

Probiotic application in fermentation fish feed materials: a review

Yuli Andriani^{1,a,*}, Rusky Intan Pratama^{1,b}

¹Fisheries Department, Faculty of Fisheries and Marine Science, University of Padjadjaran, Indonesia.

^ayuli.andriani@unpad.ac.id ^brusky@unpad.ac.id

*Corresponding author

ARTICLE INFO	ABSTRACT
<p>Keywords: enzymatic feed fermentation nutritional value probiotics</p>	<p>Feed is an essential component in fish farming because it supports the survival and growth of fish. Provision of feed, one of which can be done through the utilization of organic waste. One of the efforts used to improve the nutritional quality of feed ingredients is the fermentation technology using probiotic bacteria. The probiotic microbes used in the fermentation will overhaul the structure of the organic matter and the complex bonds contained in the feed ingredients. This right will lead to an increase in the quality of feed ingredients, and in turn, the product will be easily digested and meet the nutritional needs of fish. Search results in several studies indicate several types of microbes involved in probiotic fermentation, either singly or in consortia. Some of them are derived from the Bacillus, Lactobacillus and Saccharomyces groups. Applying probiotics in the fermentation process of feed ingredients can improve their nutritional quality. Examples of some common feed ingredients used in the fermentation process are coffee husk waste, banana peel waste, peanut shell waste, rice straw and coconut cake.</p>
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1. Introduction

Feeding is one of the critical aspects of fish farming because it supports the survival and growth of fish. Wardhani et al. (2017) explained that commercial feed has a high price, so the costs incurred by fresh fish farmers reach 75% of the total cost needed: the high feed price and the low nutritional value cause cultivation failure. Therefore, efforts are required to reduce production prices and optimize the nutritional value of feed ingredients. Thus cultivation can be successful.

According to Gandjar (1983), fermentation is a chemical change of organic substances (carbohydrates, fats, proteins, and other organic matter) under aerobic and anaerobic conditions through the work of enzymes produced by microbes. The working principle of fermentation is that the microorganisms produced will change the properties of hard-to-digest ingredients into easily digestible ingredients and increase the nutritional value of these feed ingredients.

Probiotics are cell supplementation in feed or its living environment favourable to its host (Irianto 2003). The basic principle of probiotics is to take advantage of the ability of microorganisms to break or decompose long chains of carbohydrates, proteins, and fats that constitute feed ingredients. There are two types of probiotics used in fermentation feed ingredients, namely indigenous probiotics, which result from isolation from fish body parts, and exogenous probiotics, which come from outside the fish body.

An important key factor that determines the success of intensive fish farming is the provision of sufficient and nutritious feed suitable for farmed fish. Aquaculture costs to procure feed for cultivated organisms can reach 50% of the production cost (Bautista 1981). Feed fermentation is one technique used to increase the nutritional content of waste-based feed ingredients. Through the fermentation process, a higher protein content will be obtained than before fermentation. Hence, the use of a higher price of fishmeal can be reduced. Thus, using probiotics in fermented feed ingredients is expected to reduce feed costs and increase the nutritional value of fish feed ingredients.

2. Types of Probiotics

Probiotics are live bacteria that can benefit the host by regulating the balance of bacteria in the digestive tract, increasing feed efficiency and utilization, increasing the immune response, and improving environmental quality. Adding probiotics to feed can boost immunity and affect survival. One way to enhance feed quality is to use a probiotic fermentation process. The fermentation of feed ingredients is influenced by the dosage of probiotics and the time of fermentation.

Not all bacteria can be used as probiotics, except for bacteria that can meet specific criteria. The following are the requirements for probiotic bacteria: not pathogenic, safe for consumption, able to survive and stable in storage, and can stay in the digestive tract after passing through the stomach. In other words, probiotics must resist acids and bile salts (Zurmiati, 2014). Several types of them are mentioned below.

2.1 *Lactobacillus* sp.

