Comparative Test on Bacteria in the Digestive Tract of Vannamei Shrimp (*Litopenaeus vannamei*) at Intensive and Extensive Ponds in Ujungpangkah, Gresik

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Abstract. Shrimp is one major commodities in industry fisheries with high economic value and market (high demand product). Shrimp is associated to issues of disease, one of which caused by bacteria. The amount of bacteria that attack shrimp is variable, depending on the cultivation. This research aims to understand the number and type of dominant pathogenic bacteria based on the system of extensive and intensive cultivation as well as to compare the number of pathogenic bacteria on both fishponds. The methods used in this research was comparative method by t-test of two independent samples. Samples were collected from two types of aquaculture: intensive and extensive. Three ponds were selected from each type of activity and 10 shrimps were collected from each of the selected pond as samples. The total samples collected were 60 shrimps. The observed parameters were the types of bacteria that exist in the digestive tract. The results show that the most common pathogenic bacteria are found in intensive cultivation. Intensive ponds have the number of bacteria of *Mycobacterium* sp. at 12 isolates, *Clostridium* sp. at 28 isolates, *Flavobacterium* sp. at 21 isolates, *Acinetobacter* sp. at 18 isolates, *Vibrio* sp. at 16 isolates, *Pseudomonas* sp. at 14 isolates, *Bacillus* sp. at 28 isolates, and *Staphylococcus* sp. at 9 isolates. Meanwhile, extensive ponds have the number of *Mycobacterium* sp. at 10 isolates, *Clostridium* sp. at 12 isolates, *Flavobacterium* sp. at 15 isolates, *Acinetobacter* sp. at 18 isolates, *Vibrio* sp. at 17 isolates, *Pseudomonas* sp. at 13 isolates, *Bacillus* sp. at 37 isolates, and *Staphylococcus* sp. at 18 isolates.

Keywords. shrimp, isolate, bacteria.

1. Introduction

The development of shrimp farming is one of the priorities in the development of aquaculture in Indonesia. Shrimp farming activities in Indonesia began to develop rapidly since 1987 with the application of extensive, semi-intensive and intensive technology (Ministry of Maritime Affairs and Fisheries, 2004).

The definition of Shrimp farming activities is raising shrimp in brackish water ponds for a certain period of time, harvesting and selling them for the purpose of gaining profits. Intensive cultivation
used in shrimp farming has the principle of high stocking density by feeding in high doses. Extensive cultivation is used in shrimp farming activities with the principle of low density solids without giving extensive feed (Garno, 2004). Giving the right amount of feed can make shrimp grow to a maximum size (Nuhman, 2009). Davis et al. (1992) stated that the interaction of various kinds of minerals contained in feed can increase growth. Feeds with different Ca/P ratios determine calcium carapace content and shrimp feed efficiency.

Farming activities cannot be separated from water quality management. Water quality is also one of the factors that is the significant to the success of shrimp pond farming (Dahuri et al., 2004). Control of water quality in general in aquaculture activities includes observing salinity, pH, temperature and DO (Poernomo, 1992).

Pollution of organic matter in ponds stimulates shrimp disease caused by pathogenic bacteria and viruses. One source of organic pollutants in ponds is feed. The food given to the pond is not all can be eaten by the shrimp, some of the remaining food will be suspended in the water and most of the other will settle at the bottom of the pond. The decomposition of organic food residue requires oxygen which causes increased use of oxygen in the pond environment. This condition will continue until the critical point that produces ammonia (NH3) and hydrogen sulfide (H2S) which supports developing pathogenic bacteria (irianto, 2003).

According to Irianto (2005) bacteria in fish and shrimp can be found on the surface of the external body and digestive tract. Some bacteria are pathogenic, while others are beneficial for fish because they aid digestion, synthesize vitamins and decompose organic matter in the waters.

According to Suminto et al. (2007), bacteria found in vannamei shrimp intestine, namely Enterobacter, Acinobacter, Bacillus, Lactobacillus, Mycobacterium, Flavobacterium, Clostradium, Desulfovibrio, Alkaligenes, and Vibrio. The bacteria found differ depending on the cultivation activities carried out. Based on the problems found, further research is needed on the bacteria found in the digestive tract of vannamei shrimp which are intensively and extensively cultivated.

