dan anak petaknya adalah mikroorganisme (Kontrol, Kontrol+Molases, *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant, Saccharomyces cerevisiae, Aspergillus niger*). Parameter yang diamati adalah produksi asam organik (asam laktat, asam asetat, asam propionat). Produksi asam laktat tertinggi pada perlakuan *B. coagulans* pada suhu 35°C (53,79 g/kg BK). Produksi asam asetat tertinggi pada perlakuan *L. fermentum* pada suhu 35°C (13,20 g/kg BK), sedangkan produksi asam propionat tertinggi pada perlakuan Kontrol pada suhu 35°C (0,37 g/kg BK).

Kata kunci: jerami padi, mikroorganisme, suhu, asam organik

ABSTRACT

The experiment was carried out to examine the organic acids production of rice straw fermented with some types of microorganisms at different temperatures. The experiment was designed as Split Plot-Completely Randomized Design. The main plot was temperatures treatments (25, 35, 45°C) and the sub plot were microorganisms (Control, Control+Mollases, *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant, Saccharomyces cerevisiae, Aspergillus niger*). The highest lactic acid productions was in *B. coagulans* treatment at 35°C (53.79 g/kg DM). The highest acetic acid productions was in *L. fermentum* at 35°C (13.20 g/kg DM), while the highest propionic acid productions were in Control treatment at 35°C (0.37 g/kg DM).

Keywords: rice straw, microorganisms, temperature, organic acids

INTRODUCTION

Rice straw (*Oryza sativa*) is a large portion of the world crop by-products that is not used efficiently. It has been well recognized as an alternative feed for ruminant because of abundant in stock and cheap in price so it can give more economic advantages due to the lower production cost. Rice straw has also been used as a roughage in the diet of beef and dairy cattle when drought limits the availability of other conserved forages in small farms. However, rice straw has limited nutritive value as ruminant feed because of low protein (2-7% on a dry matter basis), low digestibility and high fiber contents (Drake *et al*., 2002).

Numerous methods of physical, chemical, biological treatments have been widely studied and developed in order to improve the utilization of rice straw for ruminant feed (Trach, 1998). Microbial fermentation was studied in the past and many studies determined to improve the fibrous feed qualities (Han and Anderson, 1974). Fermentation usually implies that the action of the

microorganisms is desirable. Cellulase (a complex multienzyme system) acts collectively to hydrolyze cellulose from agricultural waste to produce simple glucose units (John, 1996). Cellulases are synthesized by cellulolytic fungi such as *Aspergillus* spesies (Milala *et al*., 2005; Vijaya and Singaracharya, 2005) and *Sacharomices* species (Lila *et al*., 2006; Callaway *et al*., 1997), and *Bacillus* species (Eun *et al*., 2006), whereas *Lactobacillus* species were used widely as additives in silage fermentation of forage to improve preservation efficiency. The epiphytic lactic acid bacteria ferment the water-soluble carbohydrates in the crop to lactic acid (Weinberg and Muck, 1996).

Fungal growth resulted from the complex interaction of several factors, such as temperature, water availability and incubation time (Pardo *et al*., 2005). The optimum-growth temperature corresponds to the usual temperature of the natural habitat of the species, and to the temperature at which the essential bacterial enzymes function best (Burdon and William, 1965). The effects of fungal growth on the chemical composition and hence nutritive value of fibrous residues is depend on the type of fungus, the nature of the residue and the conditions of growth.

Although many reports suggest fermentation method with microbes could improve quality of fibrous feed but selections of the type microbes in suitable temperature of fermentation have not been determined yet. The aim of this research was to determine the nutritive value of rice straw, especially organic acids, fermented with some types of microorganisms at different temperatures. Hypothesis of this research was the different type of microbes need different temperatures may result in differ nutritive value of fermented rice straw.

MATERIALS AND METHODS

This research was conducted in Tropical Grassland Chemical and Utility Laboratory, Faculty of Agriculture University of the Ryukyus, Okinawa, Japan. Rice straw was obtained from rice fields in Ishigaki District, Okinawa, Japan. The types of microbes used were *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulanst Saccharomyces cerevisiae,* and *Aspergillus niger.* Water was added to rice straw prior to fermentation. Molasses were added to rice straw 5% dry matter basis.

