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BACILLUS SP.FORPREVENTIONDISEASEOFCOMMON CARP

YuniarMulyani[1], Dian YuniPratiwi[1]

[1]Lecture of Fisheries, Faculty of Fisheries and Marine Science, UniversitasPadjadjaran, Indonesia

*Corresponding Author:
Yuniar Mulyani
Address: Faculty of Fisheries and Marine Science, UniversitasPadjadjaran, Indonesia
Email: yuniarmulyani@gmail.com

ABSTRACT

The protein needs of the community can be done through aquaculture activities. Commodities of common carp (*Cyprinus carpio* L.). much in demand by aquaculturist. However, in the process of cultivation, it is still often found the problem of fish mortality caused by disease. Efforts are usually made by farmers to overcome the attack of pathogenic bacteria is to use antibiotics. However, the present of antibiotics to fish can cause negative effects, especially long-term use. Efforts are made to control infections caused by bacteria that cause disease, one of which is to prevent by increasing the fish body's defense system, including by utilizing probiotics. Bacteria *Bacillus* sp. including one of the probiotic bacteria, which has been widely used as a bacterial inhibitor of pathogenic bacteria. Spores of the genus *Bacillus* can be used as commercial products that are useful for biological agents. Endospores produced from *Bacillus* bacteria have a strong resistance to chemical and physical factors. Probiotics *Bacillus* sp. can be applied in a variety of ways, one of which uses mixing feed. The provision of *Bacillus* sp. into the feed has been done by many researchers. *Bacillus* sp. can be used as a probiotic to increase endurance of common carp. Based on several studies that have been conducted, it can be concluded that giving of *Bacillus* sp. into the feed can overcome the disease attack on common carp.

KeyWords : Aquaculture, *Bacillus* sp., Disease Attack, Probiotics

Introduction

Increasing fisheries production to fulfill the protein needs of the community can be done through aquaculture. One commodity that is excellent in freshwater aquaculture is common carp (*Cyprinus carpio* L.). Commodities of carp are in great demand by farmers. However, in the process of aquaculture, fish problems are still often found to be caused by diseases originating from bacteria or viruses.

Death in fish is caused by the inability of fish to control disease because it does not have a good body defense system. The body's defense system can be seen from the value of leukocytes in the blood so that it can be used as an indicator to determine whether the fish is resistant to a pathogen. Increased leukocyte values can indicate infection, stress, leukemia, or factors such as chemicals, toxins, parasites, bacteria and viruses [1].

Efforts are made to control infections caused by bacteria that cause disease, one of which is to prevent by increasing the fish body's defense system, including by utilizing probiotics. Probiotics are live microbes that can provide benefits to the host by modifying the microbial community or associating with the host, improving nutritional value and feed utilization, increasing the host's response to the disease and improving environmental quality [2]. It is hoped that the application of probiotics can function as a biocontrol agent to reduce disease attacks. One probiotic bacteria that has been widely used in fish farming is *Bacillus* sp. Probiotics are live microbial agents that can benefit their host. Proving these probiotics can increase immunity to the disease and can increase feed digestibility [3]. Bacteria *Bacillus sp*. including one of probiotic bacteria, which has been widely used as a bacterial inhibitor of pathogenic bacteria.

Efforts are usually made by farmers to overcome the attack of pathogenic bacteria is to use antibiotics. Common types of antibiotics include ampicillin, chloramphenicol, and tetracycline. However, giving antibiotics to fish can cause negative impacts, especially long-term use, among which can cause resistance to bacteria, require quite expensive costs, can pollute the environment, and accumulate in the body of the fish so that it is harmful to humans who consume it [4]. Therefore, efforts are needed to prevent pathogen infections by increasing the immune response using compounds that are not harmful to the body of the fish, one of them is by using probiotics. One probiotic bacteria that has been widely known and used in fish farming is *Bacillus* sp.

Bacillus sp.

Bacillus sp. is a rod-shaped bacteria, belongs to a group of gram-positive bacteria, and is chemoheterotroph. Kemoheterotrof is an organism that gets its source of energy from chemical compounds, while the source of nutrients for its metabolism comes from organic materials. Most of *Bacillus* sp. motile with a characteristic lateral flagellum. In environmental conditions that are not supportive, usually, these bacteria form endospores. The metabolic pathway of *Bacillus* sp. is through aerobic respiration, where the process of breaking down organic matter into ATP is assisted by oxygen [5]. *Bacillus* sp. can live in a wide temperature range, which is between 10-50^{o}C [6].

