SINBIONESA: Synbiotic Powder Made of Indigenous Microorganisms from Goat Digestive Tract with Rice Bran and Corn Flour Fillers

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ABSTRACT
This study aims to evaluate the quality of SINBIONESA produced to meet the probiotic quality of the SNI benchmark. Identifying potential probiotic bacteria isolated from a goat's digestive tract by using molecular analysis of the 16S rRNA gene was conducted. Selected isolates were observed and tested to identify potential bacteria as probiotics, including cell shape, gram methods, positive character test, negative character test, cellulolytic activity test, optimal pH, optimum temperature, acid resistance, and resistance to bile salts. The selected bacteria were formulated with a filler of 75% rice bran and 25% corn starch, then dried by freeze-drying. SINBIONESA quality was evaluated on the moisture content (by drying technique), the total number of probiotics (by total plate count), and the expiration period (linear regression formula). The results show that SINBIONESA has three potential probiotic bacteria: *Bacillus pumilus*, *Bacillus brevis*, and *Pseudomonas diminuta*. It has a water content of 2.89% with an expiration period at room temperature storage and cold temperatures of 1 year, four months, and two years five months, respectively. Based on these results, it is stated that SINBIONESA has met the SNI quality of probiotics in Indonesia.

Keywords: *Prebiotics, probiotics, Symbiotic, Indigenous microorganism.*

1. INTRODUCTION

Efforts to improve the efficiency of the livestock business continue to be carried out. In previous years, research has been carried out on the production of fermented feed made from raw hyacinth with a high nutritional content called fermege. This feed has been tested in goats with convincing results such as [1]. It has been shown to increase goat weight, improve carcass quality, increases the number of female goat cubs, and enhance the quality of male goat sperm [2-5]. Using fermented feed also decreases the manure odor intensity, which can help farmers save costs and effort for maintenance. The advantage of fermented feed must be caused by microorganisms in feed raw materials and work during fermentation. These microorganisms degrade the complex compounds contained in the feed into simple compounds that are more easily absorbed by the goat's digestive system. This combination will function as probiotics that positively impact their health.

The benefits of probiotics for humans, mainly the Lactic Acid Bacteria (BAL) group, have been proven to improve the health of the digestive system [6]. This benefit can also be applied to livestock, especially those classified as mammals, including ruminants. Empirical facts and theoretical studies become a solid background for conducting research on the development of prebiotics, probiotics, and symbiotic for livestock based on indigenous microorganisms isolated from the manure of goats fed with fermented feed. In this study, a special symbiotic was developed for ruminants called SINBIONESA.

In the production of symbiotic, fillers are commonly used to fulfil the nutrients of the symbiotic bacteria. The filler used is generally an organic substance that meets several requirements, such as being resistant to the acidity of the gastrointestinal tract, can be fermented by the microflora of the gastrointestinal tract, and supporting the growth of these microflorae [7].

This study aims to evaluate the quality of SINBIONESA, which has been developed from several aspects following the SNI (Indonesian National Standard) for applicable probiotics. In addition, microorganisms must meet several requirements to function as probiotics. It includes being resistant to acidic and alkaline atmospheres in the digestive tract, can be attached to the mucosa or epithelial layer of the food digestive tract, having activity against pathogenic microorganisms in the digestive tract, and hydrolysis activity on bile salts [8]. Clinical trials are conducted to ensure the safety of probiotics, and the probiotic bacteria remain alive within the product's shelf life [9].

2. MATERIALS AND METHODS

This research is an exploratory study (to find potential bacteria as probiotics from the goat's digestive tract/feces) followed by experimental research (to determine the best filler composition). Bacteria from the digestive tract of the testing goat were isolated by the method of spread plate, then incubation and purification of isolates were carried out. Pure isolates were identified based on molecular analysis of the 16S rRNA gene to determine their species. Furthermore, observations were conducted each isolate to determine its potential as a probiotic. Isolates were observed and tested in several aspects, including their form (microscopic observations), bacterial gram identification, positive/negative character test, cellulolytic activity test (clear zone observation on CMC media coloured with Congo Red), optimal pH (measurement with pH meter), optimum temperature (duplation with thermometer), acid resistance (in acidic media conditions) and bile salts resistance (on bile-stained media). The three selected isolates are then propagated to 1012 cells/mL and mixed with various filler formulas (three formulas), a combination of rice bran and cornmeal. SINBIONESA powder is produced by the method of freeze-drying. The product is then evaluated in the aspects of water content, total probiotic bacteria content, and product expiration period at room temperature storage and cold temperature (ice cupboard), whose determination is carried out by the TPC (total plate count) method followed by a linear regression formula.

