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Probiotic Microbes and Their Growth in Different Carriers (A Review)

Yuli Andriani [a*], Rusky I. Pratama [a] and Ratu Safitri [b]

[a] Department of Fisheries, Faculty of Fisheries and Marine Science, Padjadjaran University, Sumedang Regency, West Java, Indonesia.
[b] Department of Microbiology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Sumedang Regency, West Java, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Probiotics have been utilized as an option to treat problems brought on by safe and ecologically friendly pathogenic microorganisms in shrimp and fish farming operations. Probiotics are currently available in powder form (dry form). Using spray drying technology, dry probiotics are produced, with the finished result being a stable powder. In order to regulate the proliferation of harmful bacteria in the digestive tract, these bacteria use a competitive mechanism. *Bacillus* sp., *Saccharomyces cereviceae*, and *Lactobacillus* sp. are among the commonly researched probiotic microorganisms. Microorganisms are blended with a number of carriers, including maltodextrin, wheat flour, and rice flour. The carrier material's main goal is to increase storage process survival. Numerous studies have demonstrated that the viability and development of the probiotic microorganisms included in each carrier material varied. The enclosed substance exhibits highly varied microbial growth. The maximum number was found in the maltodextrin carrier material for the bacteria *B. licheniformis* and *B. subtilis*, the rice flour carrier for the bacteria *L. brevis* and *L. curvatus*, and the talc carrier for the bacterium *L. bulgaricus*. These findings suggest that, following encapsulation, probiotic populations and numbers can be maintained in all types of carrier materials. Specific carriers, like tapioca, skim milk, and activated carbon, revealed a greater microbial population than the typical medium. The microbial cells were compressed in the dry carrier material, which is why there was an increase of bacteria following the encapsulation

**Corresponding author: Email: yuli.andriani@unpad.ac.id, yuliyusep@yahoo.com;*

procedure. Probiotic microbe counts increased by 40.7-90.1% for carriers made of activated carbon, 79-94.5% for those made of skim milk, and 31.7-95.7% for those made of tapioca flour. Bacterial cell death during the spray drying process of encapsulation could be the reason for the decline in the population of probiotic bacteria.

Keywords: Aquaculture; carrier; fisheries; probiotic.

1. INTRODUCTION

Fish farming activities or commonly refer as aquaculture currently apply many additional commodities to increase their productivity, one of which is the use of probiotics. Probiotics are live microbial agents that are able to provide benefits to the host by modifying the microbial community or associating with the host, thereby increasing the host's response to disease, and improving environmental quality. Furthermore, probiotics have been used as an alternative in controlling diseases caused by pathogenic microorganisms that are safe and environmentally friendly in fish and shrimp farming activities. Probiotics are defined as organisms that contribute to the balance of microbes in the intestine. According to [1], probiotics will be effective if they are able to survive well in several environmental conditions and survive in several forms of packaging. The characteristics of effective probiotics are that they can be packaged in live forms on an industrial scale, are stable and live for long periods of storage and field conditions, can survive in the intestine and are beneficial [2].

The use of probiotics in the field of fisheries is done by adding probiotics in cultivation media (water) or mixed into feed. The benefits of probiotics in aquaculture activities are: 1) As a biocontrol agent to reduce disease attacks, 2) Bioremediation to improve environmental quality, and 3) Increase the nutritional value of feed and the rate of nutrient absorption of fish or shrimp. Application of probiotic bacteria can be done with artificial feed and natural feed enrichment [3].

A microorganism is said to be a probiotic if it meets several criteria, as follows: (a) a probiotic must be non-pathogenic, which represents normal gut microbes from a particular host, and is still active in conditions of gastric acid and high concentrations of bile salts in the small intestine, (b) a good probiotic must be able to grow and metabolize quickly, so it is present in high amounts in the intestine around 10^6-10^8 CFU/ml, (c) an ideal probiotic can attach to and colonize in some parts of the intestinal tract. This will increase competition with pathogenic microbes and carcinogenic causes, (d) probiotics can produce organic acids efficiently and have antimicrobial properties against harmful bacteria, (e) coaggregation forms a normal and balanced microflora environment, (f) can stimulate increased immune defense, (g) easy to produce, able to grow in large-scale production systems, and survive during storage conditions [4].

