Original Research Article

PROBIOTIC CHARACTERIZATION OF BACILLUS AND LACTOBACILLUS ENCAPSULATED IN SOME CARRIER MATERIALS

ABSTRACT

Research has been carried out on the encapsulation of probiotics of the genus *Bacillus* and *Lactobacillus* using rice flour, maltodextrin, and talc as carriers. The purpose of this study was to obtain a type of carrier material that can maintain the character of probiotics for fishfeed in aquaculture activity. The research method used is the experimental method, and the data were analyzed descriptively and experi-mentally. The experimental method used a completely randomized design with a factorial pattern. The results showed that talc can main-tain antimicrobial activity against *E. coli* (16.3 mm*), S. typhimurium* (17.3 and 13.8 mm) and coaggregation (12.9% and 15.01%). Rice flour can maintain antimicrobial activity against *E. coli* (20 mm), *S. typhimurium* (11.7 mm), and coaggregation (14.5%). Maltodextrin can maintain antimicrobial activity against *E. coli* (20.7 and 17.2 mm), *S. typhimurium* (19.8 and 20.8 mm) and coaggregation (8.8 % and 17.35%).

Keywords: Bacillus, carrier, encapsulation, Lactobacillus, probiotic

1. INTRODUCTION

Vannamei products have a great opportunity to become a source of food for humans, because shrimp is an important source of high-quality animal protein. To increase the yield of Vannamei commodities, many biological agents have been used, one of which is the use of probiotic bacteria. According to [1], probiotics are live microorganisms which if given in sufficient quantities will have a beneficial effect on the health of the host organism.

Groups of bacteria that contain probiotics include *Bacillus* sp., *Photobacterium* sp., and *Lactobacillus* sp. [2]. Probiotic bacteria must have criteria to be applied, including being resistant to low pH, bile salts, being able to live in the digestive system, being able to produce antimicrobial compounds, being able to stick (adhesion) to intestinal cells, being mass-produced, remaining stable and viable in the environment in long periods of time under storage conditions and in the field, and have a significant impact beneficial to the host [3], [4], [5]. The minimum number of probiotic strains present in food products is 10^6 CFU/g or the number of probiotic strains that must be consumed daily is around 10^8 CFU/g, with the aim of compensating for the possible decrease in the number of probiotic bacteria while in the digestive tract [6]. In order to improve and maintain the character of these probiotic bacteria, encapsulation method can be used.

Encapsulation is a technique of coating a material so that the coated material can be protected from environmental influences. Encapsulation in bacteria can provide conditions

that can protect microbes from unfavorable environmental influences, such as heat and chemicals [7], [8], [9]. The requirements of the coating material include non-toxicity, easy application, relatively cheap price, and the encapsulated material has relatively high cell viability and relatively the same physiological properties as before it was encapsulated [9].

[10] stated that flour as a carrier for the manufacture of dry starter cultures can maintain the viability of the culture. Based on this opinion, the encapsulation carriers that can be used are rice flour, maltodextrin, and talc. Rice flour is an ingredient that contains quite high protein, namely 8.7% [11]. According to [12], maltodextrin is used in the encapsulation process to protect compounds that are sensitive to oxidation and heat, maltodextrin can protect flavor stability during the drying process. Meanwhile, talc is the dominant mineral soil type associated with kaolinite and gibbsite. The stability of talc is relatively different from clay minerals which have a smooth, slippery structure and high heat conductor [13]. The use of several types of carrier material is due to the presence of suitable nutritional content to support the growth of probiotics and the nature of the carrier material that supports the encapsulation process. In this study, the use of probiotics *Bacillus* and *Lactobacillus* encapsulated in carriers of rice flour, maltodextrin, and talc were investigate to see the probiotic character, by observing the antimicrobial activity of several pathogenic bacteria and their coaggregation properties.

2. MATERIAL AND METHODS

The materials used in this study were: fuchsin water, sea water, fresh water, 70% alcohol, 96% alcohol, aquades, PBS phosphate buffer (Sigma), carbol gentian violet, matches, probiotic culture (*Bacillus licheniformis, Bacillus subtilis, Lactobacillus brevis, Lactobacillus bulgaricus,* and *Lactobacillus curvatus*), *Escherichia coli* bacteria culture ATCC 25922, *Salmonella thypimurium,* physiological NaCl solution (0.9%), 1% HCl solution, 1% NaOH solution , maltodextrin, Nutrient Agar (NA) (Oxoid) medium, Nutrient Broth (NB) (Oxoid), deMan, Rogosa and Sharpe Agar (Oxoid), deMan, Rogosa and Sharpe Broth (Oxoid), immersion oil (Olympus), spirtus, standard Mc Farland 3, standard Mc Farland 0.5, talc, rice flour, and tissue.

