

## **Antibacterial activity of isolates from digestive tract of catfish (*clarias gariepinus*) as probiotic candidates**

**Toto Apriliyanto<sup>1\*</sup>, Nosa Septiana Anindita<sup>2</sup>, Wisnu Adhi Susila<sup>3</sup>**

<sup>1,2</sup>Faculty of Health Sciences, 'Aisyiyah University of Yogyakarta, West Ringroad, 63 Nogotirto Street, Gamping Sleman 55292, Yogyakarta Indonesia

<sup>3</sup>Head of Bacteriology Laboratory, PT Biotek Cipta Kreasi

apriyanto556@gmail.com\*; <sup>2</sup>nosa.nindita@unisayogya.ac.id; <sup>3</sup>wisnu.adhi.susila@gmail.com  
\* corresponding author

Submission date: 7 April 2021, Receipt date: 15 Mei 2021, Publication date: 1 Juli 2021

### **Abstract**

*African catfish (Clarias gariepinus) was a popular fish in the community because it provides a high level of protein at a low cost. The motile Aeromonas septicemia (MAS) disease produced by Aeromonas hydrophila (A. hydrophila) infection was one of the things that create difficulties in the cultivation of this fish. Synthetic antibiotics were commonly used in MAS disease control management efforts in fish farming, which have had an influence not only on fish but also for consumers. The application of probiotic Bacillus sp isolated from the digestive tract of catfish as a means of preventing the sickness is one option. Enzymatic tests (lipolytic, amylolytic, cellulolytic, proteolytic activity) and antagonist testing were used to assess the probiotic activity of the Bacillus sp isolates. The antagonist test is not influenced by the isolate source factor, but the ability of each isolate depends on the variant.*

**Keywords:** antibacterial, bacillus sp, clarias gariepinus, probiotic

---

### **INTRODUCTION**

Because of the high protein content of fish, African catfish (*Clarias gariepinus*) provides a viable source of animal protein for the community. Because catfish does not require running water and can be cultivated with high solids, it can be cultivated with basic technology and can grow in restricted water sources. However, raising African catfish with high solids levels can cause sickness in the fish (Sya'bani et al., 2015).

Yogyakarta's catfish consumption needs are estimated to be over 150,000 tons per year. In 2018, the community's aquaculture could only meet 91,000 tons of fish demand. Based on these findings, it can be stated that African catfish farming is still far from ideal. The culture of catfish must be carefully considered and carried out in order to ensure that the process is carried out in compliance with excellent fish farming practices. The prevalence of disease assaults on fish, which make it tough for farmers, is one of the things that become obstacles in this cultivation procedure (Anonim, 2020).

*Motile aeromonad septicemia (MAS), caused by the infection Aeromonas hydrophila, is a disease that mostly affects African catfish. Catfish and other tropical freshwater fish are susceptible to this sickness. Within one week, the mortality rate in*

---



catfish might reach 80 percent and even 100 percent. Environmental changes, such as high density, high temperature, low dissolved oxygen, inadequate nutrition, parasite infections, and physiological changes in fish, all contribute to fish contracting this disease (Ulkhayq et al., 2014). Meat quality suffers as a result of this bacterial illness, and catfish farmers lose money (Sukenda et al., 2016).

Antibiotics, including chloramphenicol and oxytetracycline, are commonly used in MAS disease control management efforts in catfish farming (Igbinsosa et al., 2012). Antibiotic misuse can result in a variety of issues in fish, including harmful bacterium resistance. Furthermore, it has the potential to contaminate the aquatic environment, with the presence of chemical residues from antibiotics in consumed fisheries products having an impact on human health. Application of probiotics is one option for preventing this condition (Ulkhayq et al., 2014).

Probiotics are living microbial organisms that benefit their hosts. The advantages of utilizing probiotics are that they can improve response to disease, improve nutrition, and help digestion. In fish, probiotics improve feed efficiency and non-specific immunity. Giving this probiotic to fish aids in healthy growth and illness resistance (Talpur et al., 2015).

Several studies have demonstrated that probiotics can strengthen the immune system of carp (Widanarni et al., 1970) and boost the survival of shrimp (Septiarini et al., 2011). *Lactobacillus* sp, *Photobacterium* sp, and *Bacillus* sp are bacteria that could be used as probiotics. Several studies have shown that using *Bacillus* sp. as a probiotic can improve fish immunity and growth (Ulkhayq et al., 2014).