Currently, the supply of probiotics in dry form (powder) has been developed. The production of dry probiotics is carried out using spray drying technology, where drying is carried out with a relatively high drying temperature with an inlet temperature of 110^{o}C and an outlet temperature of 68^{o}C, a short drying time, and the final product is a stable powder [5]. The spray drying technique converts food ingredients that are initially liquid into solid materials. The product is able to last longer because the water content is minimized thereby reducing the risk of damage to foodstuffs by microbes [6]. There are benefits and drawbacks to using spray drying. According to [7], this method has the following benefits: it is simple to use, capable of producing large quantities of capsules, and the coating material used can also be used as an ingredient in food because it is water soluble and can release the core material without any coating material precipitating. The weakness of the spray drying technique is that the humidity is quite high which causes the high process temperature required.

Encapsulation is a coating technique for a material so that the coated material can be protected from environmental influences. Encapsulation is said to be successful if the encapsulated material has relatively high cell viability and relatively the same physiological properties as before encapsulation. Previous research on the encapsulation of probiotics has been carried out by several researchers with various variations of encapsulated materials and cultures, including: encapsulation of *Bifidobacteria* and *Lactobacillus* with alginate-starch, *Lactobacillus casei* with alginate-polard flour and wheat [8].

According to [9], the application of encapsulation techniques to the protection of probiotic strains will result in high viability of microorganisms in food products and in the digestive tract. Both encapsulation techniques showed effectiveness in keeping the number of probiotic bacteria higher than the minimum level (>10^7 cfu/ml). Meanwhile, the number of non-encapsulated probiotic bacteria decreased by approximately 10^3 CFU/ml.

The advantages of probiotic products in dry preparations (powder) are that they can be distributed further between islands, have an efficient storage area and have a longer shelf life. Efforts to stabilize cells, increase the survival and stability of bacteria in production, and storage can be done by encapsulation techniques, namely coating with a carrier material. Various carriers can be used to make dry preparations of probiotics, such as maltodextrin, wheat flour, alginate, talc, and so on. Each carrier material has a different ability to support the viability of probiotic microbes. This carrier material aims to maximize survivability during the storage process. That way, if the probiotic survival ability is high, the growth of fishery commodities will be optimal. This paper intends to conduct a literature study on the growth of probiotic microbes in various carrier materials.

2. TYPES OF PROBIOTIC MICROBES

Probiotics are dietary supplements that contain beneficial bacteria needed for the repair of the gastrointestinal tract. Probiotics can also be interpreted as active products, defined by microorganisms in sufficient quantities by changing the microflora through cultivation or through colonization in the host and having health benefit implications for the host [10]. The increase in the population of harmful bacteria can cause a decrease in the growth rate of the host [11]. The increase in growth rate is thought to be due to the contribution of digestive enzymes by probiotic bacteria which can increase digestive activity. In the process of increasing digestive activity, probiotics have a mechanism to produce several exogenous enzymes for feed digestion such as amylase, protease, lipase, and cellulase [12].

The current use of probiotics, replaces the use of antibiotics. Probiotics are a form of biological control by using biological enemies for pathogenic bacteria to reduce disease populations. Utilizing antagonistic bacteria through its mechanism, by producing bacteriocin compounds that can damage the cytoplasmic structure of pathogenic bacteria so as to minimize their survival in the host body. The basic principle of working probiotics is the utilization of the ability of microorganisms to break down or decompose long chains of carbohydrates, proteins and fats that make up feed. This ability is obtained due to the presence of special enzymes possessed by microbes to break these bonds. This enzyme is not owned by fish and other aquatic creatures. The breakdown of these complex molecules into simpler molecules will facilitate further digestion and absorption by the digestive tract of fish. On the other hand, microorganisms that break down these molecules benefit in the form of energy obtained from the breakdown of these complex molecules [13].

Probiotic bacteria are non-pathogenic microorganisms that have a positive influence on the physiology and health of the host. One of the commonly used lactic acid bacteria as probiotics is *Lactobacillus* [14]. Some of the criteria possessed by probiotic bacteria are being able to live and be stable in storage, more beneficial to the host, able to survive in the ecosystem, and not being pathogenic. This bacterium also has criteria as a digestive tract probiotic, because it has advantages in terms of its ability to live at low pH and tolerance to bile salts [15].

