29-Viability Bacteria_wjpps_1564562236

by Turnitin Checker

Submission date: 05-Apr-2023 04:46PM (UTC+1000)
Submission ID: 2056402988
File name: 29-Viability_Bacteria_wjpps_1564562236.pdf (258.64K)
Word count: 3805
Character count: 20583

WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 8, Issue 8, 285-294 Research Article ISSN 2278 – 4357

VIABILITY BACTERIA AS BIOINCLUSI CAPSULAN

Kasprijo* and Tjahjo Winanto

Lecturer of Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University Purwokerto.

Article Received on
11 June 2019,

Revised on 02 July 2019,
Accepted on 23 July 2019,

DOI: 10.20959/wjpps20198-14410

***Corresponding Author**
Dr. Kasprijo
Lecturer of Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University Purwokerto.

ABSTRACT

This research was conducted in the laboratory of the Faculty of Fisheries and Marine Sciences of the Jenderal Soedirman University of Purwokerto. This study aims to determine the viability of the probiotic microcapsules from the digestive tract of grouper encapsulated by modifying the basic thermal cross-linking method, and applying the freeze dryer technique. Microcapsules made with different matrix materials include albumin, agar, and alginate. This research was conducted by laboratory experimental method. Stages of preparing probiotic microcapsules are preparations which include inclusion preparation, matrix preparation, followed by droplet making stage, cross linking polymer stage and recovery stage. How it works is a modification of Arshady's guidance (1989). This study used a complete randomized factorial design, with different matrix material treatments (A) Albumin; (B) agar and (C) Alginate; with the inclusion of Bacillus amyloliquefacient bacteria. Characteristic morphology of microcapsule with matrix of albumin, agar and alginate Color of each yellow, white and white, round shape, diameter, water content 5%, solubility and buoyancy < 30 min, matrix diameter of material albumin, agar and alginate respectively 0,98, 0.96 and 1.22 mm. The probiotic bacterial endurance (viability) test is performed by comparing the total number of probiotic bacteria before and after the encapsulation process by drying freeze proces. The total population of probiotic bacterial cultures before the encapsulation process was 5.4 x 10^8 cells / ml and after the encapsulation process with matrix albumin, agar and alginate the total amount of probiotic bacteria was 5.6 x 10^7 to 6.2 x 10^7 cells / ml. after storage of 35 days at a temperature of 4^{o}C of bacterial density 5.5 x 10^7 to 6.4 x 10^7 cells / ml.

KEYWORDS: *viability, bacillus, bioinclusi, encapsulant, and freeze dryer*.

PRELIMINARY

Probiotic bacteria in the world of fisheries is still limited to isolate, characterize, purify and multiply isolates. Based on consideration it is necessary to develop bacteria probiotics for fisheries as a development fish feed. Probiotic bacteria is expected to be able to help the digestion of feed so that will improve the efficiency of feed. Probiotics are living microorganisms that, when administered in sufficient quantities, can provide healthful effects to its host by improving the composition of the intestinal microbiota Bacillus sp is a probiotic candidate isolated from the digestive tract of the invitro test fish that has been done indicates that Bacillus sp is able to form a good cell mass and can inhibits pathogenic bacteria. In addition, Bacillus is able to pass simulated gastric acid conditions on pH 3 and 4, does not convert primary cholesterol to secondary cholesterol, and is able to hydrolyze bile salts that contribute to a decrease in blood cholesterol. Bacterial resistance (viability) is one of the important considerations in the development of probiotic products. Generally, probiotic products must contain 106-107 cfu / g live bacteria in order to provide a functional effect to the body. Probiotic resistance in the product is affected by several factors, such as pH, acid enhancement during storage (in fermentation products), production of hydrogen peroxide, oxygen and nitrogen, competition with other bacteria during fermentation and stability in both dry and frozen form (Kailasapathy 2002). Efforts to increase the resistance of probiotic bacteria can be done through microencapsulation technology. Microencapsulation is defined as the process of coating cells using hydrocolloid components that correspond to the purpose of protecting cells from the surrounding environment so that cells can be released in the digestive tract. Drying methods and encapsulation types are important factors in the microencapsulation process. Drying methods exist in the following ways: freze dryer, spray drying (Harmayani et al., 2001). Selection of encapsulation materials is important because it affects emulsion stability prior to drying, flowability, physical stability, shelf life after drying and the effectiveness of cell protection. This research used drying method with freeze dryer for microencapsulation of Baciilus sp bacteria with encapsulation of maltodextrin, carrageenan, and alginate. Maltodextrin has good coating properties because of its ability to form its low emulsion and viscosity. Carrageenan is an encapsulation material that can be used to effectively protect probiotic bacteria from the pressure of environmental conditions (Ding and Shah 2009) so that it can be used as cell immobilization medium (Chibata, 1981). Alginate has the ability to survive through the stomach without being degraded. In this regard, efforts to increase the production of fish seed can be done by first studying the preparation of probiotic microcapsule feed with different matrix materials.

