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QUALITY OF LACTOBACILLUS PLANTARUM YN 1.3, LACTOBACILLUS PENTOSUS YN 1.6 AND L.PLANTARUM YN 1.1. AFTER FREEZE DRYING UTILIZING A DIFFERENT B INDING AGENT, AS PROBIOTIC BACTERIA ISOLATED FROM GOAT MILK.

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Abstract. Probiotic are living microorganisms if consumed will be provided a therapeutic effect on healthy consumers by improving the microflora balance in digestive tracts. The utilization of probiotics could be implemented by using varied applications which would provide benefits for either human or animal health. The aims of this study were to evaluate the quality of Lactic acid bacteria (LAB) as probiotic such as Lactobacillus plantarum YN 1.3 and Lactobacillus pentosus YN 1.6 and L.plantarum YN 1.1. after freeze drying used Zinc and different binding agents. The binding agent is used ie maltodextrin, rice flour and skim milk. The results of this experiment showed that LABdecrease after freeze drying where L. plantarum YN 1.3 utilizing maltodextrin decrease 1.21 (Log cfu / g), utilizing rice flour decreased 1.66 (Log cfu / g) and utilizing skim milk LAB decrease 1.04 (Log cfu / g). LAB of L. pentosus YN 1.6 utilizing maltodextrin decrease 1.25 (Log cfu / g), utilizing rice flour decrease 2.11 (Log cfu / g) and utilizing skim milk decrease 1.00 (Log cfu / g). Although total lactic acid bacteria was decreased after freeze drying, but overall dried culture still better as probiotic bacteria. Anti microbial activity of starter culture showed that Probiotic bacteria have the ability to inhibit all of indicator bacteria such as Salmonella, E. coli, S. aureus and B. cereus as pathogens bacteria. Evaluation for color showed that dry culture used maltodextrin given the best appearance. The conclution of this research is that probiotic bacteria L. plantarum YN 1.3 with zinc and maltodextrin as binding agent is the best product after freeze dryng.

Key words: Frezee drying, L.plantarum YN 1.3, L. pentosus YN 1.6, Zinc Binding

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Introduction

Probiotics are live microbial supplements that have a positive effect on humans and animals by improving the balance of microflora in the digestive system. Salminem (1998) also reinforces the importance of probiotic viability. the amount should be sufficient agarmemberikan effect positive bagikesehatan , bias colonizes so the bias reaches the number specified during the time specified . Lee (2009) stated that bacteria probiotic has the ability to reduce the occurrence of diarrhea, acute in children are caused by bacterial pathogens and rota virus. Lactobacillus acidophilus and Bifidobacterium in ice cream probiotics can decrease the total bacterial Echericia coli in the channel intestine and increase the total bacteria acids lactic and increase the levels of Fe in the blood of animals. While Lactobacillus acidophilus in yogurt that are given to the children for three weeks can meurunkan total bacteria Salmonella as much as 5 Log cicle and bacteria Echericia coli as much as 2 log cicle in the channel intestine (Yelnetty, et al, 2009; Yelnetty, 2004).

In addition to the use of bacteria Probiotics, Zinc is a micronutrient that is found in all tissues of the body and is important for the growth of cells. WHO recommends the use of zinc during the occurrence of diarrhea for children. Combination between bacterial probiotic and Zinc are intended to look at the effectiveness of its in inhibiting bacterial pathogens during the process of drying bacteria probiotic use various types of materials binder.

The study is intended to evaluate the quality of bacterial acid lactate (BAL) as probiotics such as Lactobacillus plantarum YN 1.3, Latobacillus pentosus YN YN 1.6 and 1.1 Lactobacillus plantarum are combined with Zinc. Evaluations performed well during the process is the growth in the media as well as the viability of the bacteria after the process of drying using
materials binder that is different (maltodekstruin , Skimmilk and flour rice).