2. Research method

2.1. Time and Place
This research was conducted from October to November 2016. The vannamei shrimp (Litopenaeus vannamei) which used were collected from intensive and extensively cultivated ponds in Ujungpangkah village, Gresik. The research was conducted at the Education Laboratory of the Faculty of Fisheries and Marine of Universitas Airlangga, Surabaya.

2.2. Research materials
The equipment used includes incubator, autoclave, Erlenmeyer 500 ml tube, hot plate, aluminum foil, bunsen, petridish, plastic wrap, glass, measuring cup, test tube, cotton, pipette (0.1 ml, 1 ml and 10 ml) and micropipette, binocular microscope, glass object, microtube, ose needle. The materials used in this study were TSA non-selective agar media (Tryptone Soya Agar), oxidase strip test, TCBS (Thiosulfate Citrate Bile Salt Agar), Physiological NaCl, SIM (Solid Indole Motyl) media, material for Gram staining test (crystal violet, lugol iodine, safranin, ethyl alcohol 95%), hydrogen peroxide (H2O2) 3%, aquades.

2.3. Research method
The method used in this study was a comparative method with t-test on two independent samples. The comparative research method is to compare the similarities and differences of two or more facts and the properties of the object being studies based on certain thoughts (Nazir, 2005).

3. Research procedure

3.1. Tools preparation
The tools used included petri dish and sterilization was done on test tubes using autoclave. Petri dish was wrapped using opaque paper or heat-resistant plastic. The test tube was first covered with cotton,
then wrapped using a heat-resistant plastic. The autoclave was carried out at 121°C temperature for 15 minutes (Dwidjoseputro, 2003).

3.2. Media production
The media used for isolation was TSA saline. Ten grams of TSA was added with 3% NaCl at 7.5 grams for 250 ml of distilled water. The solution was put into a 500 ml Erlenmeyer and stirred until it went homogeneous. Erlenmeyer containing a solution was closed with cotton and newspaper to be then heated using a hot plate to boil. The media was sterilized using autoclave at 121°C temperature for 15 minutes (Rahmadani, 2013). The sterilized media was waited until the temperature dropped to be then poured into petridish as much as 10 ml. Then, the media was waited until it hardened to be able to do the next step.

3.3. Sampling
Samples were taken from two ponds, namely the intensive and extensive ponds that had many plots. The number of farm plots selected from each pond was three plots. Ten shrimps were taken from each shrimp pond, and the total shrimps collected was 60 shrimps. Shrimp collected from the farm using anco tool. The sampling point in one pond was done randomly.

The shrimps collected were 30 days old for each type of cultivation. Ponds with intensive systems have a salinity of 23 ppt, while ponds with extensive systems have a salinity of 30 ppt. Samples were placed in containers and given a sample code based on the location of the pond. Ponds with extensive systems are coded E1, E2, and E3. Ponds with intensive systems are coded I1, I2, and I3.

3.4. Isolation of shrimp gastrointestinal tract
Isolation is done using the streak plate technique. One petri dish was used to isolate the digestive tract of two shrimps. Vannamei shrimp was sprayed using 70% alcohol in order to sterilize and remove mucus from the outside of its body, followed by cutting the shrimp abdomen to follow the intestinal channel. The shrimp’s intestine was removed from its body using tweezers, while the contents of the shrimp’s intestine was removed using a scalpel. The contents of the shrimp digestive tract were then isolated into the prepared media.

The method used was the streak plate method. The streak method was done by using the ose needle to be burned in bunsen fire first until it glowed, then cooled by attaching the tip of the needle to the petri dish glass. A small portion of the shrimp’s digestive tract was taken using ose needle and then streaked into TSA saline and TCBS media in a tight manner and one vertical stroke direction to half of the media on the petri dish. The ose needle was then reheated and cooled on the inner surface of the petroleum glass. After cold streaking was carried out at the end of the first streak, the second streak was carried out in one horizontal stroke direction.

3.5. Bacteria purification
Purifying bacteria was almost the same as isolating bacteria. The bacteria to be purified were taken from the isolation of the samples that have been done before. The previously-isolated bacteria were determined by the colonies of bacteria to be purified. Four different bacterial colonies were taken from one sample of the isolated shrimp. One petri dish was divided into four parts using a permanent marker on the outside of the petri dish.

