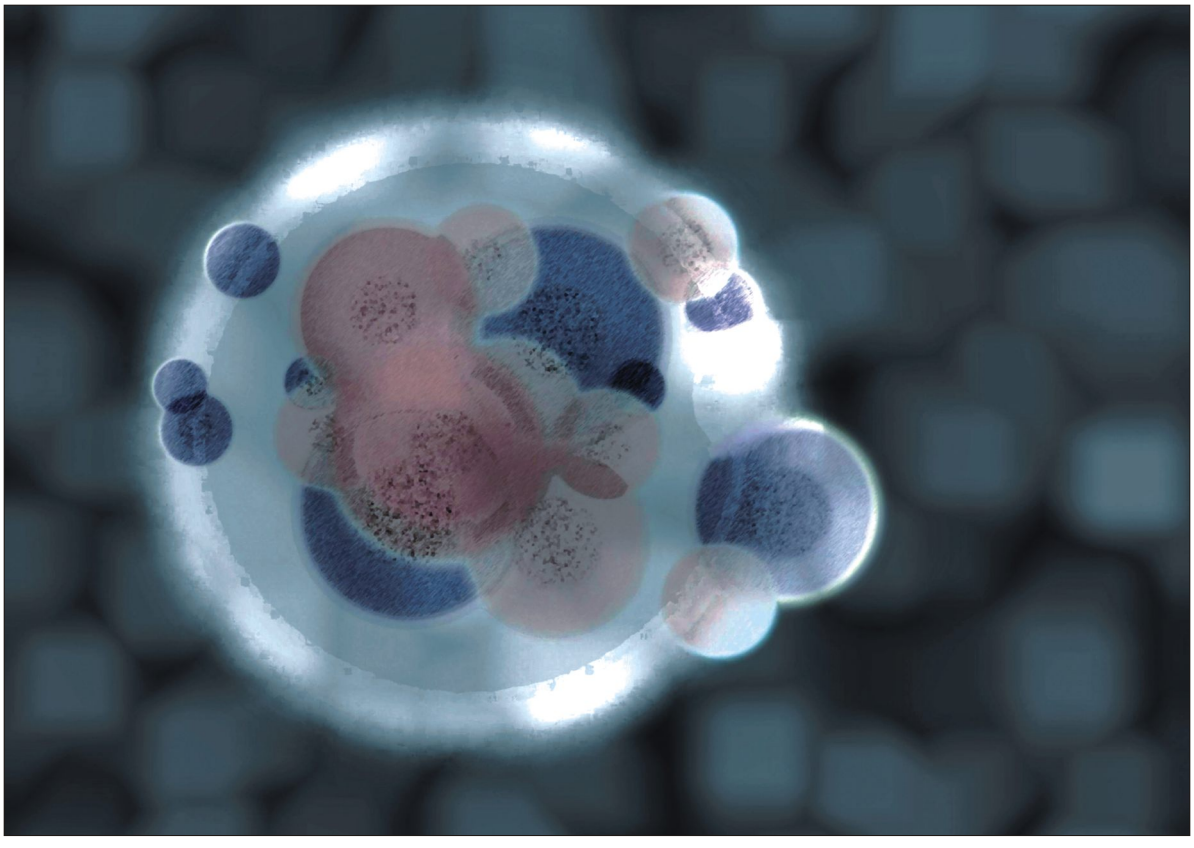

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Research Article
Effectivity of Biocatalyst of Probiotic Lignocellulolytic Bacteria as Starter of Agricultural By-Product

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Abstract

Background and Objective: Production of good quality biocatalysts is one important step in optimizing the utilization of agricultural by-products in the development of the simantri-pattern livestock business. This study has been carried out to evaluate the quality and effectivity of biocatalyst formulated by probiotic lignocellulolytic bacteria from Bali cattle rumen content and termites as the starter of agricultural by-products. **Materials and Methods:** The 5 probiotic lignocellulolytic bacteria namely (1) *Bacillus subtilis* strain *BR$_4$LG*, (2) *Bacillus subtilis* strain *BR$_2$CL*, (3) *Aneurinibacillus* sp., strain *BT$_4$LS*, (4) *Bacillus* sp., strain *BT$_3$CL* and (5) *Bacillus* sp., strain *BT$_8$XY* use for production 10 biocatalyst formula namely B_0, B_1, B_2, B_3, B_4, B_5, B_{1234}, B_{1235}, B_{1245} and B_{12345} as treatments and 6 replicated. The biocatalyst quality was evaluated by nutrient contents, bacteria population and lignocellulose activities. The effectivity of biocatalyst was evaluated by silage nutrient contents, metabolic product (totally VFA and NH3-N), dry matter and organic matter *in vitro* digestibility of rice straw silage. Analysis of variance (ANOVA) used to analyze data and followed by Honestly of Significant Difference (HSD) analysis if there are significant differences between treatments. **Results:** The utilization of probiotic lignocellulolytic bacteria was able to improve the quality and effectivity of solid biocatalysts produced shown by increasing bacteria, nutrient content and lignocellulose activity of biocatalyst produce. These biocatalysts increase the quality of nutrient contents, metabolite product and as well as dry matter and organic matter *in vitro* digestibility of rice straw silage. **Conclusion:** Biocatalyst B_{12345} is the best biocatalyst formula which has the highest quality and effectiveness compared to other biocatalysts.

Key words: Agricultural by-product, biocatalyst, livestock business, probiotic lignocellulolytic bacteria, simantri pattern, starter

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Utilization of agricultural by-products and waste are the main strategies developed in developing agricultural business "*Simantri*" (Integrated Farming System) pattern which is a national program in economic empowerment of rural communities and supporting the development of competitive and sustainable animal production systems[1,2]. The catalyst is a vital element in processing agricultural by-products or waste into useful products and economic value. Various chemical or biological catalysts are developed to optimise the utilization and treatment of agricultural by-products and waste resources. Bacteria are 1 source of biocatalyst that is widely used both in the form of a single or a consortium (a) Combination of several species/types of bacteria and/or with various other microbes) which has now been widely produced on an industrial scale[1-3].

Various starter fermentation biocatalysts have been developed for optimal use and treatments agricultural waste and by-product but the quality of product of agricultural waste and by-product are still very diverse and productivity livestock was given its product is widely various too and have not been able to produce products such as forage feeding. This is mainly due to feeding ingredients from agricultural waste and by-products rich in lignocellulose that is difficult to break down/degrade into their constituent components so many nutrients have not been utilized by livestock. Only certain microbes that can degrade lignocellulose, one of which is lignocellulolytic bacteria[4,5].

