Bacteria Application in Fermentation of Fish Feed Components: a review

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ABSTRACT

Feed is the most important component in fish farming business. The feed given to fish must have a balanced nutritional content hence that fish can grow optimally. The demand for feed derived from fish meal and soybeans has increased, causing the selling price of these feed ingredients to rise. The use of local raw materials sourced from agricultural industrial waste is a feasible solution. However, in this regard, the utilization of waste raw materials has drawbacks, one of which is the high content of crude fiber which can inhibit fish growth. The application of fermented feed ingredients with microbes is the best solution to use because it can improve the nutrition of feed ingredients, reduce crude fiber, and increase the digestibility of fish. One of the factors that influence the fermentation process is the substrate and microbes used. The bacteria commonly used for the fermentation process of feed ingredients are Bacillus subtilis and lactic acid bacteria. Fermentation of feed ingredients with bacteria has been proven to be able to produce quality feed raw materials and has an effect on increasing digestibility and fish growth.

Keyword: *bacteria, fermentation, fish feed, local raw material, substrate*

1. INTRODUCTION

Feed is the main supporting component as well as an important factor in the success of an aquaculture activity and contributes greatly to production costs. Feed costs can range from 40% - 80% of the total cost of fish farming production. The feed given to fish must have nutritional value that meets the needs of fish to grow optimally. Fish feed needs to contain sufficient and balanced protein, fat and carbohydrate nutrients to suit each type of fish. The nutrient content that is most needed by fish in high levels is protein content. Protein content in feed is usually sourced from feed ingredients such as soy flour and fish meal, but with the development of the times and fish farming technology, the demand for raw materials sourced from soy flour and fish meal has increased. This causes the selling price of raw materials for soybean flour and fish meal to be expensive. In Indonesia, these two feed raw materials are known to be imported products, so they are expensive [1]. Therefore, the action that can be taken as a solution to this problem is to utilize local raw materials that are widely available and have not been used optimally. Local raw materials that have the potential to be used as alternative feed raw materials include those originating from agricultural industrial waste and processing waste [2].

Alternative feed raw materials are feed ingredients that are easy to obtain because they are still abundant, have economic value, still contain nutrients, and their availability does not compete with food consumed by humans. However, alternative feed ingredients are known to have drawbacks, some of which are the high crude fiber content in the ingredients, the low crude protein content, and the presence of anti-nutritional substances that can inhibit the digestibility of fish so that it can result in sub-optimal fish growth. This can be overcome by further processing of the feed. An example is carrying out a fermentation process with the help of bacteria to improve the quality of the nutritional content of feed ingredients where the crude nutrient content contained in the feed ingredients decreases [3].

Therefore, it is hoped that the use of bacteria in the fermentation process of alternative feed ingredients can produce quality feed ingredients with sufficient nutritional content for fish to grow and develop. This is due to the nature of fermentation which can improve the nutritional content of feed ingredients. In addition, this utilization can provide a solution in producing feed at a lower price but still has good nutritional quality to meet the nutritional needs of fish feed.

2. FERMENTATION USING BACTERIA

Fermentation is a process for breaking down organic compounds into simpler ones with the help of bacteria or microorganisms. According to [4], fermentation is a process of chemical change of organic compounds such as carbohydrates, proteins, fats, and other organic materials in both aerobic and anaerobic conditions, through the action of enzymes produced by microbes. Enzymes produced by microbes in a fermentation process are able to degrade fiber in fish feed ingredients, so they are able to produce feed ingredients that can be used as a source of nutrition for fish growth. Several factors can affect the fermentation process of feed raw materials, namely the substrate used, the type of microbe or bacteria used, temperature, pH and oxygen [5]. The purpose of the fermentation process using bacteria or microbes is to make the feed have longer shelf life, able to reduce toxic compounds and crude fiber contained in feed ingredients [4].

3. TYPES OF FERMENTATION BACTERIA

3.1 *Bacillus subtilis*

The use of *Bacillus subtilis* bacteria in fermented feed can increase the crude protein content, for example in jackfruit waste [6]. *Bacillus subtilis* is antagonistic and widely used in controlling pathogenic bacteria that can produce protease enzymes, amylase, lipase, and chitinase where these enzymes are enzymes that break down the cell wall of pathogens.