There has been a lot of research into the usage of probiotic bacteria *Bacillus* sp. in the past. To prevent the pathogenic *A. hydrophilia* from colonizing the gastrointestinal system, this *Bacillus* sp competes with harmful bacteria for energy sources in the growth media and for adhesion sites in the gastrointestinal tract. The capacity of probiotic bacteria to cling to mucus and the surface of the digestive tract wall is the most important condition for their survival in the digestive system. In addition, *Bacillus* spable to create inhibitory compounds that can kill the disease *A. Hydrophila* (Lusiastuti, 2012).

According to the United Nations, the world's population will reach 11.2 billion people by 2100. The need for food and energy will rise as a result of this increase. PT. Biotek Cipta Kreasi (BCK), which was founded in 2018, is here to help with issues in agriculture, fisheries, food, the environment, and health. Since 2018, PT. BCK has been doing biotechnology research and development in order to generate valuable information, services, and high-quality products in the domains of agriculture, fisheries, the environment, and food (Anonim, 2020). In light of this, a study to investigate the ability of *Bacillus* sp. in preventing MAS in African catfish induced by *A. hydrophila* is required. The aims of this study are intended to give an ecologically friendly alternative for disease prevention in African catfish caused by pathogenic bacteria in catfish by using probiotic *Bacillus* sp.

## RESEARCH METHODS

The research method can be divide into several stage include,

### 1. Tools and materials

Petri dishes, Erlenmeyer flasks, test tubes, measuring cups, micropipette, measuring pipette, tweezers, scalpel, autoclave, LAF, vortex, Bunsen lamp, ose needle, digital scale, tissue, gauze, aluminum foil, cotton, and oven are among the instruments used. Healthy catfish, 70% alcohol, pond water, lipolytic media, Skim Milk Agar (SMA) media, Glutamate Starch Phenil (GSP) media, Nutrient Broth (NB), media Nutrient Agar media (NA), phosphate buffer sterile, amylyolytic media, cellulolytic media, and reverse osmosis (RO) water were among the materials used.

### 2. Sterilization of tools and materials

The equipment utilized, including as test tubes, erlenmeyer flasks, and petri dishes, are sterilized in an autoclave for 30 minutes at 121°C. Wet heating is often carried out in an autoclave or steam sterilizer, according to Istini et al., 2020. This can be easily removed with 15 minutes of pressured saturated steam at 121°C. Meanwhile, the materials were sterilized using the Tille et al., (2017) with some modifications, including RO water, lipolytic media, amylyolytic media, cellulolytic media, GSP media, NA media, and NB media sterilized at 121°C for 15 minutes.

### 3. Preparation of test

GSP media was made according to Asril & Lisafitri (2020) with the following modifications: 1 g L-glutamate, 2 g starch, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 7.4 ml phenol red, 1.2 g agar, 0.05 g magnesium sulfate per 100 ml. Each of these components was placed in a sterile Erlenmeyer with 100 mL RO water. Then it was placed on a hot plate set to 225°C. Then it was autoclaved at 121°C for 15 minutes to disinfect it.

The modified Setyati & Subagiyo (2012) approach was used to make the lipolytic medium. 0.5 ml Tween-80, 0.05 g NaNO<sub>3</sub>, 0.05 g K<sub>2</sub>HPO<sub>4</sub>, 0.025 g KCL, 0.01 g Peptone, 1 g agar, 0.025 g MgSO<sub>4</sub> were used to make lipolytic media for 50 ml. Each item was combined with 50 mL of RO water in an erlenmeyer. Then it was placed on a hot plate set to 225°C. Then it was autoclaved at 121°C for 15 minutes to disinfect it.

The modified Setyati & Subagiyo (2012) approach was used to make the cellulolytic medium. 0.02 g Carboxill Methyl Cellulose (CMC), 0.05 g NaNO<sub>3</sub>, 0.05 g K<sub>2</sub>HPO<sub>4</sub>, 0.025 g KCL, 0.01 g Peptone, 1 g agar, 0.025 g MgSO<sub>4</sub> were used to make cellulolytic media for 50 ml. Each item was combined with 50 mL of RO water in an erlenmeyer. Then it was autoclaved at 121°C for 15 minutes to disinfect it.

