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Research Article
Utilization of Lignocellulolytic Probiotic Bacteria Consortium as Microbial Protein

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Abstract

Background and Objectives: While the use of AGPs is band, the development of a competitive and sustainable livestock business faces various challenges, mainly related to the low quality and quantity of local feed. This study aimed to create a synergistic formula of lignocellulolytic probiotic bacteria for the production of microbial protein functional feed that can be used as a substitute of antibiotics growth promoters for the development of competitive and sustainable livestock. **Materials and Method:** The microbial protein feed was formulated using a superior lignocellulolytic probiotic bacteria isolated from Bali cattle rumen fluid and termites, namely [1)] *Bacillus subtilis* strain BR_4LG, *Bacillus subtilis* strain BR_2CL, *Aneurinibacillus* sp. strain BT_4LS, *Bacillus* sp. strain BT_3CL and [5)] *Bacillus* sp. strain BT_8XY. "Microbial Direct Feed" formulas were FB_0, FB_1, FB_2, FB_3, FB_4 and FB_5. The nutrient contents, bacteria population, metabolic producet, dry matter and organic matter of *in vitro* digestibility and amino acids profile were used to evaluate the quality of microbial protein feed. Analysis of variance (ANOVA) was used to analyze the data followed by HSD test for comparison among treatments. **Results:** In comparison with the control feed (FB_0), lignocellulolytic probiotic bacteria produced higher quality (nutrients contents, bacteria population, metabolite substrates and *in vitro* digestibility) of microbial proteins feed (FB_{1234}, FB_{1235}, FB_{1245} and FB_{12345}). Bacterial consortium formula FB_{12345} produced high quality microbial protein feed with the highest nutrients content, bacterial population, metabolite substrates, *in vitro* digestibility and amino acids profile. **Conclusion:** The probiotic lignocellulolytic bacteria consortium with formula FB_{12345} can produce microbial protein feeds of the highest quality.

Key words: Amino acids profile, animal feed, bacteria consortium, functional feed, microbial protein

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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

A number of challenges remain in the development of competitive and sustainable livestock farmings, including lack of good quality local feed[1] and a ban on the use of antibiotics as growth promotoers[2]. Efforts to import quality feed also face obstacles due to the high price of quality feed, especially functional feed such as amino acid sources[3]. In order to develop a microbial proteins functional feed, technology can be applied through the use of microorganisms, particularly superior lignocellulolytic probiotic bacteria from the rumen fluid of Bali cattle and termites, which have multiple functions as amino acids and probiotics[4-6].

Mudita[4] has succeeded in isolating and selecting superior lignocellulolytic bacteria from the rumen of Bali cattle and termites that have a high ability to degrade lignocellulose compounds and are highly active in the production of lignocellulose enzyme, including: [1)]*Bacillus subtilis* strain BR_4LG, [2)]*Bacillus subtilis* subtilis BR_2CL strain, [3)]*Aneurinibacillus* sp. BT_4LS strain, [4)]*Bacillus* sp. BT_3CL strain and [5)]*Bacillus* sp. Based on preliminary studies and various references, BT_8XY strains play a role as probiotics and are commonly used as direct fed microbial/DFM (microbial protein feed)[7-10]. Kubad *et al*.[7] revealed that, in developing countries, microbial protein is a very potential source of protein, both for humans and livestock. Sok *et al*.[8] exemplified that rumen bacteria body cells are composed of 50-65% protein, 1-3% fat, 3-7% annorganic/mineral and 8-12% nucleic acid and with a complete and balanced amino acid profile. Wester *et al*.[9] and Thasana *et al*.[10] revealed that the bacteria *Bacillus subtilis* and *Bacillus* sp. is a beneficial bacteria/Generally Recognized as Safe (GRAS) which play a role in the production of food or medicine.

A microbial protein performs two functions, namely provide protein/amino acids and also act as a probiotic to reduce dependence on antibiotic products in livestock business[4-6]. However, the effectiveness of a bacterial consortium is largely determined by its activity and synergy, so an effective and synergistic formulation will produce a high quality microbial proteins functional feed[5,6].