Split plot completely randomized design was used in this experiment to study the effect of rice straw fermented with microbes in different temperatures on organic acids content. Treatments were assigned as mainplot and subplot. The mainplot was temperature (25, 35, 45°C) and the subplot was microorganism (Control, Control+Mollases, *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant, Saccharomyces cerevisiae* and *Aspergillus niger*).

The methods used in the fermentation process were as follow: 1) Rice straws were chopped into small pieces at 1-2 cm length. 2) Water was added to the rice straws at ratio water:rice straw = 2:3. 3) All types of microbes were cultivated in culture media agar specific for each types in plate and the occurred cell then were transferred to increase the cell numbers in culture media broth specific for each types in a 100 ml flasks to make microbes juice. The cell was then incubated for 3 days of each types. 4) The 5% microbe juice and 5% molasses were mixed with 1 kg of the rice straw based on dry matter. The control treatments were not mixed with molasses and the other one were mixed with molasses. 5) Approximately 150 g of mixture were transferred into a plastic pouches. 6) Fermentation method: Ferment bag by plastic pouchs were sealed by vacuum sealer in groups of control, *L. fermentum*, *B. subtilis* and *B. coagulanst*. Exception in group fermented with *S. cerevisiae* and *A. niger* the ferment plastic pouches were not sealed but were loosely closed. 7) Rice straw bags were stored at 25, 35 and 45°C according to the treatment for 3 weeks.

After 3 weeks fermentation, a representative sample of rice straw (20 g fresh matter) was macerated with 70 ml of distillated water and stored at 4°C for 12 hours. The extract was filtered through No. 5A filter paper (Toyo Roshi Co. Ltd., Tokyo, Japan). The filtrate was collected to analyze the concentration of Volatile Fatty Acids (VFA). VFA content was measured by HPLC (Shim-pack SCR-102H, 300 mm x 8.0 mm i.d; column temperature, 40°C; flow rate, 0.8 ml/min, Shimadzu Co. Ltd., Kyoto, Japan). After 3 weeks fermentation, the fermented materials were dried in a 60-70°C drying oven for 48 hours to determine water content.

Effect of treatment was analyzed using the analysis of variance (ANOVA). The Duncan's New Multiple Range Test was performed to determine the significant differences among treatments. The Least Square Different (LSD) was used when interaction between temperature and

microorganisms were found (Sastrosupadi, 2000).

RESULTS AND DISCUSSION

The lactic acids, acetic acid and propionic acid production of fermented rice straw are presented in Table 1. There were temperatures, microorganisms and its interaction effects (P<0.01) on lactic, acetic and propionic acid production of fermented rice straw. Interaction between temperatures and microorganisms on lactic, acetic and propionic acid are shown in Figure 1.

The lactic acid production of Control+molasses and *L. fermentum* treatments at temperature 25°C showed higher (P<0.01) than those in others microorganism treatments. It could be explained that 25°C temperature may give a better environment for Control+mollases and *L. fermentum* to produce lactic acid. The high content of lactic acid in Control+molasses treatment might be caused by lactic acid bacteria that grow and ferment molasses to produce lactic acid. This result confirmed to the report of Gao *et al*. (2008), who isolated microbial community from fermented rice straw identified as *L. fermentum, L. plantarum* and *L. paracasei*, these species were lactic acid bacteria producing lactic acid as well as acetic acid. This result was coincided with the report of McDonald *et al*. (1991), lactic acid bacteria grow within the range 5-50°C, while for most strain grow optimally around 30°C.

B. coagulans at temperature 35°C and 45°C gave lactic acid production higher (P<0.01) than othr treatments. The high content of lactic acid in *B. coagulans* treatment was similar to the report of Woolford (1977) citated by McDonald *et al*. (1991). Woolford examined the activities of *B. coagulans* in grass with low content of water soluble carbohydrates (WSC) and found that organism fermented sugar largely produced lactic and acetic acids. *B. coagulans* at 35°C produced high lactic acid, and this confirmed the report of Davey *et al*. (1966) that *B. coagulans* optimally grow at temperature 35-55°C, except at 45°C the growth rate decreased.

S. cerevisiae and *A. niger* treatments gave lower lactic acid and acetic acid production (P<0.01) than those in other treatments in all temperatures. This might be the effect of loosely sealing which caused the lactic acid evaporation during fermentation. This is also agreed to McDonald *et al*. (1991) who reported that yeast and mold produced only small amount of lactic and acetic acids.