Probiotic bacteria work with a competition mechanism, namely by controlling the growth of pathogenic bacteria in the digestive system. Adhesive and colonizing in tissues, probiotics have been shown to be able to prevent pathogens from entering and specifically block receptor cells, especially in food-destroying bacteria such as *Bacillus cereus, Escherichia coli* and *Salmonella typhimurium* which cause diseases of the digestive system [16,17].

In general, probiotic bacteria live in the digestive tract and are of good quality with their host body, live at pH 2-4, do not cause negative things to the body, are not pathogenic, generally do not form spores, saccharolytic, generally anaerobic, do not interfere with the body's ecosystem, live and grow in the intestine [18].

According to [19] probiotics are live microbial agents that are able to provide benefits to the host by modifying the microbial community or associating with the host, improving nutritional value and feed utilization, increasing host response to disease, and improving environmental quality thresholds. Based on this understanding, the application of probiotics does not only function as a biocontrol agent to reduce disease attacks or bioremediation to improve environmental quality, but can also increase the nutritional value of feed and the rate of nutrient absorption. [20] stated that the group of microbes categorized as probiotics consist of *Bacillus* sp., *Saccharomyces cereviceae*, and *Lactobacillus* sp.

Lactobacillus sp.

The microbes applied in probiotics are from the *Lactobacillus* group (*Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus acidophilus*). *Lactobacillus* belongs to the group of lactic acid bacteria that are often discovered in fermented foods, processed fish products, meat, milk, and fruits. Thus far, it has been known that the presence of this bacterium is not pathogenic and safe for health, hence it is often used in the food and beverage preservation industry and has the potential as a probiotic product. These bacteria act as normal flora in the digestive system. Its function is to maintain the balance of acids and bases so that the pH in the colon is constant [21].

Sources of probiotics derived from aquatic organisms have been widely studied for use in aquaculture activities. Some of the probiotics studied were from the group of lactic acid bacteria, for example *Lactobacillus* sp. *Lactobacillus* sp. has great potential as a probiotic product because of its advantages over other lactic acid bacteria. [21] stated that *Lactobacillus plantarum* can be active at low pH and produce lactic acid in large quantities so that animal feed can help store energy. *Lactobacillus acidophilus* and *Lactobacillus fermentum* produce antibacterial compound that can inhibit the growth of pathogenic bacteria. *Lactobacillus* isolates have the ability to inhibit pathogenic bacteria because they produce antimicrobial compounds such as H_2O_2, lactic acid and bacteriocin that can damage the cell walls of pathogenic bacteria. Pathogenic bacteria that can be inhibited by *Lactobacillus* are *Aeromonas* sp. and *Vibrio* sp. [22].

Lactobacillus has the characteristics of rod-shaped cells with very diverse sizes and shapes, some can be very long and some are round rods. *Lactobacillus plantarum* is a type of homofermentative LAB (lactic acid bacteria) with an optimal temperature lower than 37°C. These bacteria are able to digest protein, tolerate acid and are able to produce lactic acid. Under acidic conditions*, L. plantarum* has the ability to inhibit pathogenic bacteria, spoilage bacteria and produce bacteriocin which functions as an antibiotic substance. *L. acidophilus* is resistant to gastric digestive enzymes, bile acids and in the digestive tract can also inhibit the growth of pathogenic bacteria [23].

Bacillus sp.

Bacillus sp. are rod-shaped bacteria, classified as Gram-positive bacteria in early cultures and will become Gram-negative bacteria when entering the stationary phase of growth, motile, producing spores (endospores) which are usually resistant to heat, aerobic (some facultative anaerobic species), catalase positive, and oxidation varies, and includes saprophytic heterotrophic bacteria that require organic matter to meet their energy sources. Most *Bacillus* belong to the group of mesophilic bacteria that grow at optimal temperatures between 25º-45ºC. Several bacteria from the genus *Bacillus* used were *Bacillus subtilis, Bacillus licheniformis* and *Bacillus polymyxa.*

In aquaculture activities, *Bacillus* sp. used as a bio-control agent (probiotic) because it has the ability to degrade organic compounds and use them to support growth. This is because, *Bacillus* sp. has proteolytic enzymes produced extracellularly that play a role in breaking down proteins and also has lipolytic enzymes that play a role in breaking down fats so that they are able to degrade organic waste that is broken down into simpler compounds.