Mudita *et al*.[3,6] has succeeded in isolating and selecting superior lignocellulolytic bacteria from the Bali cattle rumen content and termites that can degrade lignocellulosic compounds and produce high lignocellulose enzyme activity, namely *Pseudomonas aeruginosa* BR_9LS, *Bacillus subtilis* strain BR_4LG, *Bacillus subtilis* strain BR_2CL, *Paenibacillus* sp., strain BT_4LS, *Aneurinibacillus* sp., strain BT_5LG, *Bacillus* sp., strain BT_3CL and *Bacillus* sp., strain BT_8XY and 5 of them were allegedly able to play a role as probiotic agents, namely (1) *Bacillus subtilis* strain BR_4LG, (2) *Bacillus subtilis* strain BR_2CL, (3) *Aneurinibacillus* sp., strain BT_4LS, (4) *Bacillus* sp., strain BT_3CL and (5) *Bacillus* sp., strain BT_8XY. Those research were showed that the use of superior lignocellulolytic bacteria as liquid biocatalysts of agricultural waste feed can produce good quality inoculant (starter fermentation) and with high effectivity as lignocellulosic substrates degrader. Utilization of those inoculants on development livestock with patent *Simantri* system can increase Bali cattle productivity especially increasing weight again (from 0.473-0.655-0.752 kg/day) and decreasing of feed conversation ratio (from 8.655-7.277-7.629) and with reduction of ammonia faecal production (from 492.672-420.892-442.883 mg/day) and methane (CH_4) emission (from 27.083-23.160-23.870 mM). The liquid biocatalyst formulated by *Bacillus subtilis* BR_4LG, *Bacillus subtilis* BR_2CL, *Aneurinibacillus* sp., BT_4LS and *Bacillus* sp., BT_8XY can produce the best livestock productivity[2]. The study also showed that different formulas of lignocellulolytic bacteria resulted in different biocatalyst qualities. The effectivity of the microorganism synergism consortium determines the quality produced[2,3].

On the other hand, the form and practicality of biocatalysts also determine the acceptance of the community, especially the industry[7]. Biocatalyst in liquid form is considered less practical, has a relatively short shelf life and is constrained by transportation[8]. The production of solid biocatalysts is believed to be able to overcome these problems, so current research aimed to produce a solid biocatalyst formula that can effectively and efficiently degrade lignocellulosic agricultural waste. The results of this research will be able to become a solution for the development of a competitive and sustainable simantri pattern livestock farming.

MATERIALS AND METHODS

Time and location study: This research was carried out at Sesetan Public Laboratory and Laboratory of Nutrition and Feed, Faculty of Animal Husbandry, Udayana University from March-November, 2019.

Biocatalyst: The biocatalyst produce by probiotic lignocellulolytic bacteria isolated from Bali cattle rumen fluid and termites[3,6], namely (1) *Bacillus subtilis* strain BR_4LG, (2) *Bacillus subtilis* strain BR_2CL, (3) *Aneurinibacillus* sp., strain BT_4LS, (4) *Bacillus* sp., strain BT_3CL and (5) *Bacillus* sp., strain BT_8XY.

Experimental design: This experiment used a completely randomized designed with ten treatments and 6 replicates. The treatments were as follows:

- B_0 = Biocatalyst was formulated without probiotic lignocellulolytic bacteria
- B_1 = Biocatalyst was formulated with *Bacillus subtilis* strain BR_4LG
- B_2 = Biocatalyst was formulated with *Bacillus subtilis* strain BR_2CL
- B_3 = Biocatalyst was formulated with *Aneurinibacillus* sp., strain BT_4LS
- B_4 = Biocatalyst was formulated with *Bacillus* sp., strain BT_3CL

B_5 = Biocatalyst was formulated with *Bacillus* sp., strain *BT_8XY*

B_{1234} = Biocatalyst were formulated with *Bacillus subtilis* strain *BR_4LG*, *Bacillus subtilis* strain *BR_2CL* *Aneurinibacillus* sp., strain *BT_4LS* and *Bacillus* sp.,strain *BT_3CL*

B_{1235} = Biocatalyst were formulated with *Bacillus subtilis* strain *BR_4LG*, *Bacillus subtilis* strain *BR_2CL*, *Aneurinibacillus* sp., strain *BT_4LS* and *Bacillus* sp., strain *BT_8XY*

B_{1245} = Biocatalyst were formulated with *Bacillus subtilis* strain *BR_4LG*, *Bacillus subtilis* strain *BR_2CL*, *Bacillus* sp., strain *BT_3CL* and *Bacillus* sp., strain *BT_8XY*

B_{12345} = Biocatalyst were formulated with *Bacillus subtilis* strain *BR_4LG*, *Bacillus subtilis* strain *BR_2CL*, *Aneurinibacillus* sp., strain *BT_4LS*, *Bacillus* sp., strain *BT_3CL* and *Bacillus* sp., strain *BT_8XY*

Biocatalyst of probiotic lignocellulolytic bacteria: The solid biocatalyst of probiotic lignocellulolytic bacteria was produced through several stages of activities starting from the growth of probiotic lignocellulolytic bacterial stock, production of liquid biocatalyst bacterial culture and production of solid bacterial culture (biocatalyst).

The regrowth of probiotic lignocellulolytic bacterial stock isolates was carried out using a nutrient broth medium at abs. 0.5 λ 650 nm and subsequently incubated at 37-39°C for 3 days. The pure liquid culture of probiotic lignocellulolytic bacteria produced is then used for the production of liquid biocatalyst bacterial culture.

Production of liquid biocatalyst bacterial culture is produced for each type/species of bacteria used. Bacteria culture of liquid biocatalysts is produced by inoculating 10% of pure bacterial cultures in liquid biocatalyst growth media. The growth media of liquid biocatalyst bacterial were formulated using a combination of natural and synthetic ingredients (pro analysis) (Table 1). Before being used, the liquid biocatalyst growth medium was sterilized at 121°C for 15 min. Inoculation of pure bacterial culture in the growth medium of liquid biocatalyst bacteria was carried out aseptically under anaerobic conditions (flowing with CO_2 gas) after the temperature of the medium began to decrease (39°C). The culture of liquid biocatalyst bacteria was then incubated for 1 week at 37°C. The culture of liquid biocatalyst bacteria produced is utilized for the production of solid biocatalyst of probiotic lignocellulolytic bacteria.

Solid biocatalysts are produced by the 1st culture of biocatalyst bacteria that have grown on a liquid medium formulated according to treatment (with the same amount of each bacterial culture) and regrowth on a biocatalyst carrier medium which is produced using ingredients such as corn bran, avicel, starch, molasses, multivitamin-mineral "pignox", urea and ZA fertilizer, tannic acid, CMC and xylan, with a composition as shown in (Table 2).