MATERIALS AND METHODS

Materials and tools

Materials and tools used in this study were: groupers, liquid NA, solid NA, skim milk, aquades, Nacl, CaCo, KCL, MgSO4, violet crystals, jodin, 96% alcohol, sufranin, label paper, and stationery, autoclaves, microscopes, bunsen, scales, micro pipettes, test tubes, erlemeyer, petri dishes, object glass, ose needles, triangle grading, stirer magnets, thermometers, incubators and ice cabinets. All equipment used will be washed thoroughly and dried after it is sterilized, in an autoclave at 121 ° C, for approximately 15 minutes.

1. Sterilize tools and materials

The tool and material impregnation of all tools and materials to be used is sterilized by autoclaving at 121 ° C at 15 lb / m2 vapor pressure for 15 minutes (Marlina 2008).

2. Sampling and bacterial isolation

Probiotic bacteria isolated on digestive organs of fresh gut and group fresh fish 1 g sample. The fish were dissected for the gastrointestinal and intestine parts, then incorporated into a 0.9% NaCl physiological solution at pH 2 with the sole aim of only probiotic bacteria that could grow and develop at the pH (Feliatra et al., 2004). Furthermore, the stomach and intestines are crushed or mashed by using porcelain mortar. The smoothed sample is then diluted. The dilution series method is carried out by taking as much as 1 g of sample, put into a reaction tube containing 9 ml of distilled water so that in dilution 10-1, to obtain dilution 10-2 Carry out by taking 1 ml of dilution 10 -1 inserted into tube the reaction containing 9 ml of distilled water was diluted to 10- 5, dilution 10-4 and 10-5 was taken 1 ml then inserted into a Petri dish containing NA medium and flattened, then incubated for 48 hours, and stored at temperature -20 0C. For further tests the planting is done to inhibit the NA media.

Having obtained colonies that are able to live on heterotrophic bacterial media, then each colony obtained is made three replications. Finally of several repetitions, at least five replicates for each strain, pure isolates of potential heterotrophic bacteria as probiotics are found. Then proceed with identification of isolates. The storage of bacterial colonies was carried out at 400C and ready for use in subsequent tests. Identification of bacteria by Kasprijo (2017), by conducting a series of morphological and biochemical tests namely Gram staining test, motility test, cell form observation, cell coupling type, aerobic and anaerobic properties. Observations were also conducted on colony color, colony size, colony shape, the ability to produce catalase andoxidase, a halophilic test and cytochrome oxidase to determine

the genus of heterotrophic bacteria obtained from groupers.

3. Making Microcapsules

Preparation Tools and Materials

Tool Tools used in this research are: freeze dryer, mixer, measuring pipette scale, test tube, Erlenmeyer, petri dish, thermometer, and ice cup incubator. The materials used in this study were: bacteria *B. amyloliquefacient*, alginate, agar, albumin, aquades, 96% alcohol, label paper, and stationery.