Microbes such as bacteria, can be used both individually and in consortium for the production of microbial protein feeds[6]. This study was designed to develop a synergistic formula of lignocellulolytic probiotic bacteria for the production of microbial protein functional feed that can be used as a substitute of antibiotics growth promoters for the development of competitive and sustainable livestock.

MATERIALS AND METHODS

Location and time of study: This study was carried out at General Laboratory of Sesetan and Laboratory of Feed and Nutrition, Faculty of Animal Husbandry, Udayana University from April to October 2019.

Probiotic lignocellulolytic bacteria: The microbial protein feed was formulated using a superior lignocellulolytic probiotic bacteria isolated from Bali cattle rumen fluid and termites, 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 study used a completely randomized designed with five treatments and six replicates. The treatment were as follows:

FB_0 = Microbial protein feed was formulated without lignocellulolytic probiotic bacteria

FB_{1234} = Microbial protein feed was formulated with lignocellulolytic bacteria [1)]*Bacillus subtilis* strain BR_4LG, [2)]*Bacillus subtilis* strain BR_2CL, [3)]*Aneurinibacillus* sp. strain BT_4LS and [4)]*Bacillus* sp. strain BT_3CL

FB_{1235} = Microbial protein feed was formulated with lignocellulolytic bacteria [1)]*Bacillus subtilis* strain BR_4LG, [2)]*Bacillus subtilis* strain BR_2CL, [3)]*Aneurinibacillus* sp. strain BT_4LS and [5)]*Bacillus*. sp strain BT_8XY

FB_{1245} = Microbial protein feed was formulated with lignocellulolytic bacteria [1)]*Bacillus subtilis* strain BR_4LG, [2)]*Bacillus subtilis* strain BR_2CL, [4)]*Bacillus* sp. strain BT_3CL and [5)]*Bacillus* sp. strain BT_8XY

FB_{12345} = Microbial protein feed was formulated with lignocellulolytic bacteria [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

Microbial protein feed: There were 5 microbial protein feed formulas in this study, 4 microbial protein functional feeds were formulated using a superior lignocellulolytic probiotic bacteria isolated from Bali cattle rumen fluid and termites (FB_{1234}, FB_{1235}, FB_{1245} and FB_{12345}) and 1 control (functional feed without lignocellulolytic probiotic bacteria isolates/FB_0) which were prepared by inoculating 50 mL of probiotic lignocellulolytic bacterial isolates (according to following treatment) into every 1 kg of basal feed (natural bacterial

growth medium) and added molasses to the dry matter of functional feed products with a content of 50-60% (35-40% moisture content) (Table 1-2). Furthermore, the functional feed mixture was fermented under anaerobic conditions for 1 week. After that, using an oven, functional feed was dried at a temperature of at 40-55°C until its functional dry matter content reached at ±85% (generally takes 4-5 days). Functional feed that had been dried was ready to be used for further research.

Evaluation of nutrient content of microbial protein feed: Nutrient contents of microbial protein feed included dry matter (DM), organic matter (OM), crude fiber (CF), crude protein (CP) and ether extract (EE) were evaluated in this study. Proximate analysis was performed using AOAC methods[11]. Crude protein was analyzed by the Kjeldahl semi-micro method using Vapodest-Gerhardt equipment[12].

Evaluation of bacterial population at microbial protein feed: Using the standard plate count/viable plate count methods, total bacteria, lignocellulolytic bacteria (lignolytic, cellulolytic and xylanolytic) and lactic acid bacteria were counted in the microbial protein feed[13]. An appropriate growth medium is inoculated with serial dilutions of the sample containing viable bacteria. We used "Nutrient Agar" NA without substrate for counting total bacterial populations[14,15], NA with lignocellulosic substrates (tannic acid, CMC and xylan) for counting lignocellulolytic bacteria (lignolytic, cellulolytic and xylanolytic respectively)[15-18] and MRS media for calculating lactic acid bacteria populations[19].