Table 1: Growth medium composition of liquid biocatalyst bacterial culture in 1 L

Material composition	Quantity
Nutrient broth medium/NA (g)	5
Molasses/sugarcane (g)	50
Urea fertilizer (g)	1
ZA fertilizer (g)	1
Tannic acids (g)	0.25
CMC (g)	0.25
Xylanosa (g)	0.25
Rice straw flour (g)	0.25
Cassava flour (g)	0.25
Rice bran flour (g)	0.25
Salt "NaCl" (g)	0.25
Multi vitamin-mineral "Pignox" (g)	0.15
Water	Volume until 1 L

Table 2: Composition of materials/ingredients of solid biocatalyst bacterial culture for 1 kg

Ingredients composition of solid biocatalyst bacterial culture	Quantity
Culture of liquid biocatalyst bacteria (follow treatment) (mL)	400
Corn bran (g)	250
Maizena flour (g)	200
Sugarcane/molasses (g)	50
Urea fertilizer (g)	30
ZA fertilizer (g)	30
Nutrient broth (g)	0.5
Multivitamin-mineral "Pignox" (g)	20
Salt (g)	19
CMC (g)	0.5
Total	100

Production of solid biocatalysts is carried out by growing a culture of liquid biocatalyst bacteria on a biocatalyst carrier medium by mixing homogeneously between the biocatalyst carrier medium and a consortium of lignocellulolytic bacteria in an anaerobic condition (sprayed with CO_2 gas). After the biocatalyst mixture is homogeneous, it is put into a container (covered bucket) which is filled with plastic first, then tightly closed in the lowest possible condition (to reduce the presence of oxygen in the biocatalyst feeder). Furthermore, the biocatalyst will be fermented/incubated for 1 week at room Temperature (T 35-37°C). After 1 week of fermentation, the biocatalyst will then be dried with a step graded drying method, which begins with drying at 40°C (for 2 days), followed by drying at 45°C (for 2 days) and at 50°C (1 day)[9]. After the multistage process, the biocatalyst is ready to be used.

Quality of probiotic lignocellulolytic bacteria biocatalyst

Evaluation of total bacteria and nutrients content of probiotic lignocellulolytic bacteria biocatalyst: Evaluation of the total bacteria of solid biocatalyst using direct count

method using the growth medium "Nutrient Agar". The nutrient content of solid biocatalysts analyzed were Ca, P, S and Zn concentrations. Analysis of Ca, P and Zn content is done by 1st doing wet ashing, then Ca levels are analyzed by EDTA Method[10], P and Zn content analysis are done by 1st making a standard P curve (for P analysis) or a Zn standard curve (for Zn analysis) so the K value (the standard curve value is known)[10]. Analysis of P or Zn levels was carried out using a standard solution of P or Zn using Atomic Absorption Spectrophotometer (AAS) at a wavelength of 660 nm. While the determination of S levels was done by the Iodometry method[11].

Evaluation of lignocellulose enzyme activity of solid biocatalyst: Lignocellulose enzyme activity (International unit IU^{-1}) (μmol g^{-1} min^{-1}) of solid biocatalyst of probiotic lignocellulolytic bacteria based on degrading ability of lignocellulose (lignin, cellulose, hemicellulose) form of simple compound (release of vanillin/glucose/xylose equivalent) by 1 g solid biocatalyst each minute. In this study, the activities of the lignocellulose enzymes analyzed were the activities of ligninase, endoglucanase, exoglucanase and xylanase enzymes using tannic acid, CMC, avicel and xylan, respectively as specific substrates.

Solid biocatalysts were tested in 1% selective substrates (following enzyme activity were evaluated) in 50 mM acetate buffer pH 5.5[12]. Each substrate liquid in buffer was taken (8 mL), added 1 g biocatalyst and 1 mL aqua dest. The mixture was then shaken by shaking bath, enzyme activity was measured in 30 min, 1, 3 and 6 hrs incubation durations. Reduction sugar (glucose from CMC/avicel and xylose from xylan), or vanillin from tannic acid (lignin) produced from the reaction were the endoglucanase, exoglucanase, xylanase or ligninase enzyme activities[13,14]. For sugar reduction: 1 mL of sample was added to 3 mL DNS reagent and 1 mL aqua dest[11], for vanillin: 1 mL of the sample was added to 4 mL methanol[12]. With use spectrophotometer on wavelength (λ) 508.5 nm for reducing sugar especially glucose (cellulase, endoglucanase and exoglucanase enzyme activity), λ 509 nm for reducing sugar especially xylose (xylanase enzyme activity) and λ 279 nm for vanillin (ligninase enzyme activity).

Value of each enzyme activity (Ligninase, cellulase and xylanase) counted follow the regression equation:

$$Y = 0.00635X+0.21098 \ (R^2 = 0.929) \text{ for ligninase}$$

$$Y = 0.00622X+0.14277 \ (R^2 = 0.972) \text{ for cellulase}$$

$$Y = 0.00002X+0.20525 \ (R^2 = 0.897) \text{ for xylanase}^6$$

One unit (U) of enzyme activity was defined as 1 μmoL of vanillin/glucose/xylose equivalent released per minute under standard assay condition[15,16].

Effectivity of solid biocatalyst as starter fermentation: Evaluation of the effectiveness of probiotic lignocellulolytic bacteria biocatalysts as a starter fermentation of agricultural waste is carried out through the production of rice straw silage. Rice straw silage was formulated using a mixture of 80% rice straw and 20% rice bran (% DM basis). The fermentation process is carried out utilizing every 10 kg feed materials added 8 L of biocatalyst solution consisting of 10 g biocatalyst, 100 mL molasses and 7.890 L of clean water. The fermentation process is carried out using a plastic bucket covered (as a silo) and fermented for 2 weeks under anaerobic conditions. After 2 weeks, rice straw silage was opened and evaluated of pH (acidity), nutrient content and *in vitro* digestibility and fermentation metabolic product.

Evaluation of pH use by pH meter, nutrients contents including Dry Matter (DM), Ash/Inorganic matter, Organic Matter (OM), Crude Fibre (CF) and Ether Extract (EE) using proximate analysis (Weende) with the AOAC methods[17]. Crude protein was analyzed by the Kjeldahl semi-micro method[18,19] using Vapodest-Gerhardt equipment. The Nitrogen Free Extract (NFE) content of the sample was calculated by the formula[20], namely:

$$\text{NFE (\%)} = 100-(\text{CP+EE+CF+Ash})$$

The evaluation of dry matter and organic matter *in vitro* digestibility of silage of rice straw produced by solid biocatalyst was carried out by *in vitro* techniques using the Minson and McLeod method[21]. Where the samples were incubated in a shaking bath for 2×48 hrs as a reflection of (1) The rumen fermentative digestibility phase and (2) The enzymatic digestibility phase. The metabolite products namely VFA and NH_3-N are evaluated from the 1st step (phase) "fermentative digestion/rumen simulation" from *in vitro* feed analysis[21].

Data analysis: Data were analyzed by analysis of variance/ANOVA if there are significant differences ($p<0.05$), followed by the analysis of the Honestly Significant Different (HSD) test.