In several studies it was found that the addition of *B. subtilis* waters can improve water quality by reducing the concentration of CO_2 in the waters. The application of *B. subtilis* in shrimp ponds showed that *B. subtilis* was able to increase the survival of tiger shrimp larvae and prevent vibriosis caused by *Vibrio harveyi*. In addition, *B. subtilis* is naturally symbiotic in the digestive tract of tiger prawns. Bacillus sp. on rearing tiger shrimp larvae have a positive influence on shrimp growth because the bacteria and enzymes produced will also enter the shrimp body and help the digestive process in the shrimp digestive tract [24].

Saccharomyces cerevisiae

Saccharomyces is a unicellular fungus also called yeast, spherical or oval in shape, 5-12 micron in size, multiplies to form buds, and when mature will split into stem cells. The structure has a thick polysaccharide wall that covers the protoplasm. [25] stated that the general benefits obtained from live *Saccharomyces* culture are increasing body weight gain, ration efficiency, and feed intake. *Saccharomyces cerevisiae* can also be used as a probiotic. The yeast is used to improve livestock health, namely as a probiotic and immunostimulant. The use of *S. cerevisiae* will increase the number of beneficial microbes, in contrast to antibiotics. In addition, *S. cerevisiae* can also be used to increase the defense system against diseases caused by bacteria, fungi and viruses.

Another advantage of using *S. cerevisiae* as a probiotic is that it does not eradicate microbes and even increases the number of beneficial microbes, in contrast to antibiotics that can annihilate microbes that are both harmful and beneficial to the body, and have a resistance effect. Similarly, the use of *S. cerevisiae* as an immunostimulant which function to improve body health by increasing the defense system against diseases caused by bacteria, fungi, viruses and others, while the use of antibiotics only kills bacteria. Figure 1. shows three types of probiotin microbes according to [2] and [26].

3. MICROBIAL GROWTH IN VARIOUS CARRIERS

There are several carriers mixed with microorganisms, including rice flour, wheat flour and maltodextrin. This carrier material aims to maximize survivability during the storage process. Carrier material is a material that contains a lot of food substances in it which serves as a protector and will produce a large number of cells. Rice flour, wheat flour and maltodextrin can be used as carriers because they are safe for food, soft, and non-toxic. In addition, the properties of the three carrier materials are able to absorb water. Most of these products are in dry form. The ability of the carrier material to absorb water can reduce the risk of food damage by microbes [2].

The carrier material in the encapsulation process is very important to note. [27], stated that the coating material for spray drying will affect the resulting product, depending on the physical and chemical properties of the bacterial carrier emulator, drying conditions and spray drying used. The final product of dry probiotics is microencapsulated with the advantage of protecting the core material by the use of a coating agent. Dry powder from spray drying which contains a large number of live microorganisms is a suitable form for storage purposes and applications in the development of functional foods [28].

Maltodextrin and talc carrier materials have sufficient nutritional value to meet the needs of probiotic bacteria after encapsulation. Maltodextrin is often used as a coating material because of its good ability to inhibit oxidation reactions so that the resulting microcapsules have a better shelf life [29]. Meanwhile, talc is able to affect the viability of bacteria and is a good formulation in the storage period of probiotic bacteria [30]. Activated carbon functions more as a toxic absorber in the digestive tract, besides that in the fields of fisheries and aquaculture, this activated carbon can be used for purification, removal of ammonia and nitrite, phenol and other heavy metals. The use of a variety of these carriers will optimize the growth of vanamei shrimp [31]. Several studies have shown that each carrier material has a different ability to support the viability and growth of the probiotic microbes contained in it. The following are the results of a literature search regarding the growth of probiotic microbes in various encapsulation materials [32, 33].

Fig. 2 and Fig. 3 show that the microbial growth in the encapsulated material is highly variable. In bacteria *B. licheniformis* and *B. subtilis*, the highest number was shown in maltodextrin carrier material, while in *L. brevis* and *L. curvatus* bacteria, the highest number was shown in rice flour carrier, and *L. bulgaricus* bacteria on talc carrier. These results indicate that all types of carrier materials can maintain the population and number of probiotics after the encapsulation process.