Among the treatments, the highest (P<0.01) acetic acid production was found in *L. fermentum* treatment in all temperatures (Table 1). It was because *L. fermentum* is a heterofermentative

Table 1. Lactic Acid, Acetic Acid and Propionic Acid Production of Fermented Rice Straw at Different Temperatures

Treatments	Lactic Acid			Acetic Acid			Propionic Acid		
	25°C	35°C	45°C	25°C	35°C	45°C	25°C	35°C	45°C
	----------g/kg DM--------								
Control	36.16^{c}	39.86^{c}	20.76^{bc}	2.32^{b}	3.41^{b}	4.01^{ab}	0.00^{e}	0.37^{a}	0.10^{d}
Control+molasses	49.48^{a}	48.37^{b}	25.15^{b}	3.04^{b}	2.99^{b}	3.49^{ab}	0.27^{b}	0.29^{b}	0.28^{a}
L. fermentum	50.84^{a}	42.54^{c}	12.91^{cd}	9.71^{a}	13.20^{a}	5.26^{a}	0.28^{a}	0.27^{c}	0.24^{c}
B. subtilis	40.26^{bc}	39.19^{c}	17.41^{c}	2.00^{b}	2.84^{b}	4.20^{a}	0.22^{c}	0.36^{a}	0.27^{b}
B. coagulans	43.07^{b}	53.79^{a}	33.44^{a}	2.43^{b}	3.09^{b}	3.14^{ab}	0.21^{d}	0.22^{d}	0.24^{c}
S. cerevisiae	0.46^{d}	0.52^{d}	0.38^{e}	0.40^{c}	0.40^{c}	0.20^{c}	0.00^{e}	0.00^{e}	0.00^{e}
A. niger	0.21^{d}	0.41^{d}	0.46 ^{e}	0.14^{c}	0.21^{c}	0.10^{c}	0.00^{e}	0.00^{e}	0.00^{e}

Different supersripct in the same column indicated highly significantly different (P<0.01); DM: Dry Matter

lactic acid bacteria (Gao *et al*., 2008) that ferments WSC into lactic, acetic acids and CO_2 (Cai *et al*., 1998).

The propionic acids production at 25°C showed that *L. fermentum* gave higher (P<0.01) than that in other treatments. While, propionic acids production at 35°C showed that Control and *B. subtilis* gave higher (P<0.01) than that in other treatments. The propionic acids production at 45°C showed that Control+mollases gave higher (P<0.01) among the treatments. The unexpected result was found in this *B. subtilis* treatment. There were no previous reports explained that *B. subtilis* produced propionic acid. Propionic acids were sometimes detected in silage in small amount (McDonald *et al*., 1991). The only microorganisms presented in silage which were likely responsible for propionic acid formation were clostridia and propionic acid bacteria.

In present study, the butyric acid was not detected in all treatments. This condition could be considered as a prove of high quality fermentation. This result was in agreement with Manabu *et al*. (2006) and Cai *et al*. (2003), that high fermentation quality was indicated by high lactic acid production and lower butyric acid production. Butyric acid was a degradation product of lactic acid by clostridia (McDonald *et al*., 1991), the undesirable microorganism in feed fermentation, especially in silage, because clostridia compete with the LAB for the available sugars, and degrade protein, causing problems in reduction of feeding value and the production of biogenic amines.

The LSD tests for organic acids are shown in Figure 1. This figure figured the effect of interaction between temperatures and microorganisms. The LSD test showed that control+mollases and *L. fermentum* at 25°C and *B. coagulans* at 35°C produced the highest (P<0.01) of lactic acid among the treatments. *L. fermentum* at 35°C also showed the highest (P<0.01) production of acetic acid, while control, control+mollases and *B. subtilis* at 35°C produced the highest of propionic acid (P<0.01) among the treatments. Rice straw fermented with *L. fermentum* at 35°C showed the highest (P<0.01) production of acetic acid among the treatments (Figure 1). This result was similar to the report of Mikelsaar *et al*. (2007) that the optimal temperature for *L. fermentum* growth was 37°C, eventhough this bacteria still grow at 45°C, but it