RESULTS AND DISCUSSION

Quality of solid biocatalysts of probiotic lignocellulolytic bacteria: Utilization of probiotic lignocellulolytic bacteria both

Table 3: Bacteria population and nutrients content of solid biocatalyst of probiotic lignocellulolytic bacteria

		Nutrients content of solid biocatalysts			
Solid biocatalyst*	Totally bacteria ($\times 10^8$ CFU)	P (ppm)	Ca (ppm)	Zn (ppm)	S (ppm)
B_0	1.667^{a**}	181.069^{a}	897.946^{a}	212.992^{a}	8.980^{a}
B_1	5.767^{b}	219.169^{b}	985.265^{b}	252.515^{b}	9.061^{ab}
B_2	7.333^{b}	222.397^{b}	997.420^{bc}	257.042^{b}	9.411^{cd}
B_3	6.300^{b}	221.391^{b}	987.342^{b}	255.858^{b}	9.094^{ab}
B_4	6.567^{b}	222.177^{b}	997.182^{bc}	257.023^{b}	9.069^{ab}
B_5	6.133^{b}	219.548^{b}	995.095^{bc}	247.641^{b}	9.286^{bc}
B_{1234}	11.067^{c}	234.571^{bc}	997.491^{bc}	257.585^{b}	9.554^{cd}
B_{1235}	12.733^{c}	246.671^{c}	1007.482^{bc}	276.313^{c}	9.866^{e}
B_{1245}	12.333^{c}	223.677^{b}	998.065^{bc}	257.596^{b}	9.586^{d}
B_{12345}	13.200^{c}	247.871^{c}	1010.705^{c}	278.483^{c}	9.866^{e}
SEM***	0.638	4.228	4.693	3.859	0.056

*Solid biocatalyst of probiotic lignocellulolytic bacteria formulated from (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*, **Means in the same columns with the same superscript differ not significantly (p>0.05), ***SEM: Standard error of the treatments means

Table 4: Ligninase enzyme activity of lignocellulolytic probiotic bacteria biocatalyst

	Ligninase enzyme activity on various incubation periods (IU)			
Solid biocatalyst*	30 min	1 hr	3 hrs	6 hrs
B_0	0.207^{a**}	0.143^{a}	0.063^{a}	0.044^{a}
B_1	0.788^{bc}	0.526^{bcd}	0.227^{bc}	0.128^{bcd}
B_2	0.771^{bc}	0.514^{bc}	0.224^{bc}	0.125^{bc}
B_3	0.777^{bc}	0.525^{bcd}	0.226^{bc}	0.126^{bcd}
B_4	0.779^{bc}	0.522^{bcd}	0.225^{bc}	0.131^{cd}
B_5	0.768^{b}	0.505^{b}	0.213^{b}	0.122^{b}
B_{1234}	0.824^{bcd}	0.543^{bcd}	0.233^{cd}	0.139^{ef}
B_{1235}	0.833^{cd}	0.551^{cd}	0.234^{cd}	0.141^{f}
B_{1245}	0.797^{bcd}	0.529^{bcd}	0.227^{bc}	0.132^{de}
B_{12345}	0.859^{d}	0.562^{d}	0.244^{d}	0.143^{f}
SEM***	0.013	0.008	0.003	0.001

*Solid biocatalyst of probiotic lignocellulolytic bacteria formulated from (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*, **Means in the same columns with the same superscript differ not significantly (p>0.05), ***SEM: Standard error of the treatment means

Table 5: Endo-glucanase enzyme activity of solid biocatalyst of lignocellulolytic probiotic bacteria

	Endo-glucanase enzyme activity on various incubation periods (IU)			
Solid biocatalyst*	30 min	1 hr	3 hrs	6 hrs
B_0	6.442^{a**}	5.445^{a}	2.522^{a}	1.661^{a}
B_1	14.747^{b}	8.344^{b}	3.789^{b}	2.182^{bc}
B_2	17.114^{c}	9.701^{c}	4.163^{c}	2.254^{bc}
B_3	17.218^{c}	9.741^{c}	4.175^{c}	2.262^{bcd}
B_4	17.849^{cd}	9.826^{c}	4.347^{cd}	2.293^{cd}
B_5	14.387^{b}	8.138^{b}	3.689^{b}	2.136^{b}
B_{1234}	18.196^{cd}	10.088^{c}	4.717^{ef}	2.573^{f}
B_{1235}	18.672^{d}	10.415^{c}	4.769^{f}	2.609^{f}
B_{1245}	17.997^{cd}	9.837^{c}	4.489^{de}	2.412^{de}
B_{12345}	18.722^{d}	10.654^{c}	4.727^{ef}	2.527^{ef}
SEM***	0.282	0.202	0.056	0.032

*Solid biocatalyst of probiotic lignocellulolytic bacteria formulated from (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*, **Means in the same columns with the same superscript differ not significantly (p>0.05), ***SEM: Standard error of the treatment means

singly and/or a consortium, namely (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT8XY*, able to improve the quality of solid biocatalysts produced as indicated by the high total bacterial population, nutrients content (P, Ca, Zn and S) and the lignocellulose enzyme activity (*Ligninase, Endo-glucanase, Exo-glucanase* and *Xylanase*) compared than biocatalysts produced without bacterial isolates (Table 3-7).

On totally bacterial populations, biocatalyst production using probiotic lignocellulolytic bacteria (B_1, B_2, B_3, B_4, B_5, B_{1234}, B_{1235}, B_{1245} and B_{12345}) has a higher ($p<0.05$) totally bacteria

population compared than solid biocatalyst produce without probiotic lignocellulolytic bacteria/B_0 (5.767-13.200×10^8 vs. 1.667×10^8 CFU) Utilization consortium of combined 4 or 5 bacteria isolates, namely (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*) has produced solid biocatalyst with a higher population of totally bacteria compare than single isolate (Table 3) and formulation of biocatalyst B_{12345} has most population bacteria (13.20×10^8 vs. 1.667-12.733×10^8 CFU). This shows that the combination of several bacteria can grow well in the growth media produced and there is no competition and even shows a synergistic relationship in growth and activity so that the total bacterial population becomes high. This also showed that the growth medium produced has a high nutrient content that can meet the needs of bacterial growth so that the inoculated bacteria can grow optimally. Kamsani *et al.*[22] also showed that the combination of 2 isolates, 3 isolates and 4 different isolates that we're able to study synergistically would grow faster and have higher enzyme activity than a single isolate.

The use of a single isolate of *Bacillus subtilis* strain *BR_2CL* (B_2) was able to produce a biocatalyst with a quantitatively higher total bacteria ($p>0.05$) compared to the use of other single bacterial isolates (Table 3). This was predicted as a result of the high content of cellulose and its constituent components in the growth medium produced which can support the growth of these cellulolytic bacteria.

Highly microbial populations and their synergistic activities will also strongly support increasing the inoculant nutrient contents. The more synergistic the microbes/bacteria that grow, the higher the bacterial population, so the higher the nutrient contents of biocatalyst[23]. This condition was also seen in this study, where the biocatalyst formulated by single and/or consortium probiotic lignocellulolytic bacteria were produced biocatalysts with nutrient content such as Calcium (Ca), Phosphorus (P), Sulfur (S) and Zincum (Zn) were significantly higher ($p<0.05$) with increase percentage, respectively 21.04-36.89, 9.72-12.56, 16.27-30.75, 0.90-9.87% (unless against the content of sulfur on the treatment B_1, B_3 and B_4 did not significantly different) compared to biocatalysts without probiotic lignocellulolytic bacteria/B_0 (Table 3). Table 3 also showed that biocatalysts B_{1235} and B_{12345} are the best biocatalysts with the highest nutrient content and total bacterial population. Highly totally bacterial population be able to increase supply nutrients for bacteria body mass was rich various nutrient including an organic matter/minerals[24,25].