Particular carriers such as activated carbon, skim milk and tapioca showed a higher microbial population than the standard medium (Fig. 2). The increase in the number of microbes after the encapsulation process occurred because the microbial cells were compacted in the dried carrier material. The increase in the number of probiotic microbes was 40.7-90.1% for activated carbon carrier, 79-94.5% for skim milk carrier, and 31.7-95.7% for tapioca flour carrier. The carbon content in activated carbon, protein in skim milk, and carbohydrates in tapioca flour are a source of nutrition for probiotic microbes, hence in addition to maintaining the number of probiotic microbes, the carrier material can also play a role in increasing the number of probiotic microbes during the encapsulation process. Meanwhile, in rice flour, maltodextrin and talc microbial growth was lower than in standard medium. The decrease in the population of probiotic bacteria could be caused by bacterial cell death during the encapsulation process using the spray drying technique. The spray drying process occurred under conditions that were not in accordance with growth tolerance of probiotics *Bacillus* sp. and *Lactobacillus* sp. The drying process occurs at an inlet temperature of 100°C ± 1°C and an outlet temperature of 50°C ± 1°C, while these bacteria have an optimal growth temperature of 15-45°C [34]. *Bacillus* sp. and *Lactobacillus* sp. bacteria are mesophilic bacteria and not thermophilic, therefore they do not have stable proteins at high temperatures. When cells are exposed to high heat due to imperfect encapsulation, proteins will be damaged thus the cells die. In addition, the cells that have been completely encapsulated are able to survive during the spray drying process [6]. The decrease in the number of cells after spray drying can also be caused by dehydration and inactivation of bacterial cells due to heat. The decrease in viability may vary depending on the bacterial culture strain, treatment and composition of the encapsulation material [35]

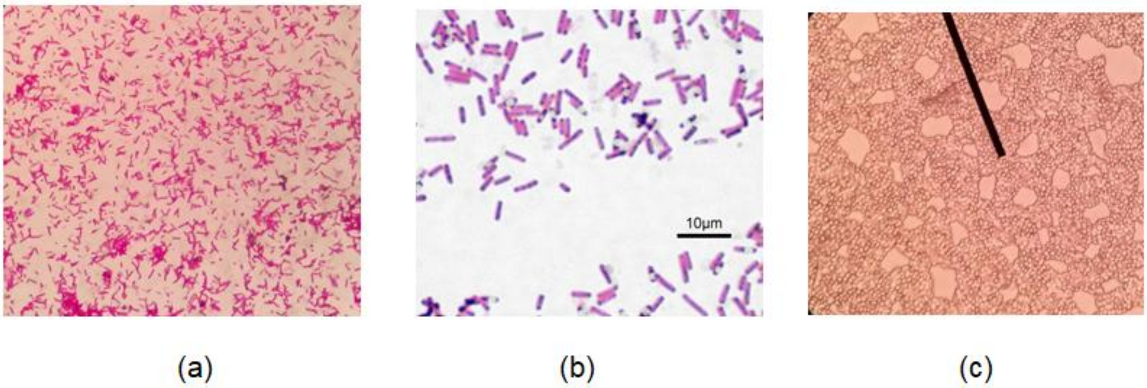

Fig. 1. Probiotic microbes: (a) *Lactobacillus plantarum* (1000x magnification), (b) *Bacillus mesentericus,* (c) *Sachharomyces cerevisiae* in 100x magnification