Lactobacillus sp. bacteria are lactic acid bacteria that can be used as probiotic microbes. Lactic acid bacteria (BAL) have long been used in the food industry for their ability to convert sugars, including lactose and other carbohydrates, into lactic acid. The genus *Lactobacillus* sp. is a gram-positive bacterium (contains a lot of peptidoglycans), does not form spores, is rod-shaped, is facultatively anaerobic (bacteria that require a small amount of oxygen for their growth), the optimum temperature of growth ranges from 30-40°C, with an optimum growth pH of 5.5 -5.8, but can generally grow at a pH below 5. Genus *Lactobacillus* sp. is divided into the homofermentative group (bacteria capable of breaking down sugars into lactic acid as the main product) and the heterofermentative group (bacteria that can break down sugars into lactic acid and other products such as alcohol, acetic, and carbon dioxide).

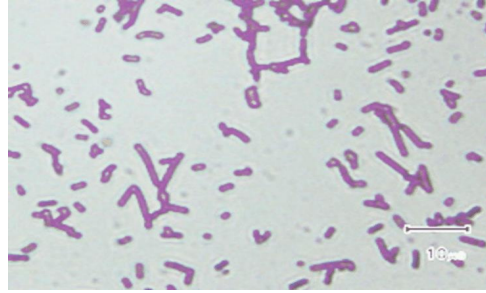


Figure 1. *Lactobacillus* sp.
Source: Wirama et al. (2015)

2.2 *Lactobacillus bifidobacterium*

Lactobacillus bifidobacterium is one of the Lactic Acid Bacteria (BAL) genera that live in the human colon. Some of the characteristics of this bacterium are Gram-positive, anaerobic, sedentary, non-spore-forming, and rod-shaped. Cells appear like the letters V or Y because they are arranged in pairs. The optimum growth temperature is around 37 - 41 °C, and the pH is between 6.5 - 7. *Lactobacillus bifidobacterium* can produce several metabolites beneficial for digestion: lactic acid, hydrogen peroxide, and bacteriocins, which can inhibit pathogenic bacteria.



Figure 2. *Lactobacillus bifidobacterium*¹

2.3 *Enterococcus* sp.

Enterococcus sp. is one of the BAL (Lactic Acid Bacteria) groups. These bacteria have a short, paired chain shape. Like other BAL groups, the genus *Enterococcus* also produces bacteriocins called enterocin. The characteristics of lactic acid bacteria, in general, are that their cells react positively to Gram staining, respond negatively to catalase, do not form spores, and glucose fermentation produces Lactic (homofermentative) acid. There are three species of *Enterococcus*: *E. cecorum*, *E. columbae*, and *E. avium*.

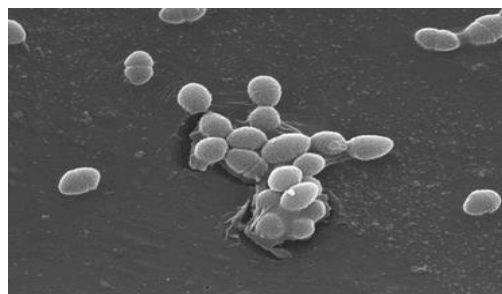


Figure 3. *Enterococcus* spp.
Source: Johnson (2018)

¹ <https://lifepack.id/lactobacillus-bifidobacterium-bifidum-r0071/>

2.4 *Bacillus* sp.

Bacillus sp. is one type of bacteria that is thought to increase the digestibility of fish. According to Fardiaz (1992), this bacterium secretes protease, lipase, and amylase enzymes. Adding probiotics through feed in optimal quantities produces the enzyme *Bacillus* sp., which is contained in probiotics and can contribute to the breakdown of complex molecules into simple molecules, making it easier for fish to digest feed.



Figure 4. *Bacillus* sp.

Source: Šarčević et al. (2018)

2.5 *Bacillus subtilis*

Bacillus subtilis is a type of probiotic microbe that can be used as a starter in the fermentation of fish by-products Lee et al. (2013).