This research was conducted at the Microbiology Laboratory and Greenhouse Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor. The method used in this study is an experimental method using a completely randomized design (CRD), which consists of two parameters with three repetitions. The types of probiotic bacteria used were *Bacillus licheniformis*, *Bacillus subtilis, Lactobacillus brevis, Lactobacillus bulgaricus,* and *Lactobacillus curvatus*. The characterization test consisted of an antimicrobial activity test and a coaggregation test. The data obtained were then analyzed using Analysis of Variance (ANOVA) with a confidence level of 95% and if there was an effect of treatment then continued with Duncan's Multiple Distance Test [14].

2.1 Probiotic Antimicrobial Activity Test against Pathogenic Bacteria

Antimicrobial activity tests were carried out before and after encapsulation. The antimicrobial activity test was analyzed experimentally using a completely randomized design (CRD) with a factorial pattern of 5 x 4 x 2 and each treatment was repeated 3 times. The parameters observed were the diameter of the inhibition zone or the clear zone formed due to the inhibition of the growth of pathogenic bacteria. The first treatment factor was the type of probiotic bacteria (B), which consisted of 5 levels. The second treatment factor is the type of carrier material (P), which consists of 4 levels. The third treatment factor was digestive pathogenic bacteria (E), which consisted of 2 levels, namely: S1 = *E. coli*; S2 = *S. typhimurium.*

The method used to test the antimicrobial activity is the agar diffusion method, namely the well [15]. Tests for antimicrobial activity were carried out by taking 0.1 gram of microcapsules and adding it to 9.9 ml of Nutrient Broth (NB), then vortexed and incubated for

48 hours at 37°C. The supernatant and pellet were obtained by centrifugation for 20 minutes at a speed of 5000 rpm. Meanwhile, pathogenic bacteria that were 24 hours old were inoculated in NB. A total of 1 ml of the pathogenic bacterial isolate was planted evenly in Nutrient Agar (NA) on a sterile petri dish and allowed to harden [16]. Then, the supernatant and pellet as much as 1 ml each were inserted into the well on NA solid media that had been inoculated with pathogenic bacteria. The media was incubated for 24 hours at 37°C. The clear zone formed indicated an inhibition of the growth of the test bacteria by the supernatant and pellet. The diameter of inhibition or clear zone (mm) around the hole was measured using a caliper. Provisions of potential probiotics can be seen by measuring the inhibition area, which is classified as follows [17]:

Table 1. Classification of Clear Zone Diameter

Clear Zone Diameter (mm)	Remark
≤ 5	Resistant
5 – 10	Less sensitive
10 – 20	Sensitive
≥ 20	Very sensitive

2.2 Probiotic Coaggregation Test against Pathogenic Bacteria

Coaggregation test was carried out before and after encapsulation. The coaggregation test was analyzed experimentally using a completely randomized design (CRD) with a factorial pattern of 5 x 4 x 2 and each treatment was repeated 3 times. The parameter observed was a decrease in the value of the relative optical density (OD) between mixed bacteria (probiotics and pathogens), and those that were not mixed. The first treatment factor was the type of probiotic bacteria (B), which consisted of 5 levels. The second treatment factor is the type of carrier material (P), which consists of 4 levels. The third treatment factor was digestive pathogenic bacteria (E), which consisted of 2 levels, namely: S1 = *E. coli*; S2 = *S. typhimurium*.

Coaggregation test is carried out by determining the magnitude of the interaction ability between bacterial cultures to stick together in the digestive tract, so that they are not easily washed out due to bowel movements [18]. The probiotic coaggregation test used the method of [18] and [19]. Microcapsules of probiotics and pathogenic bacteria were grown on NB medium for 24 hours at 37°C. The cells were harvested by centrifugation at 10,000 rpm for 10 min and then each was washed twice with phosphate buffer saline (PBS) with a pH of 7.2. The microcapsules were mixed with each bacterial pathogen with a volume of 2 ml (1:1). The control was made containing 4 ml of microcapsules and 4 ml of pathogenic bacteria. The mixed culture was put into a cuvette and seen a decrease in OD at a wavelength of 620 nm, observed for 4 hours at room temperature. The same was done for the control on a single bacterial culture. Coaggregation was expressed as a decrease in the relative OD between mixed and unmixed bacteria (single). The percentage of coaggregation was calculated using the equation according to [18].