The ose needle was heated using bunsen fire until it glowed, then cooled by attaching it to the top of the petri dish containing bacterial isolation. The needle ose was then attached to the desired bacterial colony. Bacterial colonies that have been attached to the osseous needle were transferred to the prepared TSA saline media. Streak started from the top of the petridish, zigzag line was drawn vertically according to the prepared plot. This process was carried out repeatedly until only one pure colony grew in one part.
3.6. Identification of bacteria

3.6.1. Observation of bacterial colonies

Observation of bacterial colonies can be done by growing bacterial isolates on agar. The aspects observed include: colony shape and colony color.

3.6.2. Observation of bacterial morphology

Observation of bacterial morphology can be done with Gram staining. Gram staining aims to determine which bacteria belong to the group of Gram negative bacteria and which belongs to groups of Gram positive bacteria. Gram staining used crystalline violet, iodine, ethyl alcohol and safranin. The ingredients were given alternately, starting with the making of bacterial preparations on glass objects added with physiological NaCl, followed by the addition of crystal violet and left for 1 minute. Preparations were washed with running water until clean, then iodine solution was added and left for 1 minute. The preparations were washed again with running water, ethyl alcohol was pressed for 5 seconds and washed again with running water. The preparation was washed with safranin dye for 30 seconds and continued to wash with running water.

Gram-positive bacteria are marked in purple indicating that the bacteria are able to bind the carbolic gentian violet color, while Gram-negative bacteria are marked with pink indicating that the bacteria cannot bind the gentian violet carbol color and are only colored by safranin (Hadioetomo, 1993).

3.6.3. Catalase test

Catalase test aims to determine the nature of bacteria in producing the enzyme catalase. Catalase test uses H2O23% reagent which is dropped on a glass object. Bacteria that have been grown on agar media are taken using an ose needle and placed on a glass object. Bacterial isolates were added two drops of H2O23% solution. A positive test is indicated by the formation of oxygen bubbles indicating that the organism in question produces the enzyme catalase which converts H2O23% into water and oxygen (Hadioetomo, 1993).

3.6.4. Oxidase test

The oxidase test aims to determine the presence of oxidase enzymes in bacteria using the oxidase strip test. The oxidase test uses an ose needle, a bacterial colony that is scratched on the oxidase strip test. Color changes that occur on the strip test are observed. The change in color to blue violet on the oxidid strip test was stated as positive and indicated that the bacteria were non-enteric bacteria. Whereas if there is no change in color, the oxidase strip test is declared negative (Hadioetomo, 1993).

3.6.5. O/F test (oxidative/fermentative)

A medium (oxidative/fermentative) O/F test aims to differentiate the properties of oxidative bacteria and fermentative bacteria containing oxidation or fermentation against glucose. Two test tubes are used for one fermentative and oxidative test with one of the tubes closed using paraffin so that there is no air that supports fermentation. Pure bacterial isolates were inoculated on O/F media by puncture using a straight needle, then stored in an incubator for 24 hours. Positive results are obtained if the oxidative test changes the yellow color of the open tube, and the blue color on the paraffin-covered tube. Positive results for fermentative tests change color to yellow for open tubes and closed tubes. Non-oxidative/fermentative bacteria in open and closed tubes are marked by no change in color (Kusdawati and Sudarno, 2011).

3.6.6. Motility test and indole production

Motility test aims to determine the possibility of motile in bacteria and to find out the indole production of Tryptophane. Motility tests are carried out using SIM media. Isolates inoculated into tubes containing SIM media using a straight needle. Straight needles are first sterilized before taking isolates to the petridish. Isolates in straight needles are inserted and pulled from the media upright.
The tubes were incubated at 35°C for 48 hours, then examined for bacterial growth on the lines made. Positive results are obtained if the bacteria grow to spread from the inoculation line, while the negative results if the bacteria only grow along the inoculation line (Hadioetomo, 1993).