Research Method

1. Strain Bacteria

Bacterial strains used in the study include; Lactic acid bacteria culture isolated from goat milk: Lactobacillus plantarum YN 1.1. Lactobacillus plantarum YN 1.3 and Lactobaillus pentosus YN 1.6. Pathogenic bacterial cultures used were Salmonella choleraesius JCM
3019, Staphylococcus aureus FNCC 0047, Escherichia coli FNCC 0091, and Bacillus cereus ATCC 0047, obtained from the Food and Nutrition culture collection, UGM Food and Nutrition PAU. Another ingredient is Zinc in addition to the bacteria used.

2. Media and Research Tools.

The median for analysis of lactic acid bacteria is; Glucos yeast extracts peptons (GYP), bean sprouts and coconut water, as growth media, MRS broth media for the production of cell biomass. The media for rejuvenation and stock preparation in pathogenic bacteria is the Nutrient Broth media. The culture stock is stored at min. 0 0 C in a mixture of 20% glycerol and 10% skim milk. The binding or protective material used is. Maltodextrin, Rice flour and Skim milk.

The tools used are standard tools for microbial analysis, such as incubators, autoclaves, spray driers, freeze driers, and glass tools.

Research Stages.

Making Starter Culture.

Starter culture of Lactic Acid Bacteria used was made by growing bacteria on MRS broth media at a temperature of 36 C for 24 hours. The resulting culture is stored as stock at -40C. *Making media for growth*.

The growth media used are bean sprout and coconut water extracts in a ratio of 1: 1.

Growth curve.

In this study, the growth patterns of bacteria were used to determine the optimization of the resulting biomass, the logarithmic phase of each bacterium. The best bacteria are used for the production of

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dry culture then using a binder that has been determined, using the Spray drying tool. To see the best binding agent, the total lactic acid bacteria produced is calculated.

Dry Culture Production.
As much as 50 ml of liquid culture from Lactic Acid bacteria that is used is added by glycerol as much as 5% and mixed with Zinc as much as 0.2% and after that it is added with binder as much as 25 grams each. The binder used was maltodextrin, rice flour, and skim milk which were first sterilized at 121 C for 15 minutes. All ingredients are mixed evenly and the resulting mixture is put into a closed container. The drying process is carried out using Spray drieying at 72C. After the drying process the resulting dry culture is mashed aseptically with a mortar. Analysis was performed to determine the viability of existing cells using MRS Broth media.

Anti-microbial testing.
The resulting dry culture was then tested for its ability to inhibit the growth of several types of indicator bacteria used , which are pathogenic bacteria that cause diarrhea. The best results on the various types of binders used will be continued in experimental animals.

Result and Discussion

1. Total Lactic Acid Bacteria on extracts Sprouts and coconut water.
Before the process of drying bacteria acid lactate were used in grow on media extracts the bean sprouts and water coconut . The pattern of growth of all bacteria that are used as isolates L.plantarum YN 1.1, L. Plantarum YN1.3 and L.pentosus YN 1.6 on extracts of bean sprouts and water coconuts are in incubation at a temperature of 37 0 C for 24 hours can be
seen in Figure below this.