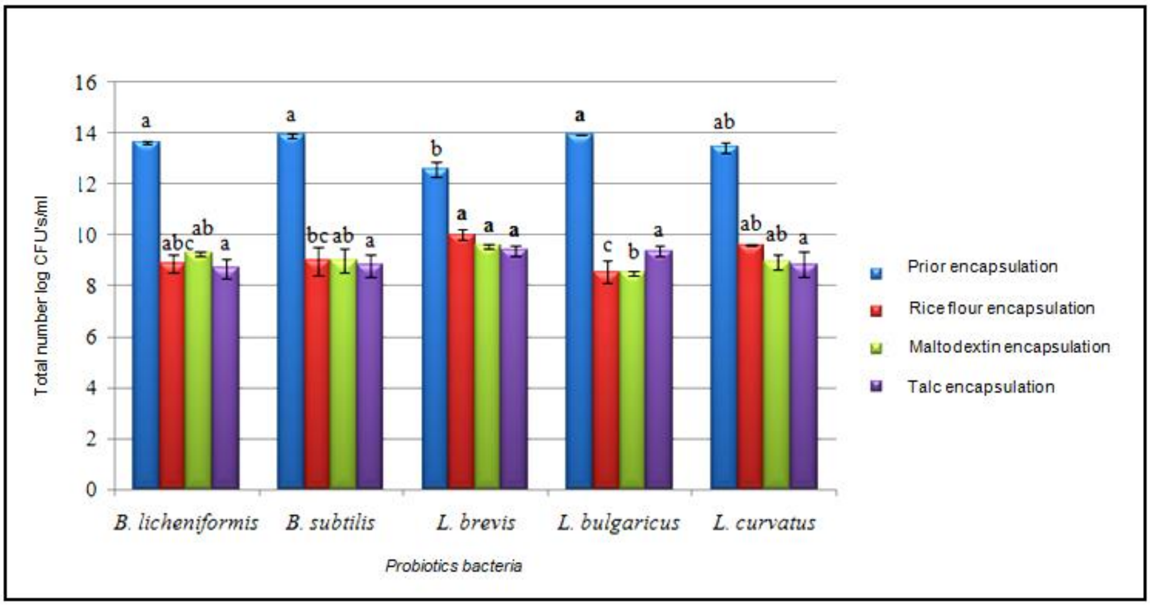

Fig. 2. Growth of *Bacillus* sp. and *Lactobacillus* sp. before and after encapsulation in rice flour, maltodextrin and talc

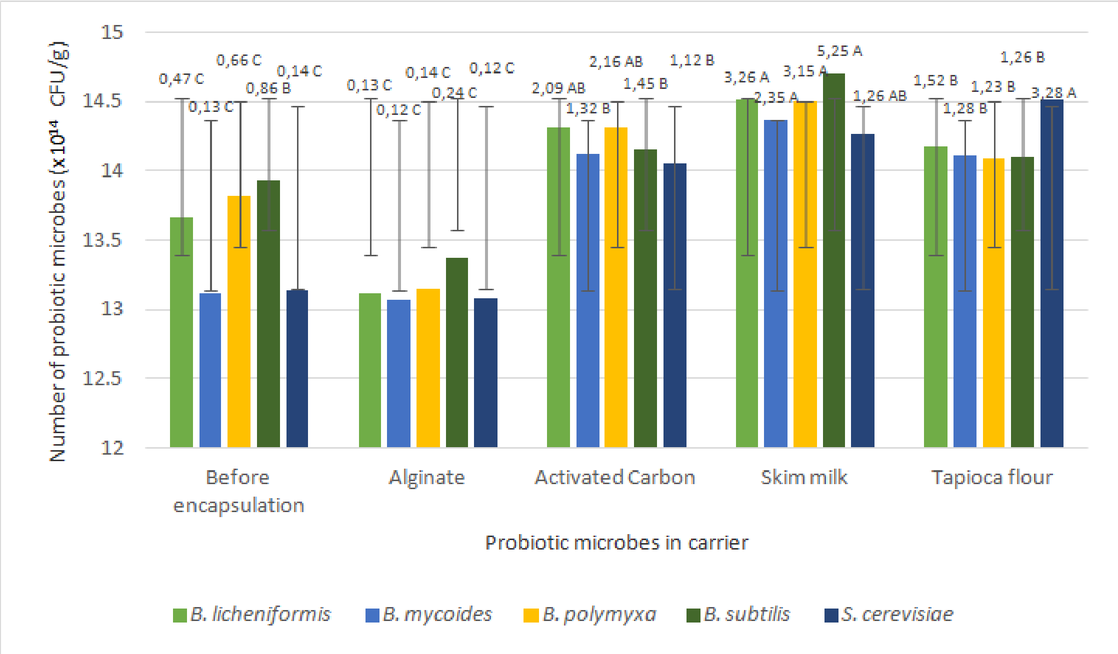

Fig. 3. Number of probiotics (CFU/g) *Bacillus licheniformis*, *B. mycoides*, *B. polymyxa, B. subtilis*, and *Saccharomyces cerevisiae* before and after encapsulation in alginate, activated carbon, skim milk and tapioca