Stages of preparing probiotic microcapsules are preparations which include inclusion preparation, matrix preparation, followed by droplet making stage, cross linking polymer stage and recovery stage. How it works is a modification of *Arshady's* guidance (1989). This study used a completely randomized design, with material treatment.

Matrices

(A1) Albumin; (A2) Agar; and (A3) Alginate;

Each treatment was repeated three times. Some parameters observed are color, shape, diameter, moisture content, and buoyancy.

3.1. Viability of Probiotics after Microencapsulation Process

Probiotic resistance test of microcapsule during *freeze dryer* is done to determine the effect of *freeze dryer* process and encapsulation material on probiotic cells that can survive. The probiotic resistance is determined by comparing the number of cells after the *freeze dryer* and before. *freeze dryer* Quantitative calculation of probiotic cells refers to the method of Lian *et al*. (2002) that is by Total Plate Count (TPC) method. A total of 0.1 mg of probiotic microcapsules homogenized in 0.9 mL sterile PBS (dilution10^{-1}). A series of dilutions is prepared up to 7 tubes (10^{-7} diluents) for serial dilution. Probiotic suspension of each tube dipipet as much as 0.05 mL and spread evenly on the NA rifampicin media. Then incubated for 24 hours at 29^{o}C inside the incubator. The number of growing colonies is calculated and multiplied by the dilution factor. The number of probiotic cells before and after the process was calculated using the formula expressed by Madigan *et al*. (2003), which are as follows:

Total bacteria = number of colony x 1 / Fp x1 / s Information.

Number of colonies = Number of colonies of probiotic bacteria Fp = Dilution factor (10-n)

S = Sample (mL)

3.2. Viability of Probiotics during Storage

Probiotic microcapsules are inserted into sterile bottles and stored at low temperature ($4^{O}C$). Quantitative quantification of probiotic viability was performed on day 0, 7, 14, 21, 28, and 35 th with TPC method. The viability of probiotics was calculated based on the method of Lian et al, (2002) that is the ratio of log of bacterial counts per gram after and before storage.

RESULTS AND DISCUSSION

1. Identification of *Bacillus amyloliquefacient* bacteria

The results of the identification through qualitative testing of enzyme activity on *B.amyloliquefacient* isolates grown on solid NA media containing soluble schemes indicate the presence of proteolytic activity (Fig. 1). Activity is marked by the emergence of clear zones around the colony. The clear zone is formed due to the activity of the protease enzyme that hydrolyzes the dissolved casein. The results showed that the isolation of probiotic candidate bacteria from digestive tract of grouper fish grown with NA media was obtained by isolates that have potential as probiotic bacteria. The morphology of observed *B. amyloliquefacient* bacteria includes the shape, edges, elevation and color of the colony.

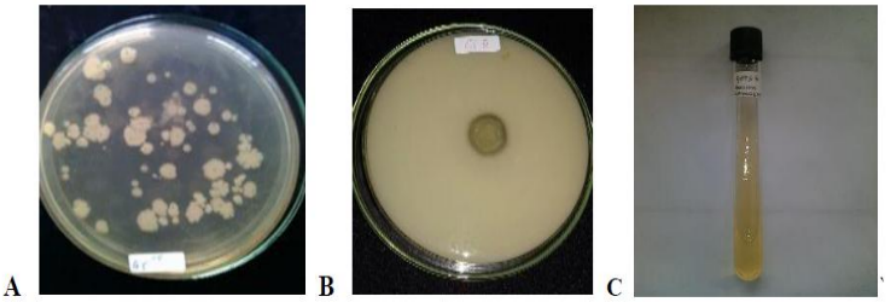

Figure 1: A. Colonies B. Clear zone, C. oblique media culture.