The increase inorganic contents (P, Ca, S and Zn) of biocatalysts produced using lignocellulolytic probiotic bacteria either singly or consortium which is in line with the increase in the population of total bacteria indicates the high contribution of inorganic materials derived from microbial body cells. The higher the total bacterial population, the higher the minerals content of the biocatalyst. Inorganic materials/mineral salts are needed as a source of anions (phosphate and sulfate) and cations (sodium, potassium, magnesium, iron and calcium) for bacterial cells for growth and activity. Gadd[23] revealed that minerals play an important role in microbial/bacterial life. Mineral/metals are directly and/or indirectly involved in all aspects of microbial growth, metabolism and differentiation. Even Hoffmann *et al.*[26] revealed that bacteria are ideal nucleating agents for mineral precipitation. So that the higher the growth and the total population of bacteria, the higher the supply of minerals from microbial/bacterial biomass.

In addition, the increase in mineral content is also suspected as a result of the degrading of lignin compounds that will form organometallic. This is possible considering the bacteria from the *Bacillus* sp., has the enzymatic ability to break down lignin compounds either through the breakdown of aromatic rings, reducing and non-reducing side chains[27]. Kajikawa *et al.*[28] revealed that rumen microbes were able to break β-aryl ether bonds from Veratrylglycerol-4-Guaiacyl Ether (VGE) and benzyl ether bonds found in lignin, while Kamsani *et al.*[22] showed bacteria isolated from the gastrointestinal tract of termites *Bulbitermes* sp., namely *Bacillus* sp., B_1, *Bacillus* sp., B_2 and *Brevibacillus* sp., Br_3, in addition to producing crude fibre degrading enzymes (cellulose and hemicellulose) also produces lignin-degrading enzymes such as *Lignin peroxidase* (Li-P), *Manganese-peroxidase* (Mn-P) and *Lacasse* (Lac.) Chandra *et al.*[29] added that the derivatization and demineralization process of lignin compounds will form organometallic which will increase the mineral metal content in feed ingredients. In this study, the bacterial isolates used were lignocellulolytic bacterial isolates where isolated from the Bali cattle rumen contents and termites, where these bacteria have been known to have the ability to degrade lignin compounds[3,6].

Evaluation of lignocellulose enzymes activity of biocatalysts showed that the utilization of probiotic lignocellulolytic bacteria can increase *Ligninase* enzyme activity (Table 4), *Endoglucanase* enzyme activity (Table 5), *Exoglucanase* enzyme activity (Table 6) and *Xylanase* enzyme activity (Table 7) compared with biocatalysts without probiotic lignocellulolytic bacteria. This shows the high quality and effectiveness of the lignocellulolytic probiotic bacterial isolates used either individually or in the consortium.

Table 4 showed that the use of lignocellulolytic probiotic bacteria on production solid biocatalysts (B_1, B_2, B_3, B_4, B_5, B_{1234}, B_{1235}, B_{1245} and B_{12345}) was able to significantly increase

Table 6: Exo-glucanase enzyme activity of solid biocatalyst of probiotic lignocellulolytic bacteria

	Exo-glucanase enzyme activity on various incubation periods (IU)			
Solid biocatalyst*	30 min	1 hr	3 hrs	6 hrs
B_0	6.764^{a**}	4.247^{a}	1.713^{a}	0.996^{a}
B_1	14.463^{b}	8.684^{b}	3.550^{b}	1.983^{b}
B_2	16.622^{d}	9.878^{c}	3.838^{bcde}	2.168^{cd}
B_3	14.796^{bc}	8.802^{b}	3.701^{bcd}	2.027^{bc}
B_4	15.780^{cd}	9.780^{c}	3.794^{bcde}	2.129^{bcd}
B_5	14.635^{b}	8.857^{b}	3.601^{bc}	1.998^{b}
B_{1234}	18.475^{ef}	10.559^{cd}	4.013^{de}	2.204^{d}
B_{1235}	19.267^{fg}	10.699^{d}	4.023^{e}	2.213^{d}
B_{1245}	18.103^{e}	10.378^{cd}	3.910^{cde}	2.174^{cd}
B_{12345}	20.471^{g}	11.050^{d}	4.017^{e}	2.231^{d}
SEM***	0.232	0.163	0.065	0.034

*Solid biocatalyst of probiotic lignocellulolytic bacteria formulated from (1) *Bacillus subtilis* strain BR_4LG, (2) *Bacillus subtilis* strain BR_2CL, (3) *Aneurinibacillus* sp., strain BT_4LS, (4) *Bacillus* sp., strain BT_3CL and (5) *Bacillus* sp., strain BT_8XY, **Means in the same columns with the same superscript differ not significantly (p>0.05), ***SEM: Standard error of the treatment means

Table 7: Xylanase enzyme activity of solid biocatalyst of probiotic lignocellulolytic bacteria

	Xylanase enzyme activity on various incubation periods (IU)			
Solid biocatalyst*	30 min	1 hr	3 hrs	6 hrs
B_0	42.494^{a**}	37.896^{a}	16.802^{a}	10.041^{a}
B_1	228.937^{b}	122.802^{b}	46.020^{b}	25.739^{b}
B_2	240.618^{bc}	142.476^{cd}	51.570^{bc}	28.284^{cd}
B_3	241.722^{bcd}	131.071^{bc}	50.558^{bc}	26.643^{bc}
B_4	237.675^{bc}	126.104^{b}	47.216^{bc}	25.862^{b}
B_5	243.377^{bcd}	146.431^{de}	52.520^{c}	28.161^{cd}
B_{1234}	251.104^{bcde}	141.740^{cd}	51.784^{bc}	29.617^{d}
B_{1235}	266.924^{de}	163.447^{fg}	60.676^{d}	34.753^{e}
B_{1245}	263.245^{cde}	155.997^{ef}	60.338^{d}	34.477^{e}
B_{12345}	277.042^{e}	175.773^{g}	64.784^{d}	39.858^{f}
SEM***	5.376	2.648	1.222	0.364

*Solid biocatalyst of probiotic lignocellulolytic bacteria formulated from (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*, **Means in the same columns with the same superscript differ not significantly (p>0.05), ***SEM: Standard error of the treatment means

ligninase enzyme activity ($p<0.05$) compared with biocatalysts without lignocellulolytic probiotic bacteria/B_0 were 0.771-0.859 vs. 0.207 IU, 0.505-0.562 vs. 0.143 IU, 0.213-0.244 vs. 0.063 IU, 0.122-0.143 vs. 0.044 IU for 30 min, 1, 3 and 6 hrs periods incubation, respectively. This showed that the bacterial isolates used in the production of biocatalysts are capable of producing ligninase enzymes which can degrade lignin compounds into simpler components through depolymerization and breakdown aromatic rings[27] with high ligninase enzyme activity values up to 6 hrs of incubation on tannic acid substrates. Table 4 also showed that the biocatalyst formula B_{12345} produces the highest ligninase enzyme activity was 0.859, 0.562, 0.244 and 0.143 IU, respectively for 30 min, 1, 3 and 6 hrs incubation periods. This further confirms that the biocatalyst formula B_{12345} is the best bacterial consortium that can work synergistically with high ligninase enzyme activity in degrading lignin complex compounds into their constituent components.

