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Method Study And Stability Testing on Liquid Probiotic Products at CV. Pradipta Paramita, Karanganyar Regency, Central Java

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Abstract

Evapond *Bacillus* is a liquid probiotic product developed to enhance the health of fish and shrimp. Maintaining its quality can be achieved through encapsulation using coating materials. This study applied an experimental method with three treatments and one replication. The encapsulation material concentration was set at 10% of the liquid volume, with variations in the ratio of maltodextrin and guar gum, namely (70%:30%), (50%:50%), and (60%:40%), each prepared in 100 ml of water. The experiment was conducted for two months to evaluate product stability after encapsulation. The results showed that the combination of maltodextrin and guar gum did not significantly affect the stability of Evapond *Bacillus*. However, the (60%:40%) ratio demonstrated relatively better performance compared to other treatments. These findings indicate that although the differences were not statistically significant, certain compositions may provide more favorable outcomes. Further studies are recommended using additional or alternative encapsulation materials to obtain more optimal and applicable results.

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Introduction

Probiotics are live microbial agents that provide benefits to their hosts by modifying the microbial community or by associating with the host, increasing disease resistance, improving nutrition, and feed utilization (Rafsyanzani & Hidayatullah, 2016). One application of probiotic products is Evapond *Bacillus*. This product is a probiotic product for maintaining and improving the health of fish and shrimp in liquid form. The quality of a product must meet applicable standards before being marketed. Good product stability should always be maintained in its initial state. Another solution offered to maintain product quality is the application of a coating agent (microencapsulation).

Encapsulation is a technique for coating a material to protect it from environmental influences. Encapsulation technology is widely used in the food, pharmaceutical, and fisheries sectors to improve stability and extend shelf life. With

a protective layer, probiotic cells are expected to survive better during storage and application in aquaculture environments. Several studies have reported the use of maltodextrin and guar gum in the microencapsulation of probiotics and other bioactive compounds (Sumanti *et al*., 2016). However, in many studies, the combination of these two materials is generally used with other additives, so the composition of each material cannot be specifically observed. Therefore, research on the direct use of the combination of maltodextrin and guar gum is still needed to determine its effectiveness as a probiotic encapsulation material.

This study evaluated the characteristics and optimal concentration of the probiotic Evapond *Bacillus* encapsulated with maltodextrin and guar gum. The encapsulation process protects the probiotic cells from environmental conditions that can reduce their viability during storage. Both materials were tested based on several parameters. These parameters included product stability, the number of viable bacteria, and the physical characteristics of the probiotic preparation. The results of this study are expected to provide information on optimal encapsulation formulations to improve the stability and quality of probiotic products used in aquaculture.

Materials and methods

Time and Place of Research

This research was conducted from October to December 2025 at the Biology and Chemistry Laboratory of CV Pradipta Paramita. The company is located in Waru Hamlet, RT 03, RW 04, Pulosari Village, Kebakkramat District, Karanganyar Regency, Central Java, Indonesia.

Research Design

This study used an experimental method to determine the concentration of the coating agent in the encapsulation of the probiotic Evapond *Bacillus*. This study used 3 treatments and 1 repetition. Each treatment had a different concentration. The concentration used was 10% of the liquid volume; the ratios for maltodextrin and guar gum were (70%: 30%), (50%: 50%), and

Table 1. Ingredients composition

Treatment	Maltodextrin	Guar gum
1	7 gram	3 gram
2	5 gram	5 gram
3	6 gram	4 gram

(60%: 40%), respectively, with a water volume of 100 ml (Pinto *et al*., 2021). The treatments were carried out once a week for 2 months. The test parameters in this study were organoleptic tests of color, odor, and swelling. Furthermore, the pH test used a pH meter, and the TPC test used a dilution of 10^6 using the pour plate method.

Research Procedures

Encapsulation Preparation

Maltodextrin and guar gum encapsulation was carried out according to established procedures. The encapsulation material composition was weighed at 7:3, 5:5, and 6:4 for each treatment, then mixed with 10 ml of distilled water and sterilized. 90 ml of liquid probiotics were added to the encapsulation material and vortexed for 5-10 minutes. The encapsulation and probiotic mixture was stored at room temperature.