The modified Setyati & Subagiyo (2012) technique was used to prepare the amylyolytic medium. 0.5 g starch, 0.05 g NaNO<sub>3</sub>, 0.05 g K<sub>2</sub>HPO<sub>4</sub>, 0.025 g KCL, 0.01 g Peptone, 1 g agar, 0.025 g MgSO<sub>4</sub> were used to make amylyolytic media for 50 ml. Each item was combined with 50 mL of RO water in an erlenmeyer. Then it was placed on a hot plate set to 225°C. Then it was autoclaved at 121°C for 15 minutes to disinfect it.

The modified Setyati & Subagiyo (2012) protocol was followed for the production of SMA media. 2.5 g lactona milk and 2 g NA were used to generate 50 ml SMA medium. Each item was combined with 50 mL of RO water in an erlenmeyer. After that, it was placed on a hot plate set at 80°C. SMA media is not sterilized, so the protein structure in the media is not damaged, and it is then allowed to cool.

1 g of NA material was used to make 50 ml of NA medium. 50 mL RO water is added to the substance. Then it was autoclaved at 121°C for 15 minutes to disinfect it. 0.4 g of NB was used to make 50 mL of NB media. 50 mL RO water is added to the substance. Then it was placed on a hot plate set to 225°C. After the components have been thoroughly combined, pour them into 10 sterile test tubes with a capacity of 5 mL each. Then it was autoclaved at 121°C for 15 minutes to disinfect it.

#### 4. Isolation of *Bacillus* sp. gastrointestinal origin of catfish

This isolation method is based on Thankappan et al. (2015), but with a few modification. Healthy catfish caught at Godean Market Yogyakarta, with intact fins, no sores on the fish's skin, and a small belly. Isolation work was done outside the lab, although all tools were utilized in an aseptic environment with 70 % alcohol sprayed on them. The catfish is killed by pricking the brain with a knife on the head, then dissecting the stomach with a scalpel to extract the large intestine. The big intestine of a catfish is filled with sterile phosphate buffer and moved to homogenize it. A sterile test tube is used to collect the liquid from the large intestine. The liquid sample was dried in the oven for 10 minutes at 80°C. The dilution was then done in stages from 10<sup>1</sup> to 10<sup>6</sup>, and roughly 50 µl was inoculated into lipolytic media in the hopes of obtaining isolates with lipolytic activity right away. Then it was incubated for 24 hours at 37°C.

#### 5. Isolation of pathogenic bacteria from catfish infected with MAS

Because the pond water at PT. Cipta Kreasi Biotech had been infected with catfish pathogenic bacteria, pathogenic bacteria were isolated from the samples. A measuring pipette disinfected with 70% alcohol was used to collect 5 ml of catfish pond water samples. The sample was then diluted 10<sup>1</sup> to 10<sup>3</sup> times before being divided into 50 µl and inoculated on GSP media, a selective medium for *A. hydrophila* and *Pseudomonas* sp. Incubated for 24 hours at 37°C. If the yellow bacterial colony is *A. hydrophila*, according to Purba et al. (2020) *Pseudomonas* sp. colonized the purple-colored bacterial colonies.

#### 6. Enzymatic test

This test was conducted prior to the antagonist test in the hopes of getting *Bacillus* sp. with probiotic properties. Lipolytic, cellulolytic, proteolytic, and amyolytic media were used in this enzymatic assay. *Bacillus* sp. pure cultures were utilized as isolates. Tween-80 solution was employed in lipolytic media, Soluble Starch 1 percent in amyolytic media, SMA media in proteolytic media, and CMC 4 percent in cellulolytic media. *Bacillus* sp. was isolated. 1 ose was obtained and inoculated at 4 quadrant plane points. Amyolytic Index (AI), Cellulolytic Index (CI), Proteolytic Index (PI), and Lipolytic Index (LI) were evaluated with activity and could be measured using the equation proposed by Djauhari et al. (2016) as follows:

$$\text{Index} = \frac{DZ - DK}{DK}$$

Ket:

- DZ = Clear zone diameter
- DK = Bacterial colony diameter

## 7. The proteolytic test

With some adjustments, testtest is based on Efendi et al. (2017). *Bacillus* sp. is isolated. One ose was collected, implanted in four quadrant locations, and incubated at 37°C for 24 hours. The formation of distinct areles around the colonies generated by *Bacillus subtilis* cultured on SMA media revealed the bacteria's proteolytic activity. The diameter of the clear zone and the diameter of the bacterial colonies were used to compute the proteolytic index.