Evaluation of *In vitro* digestibility and metabolite products: The evaluation of dry matter digestibility and organic matter digestibility from functional feed was conducted using *in vitro* techniques using the Minson and McLeod method[20] whereby the samples (microbial protein feed) were incubated in a shaking bath for 2×48 hrs as a reflection of the fermentative digestibility phase (rumen simulation) and hydrolytic digestibility phase. Using *in vitro* feed analysis, the metabolite products (totally VFA and NH_3-N) were evaluated from the first step (phase) "fermentative digestion/rumen simulation". Total VFA was analyzed using steam distillation method following the General Laboratory Procedure[21]. The NH_3-N concentration was analyzed using the phenol hypochlorite method[22].

Evaluation of amino acid profiles from functional feeds: According to the amino acid profile of the functional feeds analyzed in this study, three microbial protein feeds were of the highest quality based on protein content, crude fiber, ether extract and in vitro digestibility of organic material and dry matter, as well as metabolite products (VFA and NH3-N). An amino acid analysis was conducted using the procedure described by Marino *et al.*[23] consisting of two phases: Liquid hydrolysis and derivatization, followed by chromatographic analysis using HPLC (High Performance Liquid Chromatography).

Data analysis: The data were analyzed using one-way analysis of variance (ANOVA), if there are significantly different values ($p<0.05$), followed by HSD test for comparison among treatments[24].

RESULTS AND DISCUSSION

By using lignocellulolytic probiotic bacteria isolated from Bali cattle rumen fluid and termites, high-quality microbial protein functional feed was produced, crude protein and ether extract was significantly higher in this functional feed, crude fiber content was significantly lower ($p<0,05$) than that of the microbial protein feed produced without using

Table 1: Composition of basal feed "natural bacteria growth media"

Materials	Composition (%)
Corn bran	30.0
Wheat bran "pollard"	45.0
Soya meal	15.0
Sugarcane/molasses	8.8
Salt/NaCl	1.0
Mineral-vitamin "Pignox"	0.2
Totally	100.0

Table 2: Formulation of microbial protein feed

Microbial	Lignocellulolytic bacteria culture (mL)						Inoculant solution	
Protein feed	B_1	B_2	B_3	B_4	B_5	Basal feed media (g)	Water (mL)	Sugarcane (mL)
FB_0	-	-	-	-	-	1000	500	100
FB_{1234}	12.5	12.5	12.5	12.5	-	1000	500	100
FB_{1235}	12.5	12.5	12.5	-	12.5	1000	500	100
FB_{1245}	12.5	12.5	-	12.5	12.5	1000	500	100
FB_{12345}	10	10	10	10	10	1000	500	100

B_1: *Bacillus subtilis* strain BR_4LG, B_2: *Bacillus subtilis* strain BR_2CL, B_3: *Aneurinibacillus* sp. strain BT_4LS, B_4: *Bacillus* sp. strain BT_3CL, and B_5: *Bacillus* sp. strain BT_8XY

Table 3: The nutrient contents of microbial protein functional feed produce using lignocellulolytic probiotic bacteria

	Treatments*					
Variables	FB_0	FB_{1234}	FB_{1235}	FB_{1245}	FB_{12345}	SEM***
Dry matter (fresh basis%)	68.862[a]	68.466[a]	68.967[a]	69.065[a]	69.451[a]	0.403
Organic matter (DM%)	94.230[a]	94.277[a]	94.120[a]	94.223[a]	95.001[a]	0.470
Anorganic matter (DM%)	5.770[a]	5.723[a]	5.880[a]	5.777[a]	4.999[a]	0.470
Crude protein (DM%)	14.221[a]	18.932[b]	19.192[b]	19.237[b]	19.246[b]	0.284
Ether extract (DM%)	6.148[a]	8.350[b]	9.092[b]	8.715[b]	9.099[b]	0.297
Crude fiber (DM%)	5.087[b]	3.002[a]	2.973[a]	3.000[a]	2.886[a]	0.142
Nitrogen free extract (DM%)	64.583[a]	60.085[b]	59.091[b]	59.924[b]	60.682[b]	0.619