4. CONCLUSION

The growth of probiotic microbes varies in different carrier materials. The ability of the carrier material to support microbial growth is influenced by several other things such as the physical and chemical properties of the bacterial carrier emulator, the drying conditions and the spray drying used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Manin F. Potential of *Lactobacillus acidophilus and Lactobacillus fermentum* from the Digestive Tract of Free-range Chicken from Peatland as a Source of Probiotics. Scientific Journal of Animal Sciences. 2010;8(5).
2. Solichah A. Effects of the Consortium of *Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus plantarum and Saccharomyces cerevisiae* in Several Carriers with Spray Drying Technique on the Probiotic Character and Performance of Vannamei Shrimp (*Litopenaeus vannamei* Boone). Bachelor Thesis. Faculty of Mathematics and Natural Sciences, Padjadjaran University. 2016.
3. Rengpipat S, Rukpratanporn S, Piyatitivorakul S, Menasaveta P. Probiotics in aquaculture: a case study of probiotics for larvae of black tiger shrimp (*Penaeus monodon*). In: Flegel TW, editor. Advances in Shrimp Biotechnology 1998;177-181.
4. Suskovic, J, Kos B, Goreta J and Matosic S. Role of Lactic Acid Bacteria and Bifidobacteria in Symbiotic Effect. J. Food Technol. Biotechnol. 2001;39(3):227-235.
5. Hayati SN, Herdian H, Damayanti E, Istiqomah L and Julendra H. Amino Acid Profile of Encapsulated Earthworm (*Lumbricus rubellus*) Extract by Spray Drying Method. Indonesian Journal of Technology. 2011;34.
6. Triana E, Yulianto E and Nurhidayat N. Viability Test of *Lactobacillus* sp. March. 8 Encapsulated. Biodiversity. 2006;7(2):114-117.
7. Nurhasanah S, Komari, Hariyadi P and Budijanto S. Microencapsulation of DHA Rich Fats for Food Fortification. Bionatura Journal of Life and Physical Sciences. 2011;13(2):140-149

8. Rizqiati H, Jenie BSL, Nurhidayat N, and Nurwitri CC. Resilience and viability of the probiotic *Lactobacillus plantarum* encapsulated with skim milk and gum arabic after drying and storage. Journal Animal Production. 2008;10(3):179-187.
9. Petrovic GM, Stojavonic GS, Kadulovic NS. Encapsulation of Cinnamon Oil in Cyclodextrine. Journal Med Plants Res. 2007;14:1382-1390
10. Gayathri D, Asha and Devaraja TN. *Lactobacillus* sp. as Probiotics for Human Health with Special Emphasis on Colorectal Cancer. Indian Journal of Science and Technology. 2011;4(8)
11. Skjermo J, Salvesen I, Øie G, Olsen Y, and Vadstein O. Microbially Matured Water: a Technique for Selection of a Nonopportunistic Bacterial Flora in Water That May Improve Performance of Marine Larvae. Aquac. Int. 1997, 5: 13–28.
12. Taoka Y, Maeda H, Yoon J, Sakata T. Influence of Comercial Probiotics on The Digestive Enzyme Activities of Tilapia, *Oreochromis Niloticus*. Aquaculture Science. 2007;55(2):183-189.
13. Mustisar, Irwan JE, and Kadir E. Effects of Different Dosage and Enrichment Time of *Lactobacillus calseii* on Survival of the Zoea Stage of Crab Crab (*Portunus pelagicus*). Journal of Mitra Laut Indonesia. 2013;2(6):26-34
14. Nagao F, Nakayama M, Muto T and Okumura K. Effects of fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. Bioscience Biotechnology and Biochemistry. 2000;64(12):2706-2708.
15. Napitupulu RNR, Yulinery T, Hardiningsih R, Kasim E, and Nurhidayat N. Cholesterol binding capacity and organic acid production of selected *Lactobacillus* isolates for cholesterol lowering. Proceedings of the Indonesian Society of Microbiology Annual Scientific Meeting. Bandung, 29-30 August 2003.
16. Schrezenmeir J. and de Vrese M. Probiotics, prebiotics andsymbiotics-approaching a definition. American Journal of Clinical Nutrition. 2001;73 (suppl): 361S-364S
17. Sudarmo SM. The role of probiotics and prebiotics in the prevention and treatment of diarrhea in children. Proceedings of the Second National Congress of the Coordinating Board for Pediatric Gastroenterology. Bandung Indonesia; 2003.
18. Fuller, R. A review, Probiotics in Man and Animals. Journal of Applied Bacteriology. 1989;66: 365-378.
19. Widanarni, Puguh W, and Dinamella, W. Application of Probiotics, Prebiotics, and Synbiotics through Feed on Vaname Shrimp (*Litopenaeus vannamei*) Infected with Vibrio harveyi Bacteria. Indonesian Journal of Aquaculture. 2012;11(1):54-63
20. Linggarjati KF, Djunaedi A, and Subagiyo. Test Use of *Bacillus* sp. as a Probiotic Candidate for Rearing Crayfish (*Portunus* sp.). Journal of Marine Research. 2013;2(1):1-6.
21. Hardiningsih R, Napitupulu RNR, and Yulinery T. Isolation and Resistance Test of Several *Lactobacillus* Isolates at Low pH. Biodiversity. 2006;7(1):15-17.
22. Achmad DI, Nofiai R, Ardiningsih P. Characterization of Lactic Acid Bacteria *Lactobacillus* sp. Red from Cincalok Formulation. Tanjungpura University: Tanjungpura; 2010.
23. Syachroni. Effect of Starter Culture Combination of *Lactobacillus plantarum and Lactobacillus acidophillus* on Microbiological and Chemical Characteristics of Fermented Drinks. Thesis. Animal Products Technology Study Program, Faculty of Animal Husbandry, Hasanuddin University. Makassar; 2014.
24. Alamsyah S. Increasing the Role of Digestive Tract Microbes to Stimulate the Growth of Milkfish. Dissertation. Bogor Agricultural University Postgraduate Program. Bogor; 2006
25. Haetami K, Abun, and Mulyani Y. Study of Making Probiotics as a Feed Supplement and Its Implications on the Growth of Red Tilapia. Faculty of Fisheries and Marine Science. Padjadjaran University; 2008.
26. Supplement Global LLC. *Bacillus mesentericus*. Accessed: 3 July 2022. Available: https://www.supplementsglobal.com/product/bacillus-mesentericus/
27. Young SL, Sarda X, and Rosenberg M. Microencapsulating properties of whey proteins with carbohydrates. Journal of Diary Science. 1995;76:2878-2885.
28. Anal AK and Singh H. Recent Advances in Microencapsulation of Probiotics for Industrial Applications and Targeted Delivery. Trends in Food Science and Technology. 2007;18:240-251.