## 8. Antagonist Test

This approach was modified from Kurniasih et al. (2014), and it was used to investigate the ability of probiotic bacteria isp. to suppress the growth of harmful bacteria. *Bacillus* sp. comes in four varieties: *Bacillus* sp. 1, *Bacillus* sp. 2, *Bacillus* sp. A2, *Bacillus* sp. 4, *Bacillus* sp. First, 125 µl of pathogenic bacteria liquid culture was removed, then mixed with NA media that had not solidified (35-37°C). The mixture was then shaken to homogenize it. Furthermore, plating the NA media into a petri dish after it has been combined with pathogenic bacteria. After the pathogenic bacteria in the NA media had solidified, each *Bacillus* sp. was inoculated at four sites and incubated for 24 hours at 37°C. *Bacillus* sp. that are antagonistic to the colony will establish a clean zone around it. A ruler was used to measure the diameter of the generated clear zone.

## RESULTS AND DISCUSSION

### 1. Isolation of candidate *Bacillus* sp. from the digestive track of catfish

Tween-80 was the predominant component of the lipolytic media used in this isolation. The goal of using lipolytic media is to see if the bacteria *Bacillus* sp. It is capable of hydrolyzing lipids.

Figure 1 depicts the procedure of preparing the digestive tract of a catfish as a source of probiotic isolates *Bacillus* sp. In this isolation, only one isolate was obtained. With the initial marking of the medium from cloudy to clear, *Bacillus* sp. forms a clear zone. This demonstrates that *Bacillus* sp. can degrade lipase. *Bacillus* sp., according to (Ervina et al., 2020), can degrade lipolitic medium by displaying a hydrolysis zone around the colony. *Bacillus* sp. will hydrolyze Tween-80 to mono-oleic acid using the substance Tween-80. In order to create a clear zone surrounding the bacterial colony.



**Figure 1.** The process of isolation *Bacillus* sp

Figure 2 shows *Bacillus* sp. isolation results from the big intestine of catfish. The colonies of the isolates observed were cream in color and spherical in shape. The spherical form of the colony and the cream color of the colony indicate that the bacterium belongs to the genus *Bacillus* sp. *Bacillus* sp. colonies are characterized by a pale cream color and a circular and irregular colony shape, according to (Puspita et al., 2017). In additional tests, such as the gram test was not performed to establish that the isolate was truly *Bacillus* sp. The results of this isolation could still be classified as bacterial isolates that are similar to *Bacillus* sp.



**Figure 2.** Isolate of the digestive tract of catfish

## 2. Isolation of Pathogenic Bacteria from Catfish infected with MAS

The bacteria *Pseudomonas* sp. were prepared for the antagonist test by renewing the colonies in NB medium. One colony was first collected with ose and then cultured in NB medium. The cells were then incubated at 37°C for 24 hours.

The bacteria that were isolated were assigned to the genus *Pseudomonas* based on morphological findings (Figure 3). The colonies that formed were purple in color, according to the findings. The colony is *Pseudomonas* sp. based on the color change.

Purba et al., (2020) reported that when bacteria colonies *Pseudomonas* sp. were cultured in GSP medium, they became purple. The bacteria *Pseudomonas* sp. is responsible for the purple color shift. On GSP media, this can hydrolyze starch.



**Figure 3.** Isolate of *Pseudomonas* sp

### 3. Enzymatic testing of *Bacillus* sp. Origin of the digestive tract of catfish

The goal of this test is to see if the isolate has any enzymatic activity. Amylase, lipase, protease, and cellulose enzymes are enzymes generated by bacteria found in probiotics. These enzymes hydrolyze complex compounds, such as carbs, proteins, and lipids, into simpler molecules to aid digestion and nutrient absorption in fish (Fajri et al., 2012). Lipolytic, cellulolytic, and amylolytic assays are included in this enzymatic test. This test was used as a preliminary step in the screening process to find isolates that were similar to *Bacillus* sp. and have probiotic qualities. The following are the findings of the enzymatic test, as shown in Table 1.