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity of *Bacillus* and *Lactobacillus* Probiotics against Pathogenic Bacteria *E. coli* and *S. typhimurium*

One of the desired criteria of probiotic bacteria is their ability to inhibit pathogenic bacteria, so that they are able to compete with pathogenic bacteria to maintain the normal balance of intestinal microflora [16]. The results of ANOVA showed that there was no interaction between pathogenic bacteria and probiotics Bacillus and Lactobacillus ($P > .05$). However, the ANOVA results showed that the interaction between the type of pathogen, the form of probiotics, and the type of carrier material on the diameter of the clear zone showed

significant differences, so it was continued with Duncan's test in Table 2.

Table 2. Duncan's Test Effect of Pathogens *E. coli* and S*. typhimurium*, Probiotic Forms, and Carrier Materials on Antimicrobial Activity of *Bacillus* and *Lactobacillus* Probiotics

Pathogenic Bacteria	Carrier Material	Probiotic Form	Average Clear Zone Diameter (mm)
E. coli	Before encapsulation	Supernatant pellet	13.2 ± 3.7 ^{bc}
			19.7 ± 3.7 ^{abc}
	Rice Flour	Supernatant pellet	**22.7 ± 3.7 ^{a}**
			20.1 ± 3.7 ^{ab}
	Maltodextrin	Supernatant pellet	17.4 ± 3.7 ^{abc}
			16.0 ± 3.7 ^{abc}
	Talc	Supernatant pellet	14.6 ± 3.7 ^{abc}
			12.1 ± 3.7 ^{c}
S. typhimurium	Before encapsulation	Supernatant pellet	20.3 ± 2.5 ^{ab}
			18.0 ± 2.5 ^{abc}
	Rice Flour	Supernatant pellet	15.2 ± 2.5 ^{abc}
			15.7 ± 2.5 ^{abc}
	Maltodextrin	Supernatant pellet	14.8 ± 2.5 ^{abc}
			18.9 ± 2.5 ^{abc}
	Talc	Supernatant pellet	12.5 ± 2.5 ^{bc}
			16.5 ± 2.5 ^{abc}

Based on Table 2, it can be seen that the interaction between pathogenic bacteria, the form of probiotics, and the carrier material that produces the largest average diameter of the clear zone is *E. coli* against probiotics in the form of pellets in the carrier material of rice flour, which is 22.7 mm. That shows that the probiotics in the form of pellets in rice flour are the most effective in inhibiting the growth of *E. coli* bacteria. To determine the probiotic formula which has the greatest antimicrobial activity in inhibiting pathogenic bacteria *E. coli* and *S. typhimurium*, the average diameter of the probiotic clear zone in the encapsulated formula against pathogenic bacteria was calculated, which can be seen in Table 3.

Table 3. Average Diameter of the Clear Zone of *Bacillus* and *Lactobacillus* Probiotics against Pathogenic Bacteria

Probiotic Formula		Probiotic Form	Average Clear Zone Diameter (mm)
B. licheniformis	Before encapsulation	Supernatant pellet	16.2 ± 2.3
			19.6 ± 2.3
	Rice Flour	Supernatant pellet	16.3 ± 2.3
			17.7 ± 2.3
	Maltodextrin	Supernatant pellet	17.7 ± 2.3
			18.1 ± 2.3
	Talc	Supernatant pellet	15.9 ± 2.3