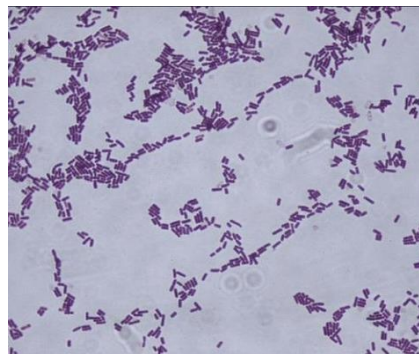


Figure 5. *Bacillus subtilis*

Source: Hussein (2019)

3. Commercial Probiotics

In its development, probiotics developed into a consortium of several types of probiotic microbes, intending to increase the effectiveness of microbial work against substrate or media. Some commercial probiotics are mixed cultures of non-pathogenic gram-positive microbial species of amylolytic, cellulolytic, proteolytic, and lipolytic harmful species. The bacterial culture contained in MEP+ is a mixture of lactic acid bacteria, including *Lactobacillus brevis*, *L. delbrueckii*, *Lactococcus lactis*, and cellulolytic bacteria, namely *Cellulomonas cellulasea* (Sukanto and Sutardi 2008). In addition, there are also commercial probiotics which are mixed cultures of microorganisms that are beneficial for improving water quality and increasing fish growth, which contain 90% *Lactobacillus* sp. as lactic acid-producing bacteria, photosynthetic bacteria, *Streptomyces* sp. and yeast (Sumarno 2010).

4. Fermentation of Feed Ingredients Using Probiotics

Fermentation is a substance-processing activity that utilizes microorganisms as a source of enzymes. Fermentation using probiotics for the feed ingredients substrate is carried out by mixing probiotic starters that have been activated using molasses, then added to the feed ingredients and closed to prevent the entry of oxygen so that the fermentation environment is anaerobic. Table 1 describes the various methods used in fermentation research using probiotics.

Table 1. Fermentation method using probiotics

Substrate	Fermentation Method	Reference
Tofu Dregs	Probiotics at a dosage of 1 ml plus 10 ml of water for fermentation and mixed with tofu pulp weighing 1 kg	Prihatini (2017)
Artemisia (<i>Artemisia annua L.</i>)	Inoculation of <i>Lactobacillus plantarum</i> as much as 1% in the pre-culture period cultured in MRS broth at 37 °C for 16 hours (2.5×10^9 CFU/ml) into sterile media containing <i>Artemisia annua L.</i> powder which has been dried for three days and crushed.	Lee et al. (2017)
Rice bran	<i>Lactobacillus</i> sp bacteria are mixed with rice bran flour in a ratio of 1/100 gram. Furthermore, microorganisms are dissolved in 20 ml of molasses by spraying with a sprayer. After fermentation is carried out for 48 hours, rice bran is steamed for 1-2 minutes to inactivate the activity of microorganisms.	Surianti et al. (2021)
Soybean Pulp	Fermentation of soybean pulp is made using bacteria 0.001 % <i>Lactobacillus</i> sp. mixed with 10% molasses. After fermentation for three weeks in an HDPE container, the material is dried in the sun until it reaches 10% humidity.	Zulhisyam (2020)
Leaf Vegetable Waste	The first sub-sample (~20 g) was mixed with 180 mL of sterile 0.8% NaCl (w/v) in a 500 mL conical flask and stirred at 30 °C for 2 h using a rotary whisk at 150× rpm. Next, the solution of the mixture is filtered through four layers of medical gauze. A mixture of substrate particles was collected by centrifugation at 4 °C for 20 min at 10,000 g ×.	Du et al. (2021)

5. Improving the Nutritional Quality of Fish Feed Ingredients through Fermentation Using Probiotics

Fermentation of feed ingredients using probiotics has been widely used in research and has improved the nutritional quality of feed ingredients. The use of probiotics in the fermentation of feed ingredients can increase nutritional content because complex bonds are simplified to be simpler through enzymatic processes by bacteria. Various studies concerning the increase in the nutritional content of some fermentation results using probiotics can be seen in Table 2.