The formulation of 5 types of bacteria with different characteristics, namely (1) *Bacillus subtilis* strain *BR_4LG* which is an isolate of ligninolytic bacteria from Bali cattle rumen fluid, (2) *Bacillus subtilis* strain *BR_2CL* is a cellulolytic bacteria from Bali cattle rumen fluid with high exoglucanase enzyme activity, (3) *Aneurinibacillus* sp., strain *BT_4LS* which is an isolate of lignocellulolytic bacteria from termites with lignocellulose enzyme activity (ligninase, cellulase and xylanase), (4) *Bacillus* sp., strain *BT_3CL* is an isolate of cellulolytic bacteria from termites with high endoglucanase enzyme activity and (5) *Bacillus* sp., strain *BT_8XY*, which is an isolate of xylanolytic bacteria from termites[6], can produce synergistic activity in the breakdown of complex compounds, where the product resulting from the breakdown of a bacterial isolate can be followed by the activity of other bacterial isolates. This also shows that its formula's there is no competition/low competition between isolates for nutrient requirements for their live or activity.

The high activity of the ligninase enzyme produced by the lignocellulolytic probiotic bacteria biocatalyst is a direct response to the high total population of bacteria present/growing in the biocatalyst (Table 3). The higher the total bacterial population, the higher the activity of the ligninase enzyme produced tends to be higher as well. In addition, several researchers also showed that the 5 types of bacterial isolates similar to those used in this study could produce ligninolytic enzyme activity. Datta *et al*.[30], Min *et al*.[31] and Lai *et al*.[32] revealed that *Bacillus subtilis* produces *Laccase* (Lac), *Dye-decolorizing peroxidase* (DyP), *Lignin peroxidase* (LiP) and *Manganese peroxidase* (MnP) which can remodel lignin compounds into their simple components through the process of dissolution, demineralization and the breakdown of side chains of aromatic compounds from lignin. Besides that *Aneurinibacillus* and *Bacillus* sp., which is also used in biocatalyst production is known to be able to produce lignin-degrading enzymes such as MnP and Lac which can degrade the side chains of aromatic lignin compounds and overhaul lignin through the process of depolymerization[33]. Martini *et al*.[34] also revealed that bacteria from the genus *Bacillus* sp., (H-10 isolate) isolated from domestic waste were able to degrade lignin "*Lindi hitam*" by 78%.

Evaluation of the activity of endoglucanase and exoglucanase, which are part of the activity of the cellulase enzyme complex showed relatively the same thing where the use of lignocellulolytic probiotic bacteria, either single or consortium was able to increase ($p<0.05$) the activity of endoglucanase and exoglucanase enzymes. Biocatalyst formula coded B_1, B_2, B_3, B_4, B_5, B_{1234}, B_{1235}, B_{1245} and B_{12345} were able to produce significantly higher endoglucanase enzyme activity compare than biocatalyst formula without probiotic lignocellulolytic bacteria coded B_0, namely 14.387-18.722 vs. 6.442 IU, 8.138-10.654 vs. 5.445 IU, 3.689-4.727 vs. 2.522 IU, 2.136-2.527vs. 1.661 IU as well as the exoglucanase enzyme activity which was also significantly higher were 14.462-20.471 vs. 6.764 IU, 8.684-11.050 vs. 4.247 IU, 3.550-4.023 vs. 1.713 IU, 1.983-2.231 vs. 0.996 IU at the incubation period of 30 min, 1, 3 and 6 hrs, respectively on CMC substrate or avicel (Table 5 and 6). In addition, Table 5 and 6 also showed that the biocatalyst formula coded B_{12345} can produce biocatalysts with the highest endoglucanase and exoglucanase enzyme activity were 18.722 and 20.471 IU, 10.654 and 11.050 IU, 4.727 and 4.017 IU, 2.527 and 2.231 IU, respectively for 30 min, 1, 3 and 6 hrs of periods incubation on CMC or Avicel.

Endoglucanase enzyme is an enzyme that is responsible for breaking the β-1,4 glucoside bonds from the inner structure (crystalline/amorphous) of cellulose compounds randomly, while the exoglucanase enzyme is responsible for the alteration of the side chains (reducing and non-reducing ends) of the cellulose structure. Various references show that the five bacterial isolates used for the production of solid biocatalyst can produce cellulase complex enzyme activity. Sadhu and Maiti[35] show that *Bacillus subtilis* and *Bacillus* sp., can produce *endoglucanase* enzyme activity with, respectively 30-35 and 54-100 mol min^{-1}. Rina[36] revealed that *Bacillus subtilis* and *Bacillus* sp., is a source of avicelase/exoglucanase with high enzyme activity. *Bacillus subtilis* is also reported to be able to produce β-glucosidase[33,37]. Maki *et al*.[38] revealed that *Bacillus subtilis* can produce endoglucanase. *Aneurinibacillus* is also reported to be able to produce various cellulase enzymes both CMCase and FPase with activities of 0.025-0.080 and 0.195-0.415 IU under various alkaline conditions, respectively (pH 7-10) with temperatures of 50-75°C[39].

Table 5 and 6 also showed that the use of a consortium of 5 lignocellulolytic probiotic bacteria (B_{12345}) was able to produce biocatalysts with the highest value of endoglucanase and exoglucanase enzyme activity. This showed that the B_{12345} formula is a synergistic bacterial consortium formula so that it can carry out the breakdown of β-1,4 glucoside bonds from the inner structure (crystalline/amorphous) of cellulose compounds and overhaul the side chains (reducing and non-reducing ends) of the cellulose structure and overhaul the side chains (reducing and non-reducing ends) of the cellulose structure for the formation of simple compounds that make up cellulose (glucose) through optimal, synergistic and continuously.

Evaluation of xylanase enzyme activity from the resulting solid biocatalyst showed that the use of probiotic lignocellulolytic bacteria was able to produce a biocatalyst with xylanase enzyme activity which was significantly higher than the biocatalyst formula without probiotic lignocellulolytic bacteria/B_0, which was 228.937-277.042 vs. 42.494 IU, 122.802-175.773 vs. 37.896 IU, 46.020-64.784 vs. 16.802 IU and 25.739-39.858 vs. 10.041 IU at incubation periods of 30 min, 1, 3 and 6 hrs on xylan substrate (Table 7). This shows the high ability of the biocatalyst produced in the breakdown of hemicellulose (xylanase) compounds into simpler components, namely xylose.

The xylanase enzyme is an enzyme that is responsible for hydrolyzing randomly building the main β-1,4-xylose bonds from the xylanase chain framework to form oligosaccharides containing xylose[40]. Furthermore, Saha[40] revealed that the total degradation of xylan compounds is the result of synergistic activity of the xylanase complex consisting of endo-1,4-β-xylanase, exo-xylanase, 1,4-β-xylosidase and several supporting enzymes such as α-L-arabinofuranosidase,

α-glucuronidase. Acetyl xylan esterase, ferulic acid esterase and p-coumarin esterase hydrolyze various xylanase components. The higher the production and enzymes activity produced, the higher the overhaul of the xylanase component occurs.