3. RESULTS AND DISCUSSION

The results of observations and tests of selected bacteria that have the potential to be probiotics are presented in **Table 1**.

Table 1. Diversity of goat digestibility indigenous bacteria that have the potential to be probiotics

Species	Form	Gram Identification	Positive Character Test	Negative Character Test	Cellulolytic Activity Test	Optimum pH	Optimum Temp.	Acid Resistance	Bile Salts Resistance
Bacillus pumilus (B1)	Rod-shape	Positive	Oxidase Motility Nitrate CMC Catalase ONPG Inositol Sorbitol	Amylum Lysine Ornithine H2S Glucose Mannitol Xylose Indole Urease VP CitrateTDA Gelatine Malonate Rhamnose Sucrose Lactose Arabinose Adonitol Raffinose Salicin Arginine	Clear zone in CMC media 11,5 mm	6	38	Withstand pH of 5 to 6 hours with a decrease in the number of isolates of not more than 3 log units /mL	0.3% Bile salt resistance (w/v) with a decrease in the number of isolates not more than 3 log/mL units after 4-hour incubation

Species	Form	Gram Identification	Positive Character Test	Negative Character Test	Cellulolytic Activity Test	Optimum pH	Optimum Temp.	Acid Resistance	Bile Salts Resistance
Bacillus brevis (B4)	Rod-shape	Positive	Oxidase ONPG VP	Motility Nitrate CMC Catalase Inositol Sorbitol Amylum Lysine Ornithine H2S Glucose Mannitol Xylose Indole Urease Citrate TDA Gelatine Malonate	Clear zone in CMC media 10,67 mm	6	40	Withstand pH of 5 to 8 hours with a decrease in the number of isolates of not more than 3 log units /mL	0.3% Bile salt resistance (w/v) with a decrease in the number of isolates not more than 3 log/mL units after 6-hour incubation
Pseudomonas diminuta (B5)	Rod-shape	Positive	Oxidase MotilityNitrate CMC VP	Catalase Inositol Sorbitol Amylum Lysine Ornithine H2S Glucose Mannitol Xylose Indole Urease Citrate TDA Gelatine Malonate Rhamnose Sucrose Lactose Arabinose Adonitol Raffinose Salicin Arginine	Clear zone in CMC media 10,08 mm	5,8	39	Withstand pH of 5 to 7 hours with a decrease in the number of isolates of not more than 3 log units /mL	0.3% Bile salt resistance (w/v) with a decrease in the number of isolates not more than 3 log/mL units after 5-hour incubation

Three bacterial isolates, namely *Bacillus pumilus*, *Bacillus brevis* and *Pseudomonas diminuta*, were then formulated with three filler compositions and evaluated based on three SNI-based symbiotic parameters, water content, total

number of probiotic bacteria and expiration period. The results are displayed in the **Table 2**.

Table 2 Water content of probiotic powder at various filler concentrations

Replicates	Formula		
	I (75% rice bran, 25% corn starch)	II (50% rice bran, 50% corn starch)	III (25% rice bran, 75%corn starch)
1	2.05	4.01	4.92
2	3.76	4.23	3.98
3	2.46	3.76	4.92
4	2.68	3.27	4.79
5	3.13	4.03	3.99
6	2.97	4.10	4.76
7	3.01	3.68	4.18
8	2.95	4.03	4.26
9	3.00	3.99	4.91
Average	2.89	3.90	4.52
Error standard	0.16	0.10	0.12
Standard deviation	0.47	0.29	0.41

It can be stated that formula I which contains 75% rice bran and 25% corn starch is the best formula because it has the smallest moisture content, thus making probiotic powders last longer. From these results, formula I is become the basis of SINBIONESA that will be produced and evaluated for quality such as total number of probiotic bacteria and expiration period (**Table 3**).