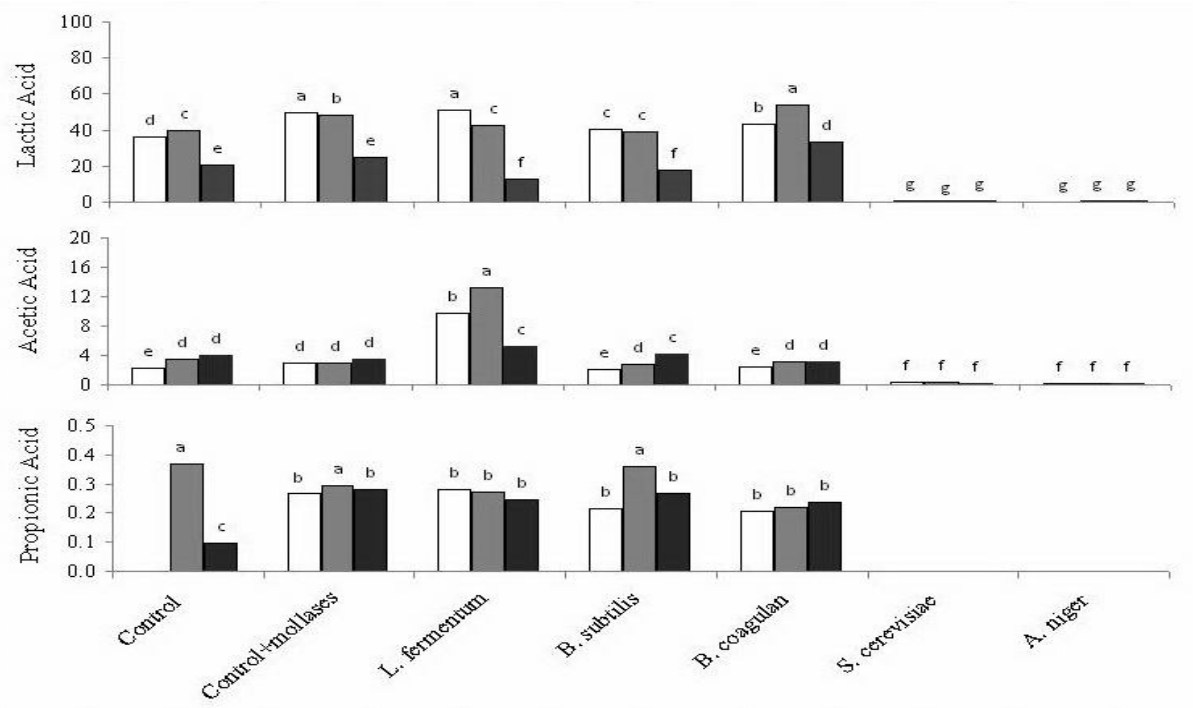

Figure 1. The Effect of Temperatures and Microorganisms Interaction on Lactic Acid, Acetic Acid and Propionic Acid Production (g/kg DM) of Fermented Rice Straw. The different letters indicates different (P<0.05). □: 25°C, ▒: 35 °C, ■ : 45 °C

does not grow at 15°C. This result was in contrast with McDonald *et al*. (1991) and Sneath *et al*. (1986) who reported that *L. fermentum* was exceptionally grows optimum at 45°C.

Control, control+molasses and *B. subtilis* at 35°C produced higher (P<0.01) propionic acid than other treatments. It had explained previously that propionic acids were sometimes detected in silage in small amount (McDonald *et al*., 1991). The only microorganisms present which were likely to be responsible for its formation were clostridia and propionic acid bacteria.

CONCLUSIONS

The highest lactic acid production was in *B. coagulans* treatment at 35°C (53.79 g/kg DM). The highest acetic acid productions were in *L. fermentum* at 35°C (13.20 g/kg DM), while the highest propionic acid productions was in Control treatment at 35°C (0.37 g/kg DM).

ACKNOWLEDGMENTS

The authors thank Directorate General of Higher Education for *Unggulan* Scholarship. Acknowledgment for Tropical Grassland Chemical and Utility Laboratory, Faculty of Agriculture University of the Ryukyus for providing facilities for the experiments. The authors also thank to Susumu Mizumachi, Miyagi Takashi and Binta Rahmi for scientific advice and excellent technical assitances.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1985. Official Method of Analysis. Association of Official Analytical Chemists, Washington, D.C.

Burdon, K. L. and R. P. Williams. 1965. Microbiology. Fifth Edition. The Mac Milan Company, New York.

Cai, Y., Y. Benno, M. Ogawa, S. Ohmomo, S. Kumai and T. Nakase. 1998. Influence of *Lactobacillus* spp. from an inoculants and of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. Appl. Environ. Microbiol. 64:2982-2987.