			11.8 ± 2.3
B. subtilis	Before encapsulation	Supernatant pellet	19.2 ± 2.8 18.5 ± 2.8
	Rice Flour	Supernatant pellet	20.6 ± 2.8 19.7 ± 2.8
	Maltodextrin	Supernatant pellet	15.8 ± 2.8 13.9 ± 2.8
	Talc	Supernatant pellet	13.4 ± 2.8 14.9 ± 2.8
L. brevis	Before encapsulation	Supernatant pellet	18.7 ± 2.6 21.0 ± 2.6
	Rice Flour	Supernatant pellet	15.8 ± 2.6 15.7 ± 2.6
	Maltodextrin	Supernatant pellet	14.6 ± 2.6 18.0 ± 2.6
	Talc	Supernatant pellet	13.3 ± 2.6 19.4 ± 2.6
L. bulgaricus	Before encapsulation	Supernatant pellet	13.2 ± 4.5 **22.4 ± 4.5**
	Rice Flour	Supernatant pellet	**24.8 ± 4.5** **21.2 ± 4.5**
	Maltodextrin	Supernatant pellet	18.2 ± 4.5 18.0 ± 4.5
	Talc	Supernatant pellet	12.4 ± 4.5 14.5 ± 4.5
L. curvatus	Before encapsulation	Supernatant pellet	16.2 ± 2.7 12.5 ± 2.7
	Rice Flour	Supernatant pellet	17.1 ± 2.7 15.2 ± 2.7
	Maltodextrin	Supernatant pellet	14.1 ± 2.7 19.0 ± 2.7
	Talc	Supernatant pellet	12.8 ± 2.7 10.8 ± 2.7

From Table 3, it can be seen that the probiotic formula that has the greatest antimicrobial activity is *L. bulgaricus* bacteria in the carrier material of rice flour, both in the form of pellets and supernatants, with an average inhibition zone diameter of 24.8 mm and 21.2 mm, respectively. In addition, the largest average diameter was also shown in *L. bulgaricus* bacteria in the form of supernatant before encapsulation, which was 22.4 mm. These results are consistent with the research by [20] who tested the antimicrobial activity of coconut water-based yogurt containing *L. bulgaricus* and *Streptococcus thermophylus* bacteria against *E. coli, Salmonella typhi, Klebsiella pneumonia*, and *Staphylococcus aureus* bacteria. The results showed that the highest antimicrobial activity in pathogenic bacteria ranged from 10-21 mm in *S. typhi* and 9-64 mm in *E. coli*. However, in general, the antimicrobial activity of probiotics in the encapsulated formula did not show a significant difference, meaning that the probiotics *Bacillus* and *Lactobacillus* in the encapsulated formula in this study were effective in inhibiting the pathogenic bacteria *E. coli* and *S. typhimurium*.

L. bulgaricus bacteria in their metabolism produce various types of organic acids that trigger a decrease in pH and produce an acidic environment. The most acid produced by *L.*

bulgaricus is lactic acid, because these bacteria are a group of lactic acid bacteria. Lactic acid can have bacteriostatic or bactericidal abilities according to its concentration in a solution. *L. bulgaricus* bacteria in addition to producing lactic acid and hydrogen peroxide are also known to produce bacteriocins known as Bulgarican. Bulgarican has the ability to inhibit both gram-positive and gram-negative bacteria [21].

[4] states that antimicrobial substrates are produced by probiotic bacteria starting from the exponential phase. However, most bacteriocins are produced in large quantities after reaching the stationary phase. Bacteriocins can damage the permeability of the cell membrane of pathogenic bacteria by forming pores in the bacterial cell, so that the cell membrane will leak which will disrupt the stability of the cell membrane. The growth of pathogenic bacterial cells will be inhibited and will experience death.

In addition to antimicrobial substrates, the influence of organic acids and hydrogen peroxide produced by probiotic bacteria, especially Lactobacillus can also inhibit the growth of pathogenic bacteria. Hydrogen peroxide produced by lactic acid bacteria will damage and oxidize pathogenic bacterial cells [4]. Bacteriocins, organic acids, and hydrogen peroxide are metabolites produced by lactic acid bacteria. These metabolites will diffuse in the growth medium with different levels of ability [22].

Meanwhile, *Bacillus* bacteria can produce more than 45 antimicrobial molecules. The antibiotic substances produced include bacteriocin, amino acids, and non-amino acids [23]. The research results of [24] showed that B. subtilis had stronger antimicrobial activity than *B. pumilus* against *E. coli, L. monocytogenes, S. typhimurium, S. dysenteriae*, and several other pathogenic bacteria. The antimicrobial activity of *B. subtilis* tends to inhibit gram-negative bacteria, for example *E. coli* and *S. typhimurium* because these bacteria can produce the amino acid subtilin, basilicin, lantibiotics, and other antimicrobial compounds. Table 4 shows the sensitivity of each pathogenic bacteria to *Bacillus* and *Lactobacillus* probiotics in the encapsulated formula.