29. Gharsallaoui A, Roudaut G, Chambin O, Voilley A, and Saurel R. Applications of Spray Drying in Microencapsulation of Food Ingredients: An Overview. Food Res Intern, 2007, 40:1107–1121
30. Sulistiani. Formulation of *Bacillus subtilis* Spores as Biological Agents and PGPR (Plant Growth Promoting Rhizobacteria) on Various Carriers. Bachelor Thesis Bogor: Bogor Agricultural University; 2009.
31. Sembiring H. Diversity and Distribution of Shrimp and Its Relation to Physical and Chemical Factors in Labu Coastal Waters, Deli Serdang Regency. University of North Sumatra: Medan; 2013.
32. Kanza AA. Characterization of *Bacillus and Lactobacillus* Probiotics Encapsulated in Several Carrier Materials and Their Effect on the Performance of Vannamei Shrimp (*Litopenaeus Vannamei* Boone, 1931). Bachelor Thesis. Faculty of Mathematics and Natural Sciences, Padjadjaran University; 2016.
33. Fahrudin SD. Effect of Encapsulation of the Consortium of *Bacillus Licheniformis, Bacillus Mycoides, Bacillus Polymyxa, Bacillus Subtilis* and *Saccharomyces cerevisiae* in Various Carrier Materials on Probiotic Character and Performance of Vannamei Shrimp (Litopenaeus Vannamei Boone, 1931). Bachelor Thesis. Faculty of Mathematics and Natural Sciences, Padjadjaran University; 2016.
34. Magfirah, Budji RG, and Sartini. Viability Test of Probiotic Isolates from the Digestive Tract of Anas domesticus Broiler Ducks Encapsulated by Spray Drying Method. Thesis. Department of Biology, FMIPA, Hasanuddin University. Makassar; 2015
35. Risqiati H, Jenie BSL, Nurhidayat N, Nurwitri C. Resistance and Viability of *Lactobacillus plantarum* Encapsulated with Skim Milk and Gum Arabic After Drying and Storage. Animal Production. 2008; 179-187.

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