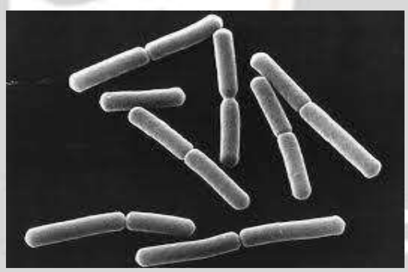

Figure 1. *Bacillus subtilis*

3.2 Lactic Acid Bacteria (LAB)

Lactic acid bacteria are bacteria that ferment sugars and carbohydrates to produce large amounts of lactic acid. Lactic acid bacteria are generally classified as Gram-positive, catalase-negative, and do not form spores. The lactic acid produced comes from the glucose fermentation process. Fermentation of lactic acid bacteria can be classified as homofermentative, in which in this type of fermentation lactic acid is the only product of fermentation. In addition, there are fermenting bacteria that are classified as heterofermentative, in which organic acids other than lactic acid (acetic acid, carbon dioxide, ethanol, etc.) are also produced in this fermentation. Several types of bacteria belonging to lactic acid bacteria are *Lactobacillus* sp., *Streptococcus* sp., *Enterococcus* sp., *Pediococcus* sp., *Tetragenococcus* sp., *Leuconostoc* sp. and *Lactococcus* sp.

The morphological characteristics of lactic acid bacteria can be observed with the naked eye and with a microscope. Visual characteristics of *Lactobacillus* sp. colonies. include colony shape, height, edge shape, internal structure, growth on oblique agar media, and motility. Fermentation using lactic acid bacteria can convert fish waste into protein. The amino acids from protein are then converted into CO_2, H_2O, lactic acid, acetic acid, ethanol, and nitrogen, namely NH_3. Because this NH_3 (ammonia) compound is alkaline, its presence can slightly increase the pH in the fermentation process. The more molasses used, the more bacteria will grow, and the NH_3 compounds produced can eradicate some of the *Lactobacillus* itself, subsequently, this can reduce its ability to break down proteins.

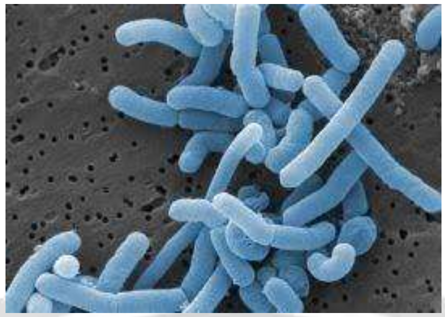

Figure 2. Lactic acid bacteria

4. TYPES OF FERMENTATION

Fermentation in its application can be differentiated based on the substrate and water content into fermentation with submerged culture (90% water content) or Liquid State Fermentation (LSF) and fermentation on solid substrates (40-75% moisture content) or Solid-State Fermentation (SSF).

4.1 Liquid State Fermentation (LSF)

Submerged culture fermentation or Liquid State Fermentation (LSF) or also called Submerged Fermentation (SmF) is a fermentation method using a liquid substrate and adding or replacing nutrients continuously in this fermentation medium. This fermentation is suitable for bacteria that require high water content. In the liquid substrate fermentation process, the initial pH must be adjusted to 5, carried out at room temperature (25-30^{o}C), and under anaerobic conditions [7].

The advantage of liquid substrate fermentation is that it is able to maintain aeration conditions, pH, and media homogeneity according to what is desired. Liquid substrate fermentation has several limitations including the cost of equipment and operations used which are more expensive than solid substrate fermentation. Various research results have shown that fermentation using bacteria with liquid substrate techniques has succeeded in improving the quality of feed ingredients (Table 1.)

Table 1. Fermentation using bacteria on a liquid substrate

Bacteria Type	Substrate	Density	Result	Reference
Bacillus licheniformis and *Lactobacillus* sp.	Shrimp shell	2% Bacillus licheniformis and 2% Lactobacillus sp.	Significant increases in essential nutrients, namely changes in lysine by 44.08%, methionine by 15.87%, calcium by 22.92%, phosphorus by 116.03%, organic acids by 45.61%, and chitosan by 68.78%.	[8]
Bacillus licheniformis	Ketapang fruit (Malabar almond)	Doses of inoculum 1, 2, and 3 %.	Crude protein increased by an average of 8.85% and decreased crude load by an average of 11.88%	[9]

4.2 Solid State Fermentation (SSF)

Solid State Fermentation is a fermentation method using simple solid media where the water content used in this method is generally low around 50-60% [10]. Fermentation on solid substrates can utilize agricultural waste, copra waste, and waste from starch processing as raw materials. As with liquid substrate fermentation, the initial pH in the solid substrate fermentation process must be 5 with a temperature of around 30^{o}C, but not necessarily under anaerobic conditions [11]. Examples of substrates that can be used in this solid-substrate fermentation method are pineapple peel waste, cassava root waste, soybean meal, and others.