Table 3 Total Place Count (TPC) SINBIONESA for determination of expiry date

Days	Number of colony CFU unit (x 10^{12})/gr			Average
	1st round	2nd round	3rd round	
1	158	149	143	150
2	148	155	143	148.67
5	157	158	138	151
12	143	162	159	154.67
19	160	142	168	156.67
29	160	140	145	148.33
39	152	135	140	142.33
51	148	130	146	141.33
65	145	122	130	132.33
130	85	113	109	102.33
195	85	108	99	97.33
260	74	99	94	89

325	44	65	62	57
390	14	33	31	26

After 390 days (about 13 months), TPC was conducted and SINBIONESA still meets the requirements as an active probiotic (with SNI not less than 10^8 CFU/gr). It is shown that SINBIONESA, which was stored for 13 months at room temperature and cold temperatures, experienced a decrease in the total number of probiotics by 54.71% and 14.61%, respectively (**Table 4**). Storage at cold temperatures can extend the shelf life of SINBIONESA. Furthermore, SINBIONESA was observed to expire after a storage period of 500.5062 days (about 16.68 months or 1 year 4 months) at room temperature and expire after a storage period of 910.3466 days (approximately (about 30.35 months or 2 years 5 months) at cold temperatures (ice cupboard temperature) (**Table 5**). Based on these data, SINBIONESA produced has met the SNI standard (minimum storage period of 3 months) from the aspect of the expiry period.

Table 4 Percentage decrease in the number of probiotics in SINBIONESA stored at room temperature and cold temperatures based on the linear regression formula.

Days	Room temperature storage		Low temperature storage	
	Total amount of probiotics	Decreasing amount of probiotics (%)	Total amount of probiotics	Decreasing amount of probiotics (%)
1	152.8	23.60	185.3	7.40
2	151.5	0.85	187.5	-1.19
5	149.8	1.96	184	0.70
12	152.3	0.33	184.3	0.54
19	151.3	0.98	184.5	0.43
29	149.3	2.29	181.8	1.89
39	144.3	5.56	180.3	2.70
51	139.8	8.51	175.8	5.13
65	133.8	12.43	168.8	8.90
130	101.5	33.57	159	14.19
195	96.5	36.85	148.3	19.97
260	88.5	42.08	135	27.15
325	56.3	36.38	121.8	9.78
390	25,5	54.71	104	14.61

Bacillus pumilus has been shown to be effective as a probiotic in poultry which shown to significantly increase the performance of vegetative growth in poultry [10]. Moreover, research related to the use of *Bacillus brevis* as a probiotic has also been widely carried out. It has been shown to be able to fight a number of pathogenic *Vibriobacteria* in fish, this bacterium can also synthesize proteins that are epidermal tissue growth factors [11-12]. The use of *Pseudomonas diminuta* as a probiotic has been carried out by Farizki et al (2020) mainly to degrade total organic matter, ammonia and total vibrio bacteria content often found in pond shrimp [13].

In SINBIONESA, rice bran or fine bran was chosen as a filler because it has high content of Non-Nitrogen Extract Materials (BETN) which crucial as an energy source. Furthermore, bran contains starch granules that can absorb up to 30% of water which will be tightly arranged and slightly permeable to water after drying. Based on this, it is hoped that the addition of fine bran fillers can reduce the hygroscopic properties of SINBIONESA. Moreover, it has been proven that snail flour filled with bran has produced flour of good quality [14]. And as a filler, bran is sufficient ingredient because it can fill the cavities of powder, as well as an additional nutrient.

Table 5 SINBIONESA expiration period at room temperature and cold temperature storage based on the linear regression formula.

Product	Room temperature storage (days)	Low temperature storage (days)
SINBIONESA	500.5062	910.3466

In addition to bran, another filler that has been chosen is corn starch. It was chosen because of its nutritional content that can meet the needs of microorganisms contained in SINBIONESA. The best fish meal has been produced using cornmeal

fillers [15]. Cornmeal is commonly used as a protector and filler in the pharmaceutical industry for tablets such as aspirin and other drugs. In SINBIONESA, cornmeal can also be a protector during the drying process. The selection of bran and corn flour fillers because it is affordable and easy to obtain which make SINBIONESA powder can be produced easily and adopted/adapted by thefarming community.