Cai, Y., F. Yasuhito, M. Masaru, O. Masuhiro, Y. Norio, K. Akira and M. Toshiharu. 2003. Application of lactic acid bacteria (*Lactobacillus plantarum* Chikuso-1) for silage preparation of forage paddy rice. Grassl. Sci. 49 (5):477-485.

Callaway, E. S. and S. A. Martin. 1997. Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035–2044.

Davey, C. B., R. J. Miller and L. A. Nelson. 1966. Temperature-Dependent Anomalies in the Growth of Microorganisms. Am. Soc. Microbiol. 91 (5): 1827-1830.

Drake, D. J., G. Nader and L. Forero. 2002. Feeding Rice Straw to Cattle. ANR Publication 8079. University of California.

Eun, J. S., K. A. Beauchemin, S. H. Hong and M. W. Bauer. 2006. Exogenous enzymes added to untreated or ammoniated rice straw: effects on in vitro fermentation characteristics and degradability. Anim. Feed Sci. Technol. 131: 86-101.

Gao, L., H. Yang, X. Wang, Z. Huang, Y. Igarashi and Z. Cui. 2008. Rice straw fermentation using lactic acid bacteria. Bioresource Technol. 99: 2742-2748.

Han., Y.W. and A.W. Anderson. 1974. The problem of rice straw wastes a possible feed through fermentation. J. Economic Botany. 28(3):338-344.

John, S. E. 1996. An industrial application of cellulases. J. Biotechnol. and Bioengineering. Press syndicate of the University of Cambridge. Third Edition. pp: 68-83.

Lila, Z. A., N. Mohammed, T. Takahashi, M. Tabata, T. Yasui, M. Kurihara, S. Kanda and H. Itabashi. 2006. Increase of ruminal fiber digestion by cellobiose and a twin strain of *Saccharomyces cerevisiae* live cells in vitro. J. Anim. Sci. 77: 407-413.

Manabu, T., N. Yutaka, P. Jinsoo, M. Toshihiro, S. Masataka and M. Yasuhisa. 2006. Fermentation Quality of Floating Rice Plant (*Oryza sativa* L.) Silages as Affected by Mixing with Phasey Bean (Macroptilium lathyroides (L.) Urb.). Japanese J. Grassl. Sci. 52 (3): 155-160.

McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. The Biochemistry of silage. Second Edition. Chalcombe Publication.

Mikelsaar, M., M. Zilmer, T. Kullisaar, H. Annuk and E. Songisepp. 2007. Strain of micro-organism *Lactobacillus fermentum* ME-3 as novel anti-microbiol and anti-oxidative probiotic. United States Patent 7244424. University of Tartu. Http://www. freepatentsonline.com/724424.html. Strain of

micro-organism *Lactobacillus fermentum* ME-3 as novel anti-microbiol and anti-oxidative probiotic. United States Patent 7244424. University of Tartu.ROOF Accessed date: January 6th, 2009.

Milala, M. A., A. Shugaba, A. Gidado, A. C. Ene and J. A. Wafar. 2005. Studies on the use of agricultural wastes for cellulose enzyme production by *Aspergillus niger*. Res. J. Agri. and Biological Sci. 1 (4): 325-328.

Pardo, E., S. Marin, V. Sanchis and A. J. Ranmos. 2005. Impact of relative humidity and temperature on visible fungal gowth and OTA production of ochratoxigenic *Aspergillus ochraseus* isolates on grapes. Food Microbiol. 22:383-389.

Sastrosupadi, A. 2000. Rancangan Percobaan Praktis Bidang Pertanian. Kanisius, Yogyakarta.

Sneath, P. H. A., N. S. Mair, M. E. Sharpe and J. G. Holt. 1986. Bergey's Manual of Systematic Bacteriology. Vol. 2. Williams&Wilkins, United State of America.

Trach, N. X. 1998. The need for improved utilization of rice straw as feed for ruminants in Vietnam: An overview. Livestock Research for Rural Development**.** 10 (2). On-line Edition

Vijaya, C. and M. A. Singaracharya. 2005. Cellulolytic and lignolytic enzymes produced during solid state fermentation of paddy straw by fungi. Indian J. Microbiol. 45 (1): 75-77.

Weinberg, Z. G. and R. E. Muck. 1996. New Trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol, 19:53-68.