**Table 1.** Enzymatic index

No.	Treatment	Result
1.	Lipolytic Test	37%
2.	Amylolytic Test	35%
3.	Cellulolytic Test	78%
4.	Proteolytic Test	33%

The results in Table 1 reveal that isolates closely related to *Bacillus* sp. that has lipolytic activity. This may be seen in the media, which has become clearer after being hazy at first. This indicates that this isolate can make lipase enzymes.

Isolates related to *Bacillus* sp. have an amylolytic activity in addition to lipolytic activity (Figure 4). The amylolytic index value indicated by the formation of a clear zone around the colony was 35%. The diameter of the amylolytic index varies, depending on the bacterial species (Setyati & Subagiyo, 2012). This is due to variances in each isolate's capacity to hydrolyze starch, which are driven by differences in the environment and genes possessed by each bacterium. After dropping a 1 percent iodine solution into the clear zone, the iodine solution is absorbed by the selective media surrounding the clear zone, resulting in a dark blue tint.



Figure 4. Lipolytic activity

The results of the cellulolytic activity tests (Figure 5) revealed that isolates that were closely related to *Bacillus* sp were able to hydrolyze cellulose at a rate of 78%. The use of 0.4 percent CMC material to induce turbidity in the media such that when bacteria have cellulase activity, a clear zone is formed, according to Rahayu et al., (2014).

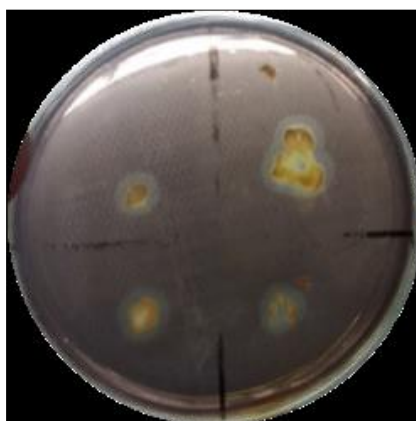


Figure 5. Cellulolytic activity

Based on the ability to manufacture protease enzymes, the proteolytic test findings demonstrate that *Bacillus* sp. possesses proteolytic activity, which is defined by the creation of a clear zone surrounding the bacterial colony with a proteolytic index of 33%. The protein hydrolysis process results in the formation of a clear zone.

According to Yuniati et al (2015), the *Bacillus* sp. species is one of the protease-producing bacteria. The protease activity of isolates with bacteria that were close to *Bacillus* sp. was determined using this proteolytic test.

#### 4. The antagonist test

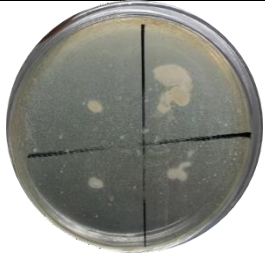


The goal of this test is to see if isolates whose bacteria are similar to *Bacillus* sp. can prevent the growth of fish pathogenic bacteria. This test was performed on *Pseudomonas* sp., and it was based on the results of pathogenic bacteria isolation acquired by *Pseudomonas* sp., which was marked with a purple isolate color. Purba et al., (2020) reported that when bacteria colonies *Pseudomonas* sp were cultured in GSP medium, they became purple. The bacteria *Pseudomonas* sp. is responsible for the purple color shift. On GSP media, this can hydrolyze starch.



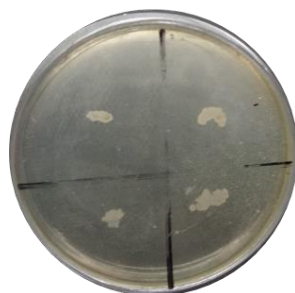
Table 2 shows the results of *Bacillus* sp. tests against *Pseudomonas* sp. The absence of a distinct zone surrounding the colony suggested that the isolates that were close to *Bacillus* sp. did not exhibit antibacterial activity, as evidenced by the results of the antagonist test. Candidate probiotic bacteria must be able to attach to the intestinal mucosal layer and use mucus as a source of nourishment for colonization, according to Djauhari et al., (2016). They must also be able to multiply in the digestive system of fish.