After mixing microorganisms and fillers to form SINBIONESA powder, the mixture is then dried by freeze-drying. This method was chosen because it can maintain the number of viable microorganisms contained in the material being dried. This process is based on the sublimation process, the evaporation of water directly from its solid form carried out at low temperatures. Thus, the viability of microorganisms in the material is well maintained. SINBIONESA powder has several advantages compared to liquid SINBIONESA. It is more durable, easy handling and the risk of contamination when storage is low, unlike liquid SINBIONESA which need to be subcultures frequently. Liquid SINBIONESA is also easily contaminated which will affect its quality [15]. The quality of SINBIONESA powder is based on three most important parameters: water content, the total number of probiotics in the product and the length of shelf life or expiration. According to Rukmi, et al., (2005) the viability of microorganisms in SINBIONESA during storage is the most important aspect [16]. In this study SINBIONESA with the smallest water content, the total number of probiotics meeting SNI and its shelf life was the longest is the best. The shelf life of SINBIONESA determines the total number of microorganisms contained in it.

The results were obtained that the product with the smallest moisture content was SINBIONESA with the most amount of bran filler, namely formula I which contains 75% bran and 25% corn flour by 2.89%. SINBIONESA powder with the filling agent also has the best shelf life, namely during the storage period, it can still maintain a high number of microorganisms. Bran is a nutrient-rich material such as protein, cellulose, lipids that are beneficial as suppliers of energy, carbon and cell-building compounds. Therefore, its presence in SINBIONESA powder in large quantities is very beneficial to the population of microorganisms contained in it. Bran also contains antioxidant compounds such as beta carotene and lycopene that protect cells from adverse oxidation reactions [17]. The addition of rice husks and bran to the manufacture of probiotic powder for food additives in livestock gives good results and can maintain the number of bacteria for a longer time [18].

Cornmeal is an endosperm or food reserve for prospective embryos inside the seeds that will grow into corn individuals. Yellow corn starch contains energy of 355 Kcal, protein 9.2 g, carbohydrates 73.7 g, fat 3.9 g, calcium 10 mg, phosphorus 256 mg, and iron 2 mg. In addition, yellow corn flour also contains vitamin A as much as 510 IU, vitamin B1 0.38 mg and vitamin C 0 mg [19]. These compounds support the life of microorganisms contained in the SINBIONESA powder. Water absorption capacity is 40.53% – 65.33% in fermented corn flour, amylose content is 33.10%, and has a water absorption capacity of 117.80% [20]. Based on the properties of cornmeal, to get SINBIONESA powder with ideal water content by using corn flour as a filler, it requires calculating the right level.

The shelf life of SINBIONESA powder is based on the total number of microorganisms contained in it which are known to perform TPC (Total Plate Count) in a certain storage period at two types of temperatures, namely room temperature and cold temperature/ice cupboard in cold temperature storage the total probiotic amount in SINBIONESA is higher when compared to the total number of probiotics in SINBIONESA stored at room temperature. This is due to the fact that at lower temperatures the metabolic speed of the organism also decreases. This means that there is a decrease or saving in the use of nutrients contained in SINBIONESA media, thus SINBIONESA media can support the life of microorganisms contained in it longer.

The quality of SINBIONESA powder, especially maintaining its expiration period can be done in various ways which include providing fillers that can maintain the life of microorganisms in basal metabolism, storing SINBIONESA at cold temperatures so that the metabolic process of microorganisms in it is slow, keeping SINBIONESA dry, or low water content, handling

SINBIONESA well when used so that it is not contaminated with other microorganisms or other materials which can disrupt its stability [21]. SINBIONESA powder produced in this study, if it has gone through an expiration period, can actually still be utilized with a larger dose of administration to the fodder and beverages. SINBIONESA that has passed the expiry period can also be used to speed up the fermentation process of feed ingredients. The fermentation process will take place at a slower speed if SINBIONESA powder is used which has passed its expiration period, but the process is still faster when compared to the speed of the fermentation process without the addition of SINBIONESA.

4. CONCLUSION

SINBIONESA product contains three types of probiotic bacteria, *Bacillus pumilus*, *Bacillus brevis* and *Pseudomonas diminuta* in the oil palm with filler of 75% rice bran and 25% corn flour. The quality of SINBIONESA produced has met SNI standards in terms of water content of 2.89% (SNI << 14%), the total number of probiotics 10^{12} cell/g (SNI >> 10^{7}cfu/g) and expiration periods at room temperature storage and cold temperatures, 1 year 4 months and 2 years 5 months (SNI >> 3 months), respectively.

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