Table 7 also shows that B_{12345} is the biocatalyst with the highest xylanase specific activity at all incubation periods (277.042, 175.773, 64.784 and 39.858 IU). This shows that the utilization of superior bacteria from Bali cattle rumen fluid and termites, namely *Bacillus subtilis* strain *BR$_4$LG*, *Bacillus subtilis* strain *BR$_2$CL*, *Aneurinibacillus* sp., strain *BT$_4$LS*, *Bacillus* sp., strain*s* *BT$_3$CL* and *Bacillus* sp., *BT$_8$XY* strain is proven to be able to produce high quality and synergistic biocatalysts which has the potential to be a starter for feed-based agricultural by-products and waste.

Table 4-7 also showed that in general, an increase in the incubation period results in a decrease in the value of the enzyme activity produced every minute by each biocatalyst produced. This may be caused by several factors, namely (1) Due to the decrease in the existing/remaining substrate so that the rate of enzyme activity is getting lower, (2) Due to the accumulation of products resulting from previous enzyme activities that cannot be further overhauled by the other enzyme, resulting in the cessation of enzyme activity as a whole.

Effectivity of solid biocatalysts of probiotic lignocellulolytic bacteria: Utilization of probiotic lignocellulolytic bacteria namely, *Bacillus subtilis* strain *BR$_4$LG*, *Bacillus subtilis* strain *BR$_2$CL*, *Aneurinibacillus* sp., strain *BT$_4$LS*, *Bacillus* sp., strain *BT$_3$CL* and *Bacillus* sp., strain*s* *BT$_8$XY* in the production of solid biocatalysts are proven to be able to produce high-quality biocatalysts with high nutrient content, totally bacteria and lignocellulose enzymes activity. Further evaluation of the effectiveness of solid biocatalysts as a starter for fermentation of rice straw can produce high-quality silage with better quality of nutrients content, acidity, metabolite products and dry matter and organic matter *in vitro* digestibility (Table 8, 9).

Table 8 shows that the use of lignocellulolytic probiotic bacteria biocatalysts was able to produce rice straw silage with higher ($p<0.05$) crude protein and ether extract content, respectively 8.860-9.869 vs. 6.483% and 2.627-4.210 vs. 1.522% and lower ($p<0.05$) crude fiber content of 14.816-15.628 vs. 17.921% compared to rice straw silage produced with biocatalyst without lignocellulolytic probiotic bacteria. While the content of dry matter, organic matter and nitrogen-free extract of rice straw silage produced varied in all treatments. Silage of rice straw produced by solid biocatalysts formulated using combine 4 or 5 bacterial isolates i.e.: (1) *Bacillus subtilis* strain *BR$_4$LG*, (2) *Bacillus subtilis* strain *BR$_2$CL*, (3) *Aneurinibacillus* sp., strain *BT$_4$LS*, (4) *Bacillus* sp., strain *BT$_3$CL* and (5) *Bacillus* sp., strain *BT$_8$XY* have higher crude protein and extract ether and with lower crude fibre.

The high content of crude protein and ether extract from rice straw silage produced biocatalyst formulated using a consortium of probiotic lignocellulolytic bacteria is a direct response to the high bacterial population, nutrient content and lignocellulose enzyme activity of the biocatalyst used (Table 3-7). The high bacteria population from solid biocatalyst produced by consortium bacteria (B_{1234}, B_{1235}, B_{1245}, B_{12345}) will be increasing the supply of protein and fat which resources from bacteria cell mass so that the protein and fat (extract ether) content of rice straw silage will increase, while the reduction of crude fibre content from rice straw silage is also a direct response from the high bacterial population that is known to have high lignocellulose enzyme activity (such as ligninase, endoglucanase, exoglucanase and xylanase) so that the breakdown (degrade) of lignocellulose compounds which are the main components of crude fibre will take place more effectively so that the crude fibre content of silage will decrease.

Table 8: Nutrients content of rice straw silage produce by solid biocatalyst of probiotic lignocellulolytic bacteria

Rice straw silage*	Dry matter (fresh basis %)	Organic matter (DM %)	Crude protein (DM %)	Crude fiber (DM %)	Ether extract (DM %)	Nitrogen free extract (DM %)
RSB_0	29.136^{ab}**	79.836^{a}	6.483^{a}	17.921^{b}	1.522^{a}	46.189^{b}
RSB_1	32.022^{b}	80.090^{a}	8.860^{b}	15.469^{a}	2.627^{ab}	45.595^{ab}
RSB_2	28.622^{ab}	80.118^{a}	9.132^{bc}	15.491^{a}	2.840^{b}	44.906^{ab}
RSB_3	27.181^{ab}	80.466^{ab}	8.846^{b}	15.485^{a}	3.289^{bc}	43.289^{ab}
RSB_4	27.694^{ab}	80.535^{ab}	8.908^{b}	15.628^{a}	3.328^{bc}	43.489^{ab}
RSB_5	26.676^{a}	80.629^{ab}	8.907^{b}	15.600^{a}	3.343^{bc}	43.970^{ab}
RSB_{1234}	29.353^{ab}	80.952^{ab}	9.621^{cd}	15.435^{a}	4.199^{c}	42.765^{a}
RSB_{1235}	29.806^{ab}	82.845^{b}	9.869^{d}	14.922^{a}	4.210^{c}	44.706^{ab}
RSB_{1245}	29.041^{ab}	81.787^{ab}	9.666^{d}	15.293^{a}	3.381^{bc}	45.251^{ab}
RSB_{12345}	31.568^{ab}	81.987^{ab}	9.676^{d}	14.816^{a}	3.783^{bc}	45.486^{ab}
SEM***	1.095	0.550	0.101	0.294	0.257	0.783

*Rice straw silage produced by solid biocatalyst of probiotic lignocellulolytic bacteria formulated by, (1) *Bacillus subtilis* strain *BR$_4$LG*, (2) *Bacillus subtilis* strain *BR$_2$CL*, (3) *Aneurinibacillus* sp., strain *BT$_4$LS*, (4) *Bacillus* sp., strain *BT$_3$CL* and (5) *Bacillus* sp., strain *BT$_8$XY*, **Means in the same columns with the same superscript differ not significantly ($p>0.05$), ***SEM: Standard error of the treatments means

Table 9: Acidity (pH), VFA, NH_3-N and *in vitro* digestibility of rice straw silage produced by solid biocatalyst of probiotic lignocellulolytic bacteria

Rice straw silage*	Product metabolic and *in vitro* digestibility				
	Acidity (pH)	VFA (mM)	NH_3-N (mM)	DM digestibility (%)	OM digestibility (%)
JPB_0	4.713^{b}	116.236^{a}	10.570^{a}	47.198^{a}	48.570^{a}
JPB_1	4.634^{ab}	174.927^{bc}	11.608^{ab}	49.873^{ab}	52.041^{abc}
JPB_2	4.605^{ab}	166.968^{bc}	11.605^{ab}	49.026^{ab}	52.676^{bcd}
JPB_3	4.620^{ab}	158.238^{b}	11.663^{b}	50.107^{bc}	52.274$_{bc}$
JPB_4	4.618^{ab}	169.142^{bc}	11.641^{b}	49.910^{b}	50.750^{ab}
JPB_5	4.624^{ab}	166.841^{bc}	11.644^{b}	48.828^{ab}	50.331^{ab}
JPB_{1234}	4.599^{ab}	179.120^{bc}	11.6845^{b}	51.177^{bcd}	53.593^{bcd}
JPB_{1235}	4.592^{ab}	179.270^{bc}	12.230^{bc}	52.758^{cd}	55.575^{cd}
JPB_{1245}	4.599^{ab}	175.382^{bc}	12.0891^{bc}	50.468^{bcd}	53.800^{bcd}
JPB_{12345}	4.545^{a}	187.652^{c}	13.135^{c}	52.923^{d}	56.030^{d}
SEM	0.060	4.709	0.216	0.559	0.730