Media Preparation

The stages of the Evapond *Bacillus* product stability test begin with the preparation of tools and media first. Petridish is sterilized using 96% alcohol and wrapped with aluminum foil, then oven at 171˚C for 2 hours 45 minutes. Prepare the media by weighing TSA media with a composition of 3 grams of TSB and 1.3 grams of agar for 100 ml of distilled water, cook and put it into durian, cover and sterilize using an autoclave for 121˚C for 18 minutes. Prepare 90 ml of physiological saline in a 250 ml enlemeyer cover with aluminum foil then wrap. Prepare 5 test tubes containing 9 ml of physiological saline and sterilize the tips in the autoclave.

Total Plate Count (TPC) Parameter Measurement

The media and tools that are ready are then placed in the BSC to carry out TPC, before starting, the gloves are cleaned using 70% alcohol, and the BSC work area is disinfected aseptically. Enter the tools to be used, first cleaned with alcohol. Then enter 10 ml of the Evapond *Bacillus* sample using a 5 ml pipette into a 90 ml garfis then vortex, this is a dilution of 10^{-1}. Take 1 ml of the 10^1 dilution into a 9 ml test tube, then vortex, do the same until the dilution of 10^6, and pay attention to always replace the tip at each dilution. Mark the petri dish with information to facilitate the TPC process. Take 0.1 ml of the lowest dilution 10^6 and place it in a petri dish that has been described for each dilution, and this treatment is allowed to use only one tip because it starts from the lowest dilution. The dilution has been done, then enter the TSA agar media whose temperature is already average (not hot/cold) using the pour plate method into 6 petridishes and 1 control, then homogenized to form the number 8. Wait until it solidifies and place it in the

incubator with the petridish upside down for 2 days. Next, TPC observations are carried out using a studio box and a counter to help and facilitate the calculation of *Bacillus* bacterial colonies. The principle of this method is that if living microbial cells are grown on agar media, the cells will multiply and form colonies that can be seen directly without using a microscope (Rizki & Jumadewi, 2022). The method of fertilizing the culture in a plate count is by the pour plate method. Based on SPC, the number of colonies is adjusted if it has been obtained.

Organoleptic Observation

Organoleptic testing is a testing method that uses the human senses as the primary tool to assess the quality of a product, including the quality specifications of appearance, odor, taste, and consistency/texture, as well as several other factors needed to assess the quality of the product (Ismanto, 2023). Organoleptic parameters for testing the stability of the Evapond *Bacillus* product use the senses of sight and smell, including odor, color, and swelling tests. Each treatment was placed in a 250 ml sample bottle and then placed at room temperature during the storage period test. Samples for organoleptic test observations were poured into 50 ml Duran glasses to observe changes in color and odor.

pH Measurement

The pH of the Evapond *Bacillus* product was measured over 8 weeks using a laboratory pH meter. The pH range for the probiotic *Bacillus* in acidic gastric conditions ranges from 2 to 4 (Khorasgani *et al.*, 2023). The *Bacillus* species itself is capable of surviving in extreme environments. To be effective in fish growth, the probiotic product must remain viable within the host through the stressful conditions of the bile-filled upper intestine and the acidic pH of the stomach (Makete, 2017).

Determination of Shelf Life

Shelf life is the duration of a product's safety, quality, and nutritional attributes under specific storage conditions (Tarlak, 2023). Storage is a crucial aspect of probiotic preparations prior to use, as storage conditions directly affect the biological viability and effectiveness of the preparation. Shelf life determination in this study used a real-time storage test method. A real-time storage test is a real-time stability test, where a product is stored under recommended storage conditions and monitored until it fails to meet specifications. Factors such as temperature, water activity, oxygen content, composition of the probiotic preparation, storage time, and pH level are all important during the storage process (Wang et al.,