3.6.7. **TSIA test (Triple Sugar Iron Agar)**
The TSIA test (Triple Sugar Iron Agar) aims to distinguish types of bacteria based on their ability to break glucose, lactose, and sucrose. The TSIA test also serves to determine the possibility of bacteria producing H2S gas. The TSIA media used is divided into two parts, slant and butt. Pure bacterial colonies are inoculated into the TSIA media using ose needles and stem needles. The stem needle is used to stab the bacteria perpendicular to the butt, while the needle is used to inoculate the bacteria zig zag into the slant section. Educational at 29°C for 24 hours. The changes observed were the color changes in the slant to red and butt to yellow, the bacteria were able to ferment glucose, whereas if the two parts were yellow the bacteria were able to ferment sucrose and lactose (Yusuf, 2009).

3.6.8. **Sugar test**
Sugar test aims to determine the ability of bacteria to degrade sugar and produce organic acids derived from each type of sugar, namely glucose, sucrose, maltose, arabinose, mannitol, and inositol. Pure bacterial colonies are inoculated into each medium using an ose needle, then stored for 24 hours. Positive results are obtained if each media changes color to yellow, while negative results if there is no change in color. Color changes occur because bacteria form acids from glucose fermentation (Yusuf, 2009).

3.7. **Determination of bacterial genus**
Determination of the genus of bacteria is useful for processing data. Bacteria that have been tested, are grouped according to the test results obtained. The identification table in the cowan and steel book's Manual for Identification of Medical Bacteria is the basis for identification. Bacteria are grouped based on Gram staining, followed by the shape test, catalase test, motility test and oxidase test. The results obtained from grouping can be continued with the determination of the genus from the bacteria obtained. Bacteria taken for data are only pathogenic bacteria, while non-pathogenic bacteria are removed. This aims to facilitate the calculation of the dominant pathogenic bacterial colonies in cultivated shrimp.

3.8. **Calculation of bacterial colonies number**
Bacteria that have been determined by the genus are grouped according to the given code. Bacteria obtained from intensive ponds are grouped to calculate the dominant bacterial colonies. Bacteria obtained from extensive ponds are also grouped for calculation of the dominant bacterial colonies. Grouping is intended to make it easier to compare the number of bacterial colonies found in shrimp with extensive intensive farming.

3.9. **Data analysis**
After collecting data from the results of the biochemical test activities, analysis of research data was carried out by matching the data in the Bergey's Manual of Determinative Bacteriology and Cowan and Steel's Manual for the Identification of Medical Bacteria. The data that has been obtained is then processed using two independent sample t tests.

4. **Results and discussion**

4.1. **Isolation and domination of bacteria in vannamei shrimp’s intestine**
The streak method was used to isolate shrimp intestines, one shrimp was streaked on two petridishes, one shrimp containing TSA media and one petridish containing TCBS media. The results of isolation on TSA saline and TCBS media can be seen in Figure 5.1.
The observation of bacteria found in the contents of the vannamei shrimp intestine from two different pond systems of thirty samples showed 30 bacterial isolates from intensive ponds and 30 bacterial isolates from extensive ponds which overall had almost the same colony morphology.

4.2. Identification of intensive and extensive pond pathogen bacteria
The results of bacterial purification in vannamei shrimp in intensive ponds and extensively show 300 isolates. 300 isolates were classified according to colony color and morphological form. From this classification 8 groups of isolates were found that had similar characteristics.

Table 1. Bacterial biochemical test results

| Characteristics | Results of bacteria identification |
|-----------------|-----------------------------------|
|                 | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| Gram test       |    | +  | -  | -  | -  | -  | +  | +  |
| Cell morphology | Bacil | Bacil | Bacil | Bacil | Bacil | Bacil | Bacil | Coccus |
| oxidase         | +  | -  | +  | +  | +  | +  | +  | -  |
| Catalase        | +  | -  | +  | +  | +  | +  | +  | +  |
| O/F             | F  | F  | F  | O  | F  | -  | O  | F  |
| MOtility        | +  | D  | -  | -  | +  | +  | +  | -  |
| Indole          | -  | +  | +  | -  | +  | -  | -  | +  |
| Ornithine       | +  | +  | +  | -  | +  | +  | +  | +  |
| TSIA            | K/K | K/A | K/A | K/A | K/A | K/A | K/K | A/A |
| Simm. Citrate   | +  | +  | -  | +  | -  | +  | +  | +  |
| Glucose         | +  | -  | +  | +  | -  | +  | +  | +  |
| Lactose         | +  | +  | -  | -  | -  | -  | -  | +  |
| Sucrose         | -  | -  | -  | -  | +  | +  | +  | +  |
| Mannitol        | +  | +  | +  | -  | +  | -  | -  | +  |
| Identification result | A | B | C | D | E | F | G | H |