The quantitative protease enzyme test results were carried out in the NA medium that enzyme activity began to appear at 8 hours after incubation. Enzyme activity grown on optimal NA medium occurred at 48 hours incubation and decreased activity after incubation 48 Increase in enzyme activity has the assumption that the monosaccharide component is utilized by bacteria as a source of nutrients. In the next incubation phase there is a decrease in activity that can be caused by the accumulation of the product on one side, or there is saturation on the other side that occur together and can also occur blockage by the substrate on the active part of the enzyme so that the catalyst function stops. Each type of bacteria produces different

protease activity. *Bacteria B. amyloliquefacient* have higher activity compared with the result of *B. subtilis* modification of substrate type can be done in increasing enzyme product to be more economical in its application (Haq et al.2003). Based on the results of research conducted, it was found that the bacteria isolated from the digestive tract of tiger grouper fish can grow and develop on NA culture medium with pH 2, which is the main indicator of probiotic bacteria. Colonies of bacteria growing on the media are present in different shapes, colors, edges, sizes, and colonic (elevation) surfaces. From the observation of bacterial morphology that approximates this genus has the following morphological characteristics in accordance with Feliatra (2004), namely: the color of milk or milky white colonies, the shape of round colonies with edges of wrinkles. Cells are rod and straight, measuring from 0.5 to 2.5 x 1, 2 to 10 µm, and are often arranged in pairs or chains, with round or square edges. Gram + cell stain, motile, catalase and oxidase positive, methyl red negative, optimum at 30-37^{0}C and grow well in 1-3% NaCl. According to Holt et al., (1994), Bacillus sp. Gram + and usually motile by peritrichous flagellum. Endospores are oval, sometimes round or cylindrical and highly resistant to unfavorable conditions. They are no more than one spore per cell and sporulation can not stand the open air. This bacterium is aerobic or facultative anaerobic. Physiological abilities are diverse; very sensitive to heat, pH and salinity; kemoorganotrof with fermentation or respiratory metabolism. Normally catalase and oxidase are positive.

2. Preparation of Probiotic Microcapsules

The observation of microcapsule morphology characteristic shows that the color of microcapsule is influenced by its basic material type. Characteristic morphology of microcapsules with matrix material albumin, agar and alginate with inclusion of *B. amyloliquefacient* bacteria (Table 1).

Table 1: Characteristics of microcapsule morphology with matrix material albumin, agar and alginate with bacterial inclusion of *B. amyloliquefacient*.

Matrix type	color	Shape	Buoyancy	Moisture	Diameter
Albumin	yellow	round	< 30 minutes	5 %	0,98 mm
Agar	white	round	<30 minutes	5 %	0,96mm
Alginat	white	round	<30 minutes	5%	1,22 mm

Pahlavi et al., (2008), said that the color of the microcapsules is influenced by the color of the origin of the basic ingredients used. The same is also observed in this study, in general the color of microcapsules made like the color matrix used is clear colored agar, but after mixed

with the material core or the color inclusion of the microcapsules to be changed and the color specifications Similarly to the aroma, the aroma of the base material is completely unchanged, although it is mixed with other ingredients and undergoes changes during drying. The color of feed is usually in addition to the attraction of fish, also attract buyers. The preference of larvae and the seeds of grouper fish is strongly influenced by the color of the artificial feed given. Bright colors and contrast with the color of the water and the basic color of the place of care, can stimulate the natural instinct to hunt and catch the feed given (BBL 2004). The size and shape of the feed is generally the same, because the method used the same. In the manufacture of artificial feed, the main factor to be considered is the size of the feed, the size of the feed should be appropriate (smaller) with a fish mouth openings are cultivated. The preference of larvae to feed depends on the size and species. Each type has different abilities, in sorting and picking out preferred foods. In principle, micro feed, should have a size that corresponds to the mouth opening of the larvae, quickly digested, contains high nutritional value, potentially mass-scale culture, fast growing with high density and does not produce toxic substances (Coutteau 1996; Ponis *et al*., 2006).