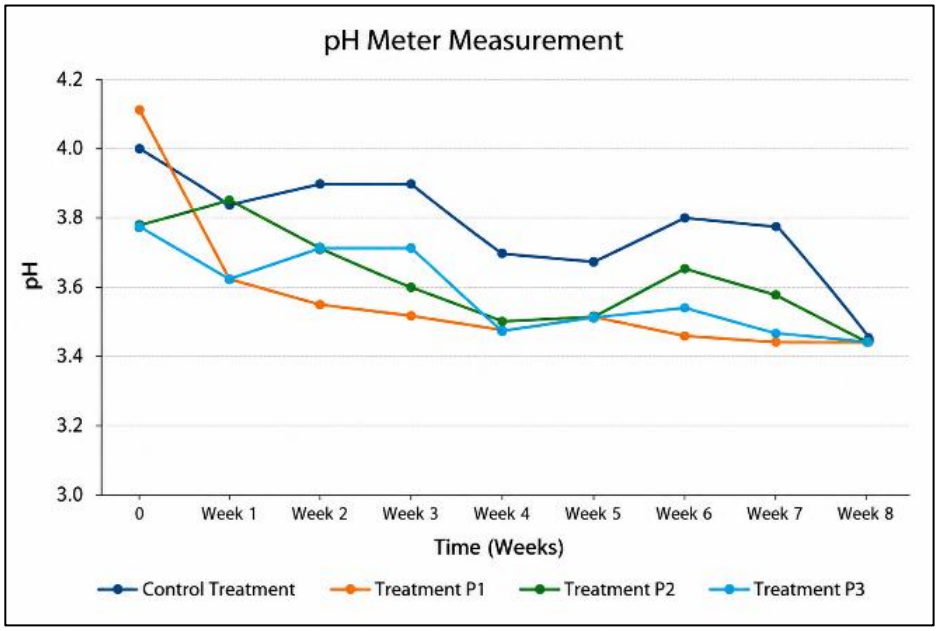

Figure 1. pH meter measurement results

2022). The shelf life of *Bacillus* probiotics was observed over a 2-month study, placed at room temperature.

Results

Effect of Maltodextrin and Guar Gum Addition pH Measurement

The results of pH measurements of Evapond *Bacillus* products in each treatment experienced fluctuations during the 8 weeks of research.

Another study showed that *Bacillus* growth at pH 2-3 had high viability of 85.15% and 87.09%, respectively, while at pH 3 the survival rate was slightly higher, at 91.11% and 90.72% (Awotundun & Olanbiwoninu 2025). This study showed that the more acidic the environment, the lower the bacterial growth. Each bacterial species has its optimal limits in acidic and alkaline pH conditions. *Bacillus* sp. is classified as a bacterium that has a high level of tolerance to low pH. The pH meter measurements for each treatment are stable because they remain within the stomach's acidic range.

Organoleptic Observation

The encapsulated Evapond *Bacillus* product did not experience any significant color or odor changes. The results showed that the Evapond *Bacillus* product had a characteristic fermented odor and a blackish-brown color.

The distinctive odor of fermentation is caused by *Bacillus* producing hundreds of volatile organic compounds with aromatic properties, including alcohols, aldehydes, ketones, volatile acids, alkenes, sulfur

Table 2. Organoleptic observation results

Time	Treatment			
	Control	**P1**	**P2**	**P3**
H0	Typical fermentation odor and blackish-brown color	Typical fermentation odor and blackish-brown color	Typical fermentation odor and blackish-brown color	Typical fermentation odor and blackish brown color
Week 1	Typical fermentation odor and blackish-brown color	Typical fermentation odor (+) and blackish-brown color	Typical fermentation odor (+) and blackish-brown color	Typical fermentation odor and blackish brown color
Week 2	Typical fermentation odor and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+) and blackish-brown color	Typical fermentation odor (+) and blackish brown color
Week 3	Typical fermentation odor and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color
Week 4	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color	Typical fermentation odor (+)(+) and blackish brown color
Week 5	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color	Typical fermentation odor (+)(+) and blackish brown color
Week 6	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color	Typical fermentation odor (+)(+) and blackish brown color
Week 7	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color	Typical fermentation odor (+)(+) and blackish brown color
Week 8	Typical fermentation odor and blackish-brown color	Typical fermentation odor and blackish-brown color	Typical fermentation odor (+)(+) and blackish brown color	Typical fermentation odor (+)(+) and blackish brown color

compounds, and benzenoids. Volatile organic compounds originate from glucose metabolism through the Embden–Meyerhof (glycolysis), Entner–Doudoroff, and fermentation pathways. One of the compounds produced by *Bacillus* is pyrazine, which provides a nutty/fermentation flavor (Caulier *et al*., 2019). This study is in line with the literature that found that *Bacillus* fermentation produces a distinctive aroma from a combination of alcohols, phenols, aldehydes, and organic acids with a soft, slightly earthy/nutty odor, without a foul odor (Gao *et al*., 2018).