One type of genus *Bacillus* namely *B. subtilis* can inhibit other pathogenic bacteria through the mechanism of antibiosis, competition and growth promoters. Bacillus subtilis can produce antibiotics that are toxic to other microbes. Antibiotics produced include streptovidin, bacitracin, surfactin, fengisin, iturin A, polymixin, diphydidine, subtilin, subtilosin, protein [7]. *Bacillus* sp. can produce bacteriocin (antimicrobial peptides) which can increase immunostimulants. Bacteriocin is a polypeptide or protein antimicrobial agent produced by bactericidal microorganisms. Bacteriocin can kill the target cell by inserting on the target membrane so that the function of the cell membrane becomes unstable and the cell undergoes lysis [8].

Bacteria *Bacillus* sp. is a gram-positive bacteria that has been widely developed and can control pathogenic bacteria. These bacteria can form endospores and can survive longer in environmental conditions that are not favorable for growth [9]. Bacteria *Bacillus* sp. can control pathogenic bacteria and can inhibit the growth of other bacteria. Therefore *Bacillus* sp. can increase the non-specific immune system in fish [3]. This is indicated by the increase in total leukocytes that act as nonspecific immunity.

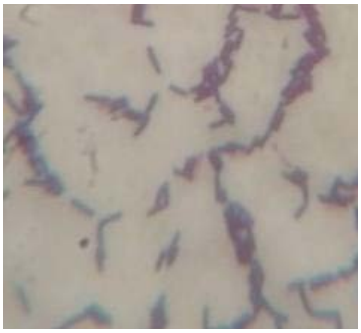

Figure 1. *Bacillus* sp.

Bacillus sp. is one of the bacteria that has been developed because it can prevent the pathogenic bacteria of the fish. Bacteria *Bacillus* sp. is a gram-positive bacterium shaped like a rod, measuring 0.3-2.2 µm x 1.2-7.0 µm. These bacteria can also grow in aerobic conditions or can be facultative anaerobic. Spores of the genus *Bacillus* can be used as commercial products that are useful for biological agents [10]. Endospores of the genus Bacillus are round, oval, elliptical, or cylindrical formed in vegetative cells, these bacterial endospores are different from other types of bacteria that form exospores. Endospores produced from *Bacillus* bacteria have a strong resistance to chemical and physical factors, such as extreme temperatures, alcohol, and others [11].

Type of bacteria *Bacillus* sp. can show different colony forms on each agar media. The color of these bacterial colonies is generally white to yellowish or gloomy white, the edges of the colony vary but are generally uneven. The shape of the colony and its size varies greatly depending on the type and each bacterium has a different resistance to face environmental conditions such as heat, acid, salinity, and others [11].

Probiotic bacteria *Bacillus* sp. can excrete protease, lipase, and amylase enzymes. Exogenous enzymes can help endogenous enzymes in the host to hydrolyze feed nutrients, thereby increasing the availability of nutrients that are readily absorbed from the digestive tract to enter blood vessels for subsequent metabolic processes [12]. So that *Bacillus* sp. can have a positive influence on growth. Provision of *Bacillus* sp. the supplementation method in feed can provide the best growth, general response, and resistance to viral infections [13], and improve feed conversion ratio [12].

Bacillus species are suitable for producing enzymes, cannot produce toxins, are easily grown, and do not require expensive substrates. The ability of *Bacillus* to survive at high temperatures, no metabolic byproducts, and its ability to produce large amounts of extracellular protein, thus making *Bacillus* a favorite organism for industry [14]. But there are types of bacteria in the genus *Bacillus*, namely *B. cereus* and B. Anthracis which can produce toxins that are different from other types of *Bacillus* bacteria so they must be aware of their existence.

Probiotic bacteria in the digestive tract have important protective functions for suppressing pathogenic bacteria and viruses, stimulating endurance and altering intestinal metabolic activity, as well as increasing or decreasing enzyme activity and stimulating immunity through increased antibody levels or macrophage activity [15].

Bacillus has interesting physiological properties because each type has different abilities, including (1) able to degrade organic compounds such as protein, starch, cellulose, hydrocarbons, and agar, (2) able to produce antibiotics; (3) plays a role in nitrification and denitrification; (4) nitrogen-fixing; and (5) are chemolithotrophic, aerobic or facultatively anaerobic, acidophilic, psychoprifilic, or thermophilic [16].