*Lignocellulolytic probiotic bacteria isolates used in microbial protein feed formulations, namely B_1 "*Bacillus subtilis* strain BR_4LG" B_2 "*Bacillus subtilis* strain BR_2CL", B_3" *Aneurinibacillus* sp. strain BT_4LS ", B_4" *Bacillus* sp. strain BT_3CL " and B_5 "*Bacillus* sp. strain BT_8XY", **The same letter on the same line is not significantly different ($p>0.05$) and ***SEM: Standard error of the treatments and means

Table 4: Acidity and bacteria population of microbial protein feed

	Microbial protein feed					
Variables	FB_0	FB_{1234}	FB_{1235}	FB_{1245}	FB_{12345}	SEM***
Acidity (pH)	5.164[a]	5.106[a]	4.773[a]	5.052[a]	4.798[a]	0.150
Totally bacteria ($\times 10^7$ CFU g^{-1})	3.333[a]	22.133[b]	25.467[b]	24.667[b]	26.400[b]	1.490
B. Lignolytic ($\times 10^7$ CFU g^{-1})	2.333[a]	18.533[b]	19.267[b]	18.600[b]	20.067[b]	0.813
B. Cellulolytic ($\times 10^7$ CFU g^{-1})	2.600[a]	20.200[b]	22.333[b]	21.200[b]	22.467[b]	0.932
B. Xylanolytic ($\times 10^7$ CFU g^{-1})	3.133[a]	20.733[b]	22.867[b]	22.333[b]	23.267[b]	1.197
B. Amylolytic ($\times 10^7$ CFU g^{-1})	3.133[a]	21.600[b]	23.600[b]	22.800[b]	24.467[b]	1.237
Lactic acid bacteri ($\times 10^7$ CFU g^{-1})	3.000[a]	21.733[b]	25.200[b]	23.800[b]	25.933[b]	1.137

*Probiotic lignocellulolytic bacteria isolates used in microbial protein feed formulations, namely B_1 "*Bacillus subtilis* strain BR_4LG" B_2 "*Bacillus subtilis* strain BR_2CL", B_3" *Aneurinibacillus* sp. strain BT_4LS ", B_4" *Bacillus* sp. strain BT_3CL" and B_5" *Bacillus* sp. strain BT_8XY", **The same letter on the same line is not significantly different ($p>0.05$), ***SEM: Standard error of the treatments and means

lignocellulolytic probiotic bacteria (FB_0), however, no significant difference ($p>0,05$) was found in dry matter, organic matter and anorganic matter. A microbial protein feed produced using the formula FB_{12345} had a high nutrient content, especially crude protein and ether extract (19.246% and 9.099%) and the lowest crude fiber content (2.886%) (Table 3).

Based on the high and synergistic work activity of the functional feed formula, the higher crude protein and ether extract content indicated a high growth rate of the bacterial consortium (Table 3). In the functional feed formula, there was low competition between the lignocellulolytic probiotic bacteria consortium, which was reflected directly in the high bacterial population (Table 4). As a result, it significantly increased the supply of nutrient-rich microbial (bacterial body cells) and especially increased the protein and fat content of the functional feed formula of microbial protein (P_{1234}, P_{1245}, P_{1235} and P_{12345}). Mulder *et al.*[25] showed that microbial body cells (microbial biomass) contain important nutrients, (protein, fat/EE, minerals, vitamins) so their use as feed can provide livestock with higher levels of nutrients. Matassa *et al.*[26] reported that microorganisms including bacteria are a very potential source of various nutrients, especially protein and amino acids, for both humans and livestock. Sok *et al.*[8] found that rumen bacteria and protozoa have a high quality and balance composition of amino acids. While the content of dry matter, organic matter and inorganic matter was not significantly different ($p>0.05$) (Table 3). A consortium of lignocellulolytic probiotic bacteria was used to ferment functional feed formulas. As a result, there was no loss of nutrients during fermentation and the dry matter and organic matter content of the functional feed tends to increase quantitatively.