Table 4. Sensitivity of Pathogenic Bacteria to *Bacillus* and *Lactobacillus* Probiotics

Probiotic Formula		Probiotic Form	Average Clear Zone Diameter (mm)	
			E. coli	***S. typhimurium***
B. licheniformis	Before encapsulation	Supernatant	12.3 (S)	20.2 (SS)
		pellet	20 (S)	19.3 (S)
	Rice Flour	Supernatant	22.0 (SS)	10.7 (S)
		pellet	19.2 (S)	16.2 (S)
	Maltodextrin	Supernatant	19.2 (S)	16.2 (S)
		pellet	15.1 (S)	21.0 (SS)
	Talc	Supernatant	16.3 (S)	15.5 (S)
		pellet	6.3 (KS)	17.3 (S)
B. subtilis	Before encapsulation	Supernatant	14.7 (S)	23.8 (SS)
		pellet	19.3 (S)	17.8 (S)
	Rice Flour	Supernatant	**25.3 (SS)**	15.8 (S)
		pellet	21.0 (SS)	18.3 (S)
	Maltodextrin	Supernatant	15.5 (S)	16.2 (S)
		pellet	14.6 (S)	13.3 (S)
	Talc	Supernatant	16.3 (S)	10.5 (S)
		pellet	15.9 (S)	13.8 (S)

L. brevis	Before encapsulation	Supernatant	12.5 (S)	25 (SS)
		pellet	20.8 (SS)	21.3 (SS)
	Rice Flour	Supernatant	20.0 (SS)	11.7 (S)
		pellet	19.8 (S)	11.7 (S)
	Maltodextrin	Supernatant	18.7 (S)	10.5 (S)
		pellet	16.7 (S)	19.3 (S)
	Talc	Supernatant	15.8 (S)	10.7 (S)
		pellet	17.0 (S)	21.8 (SS)
L. bulgaricus	Before encapsulation	Supernatant	14.7 (S)	11.8 (S)
		pellet	23.3 (SS)	21.5 (SS)
	Rice Flour	Supernatant	22.7 (SS)	**27.0 (SS)**
		pellet	22.0 (SS)	20.3 (SS)
	Maltodextrin	Supernatant	20.7 (SS)	15.7 (S)
		pellet	16.2 (S)	19.8 (S)
	Talc	Supernatant	9.5 (KS)	15.3 (S)
		pellet	10.7 (S)	18.3 (S)
L. curvatus	Before encapsulation	Supernatant	11.7 (S)	20.7 (SS)
		pellet	14.9 (S)	10.1 (S)
	Rice Flour	Supernatant	23.5 (SS)	10.7 (S)
		pellet	18.3 (S)	12.2 (S)
	Maltodextrin	Supernatant	12.8 (S)	15.3 (S)
		pellet	17.2 (S)	20.8 (SS)
	Talc	Supernatant	15.2 (S)	10.5 (S)
		pellet	10.5 (S)	11.0 (S)

*Note: *(SS) = very sensitive pathogenic bacteria*
(S) = sensitive pathogenic bacteria
(KS) = less sensitive pathogenic bacteria

Based on Table 4, it can be seen that the probiotics *Bacillus* and *Lactobacillus* in the carrier material of rice flour, both pellets and supernatants have a larger clear zone diameter and can inhibit *E. coli* bacteria better than other carriers, with a clear zone diameter range of 18.3 mm (sensitive) to 25.3 mm (highly sensitive). The bacteria that had the largest diameter of the inhibition zone was *B. subtilis* in the carrier material of rice flour in the form of pellets. Meanwhile, for *S. typhimurium*, it was seen that the *L. bulgaricus* formula in pellet-shaped maltodextrin carrier had a larger clear zone diameter than the other formulas, which was 27.0 mm. Overall, the diameter of the clear zone produced by *Bacillus* and *Lactobacillus*

probiotics in the encapsulated formula in inhibiting the growth of E*. coli* and *S. typhimurium* bacteria ranged from 6.5 mm to 27.0 mm, which indicated that all probiotic formulas could inhibit the pathogenic bacteria *E. coli* and *S. typhimurium*.