Research that has been done

Bacillus sp. has the potential as a biocontrol agent or also called probiotics. *Bacillus* sp. has been proven beneficial for health and disease prevention in fish culture systems, which can stop pathogens from multiplying in the digestive tract and aquatic aquaculture environments. By research which states that *Bacillus* sp. can fight *Vibrio* sp. in groupers thus increasing survival because it focuses on the multi-factor effects of probiotics, such as the production of enzymes, nutrient components, and

space [17]. Besides, *Bacillus* sp. into the rearing pond and providing feed which has been added by Bacillus sp., which shows an increase in immune activity and antioxidant ability of grass carp [18].

Bacillus sp. as a probiotic it is thought to be able to suppress disease in the body of common carp through antagonistic activity and an increase in immune response to increase the survival rate of common carp [19]. The addition of probiotics *Bacillus* sp. can increase the total leukocytes of rainbow trout infected by *Streptococcus agalactiae* [20]. Increased total leukocytes are associated with the performance of the fish's immune system in reducing pathogen attack. Leukocytes function to protect the body of fish from foreign objects, including phagocytic cells to prevent pathogens from spreading virulence factors in the body of the fish [3]. In line with other studies which state that the feed added *Bacillus* sp. proven to reduce the risk of pathogenic bacterial infection so that the fish Rohu (*Labeorohita*) can develop the capacity to protect themselves from various diseases [21]. This can be seen from the increased total leukocytes in Rohu fish that are fed with *Bacillus* sp.

Other research shows that *Bacillus* sp. in the form of more stable spores during fish food processing including the addition of *Bacillus* sp. into feed and storage in refrigerators [22]. *Bacillus* sp. which enters the body of the fish through the feed will attach to the digestive tract and form a colony that produces bacteriocin and forms the immune system [23]. *Bacillus* sp. Colonization in the epithelial cells of the digestive tract and mucosal surface is an important thing that is done by probiotic bacteria to prevent adhesion of pathogenic bacteria and invasion of intestinal spaces, and prevent inflammatory reactions [24]. The mechanism of action of *Bacillus* sp. in boosting the immune system by stimulating the formation of leukocyte cells namely lymphocytes, monocytes and neo-trophils [25]. Lymphocytes will proliferate and form B lymphocytes and T lymphocytes which will differentiate into effector cells or phagocytic cells. Phagocytic cells formed include monocytes and neutrophils which will phagocyte foreign bodies or kill pathogens [26].

Furthermore, in the study of Djauhari et al. 2016, the addition of *Bacillu*s sp. into feed can increase carp's protection and resistance to pathogenic bacteria, which is indicated by increased survival after being challenged by *Aeromonas hydrophila* [27]. *Bacillus* sp. added to feed as much as 10^8 CFU / ml can increase the nonspecific immune activity of grass carp [18]. In line with the research of Liu et al. 2012, *Bacillus* sp. with a density of 108 CFU / ml in feed can increase the growth and immune response of groupers (*Epinepheluscoioides*) [28]. Zokaeifar et al. 2008 also stated that *Bacillus* sp. with a density of 10^8 CFU / ml can increase the immune response and growth of shrimp (*Litopenaeusvanamei*) [29]. This is consistent with other studies that explain that the density of *Bacillus* added to feed ranges from 10^7-10^8 CFU / ml with a 2-4 week application period to improve the immune response and survival of fish [30].

Some studies also show that the bacteria *Bacillus* sp. able to improve the carp immune system [31], improve feed efficiency in catfish [32], improve the survival of catfish [3], and increase the non-specific immune response in tilapia [33].

Probiotic doses are generally very varied from 10^6-10^{10} CFU / g feed, this is related to the type of host and the level of fish immunity [34]. Probiotics *Bacillus* sp. can be applied in a variety of ways, one of which uses mixing feed. Provision of *Bacillus* sp. in the feed has been done a lot by previous researchers, as evidenced by probiotics *Bacillus* sp. can increase the nonspecific immune system of fish such as increasing the immune of catfish [3] tilapia, common carp [19].

Addition of *Bacillus* sp. into feed with a density of 10^8 cells / mL can increase the immune system of African catfish, giving probiotics Bacillus firmus with a dose of 10^9 CFU / mL can increase the body's resistance of African catfish seeds [35], the addition of Bacillus sp. with a density of 10^8 CFU / mL can increase shrimp endurance [29], administration of synbiotics with a dose of 10^6 CFU / mL probiotics can improve immune response and resistance to *Chromileptesaltivelis*.

Conclusion

Bacillus sp. can be used as a probiotic to increase endurance of carp. Based on several studies that have been conducted, it can be concluded that giving of *Bacillus* sp. into the feed can overcome the disease attack on common carp.

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