Formulas FB_{1234}, FB_{1235}, FB_{1245} and FB_{12345} exhibited high synergy of probiotic lignocellulolytic bacteria, which was also reflected in the reduction of crude fiber content of their microbial protein feeds. The high population of lignocellulolytic bacteria (lignolytic, cellulolytic and xylanolytic) has a high lignocellulose enzyme activity (ligninase, endoglucanase, exoglucanase or xylanase)[9,25,26], the lignocellulosic fiber was converted into its constituent components (simple compounds) in order to reduce the crude fiber content of the microbial protein feed. One interesting finding from this study is that the high level of crude fiber overhaul does not occur simultaneously with the increase in extract without Nitrogen-free extract (NFE). This is probably due to the fact that The compounds that comprise crude fiber, namely lignocellulose, are not all carbohydrate compounds, so the product produced is not only NFE. One of the constituent components of the cell wall/crude fiber is lignin, which

Table 5: Metabolic product and dry matter and organic matter *In vitro* digestibility of microbial protein feed

	Treatments*					
Variables	FB_0	FB_{1234}	FB_{1235}	FB_{1245}	FB_{12345}	SEM***
VFA (mM)	155.14[a]	187.29[b]	189.26[b]	185.17[b]	189.24[b]	1.855
NH_3-N (mM)	12.965[a]	15.094[b]	15.172[bc]	15.736[bc]	16.795[c]	0.327
DM digestibility (%)	67.768[a]	70.971[b]	71.286[b]	70.811[b]	71.531[b]	0.223
OM digestibility (%)	68.358[a]	71.639[b]	71.867[b]	71.590[b]	72.174[b]	0.260

*Probiotic lignocellulolytic bacteria isolates used in microbial protein feed formulations, namely B_1 "*Bacillus subtilis* strain BR_4LG" B_2 "*Bacillus subtilis* strain BR_2CL, B_3 "*Aneurinibacillus* sp. strain BT_4LS ", B_4 "*Bacillus* sp. strain BT_3CL" and B_5 "*Bacillus* sp. strain BT_8XY", **The same letter on the same line is not significantly different ($p>0.05$), ***SEM: Standard error of the treatments and means

contains aromatic compounds, so degradation can result in compounds containing nitrogen (NPN/crude protein) and minerals[27-29]. This also indicates that the formula FB_{1234}, FB_{1235}, FB_{1245} and FB_{12345} have a high ability to degrade lignin compounds into simpler components. Probably the low NFE content was due to NFE being formed from the overhaul of crude fiber (cellulose and hemicellulose) that has been used by microbes (bacteria) for their growth (formation of the carbon skeleton of microbial body cells)[30] resulting in a low NFE content of the feed.

The high quality and effectiveness of lignocellulolytic probiotic bacterial isolates used in the production of microbial protein feed (FB_{1234}, FB_{1235}, FB_{1245} and FB_{12345}) are also evident in the growth and work synergy of the bacterial consortium, resulting in significant increases in the total bacterial population, lignocellulolytic bacterial population (lignolytic, cellulolytic and xylanolytic), amylolytic bacteria population and lactic acid bacteria population ($p<0,05$) (Table 4). Since the bacteria used are probiotic bacteria (lactic acid bacteria), the degradation of organic matter, especially components containing carbon skeletons (carbohydrate/CHO, fat/ether extract/EE, crude protein/CP) will result in glucogenic compounds (propionate and lactate acid) to maintain a low acidity of the product which was reflected by the non-significant differences among the pH values ($p>0,05$) (Table 4).

The high quality and effectiveness of the consortium of lignocellulolytic probiotic bacteria isolates (Table 4) and synergistic of the enzymes activity (ligninase, endoglucanase, exoglucanase, xylanase, amylase and lactate reductase)[9,26-29] increased the overhaul of complex compounds especially lignocellulosic compounds from basal ingredients of microbial protein feed which was shown by a decrease in crude fiber content (Table 3) that become simple compounds in order to increase the metabolite products (VFA and NH_3-N) as well as the in-vitro digestibility of dry matter and organic matter from microbial protein feed (Table 5).