*Rice straw silage produced by solid biocatalyst of probiotic lignocellulolytic bacteria formulated by, (1) *Bacillus subtilis* strain *BR_4LG*, (2) *Bacillus subtilis* strain *BR_2CL*, (3) *Aneurinibacillus* sp., strain *BT_4LS*, (4) *Bacillus* sp., strain *BT_3CL* and (5) *Bacillus* sp., strain *BT_8XY*, **Means in the same columns with the same superscript differ not significantly ($p>0.05$), ***SEM: Standard error of the treatment means

Lignocellulose enzyme activity, whether ligninase, endoglucanase, exoglucanase or xylanase produced by biocatalysts of probiotic lignocellulolytic bacteria, has remodelled the crude fibres of rice straw silage components as indicated by a decrease in crude fibre content from silage and the formation of simple compounds (metabolite compounds) in the form of VFA and NH_3-N accompanied by a decrease in pH of silage produced.

The high overhaul of crude fibre components and other nutrient components of rice straw silage produced using the biocatalyst of probiotic lignocellulolytic bacteria has resulted in increased production of organic acids (VFA) which have an impact on reducing the pH of the silage produced (Table 9). In that table, it appears that all rice straw silage has a low pH (4.545-4.713). This shows that the fermentation process can take place well. The availability of soluble carbohydrates, both from the component of silage (rice bran) and/or the component of the starter solution (molasses) also supports the ongoing process of good ensilage so that the resulting silage has a low pH (acid). The data in Table 9 also showed that the use of biocatalysts produced using probiotic lignocellulolytic bacteria can produce silage of rice straw with lower pH even the use of solid biocatalyst B_{12345} produces silage of rice straw with a significantly lower pH ($p<0.05$) compared to silage of rice straw produced using biocatalyst without bacterial isolate/B_0. This is a direct response from the high bacterial population (Table 3) and high lignocellulose enzyme activity (Table 4-7) of biocatalysts produced using 5 superior probiotic lignocellulolytic bacterial isolates (B_{12345}) resulting in the process of breakdown/degrading complex compounds into simpler components and organic acids (VFAs) being high so that the silage pH becomes low. The results of this study were in line with the statement of Robinson[41], who revealed the use of microbes that can produce enzymes with high enzyme activity will accelerate the process of breaking down complex compounds into their constituent components.

Chandra *et al.*[29] revealed that in an anaerobic environment (fermentation process), ligninolytic bacteria with ligninase enzymes *Lignin peroxidase* (Li-P), *Manganese peroxidase* (Mn-P), *Versatile peroxidase* (VP), *Laccase* (Lac) and *Dye-decolorizing peroxidase* (DyPs) and various other ligninase enzymes will overhaul lignin compounds to form hydroxyl/phenol compounds (aromatic alcohols), carboxyls (including VFA), amines (including NH_3), organic minerals (organometallic), CO_2, H_2O and CH_4, while cellulolytic and hemicellulolytic bacteria and supported by non-saccharolytic bacteria with individual enzyme activity and/or multi enzymes "cellulosome" will degrade cellulose and hemicellulose to form simple sugars (glucose, xylose, mannose, etc.) which will soon be fermented to form organic acids/VFA, H_2O, CO_2 and CH_4. This is what causes the concentration of VFAs produced by rice straw silage from biocatalyst to be high (Table 9).

For the production of NH_3-N from rice straw silage, the results showed that rice straw silage produced with solid biocatalysts of probiotic lignocellulolytic bacteria, both single and/or consortium (RSB_1, RSB_2, RSB_3, RSB_4, RSB_5, RSB_{1234}, RSB_{1235}, RSB_{1245} and RSB_{12345}) were able to significantly increase ($p<0.05$) 9.79-24.27% NH_3-N concentration yields were compared with rice straw silage produced with biocatalysts without bacterial isolates/RSB_0 (Table 9). The high nutrient degradation of rice straw silage components both crude fibre (especially lignin compound) and protein components and supported by high crude protein content of rice straw silage contributes positively to the increased production of NH_3-N from rice straw silage fermented by probiotic lignocellulolytic bacteria biocatalysts. Besides that, the high bacterial population of biocatalysts also contributes to the high

concentration of NH_3-N produced which is a direct contribution from the breakdown of bacterial body cell mass was rich in protein and other N components[20].

Utilization of probiotic lignocellulolytic bacteria biocatalysts as a starter in the fermentation process is be able to produce rice straw silage with *in vitro* Digestibility of Dry Matter (DDM) and organic matter digestibility/DOM higher ($p<0.05$) respectively 3.45-12.13 and 3.63-15.36% compared than rice straw silage produced using biocatalysts without bacterial isolates that have DDM 47.198% and DOM 48.570% (Table 9). In the table, it also appears that RSB_{12345} has the highest *in vitro* digestibility of dry matter and organic matter. The high *in vitro* digestibility of dry matter and organic matter produced by rice straw silage produced using biocatalysts with 5 of probiotic lignocellulolytic bacteria isolates (B_{12345}) is a direct response to the quality of the solid biocatalyst which has a high nutrient content, bacterial populations and lignocellulose enzyme activity and supported by low crude fibre content and high crude protein and extracts ether from rice straw silage RSB_{12345}.

CONCLUSION

Based on the results of the study it can be concluded that the use of probiotic lignocellulolytic bacteria can produce solid biocatalysts with high quality and effectivity as a starter for fermentation of agricultural waste and by-products. Utilization of a consortium of 5 probiotic lignocellulolytic bacteria isolates such as *Bacillus subtilis* strain BR_4LG, *Bacillus subtilis* strain BR_2CL, *Aneurinibacillus* sp., strain BT_4LS, *Bacillus* sp., strain BT_3CL and *Bacillus* sp., strain BT_8XY can produce high-quality and effectivity of solid biocatalysts as starter fermentation for agricultural waste and by-product.

SIGNIFICANCE STATEMENT

This study discovered the solid biocatalysts were formulated utilizing a consortium of 5 superior probiotic lignocellulolytic bacteria, namely, *Bacillus subtilis* strain BR_4LG, *Bacillus subtilis* strain BR_2CL, *Aneurinibacillus* sp., strain BT_4LS, *Bacillus* sp., strain BT_3CL and *Bacillus* sp., strain BT_8XY that can be beneficial for optimizing the development of simantri-pattern animal livestock that utilizes feed based on waste and by-products agricultural. This study will help researchers to uncover critical areas of a synergistic consortium formulation of lignocellulolytic probiotic bacteria that many researchers were not able to explore. Thus a new theory about biocatalyst formulations utilizing a consortium of 5 lignocellulolytic probiotic bacteria may be arrived at.

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