E. coli and *S. typhimurium* are gram negative bacteria. These bacteria are natural microflora in the digestive tract. However, if the amount exceeds the normal limit, these bacteria can cause disease in the digestive tract. The results of this antimicrobial activity test indicate that the probiotic bacteria *Bacillus* and *Lactobacillus* in the encapsulated formula have met one of the requirements for the characteristics of probiotics, which is to have the ability to inhibit the growth of pathogenic bacteria.

3.2 Coaggregation of Bacillus and Lactobacillus Probiotics against Pathogenic Bacteria *E. coli* and *S. typhimurium*

Coaggregation according to [19] is the result of cell-to-cell interactions between different cell types. Macroscopically they can usually be detected as clumps when different cell types are mixed. Coaggregation is one way of testing to support the attachment of these bacteria to the surface of the intestinal mucosa. Coaggregation aims to determine the ability of bacteria to interact with other bacteria to stick together. Coaggregation is an interaction between bacterial cells with different types [4]. The purpose of coaggregation is to determine the interaction ability of a bacterium with other bacteria to stick together. In this study, we tested the coaggregation properties between probiotic bacteria *Bacillus* and *Lactobacillus* with pathogenic bacteria *E. coli* and *S. typhimurium*. The results of ANOVA showed that there was an interaction between types of probiotics and pathogenic bacteria (P = .05). This shows that pathogenic bacteria have a significant effect on probiotic bacteria. Based on these results, Duncan's Multiple Distance Test was carried out with a 5% significance level which is presented in Table 5.

Table 5. Duncan's Multiple Range Test Means of Coaggregation of *Bacillus* and *Lactobacillus* Probiotics against Pathogenic Bacteria

Probiotic Formula		Average Coaggregation Value (%)	
		E. coli	***S. typhimurium***
B. licheniformis	Before encapsulation	2 ^{Fb}	10.3 ^{FGHa}
	Rice Flour	8.3 ^{ABCDEb}	14.0 ^{CDEFa}
	Maltodextrin	7.9 ^{BCDEb}	14.0 ^{CDEFa}
	Talc	7.5 ^{CDEb}	13.0 ^{EFGa}
B. subtilis	Before encapsulation	11.8 ^{ABb}	21.6 ^{ABa}
	Rice Flour	11.3 ^{ABCb}	**21.8** ^{Aa}
	Maltodextrin	11.7 ^{ABb}	17.6 ^{BCa}
	Talc	10.6 ^{ABCDb}	15.0 ^{CDEa}
L. brevis	Before encapsulation	5.2 ^{EFb}	14.8 ^{CDEa}
	Rice Flour	7.1 ^{DEb}	14.5 ^{CDEa}

	Maltodextrin	6.8 DEb	13.7 DEFa
	Talc	6.0 DEb	13,2 EFGa
	Before encapsulation	0 Gb	10.3 FGHa
L. bulgaricus	Rice Flour	9.3 ABCDa	9.6 GHa
	Maltodextrin	8.8 ABCDEa	8.7 Ha
	Talc	8.0 ABCDEa	8.4 Ha
	Before encapsulation	8.3 ABCDEb	15.5 CDEa
	Rice Flour	**11.9 Ab**	16.2 CDEa
L. curvatus	Maltodextrin	10.1 ABCDb	17.3 CDa
	Talc	11.2 ABCa	14.2 CDEFa

*Note: *The average number of bacteria followed by the same capital letter (vertical/perpendicular direction) and the same lowercase letter (horizontally/horizontally) shows that the values are not significantly different according to Duncan's Multiple Distance Test.*

Based on Table 5, it can be seen that the largest coaggregation value was a mixture of formula *B. subtilis* in rice flour carrier and *S. typhimurium* bacteria, which was 21.8%. This indicated that the bacteria had the greatest decrease in the population of mixed bacteria compared to single, so it was hoped that this formula would be the most effective in inhibiting the growth of *S. typhimurium*. The lowest value was shown in the mixture between the *L. bulgaricus* formula before encapsulation with *E. coli* bacteria, which was 0%, meaning that the coaggregation pair had the smallest decrease in the mixed population or the large mixed culture population, indicating that the bacterial pair did not inhibit each other's growth.