Chandra *et al.*[29] reported that in anaerobic environment lignolytic bacteria with their ligninase enzymes (lignin peroxide/Li-P, manganese peroxidase/Mn-P, versatile peroxidase/VP, lacase/lac and dye-decolorizing peroxidase/DyPs and various other ligninase enzymes) remodeled lignin compounds to form hydroxyl/phenol compounds (aromatic alcohol), carboxyl (including VFA), amines (including NH_3), organic minerals (organometallic), CO_2, H_2O and CH_4, while cellulolytic and hemicellulolytic (xylanolytic) bacteria and supported by non-saccharolytic bacteria (amylolytic and lactic acid bacteria) with individual and/or multi-enzyme activity of cellulosomes degraded cellulose and hemicellulose to form simple sugars (glucose, xylose, mannose, etc.) which was fermented immediately to form organic acids (VFA) , H_2, CO_2 and CH_4.

The high bacterial population and various enzyme activities[9,25,26] have significantly increased the production of metabolites and digestibility of dry matter and organic matter from the functional feed (Table 5). Hence, the formulation of the lignocellulolytic probiotic bacteria consortium derived from the rumen fluid of Bali cows and termites works synergistically and with a low level of competition. The high synergy of the functional feed formula showed that the bacterial isolates used have high quality as a fermentation starter/biocatalyst[26].

The results of the evaluation of the quality of the microbial protein feed products produced, mainly based on the parameters of feed nutrient content, bacterial population, metabolite products and product in-vitro digestibility, indicate that the formula FB_{12345}, FB_{1235} and FB_{1234} are the three best formulas for producing high quality protein microbial feed products. The results of the amino acid profile analysis also showed that the three functional feed formulas had a high amino acid content (Table 6).

Table 6 shows that the microbial protein feed with formula FB_{12345} has a higher concentration of amino acids (except for Leucine which is slightly lower than FB_{1235}) than other microbial protein feed formulas. This further confirms that FB_{12345} is the best formula for producing microbial protein feed due to high quality and synergy of a consortium of five superior lignocellulolytic probiotic bacteria derived from Bali cattle rumen fluid and termites that produce high-quality

Table 6: Profile of amino acids the best quality of feed microbial protein

	Microbial protein feed		
Amino acids	FB_{12345} (%)	FB_{1235} (%)	FB_{1234} (%)
Aspartic acids	1.69	1.43	1.37
Glutamic acids	2.96	2.23	2.19
Serin	0.94	0.72	0.71
Glisine	1.11	0.91	0.88
Histidin	1.13	1.03	0.94
Arginin	0.93	0.88	0.82
Threonine	0.79	0.71	0.62
Alanine	0.69	0.53	0.59
Proline	0.92	0.84	0.82
Tyrosine	1.05	0.98	0.91
Valine	0.73	0.62	0.60
Methionine	0.88	0.86	0.76
Cysteine	0.76	0.68	0.62
Isoleucine	1.07	0.92	0.88
Leucine	1.21	1.22	1.09
Phenylalanine	0.80	0.71	0.73
Lisine	1.26	1.17	1.11

microbial protein feeds with the highest nutrient contents, bacterial population and metabolite and *in vitro* digestibility (Table 3-5). Nasseri *et al.*[31] also reported that *Bacillus subtilis*, *Bacillus* sp. and *Bacillus megaterium* are sources of single-cell proteins and with a high, complete and balanced amino acid content. This also indicates that the use of a consortium of superior lignocellulolytic probiotic bacteria derived from the rumen of Bali cattle and termites can be developed as a source of functional feed and amino acids.

CONCLUSION

By using lignocellulolytic probiotic bacteria, high quality microbial protein feeds could be produced. FB_{12345} is the best formula for producing microbial protein feed due to high quality and synergy of a consortium of five superior lignocellulolytic probiotic 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.

SIGNIFICANCE STATEMENT

This study discovered the high quality microbial protein feed formula using a consortium of 5 superior lignocellulolytic probiotic bacteria, 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 that can be useful in developing competitive and sustainable livestock farming due to the restrictions on antibiotic growth promoters and the limited availability of feed. 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 microbial protein feed formulations utilizing five lignocellulolytic probiotic bacteria isolates may be arrived at.

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