Observation of the swelling test

Oxygen is a key factor influencing the viability (activity of live strains) and stability of probiotic strains, whether bacterial or yeast. The effects are directly related to the technological production process, which must ensure the viability of probiotic strains during all phases of production and throughout the product's shelf life (Katona *et al*., 2023). Maltodextrin and guar gum encapsulation were chosen as the solution to serve as a coating agent for *Bacillus* to maintain microbial viability, stability, and product packaging during storage.

Based on the Evapond *Bacillus* swelling test data, no products experienced swelling during the study period. This data can refer to two possibilities: the coating material used is able to maintain stable microbial activity due to the selection of maltodextrin and guar gum as a protective matrix that has properties that function as an important barrier against oxygen and carbon dioxide permeability (Abbas *et al*., 2023). The second possibility is that no swelling occurred because the packaging during the study used a volume of 250 ml, while the liquid sample was only 100 ml. This could have occurred because there was still empty space for oxygen to occupy, so that there was no pressure due to microbial activity (Puligundla *et al.*, 2012).

Total Plate Count (TPC) Observation

Evapond *Bacillus* Day 0 is the optimal period for *Bacillus* bacterial growth. A decrease in the number of *Bacillus* bacterial colonies occurs in the following week. The effect of maltodextrin and guar gum encapsulation concentrations gives different results in each treatment. Treatment

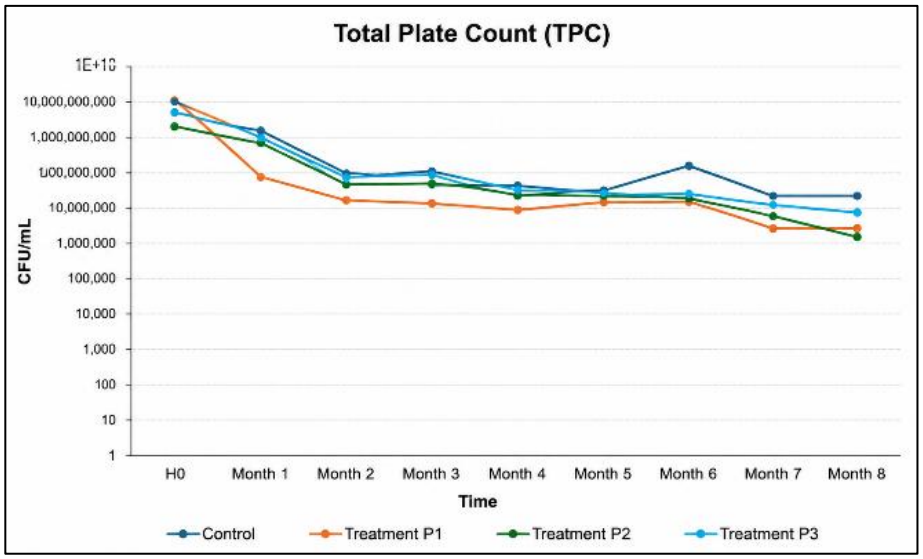

Figure 2. TPC observation results

P1 with a high maltodextrin concentration and low guar gum should be added with sugar/sucrose, because the addition of sugar/sucrose can increase bacterial viability. Encapsulation of maltodextrin and guar gum without the addition of sugar only produces 2-3% viability (Novelina *et al.,* 2025). A concentration of 60%:40% is still quite good compared to treatments P1 and P2. P3 is a fairly good treatment because the concentration of maltodextrin used is not too high; excessive maltodextrin can produce a matrix that is too stiff, reducing the ability of bacteria to rehydrate, while dominant guar gum can produce excessive viscosity.