Coaggregation was determined based on the decrease in the relative optical density (OD) value between mixed bacteria and unmixed (single) bacteria. A high percentage of coaggregation means that there is a decrease in the mixed bacterial population that is greater than the average decrease in a single population. The low coaggregation value indicated that the combination of bacteria did not inhibit each other's growth. In testing the coaggregation of probiotic and pathogenic bacteria, it is expected that the coaggregation value is high because there will be a decrease in the population in the mixed culture and it is hoped that the bacteria that die when mixed are pathogenic bacteria, so that the dominance of probiotic bacteria will occur. Probiotics will produce metabolites that can inhibit pathogenic bacteria. Therefore, coaggregation can increase the elimination of pathogens by destroying the pathogen while in the digestive tract [19].

According to [4], the coaggregation value is determined from the decrease in the absorbance value between bacteria mixed with bacteria in a single form. In this study, a test was conducted between probiotic bacteria and pathogenic bacteria. The greater the coaggregation value, the inhibition between probiotic bacteria and pathogenic bacteria will increase.

4. CONCLUSION

Based on the results of the study, it can be concluded that the carrier talc can maintain the probiotic character of *B. licheniformis* and *B. subtilis* bacteria, the rice flour carrier can maintain the probiotic character of *L. brevis* bacteria, and maltodextrin carrier can maintain the probiotic character *of L. bulgaricus* and *L. curvatus* after the encapsulation process.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. CORCIONIVOSCHI N, DRINCEANU D, STEF L, LUCA I, JULEAN C, MINGYART O. PROBIOTICS - IDENTIFICATION AND WAYS OF ACTION. INNOVATIVE ROMANIAN FOOD BIOTECHNOLOGY 2010:6:1-11

2. LINGGARJATI KF, DJUNAEDI A, SUBAGIYO. TEST USE OF BACILLUS SP. AS A PROBIOTIC CANDIDATE FOR REARING CRAYFISH (PORTUNUS SP.). JOURNAL OF MARINE RESEARCH. 2013: 2(1): 1-6

3. HARDININGSIH R, NAPITUPULU RNR, YULINERY T. ISOLATION AND RESISTANCE TEST OF SEVERAL LACTOBACILLUS ISOLATES AT LOW PH. BIODIVERSITAS 2006:7(1): 15-17

4. HUNAINAH. CHARACTERIZATION OF ENTEROCOCCUS BACTERIA FROM COW COLOSTRUM AS PROBIOTICS FOR DIGESTION" BACHELOR THESIS. BIOLOGY STUDY PROGRAM, FACULTY OF MATHEMATICS AND NATURAL SCIENCES, PADJADJARAN UNIVERSITY. JATINANGOR. 2014.

5. ONAYANTI, N. VIABILITY TEST OF PROBIOTICS FROM THE DIGESTIVE TRACT OF ANAS DOMESTICUS BROILER DUCKS ENCAPSULATED BY CROSS LINK METHOD "BACHELOR THESIS. HASANUDDIN UNIVERSITY. MAKASSAR. 2015

6. SHAH NP. FUNCTIONAL CULTURES AND HEALTH BENEFITS. INTERNATIONAL DAIRY JOURNAL. 2007:17:1262- 1277

7. YOUNG SL, SARDA X, ROSENBERG M. MICROENCAPSULATING PROPERTIES OF WHEY PROTEINS WITH CARBOHYDRATES. JOURNAL OF DIAIRY SCIENCE 1995:76: 2878-2885

8. FRAZIER WC, WESTHOFF DC. FOOD MICROBIOLOGY 4TH ED. NEW YORK: MCGRAW HILL INC. 1998.

9. TRIANA E, YULIANTO E. NURHIDAYAT N. VIABILITY TEST OF LACTOBACILLUS SP. MARCH. 8 ENCAPSULATED. BIODIVERSITAS 2006:7(2): 114-117

10. RUKMI WD, ZUBAIDAH E, MARIA M. PREPARATION OF DRY STARTER CULTURE OF MIXED LACTIC ACID BACTERIA AND SACCHAROMYCES CEREVISIAE FOR THE FERMENTATION PROCESS OF INSTANT CEREAL PRODUCTS. JURNAL TEKNOLOGI PERTANIAN. 2012: 4(1): 56-69

11. WIDJAJASEPUTRA AI, HARIJONO, YUNIANTA, ESTIASIH T. THE EFFECT OF RICE FLOUR AND WATER RATIO ON THE CHARACTERISTICS OF WET SPRING ROLLS" JURNAL TEKNOLOGI DAN INDUSTRI PANGAN 2011:22(2): 184-189.