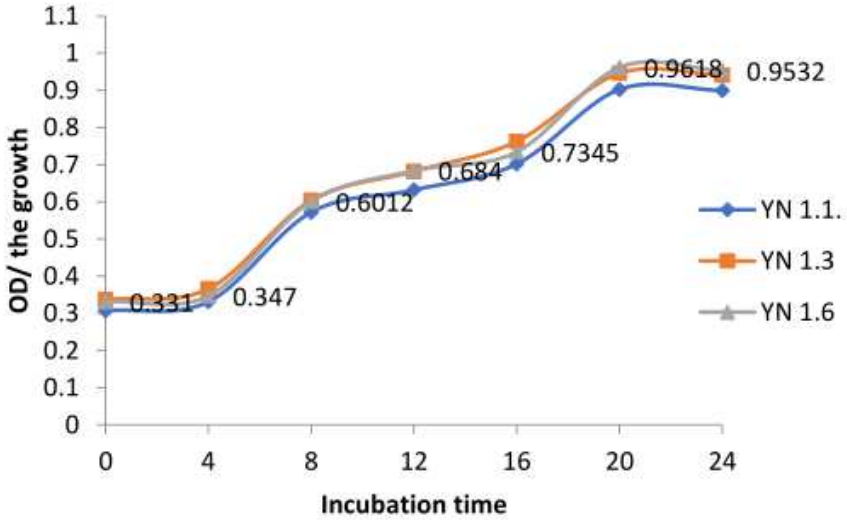

Figure 1. The Growth of L.plantarum YN 1.1, L.plantarum YN1.3 And L.pentosus YN 1.6 isolate using Bean sprout ectract and coconut water.

The growth pattern of Lactic acid bacteria during fermentation using bean extracts and coconut water was presented in Figure 1. It was shown that the growth of Lactic Acid Bacteria during 4 hours fermentation all of isolate not increased Lactic acid bacteria numbers. Logarithmic phase constant after 4 hours to 20 hours fermentation, where is Lactic acid bacteria number increase rapidly. The Number of Lactic acid bacteria to be considered with the Total plate count method and showed that the number of L.plantarum YN 1.1 was
8.25x10 8 Cfu / ml, L.plantarum YN 1.3 was 9.75x10 8 Cfu / ml and number of L .pentosus. YN 1.6 was 9,20x10 8 Cfu / ml.

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According to Mary et al., (1985), that bacteria cell at early stationary phase or phase logaritmic was better for using for dryng culture. At this time the cell is constant and lasting againts of chancing environment. The harves cell of isolates for drying culture would be done at 20 hours fermentation. The result indicated that Lactobacillus plantarum YN 1.1. the lowest number of viable bacteria among all isolates.

2. Chancing pH on Exctract Bean sprout and Water coconut media.
During incubation and growth of L. pentosus isolate YN 1.6. , L. brevis YN 1.1 , L. plantarum YN 1.3, showed that these probiotic bacteria produced similar pH patterns. The alteration of pH during the incubation process are shown in Figure 2.

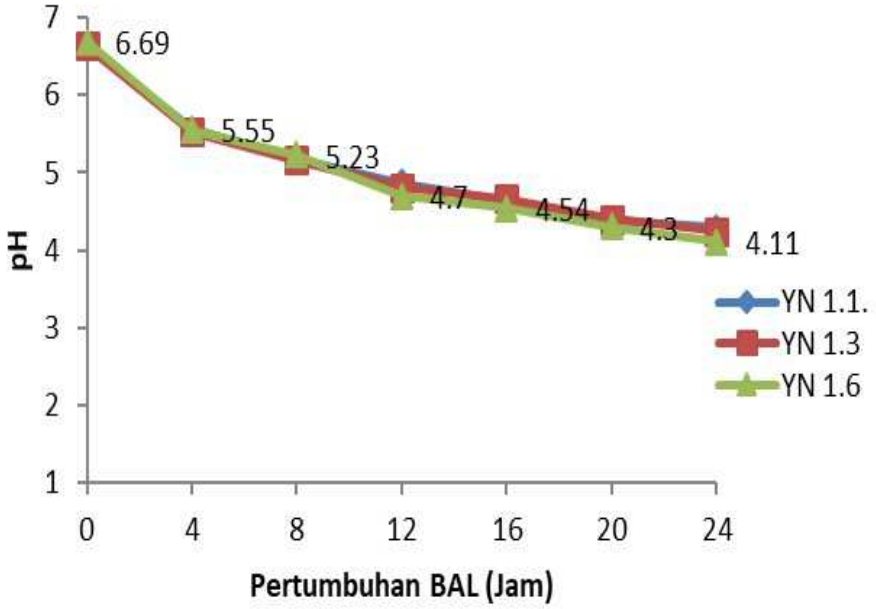

Figure 2 . Changes in pH during LAB growth of Isolate Yang Used (L.plantarum YN 1.1, L.plantarum YN 1.3 And L.pentosus YN 1.6).