The result of organoleptic observation on bacterial encapsulation of *B. amyloliquefacient* with albumin matrix, agar and alginate produced is rounded. Average size of microcapsule size with albumin, agar and alginate matrices 0,98, 0,96 and 1,22. The result of this diameter size is smaller than the result of diameter of the research, that is 2.5 mm (Krasaekoopt, 2004) but bigger than the result of size of encapsulation diameter done by Anal and Singh (2007) that is 1-4 μm. The size of the microencapsulation diameter affects the matrix's ability to protect the bacteria inside it. The diameter of the size is influenced by the concentration of marik concentration, the greater the concentration will be the greater the diameter produced this is due to the biopolymer composition used in the process of encapsulation will affect the diameter and shape produced. Drying can increase the stability of bacterial culture in encapsulation in long storage time, (Krasaekoopt et al, 2003). Other reasons for the difference in diameter size, according to the study The process of drying the probiotic encapsulation can be done in various ways, including dry spray, dry freeze (Solanki 2013) viabiltas bacteria with different matrix (Table 2) Encapsulation technique is a way to protect bacteria from factor factor environment, and increase the viability of microorganisms in the digestive tract. Encapsulation makes the environment where bacteria will survive during processing, storage, until expelled in the small intestine of the gastrointestinal tract (Chávarri et al., 2012).

The survival rate of bacterial culture before and after depends on several factors such as species, culture strain, drying conditions, inoculum, medium and coating agent used. Kailasapathy (2006) stated that encapsulated probiotic bacteria showed that bacterial inhibitor was only in 100 x count, otherwise unencapulated L. Acidophilus probiotic bacteria showed 10,000 x reduction and 1000 x decrease for B. lactis bacteria. (Kailasapathy 2006). Hariyadi (2013) says that an important factor to be able to maintain the "premium" quality of frozen-dried products is packaging. The frozen dried product has a very hygroscopic nature-easy to absorb water. Therefore, it is necessary to process the packaging and selection of appropriate packaging materials; so it will be able to protect the product from the possibility of absorbing water. To further extend shelf life, especially for oxygen-sensitive dry products, often packed with vacuum packaging. It is important to note that the packaging process should be carried out as soon as the frozen drying process is over.

Table 2: Bacterial viability of *B. amyloliquefacient* with albumin, agar and alginate matrix at storage temperature 4^{o}C.

No	Matriks	Populaton bakteria before encapsulation	Bacterial population (average) after encapsulation (days)					
			0	7	14	21	28	35
1	Albumin	6,3 x 10^{8}	5,5 x 10 7	5,4 x 10 7	5,6 x 10 7	5,6 x 10 7	5,4 x 10 7	5,2 x 10 7
2	Agar	6,3 x 10^{8}	5,3 x 10 7	5,2 x 10 7	5.4 x 10 7	5,5 x 10 7	5,2 x 10 7	5,2x 10 7
3	Alginat	6,3 x 10^{8}	5,1 x 10 7	5,0 x 10 7	5,2 x 10 7	5,3 x 10 7	5,3 x 10 7	5,0 x 10 7

CONCLUSION

Based on the morphological properties, the biochemistry and isolate isolate physiology obtained from the grouper's digestive tract (*E. fuscogutatus*) are Bacteria *B. amyloliquefacient* The highest proteolytic activity enzyme is produced by *B. amyloliquefacien* bacteria by achieving the value of the protease activity index 1.8 on 24 hours storage. Based on cell viability value of *B. amyloliquefacient* bacteria on albumin matrix encapsulation, agar and alginate with freeze dryer drying with 35 day storage time at 4 o C temperature did not significantly decrease.

BIBLIOGRAPHY

1. Anal, A.K., and Singh, H. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends Food Science and Technology,* 2007; 18(5): 240–251.
2. Arshady, R. Microspheres and Microcapsules: A Survey of Manufacturing Techniques

Part 1; Suspension cross-linking. *Polymer Engineering and Science*, 1989; 29(24): 1746–1757.