12. LAILIYAH N. EFFECT OF TOTAL MALTODEXTRIN AND DRYING TIME ON ORGANOLEPTIC PROPERTIES OF POWDERED SOY MILK YOGHURT. 2014 E-JOURNAL BOGA: 3(1): 65-78.

13. PUTRI, D, MUNIF A, MUTAQIN KH. STORAGE TIME, PHYSIOLOGICAL CHARACTERIZATION, AND VIABILITY OF ENDOPHYTIC BACTERIA BACILLUS SP. IN FLOUR FORMULA. JURNAL FITOPATOLOGI INDONESIA 2016:12(1): 19-26.

14. GOMEZ KA, GOMEZ AA. STATISTICAL PROCEDURES FOR AGRICULTURAL RESEARCH SECOND EDITION. UI PRESS. JAKARTA. 1995.

15. SARI RA, RISA N, PUJI A. CHARACTERIZATION OF LACTIC ACID BACTERIA OF THE GENUS LEUCONOSTOC FROM ALE-ALE PASTE FORMULATIONS ON A LABORATORY SCALE. JKK 2012:1(1): 14-20.

16. SUSANTI I, KUSUMANINGTYAS RW, ILLANINGTYAS F. TEST OF PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA AS CANDIDATE FUNCTIONAL FOOD INGREDIENTS. JURNAL TEKNOLOGI DAN INDUSTRI PANGAN 2007:18(2): 89-95

17. FITRI L, BETTY MB. SCREENING OF ANTIMICROBIAL PRODUCING STRAINS ISOLATED FROM THE SOIL OF GRASSLAND RHIZOSPHERE IN POCUT MEURAH INTAN FOREST PARK, SEULAWAH, ACEH BESAR. BIODIVERSITAS 2010:11(3): 129-132

18. MANIN. POTENTIAL OF LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS FERMENTUM FROM DIGESTIVE TRACT OF FREE-RANGE CHICKEN FROM PEATLAND AS A SOURCE OF PROBIOTICS. JURNAL ILMIAH ILMU-ILMU PETERNAKAN 2010: 13(5): 221-228

19. SUMARNI D. CHARACTERISTICS OF ATTACHMENT AND COAGGREGATION OF INDIGENOUS LACTIC ACID BATTERIES AND YOGURT AS PROBIOTIC CANDIDATES IN SMALL INTESTINES OF RATS IN VITRO. BACHELOR THESIS. BOGOR AGRICULTURAL INSTITUTE. BOGOR. 2011.

20. LINDAWATI SA, HANIYAH YS, MIWADA IN, INGGRIATI NWT, HARTAWAN M, SUARTA IGD. COCONUT WATER BASED YOGURT ANTIMICROBIAL ACTIVITY INHIBITS PATHOGENIC BACTERIA IN VITRO. MAJALAH ILMIAH PETERNAKAN. 2014:17(2): 51-55.

21. FITRIANARNI D, IBRAHIM M, TRIMULYONO G. ANTIBACTERIAL ACTIVITY OF COW'S MILK YOGHURT AND SOY MILK YOGHURT AGAINST SHILGELLA DYSENTERIAE IN VITRO. LENTERA BIO 2014:3(1):97-102

22. SARI R. CHARACTERIZATION OF PROBIOTIC BACTERIA DERIVED FROM THE DIGESTIVE TRACT OF BROILERS. BACHELOR MINITHESIS FROM HASANUDDIN UNIVERSITY. MAKASSAR, 2012.

23. DIANTI. CHARACTERIZATION OF PROBIOTIC BACILLUS BACTERIA FROM COW COLOSTRUM IN SEVERAL DRY INOCULUM FORMULAS. BACHELOR THESIS. FACULTY OF MATHEMATICS AND NATURAL SCIENCES, PADJADJARAN UNIVERSITY. JATINANGOR, 2014.

24. OUOBA LII, DIAWARA B, JESPERSEN L, JAKOBSEN M. ANTIMICROBIAL ACTIVITY OF B. SUBTILIS AND B. PUMILUS DURING THE FERMENTATION OF AFRICAN LOCUST BEAN (PARKIA BIGLOBOSA) FOR SOUMBALA PRODUCTION. JOURNAL OF APPLIED MICROBIOLOGY. 2007:102:963-970