The pattern of decreasing pH during lactic acid bacteria growth shows that all of isolates have a similar pattern where is each of the incubation periods increases the pH value to decrease. It is believed that pH values reduce due to milk sugar hydrolysis into lactic acid. Beside lactic acid other acids such as acetic acid, propionic acid and butyric acid were also produced. Tharmuraj and Shah, (2009), noted that Lactobacillus plantarum produces organic acids during fermentation as for lactic acid, acetic acid, proponic acid, butiric acid and benzoic acid. All of this organic acid to cause pH decrease. The result of this study showed that L.plantarum YN 1.3 and L.plantarum YN 1.6 the greates thorought decreased pH during incubation.

3. Production of Dried Culture from LAB mixed with Zinc. Using Various Types of Binders
1. Total BAL Before dryng
Isolates were used subsequently for the production of culture dried namely that have the ability that is good. From the results previously demonstrated that isolates L.plantarum YN 1.3 and L. pentosus YN 1.6 is the isolates were selected to proceed to the manufacture of culture arid which interfere with Zinc and 3 types of materials binder or as a filler, namely (1). Rice powder (2) Skim milk powder (3) Maltodextrin. The yield culture using binding filler befor dried showed on Figure 3.

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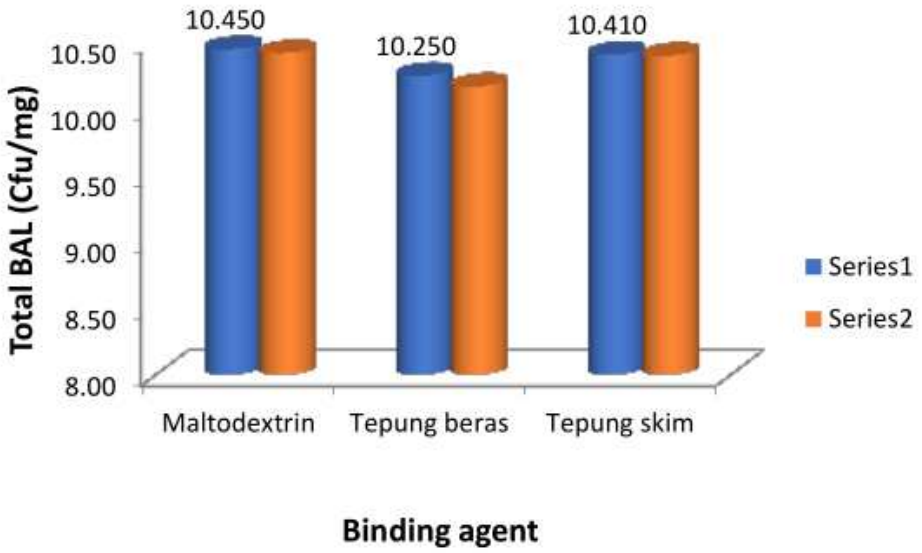

Figure 3 . Total Lactic acid Bacteria (L.plantarum YN 1.3 and L.plantarum YN 1.6). before dryed using different bindings.

The results in Figure 6 showed that there was a decrease in total bacteria on isolates, after exposure to rice powder as binding consideration from Maltodextrin and skim milk powder. The decreased on total bacterial of L. plantarum YN 1.3 and Lp entosus YN 1.6 used rice powder much more than isolate used Maltodextrin and skim milk powder.
The use of binders in the manufacture of dry culture results in the growth of lactic acid bacteria that differ from one another. The use of maltodextrin binder is the best binder seen from the total lactic acid bacteria produced. Rice flour binding material is the lowest binding quality or filler, which results in the smallest BAL growth. While based on the isolates used, it is known that L. plantarum YN 1.3 produces better total BAL (Log 10.45 cfu / g) on the maltodextrin binding agent, (log 10.25 cfu / g) on the rice flour binder and (log 10,41 cfu / g) on the skim milk binder. While in isolates L.pentosus YN lower total LAB 1.6 on all material binder compared with L.plantarum YN 1.3.