*Bacillus* 1 and *Bacillus* 2 candidate isolates were isolated from soil, whereas *Bacillus* 4 and *Bacillus* A2 candidate isolates were isolated from the large intestine of catfish. The isolates from the two isolation sources were shown to have no ability to suppress the growth of the pathogenic bacteria *Pseudomonas* sp. based on the results of the antagonist test. As a result, the nature of this antagonist is unaffected by the isolate's source factor, although the ability of each isolate is variant dependant.

Table 2. Antagonist test

Variant <i>Bacillus</i> sp.	Antagonist test	Activity
<i>Bacillus</i> 1		-
<i>Bacillus</i> 2		-
<i>Bacillus</i> A2		-

*Bacillus*  
4



## CONCLUSION

Based on the findings of PT. Biotech Cipta Kreasi investigation of *Bacillus* sp. potential antibacterial activity as a probiotic, it can be concluded that *Bacillus* sp. candidate isolates isolated from the large intestine of catfish did not have antibacterial activity against *Pseudomonas* sp. This antagonist is not influenced by the isolate source factor, but the ability of each isolate depends on the variant.

## ACKNOWLEDGMENTS

With the help of a few people, this Field Work Practice task can be completed successfully. The researcher would like to express his gratitude to PT. Cipta Kreasi Biotech, as a partner for street vendors, as well as the University of 'Aisyiyah Yogyakarta for overseeing lecturers for street sellers.

## REFERENCES

- Anonim. Produksi Perikanan Lokal Baru Mampu Penuhi 60% Kebutuhan DIY, Sisanya dari Luar Daerah. 2019. <https://jogja.tribunnews.com/amp/2019/02/11/produksi-perikanan-lokal-barumampu-penuhi-60-persen-kebutuhan-di-diy-sisanya-dari-luar-daerah?page=2> (Diakses pada 10 Desember 2020).
- Anonim. Profil-Biotek Cipta Kreasi. 2018. <https://biotek.co.id/home>. (Diakses pada 23 November 2020).
- Asril, M., & Lisafitri, Y. (2020). Isolasi Bakteri Pelarut Fosfat Genus *Pseudomonas* dari Tanah Masam Bekas Areal Perkebunan Karet di Kawasan Institut Teknologi Sumatera. *Jurnal Teknologi Lingkungan*, 21(1), 40–48. <https://doi.org/10.29122/jtl.v21i1.3743>
- Djauhari, R., Widanarni, Sukenda, Suprayudi, M. A., & Zairin, M. (2016). Characterization of *Bacillus* sp. NP5 and its application as probiotic for common carp (*Cyprinus carpio*). *Research Journal of Microbiology*, 11(4–5), 101–111. <https://doi.org/10.3923/jm.2016.101.111>
- Efendi, Yusra, & Oktavianis, V. (2017). Optimasi Potensi Bakteri *Bacillus subtilis* Sebagai Sumber Enzim Protease. *Jurnal Aquatika Indonesia*, 2(1).
- Ervina, E., Ekowati, C. N., Sumardi, S., & Rosa, E. (2020). Lipolytic-screening of *Bacillus* genera as Biocontrol candidate In Coffee Plantation. *Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati*, 7(1), 31–34. <https://doi.org/10.23960/jbekh.v7i1.12>