Table 2. Studies on increasing the nutritional content of feed ingredients through the fermentation process

Types of Feed Ingredients	Types of Fermentation Probiotics	Benefits of Fermentation of Probiotics	Reference
Coffee Skin (<i>Coffea sp.</i>)	Cellutotic bacteria	Increase in protein from 12.15% to 13.93% and a decrease in fibre from 34.37% to 30.8%	Fatmawati (2019)
Agricultural waste with hyacinth supplements	Lactic acid bacteria, Productive, effective microbes plus (MEP+)	A fermented agricultural waste feed with as much as 10% hyacinth supplements and MEP+ probiotics provided the highest BAL population density with an average BAL population of 5.85 x 10 ⁶ CFU/g.	Setyawan et al. (2014)

Rice Straw	<i>Starbio Probiotics</i> <i>Probian</i>	The addition of a starter using banana weevil MOL significantly reduced the levels of Organic Matter (70.59%) and Crude Fiber (18.87%) and tended to increase Crude Protein levels (8.46%)	Suningsih et al. (2019)
<i>Kepok</i> Banana Peel	Probiotic EM-4	There are significantly different results in water content, while there is no real difference in crude fat content.	Rokhim (2019)
Peanut shells	BioMC4 Probiotics	The analysis of related articles showed that there was an increase in dry weight content from 52.3428% to 88.1613%, ash content from 5.0814% to 8.2889%, crude protein content from 6.8627% to 7.9928%, crude fat from 0.9934% to 1.9789%, calcium from 3.0595% to 3.7497%, ME from 498.0747% to 1772.5249%. The highest increase in levels was indicated by BETN levels from 2.9983% to 35.4657%.	Kurnijasanti (2016)
Chicken Manure, Bran, and Coconut Meal	Probiotics	Organic fertilizer of chicken manure, bran, and coconut meal fermented with probiotic bacteria has had a different influence ($P < 0.05$) on <i>Daphnia</i> sp's growth pattern and biomass production.	Izzah (2014)
White Soybean Flakes	<i>Lactobacillus brevis</i>	Lactic acid fermentation increases the nutritional value of soy white flakes.	Refstie et al. (2005)

The crude fibre decrease in the feed ingredients fermentation is caused by changes in the cell wall resulting from microbes-induced hydrolysis that can break down lignocellulose and lignohemicellulose bonds and dissolve silica along with lignin contained in the cell wall of fibrous feed ingredients Komar (1984). The decrease in crude fibre in feed ingredients helps increase the digestibility of feed ingredients by the organism.

The fermentation process generally increases the moisture content in the feed ingredients. The increase in water content is due to the longer fermentation time; hence the activity of probiotic microbes increases, increasing the water content produced. This is because, in the fermentation process, there is a breakdown process of glucose into carbon dioxide (CO_2) and water (H_2O) that will increase the moisture content of dry matter Fardiaz (1992).

The increase in crude protein in the fermentation yield of feed ingredients is caused by the rise in mould mycelium in the substrate. According to Indrayati dan Rakhmawati (2013), this is due to the mould containing nucleic acids that can increase nitrogen as a source of single-cell protein. The protein content decreased in fermented feed ingredients is due to the activity of proteolytic microbes that degrade the proteins in fermented feed ingredients for their growth and development.

6. Conclusion

Based on the review results of several studies, various probiotic bacteria and their application methods have been widely used in the fermentation research of feed ingredients. Probiotic bacteria used in fermentation are indigenous and exogenous bacteria derived from fish. Ingredients obtained from waste residues or feed ingredients not widely used to meet people's daily needs are used as feed ingredients for fermentation. Fermentation with probiotics has many advantages, including; increasing the nutritional value and efficiency of feed ingredients contained in fish feed, thus, the costs incurred for feed can be reduced, and the growth of fish becomes more effective. In addition, fermentation has benefits for increasing the immunity of aquaculture organisms to diseases.

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