3. BBL. 2004. Pembenihan Ikan Kerapu, Departemen Kelautan dan Perimanan, Ditjen, Perikanan Budidaya, Balai Budidaya Laut Lampung, Seri Budidaya Laut, 13: 106pp.
4. Chávarri, M., Izaskun Marañón dan María Carmen Villarán. 2012. Encapsulation technology to protect probiotic bacteria. *In Probiotics Chapter 23 InTechDOI: 10.5772/50046*.
5. Chibata, I. Immobilized microbial cells with polyacrylamide gel and carragenan and their industrial application, In immobilized cells (ed K. Venkatsubramanian) Am. Chem. Soc. Symp. Ser, 1981; 106: 187-202.
6. Coutteau P. Micro-Algae. In: Lavens P, Sorgeloos P. Manual on The Production and Used of Live Food for Aquaculture. FAO Fisheries Technical Paper, 1996; 361(2): 7-43.
7. Ding. W.K., and Shah N.P. Effect of Various Encapsulating Materials on the Stability of Probiotic Bacteria. Journal Of Food Science. 2009; 74: 100-107.
8. FAO/WHO. 2002. Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada, April 30 and May 1.
9. Feliatra., Efendi, I., dan Suryadi, E. Isolasi danIdentifikasi Bakteri Probiotik dari Ikan Kerapu Macan(*Ephinephelus fuscogatus*) dalam Upaya Efisiensi PakanIkan. Jurnal Natur Indonesia. 2004; 6(2): 75-80.
10. Hariyadi, P. 2013. Pengeringan Beku dan Aplikasinya di Industri Pangan. Diunduh dari http://seafast.ipb.ac.id/lectures/ itp530/11-itp%20530-pengeringan-beku. Pdf
11. Harmayani, Erni, Ngatirah, Endang S. Rahayu, dan Tyas Utami. Ketahanan dan viabilitas probiotik bakteri asam laktat selama proses pembuatan kultur kering dengan metode freeze dan spray drying. Jurnal Teknologi dan Industri Pangan 2001; XII(2): 126-132.
12. Marlina. 2008. Identifikasi bakteri *Vibrio parahaemolitycus* dengan metode *BIOLOG* dan deteksi Gen *ToxR* nya Secara PCR. *Sains dan teknologi farmasi,* 13.
13. Kailasapaty, K. Microencapsulation of probiotic bacteria: technology and potential applications. *Curr. Issues Intest. Microbiol*. 2002; 3: 39-48.
14. Krasaekoopt, W., Bhandari, B., and Deeth, H. Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 2003; 13: 3– 13.
15. The influence of coating materials on some properties of alginate MLN and survivability of microencapsulated probiotic bacteria. International Dairy Journal, 2004; 14: 737–743.
16. Pahlevi, Y,W,, T, Estiasih dan E, Saparianti, Mikroenkapsulasi Ekstrak Karoten Dari

Spora Kapang Oncom Merah (Neurospora sp,) Dengan Bahan Penyalut Berbasis Protein Menggunakan Metode Pengeringan Semprot, Jurnal Teknologi Pertanian 2008; 9(1): 31-39.

17. Ponis E, Probert I, Veron B, Le Coz J.R, Mathieu M, and Robert R. Nutritional value of six Pavlovaceae for Crassostrea gigas and Pecten maximus larvae. Aquaculture 2006; 254: 544- 553.
18. Solanki 2013 Development of mikrocapsulation delivery system for along -term. Preservation of probiotics as biotherapentic V.2013. artile Id 620719, 21 p.

29-Viability Bacteria_wjpps_1564562236

ORIGINALITY REPORT

8%	7%	2%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.pjbs.org Internet Source	3%
2	Submitted to University of California, Merced Student Paper	3%
3	www.slideshare.net Internet Source	1%
4	www.intechopen.com Internet Source	1%
5	central.bac-lac.gc.ca Internet Source	1%
6	journal.lemigas.esdm.go.id Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		