The decrease in TPC values in each treatment could occur due to environmental stress that can change probiotic cells into a viable but non-culturable (VBNC) state, where the cells remain metabolically active but are not detected by the number of CFU (Arroyo-moreno *et al*., 2025). Environmental stress is influenced by several factors, such as temperature during the storage process, extreme acidic pH. The results of pH value research indicate that bacterial growth viability decreases as pH levels become more acidic (Awotundun & Olanbiwoninu 2025).

Shelf Life

The shelf life of Evapond *Bacillus* products was observed until each treatment failed to meet specifications. Total Plate Count (TPC) was the main focus in determining the shelf life of this product, because in probiotic products, the bacteria contained were at least 10^6 CFU/ML (Sebastian *et al*., 2023). The shelf life of a probiotic product is usually determined based on its absolute viability (CFU) relative to its effective threshold, not the percentage decrease from its initial count. Based on Total Plate Count (TPC) data, the treatment for Evapond *Bacillus* has a shelf life of 49 days (Hairani & Meryandini, 2024) Treatment for P1 with a concentration of 70%:30% has a shelf life of 14 days, P2 with a concentration of 50%:50% has a shelf life of 28 days, and P3 with a concentration of 60%:40% has a shelf life of 42 days. The difference in shelf life in encapsulation also depends on the concentration used between maltodextrin and guar gum encapsulation, which provides nutrition to the bacteria. Additionally, storage temperature affects viability. The recommended temperature for probiotics is 4°C, and storing probiotics at 4°C maintains bacterial viability (Hairani & Meryandini, 2024).

Discussion

Microbes are small organisms (micro) and are classified as prokaryotes, such as bacteria and viruses, and eukaryotes,

such as algae and protozoa (Pitt & Barer, 2012). Not all microbial growth negatively impacts the surrounding environment, as most can be utilized. One such utilization is the production of probiotic products using bacteria as the main component. *Bacillus* bacteria, which have the ability to survive in harsh conditions, are known as *Bacillus* bacteria. *Bacillus* bacteria have been used as probiotic bacteria due to their key probiotic properties, including multi-antibiotic resistance, digestive enzyme production, vitamin synthesis, and immunomodulatory effects (My *et al* . 2022). One of the probiotic products from this company is Evapond *Bacillus*.

One of the encapsulation methods for probiotics that can be used is maltodextrin. The composition of maltodextrin is called complex carbohydrates, and it is derived from oligosaccharides, which provide energy for the growth of bacteria (prebiotics). Maltodextrin was chosen as the coating material for Evapond *Bacillus* because it is a derivative of oligosaccharides, which provide energy sources for the growth of bacteria (prebiotics). The reasons maltodextrin is widely used are: it is easy to find, easy to handle, has rapid dispersion ability, is highly soluble, forms a matrix, has a low tendency to brown, inhibits crystallization, has high binding power, low viscosity, and is stable in oil and water emulsions. Maltodextrin has a good ability to inhibit oxidation reactions, so the resulting microcapsules have a better shelf life (Sumanti *et al.*, 2016). The second coating material is guar gum, which is a selected polysaccharide derived from *Cyamopsis tetragonolobus* seeds, the Leguminosae family. Guar gum is widely used in industry as a binder, disintegrant, suspending agent, thickening agent, and stabilizer (Ji *et al.,* 2009).

The encapsulation method studied in this study did not show significant results on *Bacillus* viability. The use of maltodextrin and guar gum as encapsulation materials is suspected to be less than optimal when applied without the addition of a coating agent or other coating materials. Furthermore, the addition of sugar or dextrose has also been reported to help increase bacterial viability during storage. Therefore, this study still requires further review. Due to time constraints, the method used has not yet reached the freeze-drying stage.

Conclusion

The encapsulation of maltodextrin and guar gum used at each concentration yielded different results for each tested parameter. Overall, the encapsulation of

maltodextrin and guar gum did not significantly impact the stability and shelf life of Evapond *Bacillus* products. The encapsulation with a concentration of 60%:40% performed well, as the product's shelf life was 42 days at room temperature.

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