2. BAL Total After Drying on Various Binding Materials.
The process of drying m enggunakan material filler which differperformed by using a spray dry at a temperature of 72 0 C. Based on the test results from two isolates growth L.plantarum YN 1.3 and L.pentosus YN 1.6 after the process can be seen in Figure 4 below it.

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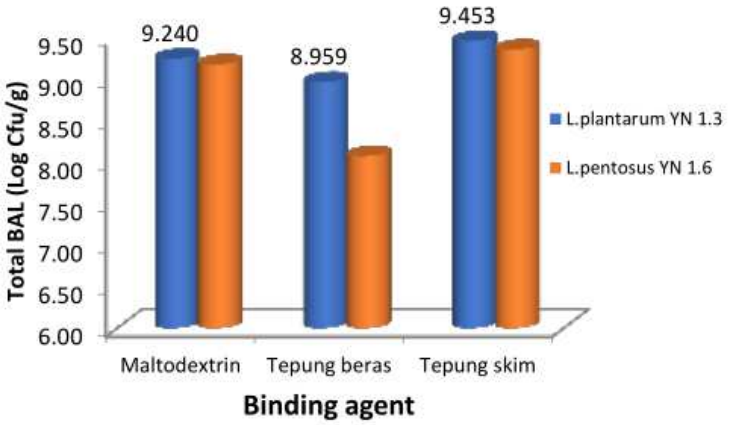

Figure 4. Total BAL In Media Production Se have Prose Drying

From the picture above it can be seen that the use of rice flour produces the smallest total lactic acid bacteria compared to other binders. Both the L.plantarum YN1.3 isolate and L.pentosus YN 1.6. Whereas the binding material of maltodextrin and skim milk media showed that the total growth of lactic acid bacteria from the isolates used was better than Rice flour.
Effect of drying on total lactic acid bacteria using various media as a binder in L.plantarum YN 1.3 and L.pentosus YN 1.6 isolates in Table 1, caused a decrease in the number of living cells to more than 1 log cicle in both isolates, even in isolates L. pentosus YN 1.6. After drying using rice flour as a binding material for living cells decreased 2 log cicle.

Table 1. Effect of Drying on Total Lactic Acid Bacteria (cfu / g) samples On Maltodextrin Production Media, Rice Flour, Skim Milk.

Binding agent	Before Drying		After Drying	
	L.plantarum YN 1.3 (cfu/g)	L. pentosus YN 1.6 (cfu/g)	L.plantarumYN 1.3 (Cfu/g)	L.pentosus YN 1.6 (Cfu/g)
Maltodekstrin	$2,82x10^{10}$	$2,63x10^{10}$	$1,73x10^{9}$	$1,48x10^{9}$
Rice powder	$1,80x10^{10}$	$1,49x10^{10}$	$1,17x10^{9}$	$9,10x10^{8}$
Skimed Milk	$2,62x10^{10}$	$2,54x10^{10}$	$1,63x10^{9}$	$1,23x10^{9}$

Before fermentation of L. pentosus 1.6 isolates , the total number of colonies was 1.49x10 10 cfu / g after drying the total bacteria produced dropped to 9.10x10 8 cfu / g.
The use of maltodextrin as a binding agent did not seem to significantly affect the total lactic acid bacteria produced after drying in both isolates in both L. plantarum YN 1.3 and L.pentosus YN 1.6 isolates.
The decrease that occurs in the use of maltodextrin as a binding agent in the manufacture of dry culture is not too large cell viability has decreased only less than 1 log cicle. Or down 1 logarithmic number from 2.82x10 10 cfu / g to 1.73x10 9 cfu / g, compared to L.pentosus YN
1.6, which has decreased to 2 log cicles. From the results obtained, it is known that L.plantarum YN 1.3 is more resistant to the effects of drying and has the best ability. Viability of isolates

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using maltodextrin and skim milk shows that the media used can protect cells from damage caused by drying. drying and dehydration.