One of the advantages of solid substrate fermentation is its simpler application compared to liquid substrate fermentation. In addition, the operational costs and equipment needed in liquid substrate fermentation are cheaper. While the disadvantages of fermenting on solid substrates include maintaining the fermentation conditions according to what is desired, for example related to homogeneity of the media and aeration which are very difficult to do. Various research results have shown that fermentation using bacteria on solid substrate techniques has succeeded in improving the quality of feed ingredients (Table 2.)

Table 2. Fermentation employing bacteria on solid substrates

Bacteria Type	**Substrate**	**Density**	**Result**	**Reference**
Bacillus pumillus	SE5 Soybean meal	1 x 10 cells/g	Increase protein by 6.2%. Lowering glycinin to 4.4 mg/g and β-coglysinin to 68.9 mg/g	[12]
Bacillus mycoides and Bacillus megaterium	Cassava tuber skin waste	5%	Reducing ash content by 68.15%, crude fiber by 51.23%, lignin by 74.24%, cyanide acid by 97.93%, cellulose by 52.31%. In addition, it increased crude protein by 135.4%, fat by 38.19% and glucose by 43.97%.	[13]
Bacillus megaterium	Cassava pile	Bacterial density of 10^{11} cfu/mL with concentrations of 3%, 6% and 9%.	Improvement of the nutritional quality of the cassava pile increased the simple sugar content to 17.07 mg/g and dissolved protein to 125.18 μg/g. In addition, reducing crude fiber to 9.42%.	[14]
Bacillus subtilis	Chicken feather waste	5 mL/2 g, 10 mL/2 g, and 15 mL/2 g.	Crude protein content increased to 80.5% and crude fat content to 10.48 ± 0.24%. Lowering the ash content to 0.39 ± 0.01%, crude fiber content to 0.04 ± 0.00%.	[15]
Bacillus sp	*Indigofera zollingeriana*	200 mL *Bacillus* sp.	The use of Bacillus sp. increasing protein to 23%, fat to 3.8%, ash content to 12.4% and BETN to 55.6%. As well as reducing crude fiber to 5.2%	[3]
Lactobacillus sp. and *Lactococcus* sp.	Peanut shell waste.	The applied dose is 1% of each *Lactobacillus* sp. and *Lactococcus* sp.	Application of *Lactococcus* sp. increased BETN by 4.48% and reduced crude fiber by 7.7%, while the application of *Lactobacillus* sp. increased BETN by 3.92% and	[16]

			decreased crude fiber by 3.8%.	
Bacillus licheniformis B2560 and *Bacillus subtilis*	Chicken feather flour	5 ml, 10 ml and 15 ml	*Bacillus licheniformis* increased amino acids starting from aspartic acid to 4.52%, glutamic acid 6.81%, serine 7.41%, histidine 0, 28%, 6.31% glycine, 3.3% threonine, 4.85% arginine and 2.64% alanine. While the use of *Bacillus subtilis* increased aspartic acid to 4.3%, glutamic acid 6.98%, serine 6.83%, histidine 0.29%, glycine 3.97%, threonine 3.4%, arginine 4.62%, and 2.55% alanine.	[17]
Bacillus sp. and *Lactobacillus sp.*	*Indigofera zollingeriana*	200 mL probiotic *Lactobacillus* sp. and 200 mL of *Bacillus* sp. powder solution.	The application of *Lactobacillus* sp. reduced the ash content to 12% and crude fiber to 5.5%. Increase protein to 27.5% and fat to 5.5%. While the bacteria *Bacillus* sp. increasing protein to 28.3% and fat to 4.9%. Reducing the ash content to 12.2% and crude fiber to 6.6%.	[18]

5. CONCLUSIONS

Fermentation using bacteria is able to alter complex compounds into simpler compounds. This process is assisted by enzymes produced by bacteria that are able to degrade fiber in feed ingredients. The types of bacteria commonly used for fermenting fish feed ingredients are *Bacillus mycoides, Bacillus megaterium, Lactobacillus* sp., *Lactococcus* sp. The utilization of solid and liquid substrates can affect the results of fermentation and has its own advantages and disadvantages such as the instruments used, operating costs and adjusting conditions for the fermented material. The results of fermentation employing bacteria are proven to be able to improve the quality of feed and nutrients needed by fish and have a positive effect on fish growth as well, mainly due to the increase in fish digestibility.

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