- Fajri MA, Adelina, Aryani N. Penambahan Probiotik Dalam Pakan Terhadap Pertumbuhan dan Efisiensi Pakan Benih Ikan Baung (*Hemibagrus nemurus*). Pekanbaru Riau. 2016
- Igbinosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M., & Okoh, A. I. (2012). Emerging Aeromonas species infections and their significance in public health. *The Scientific World Journal*, 2012. <https://doi.org/10.1100/2012/625023>
- Istini. Pemanfaatan Plastik Polipropilen Standing Pouch Sebagai Salah Satu Kemasan Sterilisasi Peralatan Laboratorium. *Indonesia Journal Of Laboratory*. 2020; 2 (3): 41-46.
- Kurniasih, T., Lusiastuti, A. M., Azwar, Z. I., & Melati, I. (2014). Isolasi Dan Seleksi Bakteri Saluran Pencernaan Ikan Lele Sebagai Upaya Mendapatkan Kandidat Probiotik Untuk Efisiensi Pakan Ikan. *Jurnal Riset Akuakultur*, 9(1), 99. <https://doi.org/10.15578/jra.9.1.2014.99-109>
- Lusiastuti, A. M. (2012). Seleksi Kandidat Probiotik Anti Aeromonas Hydrophila Untuk Pengendalian Penyakit Ikan Air Tawar. *Jurnal Riset Akuakultur*, 7(2), 269. <https://doi.org/10.15578/jra.7.2.2012.269-277>
- Purba, A., Riauwaty, M., & Syawal, H. (2020). Sensitivitas Larutan Daun Ketapang (*Terminalia catappa* L.) terhadap Bakteri Aeromonas hydrophila. *Jurnal Perikanan Dan Kelautan*, 25(2), 116–122.
- Puspita, F., Ali, M., & Pratama, R. (2017). Isolasi dan Karakterisasi Morfologi dan Fisiologi Bakteri Bacillus sp. Endofitik dari Tanaman Kelapa Sawit (*Elaeis guineensis* Jacq.). *Jurnal Agrotek*, 6(2), 44–49.
- Rahayu, A. G., Yahyani, Y., & Puspita, F. (2014). Uji Aktivitas Selulolitik dari Tiga isolat bakteri bacillus sp. Galur Lokal Riau. *JOM FMIPA Unri*, 1(2), 319–327.
- Setyati, W. A., & Subagiyo. (2012). Isolasi dan Seleksi Bakteri Penghasil Enzim Ekstraseluler (proteolitik, amilolitik, lipolitik dan selulolitik) yang Berasal dari Sedimen Kawasan Mangrove. *Jurnal Ilmu Kelautan*, 17(3), 164–168.
- Septiarini W, Padaga, Oktaviani. Aktivitas Antimikroba Bakteri Asam Laktat (BAL) yang Diisolasi Dari Feses Orangutan (*Pongo pygmaeus*) Terhadap Penghambatan Pertumbuhan Bakteri Enterik Patogen Secara *In-Vitro*. *Skripsi*. Universitas Brawijaya. Malang. 2011
- Sukenda, , Rafsyanzani, M. M., Rahman, , & Hidayatullah, D. (2016). Kinerja probiotik Bacillus sp. pada pendederan benih ikan lele Clarias sp. yang diinfeksi Aeromonas hydrophila. *Jurnal Akuakultur Indonesia*, 15(2), 162. <https://doi.org/10.19027/jai.15.2.162-170>
- Sya'bani, N., Yustiati, A., & Lusiastuti, A. (2015). Frekuensi Penambahan Probiotik Bacillus SP. Dan Staphylococcus SP. Pada Media Pemeliharaan Benih Ikan Lele Dumbo (*Clarias Gariepinus*) Untuk Ketahanan Terhadap Aeromonas Hydrophila. *Jurnal Perikanan Dan Kelautan Unpad*, 6(2), 130–140.
- Talpur AD, Munir M, Mary A, Hasim R. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and

- disease resistance against *Aeromonas hydrophila* in snakehead *Channa striata* fingerlings. *Aquaculture*, 2015; 426-427: 14-20
- Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., & Anbarasu, K. (2015). Characterization of *Bacillus* spp. From the Gastrointestinal Tract of *Labeo rohita*—Towards to Identify Novel Probiotics Against Fish Pathogens. *Applied Biochemistry and Biotechnology*, 175(1), 340–353. <https://doi.org/10.1007/s12010-014-1270-y>
- Ulkhag, M. F., Widanarni, & Lusiastuti, A. M. (2014). Aplikasi probiotik *Bacillus* untuk pencegahan infeksi *Aeromonas hydrophilla* pada ikan lele. *Jurnal Akuakultur Indonesia*, 13(2), 105–114.
- Widanarni, W., Wahjuningrum, D., & Puspita, F. (1970). Aplikasi Bakteri Probiotik melalui Pakan Buatan untuk Meningkatkan Kinerja Pertumbuhan Udang Windu (*Penaeus monodon*). *Jurnal Sains Terapan*, 2(1), 19–29. <https://doi.org/10.29244/jstsv.2.1.19-29>
- Yuniati R, Nugroho TT, Puspita F. Uji Aktivitas Enzim Protease dari Isolat *Bacillus* sp. Galur Lokal Riau. *Kampus Binawidya Pekanbaru*. 2015; 1(2).