4. Effect of Drying Of Color Products.
The effect of drying on the various binding media used can be seen inthe Figure below.

Figure 5. The effect of drying using various media on Color From Dry Culture, Rice Flour (A), Maltodextrin(B), Skim milk (C).

Figure 5 shows that color culture dried are generated as a result of drying using Spray Dry using median filler (maltodextrin, rice flour and skim milk), affect the appearance of the color of the product are different. In the picture above the use of skim milk causes the color of the resulting culture to be darker, compared to the use of maltodextrin and rice flour. Skim milk media which are used as a binder produce the darkest color in the final product of dry culture. Skim milk is rich will be proteins but suffered damage as a result of the process of cleaning spray that is done.
Maltodextrin and starch rice rich will carbohydrates and more resistant to the process of cleaning spray that is done. From the color changes caused in each media due to the drying process, it can be concluded that maltodextrin and rice flour produce clearer colors to be used in the production of further dry culture.

6. Anti-bacterial activity of dry culture.
Examining on antibacterial activity conducted with 3 replications. The results of the inhibitory ability are measured based on the clear zone produced which is the average yield of the three replications performed. Anti-microbial used is derived from dry culture with predetermined media (maltodextrin, rice flour, skim milk). In testing this antimicrobial activity using indicator bacteria from pathogenic bacteria such as Salmonella, Escherichia coli.
Staphyllococcus Aureus and Bacillus cereus. The effect of the dry culture used (L plantarum YN.1.3, and L. pentosus YN 1.6), on the test bacteria can be seen in Table 2.
Table 2. Diameter of clear zone (mm) using dry culture from L.plantarumYN 1.3 and L. pentosus YN 1.6.
Results of anti-microbial testing on dried cultures of each isolate L.plantarum plus Zinc use a filler that is different can be in seen in Table 1 show that the lactic acid bacteria L.plantarum YN 1.3 and L pentosus YN 1.6 are used have an antagonistic activity which varies with indicator bacteria. In general, all cell cultures from L. plantarum used have the ability to inhibit the growth of test bacteria. The ability of the lactic acid bacteria used shows that the cell culture of L. plantarum YN 1.3. has a greater inhibition than cell culture from L. pentosus YN 1,6. Inhibitory ability of L.plantarum YN 1.3. the best for Staphylococcus aureus is 21.3 mm, while for inhibition of Bacillus cereus bacteria is 11.6 mm. L. plantarum YN 1.3 also has the ability to inhibit other bacterial pathogens such as Salmonella and Eschericia colli although the inhibition is not as large as the inhibition of Staphylococcus aureus bacteria . From the picture above it appears that the color that

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occurs due to drying on the media used (maltodextrin, rice flour and skim milk), affects the appearance of the resulting color. In the picture above the use of skim milk causes the color of the resulting culture to be darker, compared to the use of maltodextrin and rice flour used. Skim milk media which are used as a binder produce the darkest color in the final product of the dry culture produced. From the color changes caused in each media due to the drying process, it can be concluded that maltodextrin and rice flour produce clearer colors to be used in the production of further dry culture. Based on the preliminary results carried out then for further testing with the use of experimental animals, L.plantarum YN 1.3 isolates were used and Zinc was added with a maltodextrin binding agent.

Conclusions

From the results of the research conducted can be concluded,

1. Growth of Bacteria of acid lactic who used the L.plantarum YN 1.1, L.plantarum YN 1.3 and *L.pentosus* YN 1.6, using the media extracts of sprouts and water coconut show that L.plantarum YN 1.1. generate growth that is lower than isolate other.
2. Isolates L.plantarum YN 1.3 are added Zinc uses maltodextrin binder has the ability to inhibit the growth of pathogenic bacteria (Salmonella, E. coli, S. aureus and B.cereus).
3. Material maltodextrin is the filler that is best as an ingredient binder compared with materials other (flour rice and skim milk) seen from the color that is produced as well as the viability of the bacteria that is generated.

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