Information:
A: *Plesiomonas* sp.  
B: *Clostridium* sp.  
C: *Flavobacterium* sp.  
D: *Acinetobacter* sp.  
E: *Vibrio* sp.  
F: *Pseudomonas* sp.  
G: *Bacillus* sp.  
H: *Staphylococcus* sp.
Identification is an activity carried out to determine the types of certain organisms with the observation, testing. The identification of microorganisms begins with testing of colonies, observing morphological characteristics, testing on test media and biochemical tests (Subyakto, 2009). In intensive and extensive aquaculture activities, 8 types of bacteria were found to attack vannamei shrimp. The results of the bacteria found are almost the same because of the similarity in the type of water used. The number of bacteria found in each type of cultivation does not have a significant difference.

Table 2. The results of identification of all bacteria in intensive and extensive farming.

| No | Bacterial species  | Quantity |
|----|--------------------|----------|
|    |                    | Intensive | Extensive |
| 1  | Plesiomonas sp.    | 12        | 15        |
| 2  | Clostridium sp.    | 28        | 18        |
| 3  | Flavobacterium sp. | 21        | 14        |
| 4  | Acinetobacter sp.  | 18        | 18        |
| 5  | Vibrio sp.         | 16        | 15        |
| 6  | Pseudomonas sp.    | 14        | 14        |
| 7  | Bacillus sp.       | 28        | 37        |
| 8  | Staphylococcus sp. | 9         | 17        |
| 9  | Bacteria that do not grow | 4 | 10 |
|    | Total bacteria     | 146       | 140       |

*Plesiomonas* sp. Bacteria have 12 isolates for intensive cultivation consisting of 3 isolates from farm 1, 3 isolates from farm 2, and 6 isolates from farm 3. Whereas an extensive number of 15 isolates consisted of 4 isolates from pond 1, 6 isolates from farm 2 and 5 isolates from ponds 3. *Plesiomonas* sp. including pathogenic microbes found in the digestive tract. This bacterium is commonly found in tropical climates (Molinari dkk, 2003).

*Clostridium* sp. Bacteria 28 isolates in intensified cultivation consisted of 9 isolates from farm 1, 9 isolates from farm 2, and 10 isolates from farm 3. While 12 isolates in extensive cultivation consisted of 6 isolates from farm 1, 6 isolates from farm 2 and 5 isolates from farm 3. The difference in the number of colonies from these two farms is quite high. *Clostridium* sp. Bacteria can cause food poisoning, these bacteria produce entotoxins which can attack the digestive tract and cause gastrointestinal symptoms (Sari, 2010).

*Flavobacterium* sp. in intensive cultivation has 21 isolates consisting of 9 isolates from pond 1, 6 isolates from farm 2, and 5 isolates from pond 3. Whereas in extensive cultivation there were 15 isolates consisting of 6 isolates from farm 1, 5 isolates from farm 2, 3 isolates from ponds 3. Flavobacterium sp. bacteria is a type of bacteria that has the potential as a probiotic because it has the ability to degrade fats, proteins, and carbohydrates (Suminto et al, 2007).

Bacteria *Acinetobacter* sp. in intensive cultivation there were 18 isolates consisting of 7 isolates from pond 1, 6 isolates from farm 2, and 5 isolates from pond 3. Whereas in extensive cultivation there were 18 isolates consisting of 7 isolates from pond 1, 6 isolates from farm 2, and 5 isolates from ponds 3. According to Jawetz (2005) the bacteria *Acinetobacter* sp. including the class of Gram negative bacteria.

*Vibrio* sp. bacteria in intensive cultivation there were 16 isolates consisting of 6 isolates from farms 15 isolates from farm 2, and 5 isolates from ponds 3. Whereas in extensive cultivation there were 15 colonies consisting of 5 isolates from farm 1, 5 isolates from farm 2 and 5 isolates from ponds 3. *Vibrio* sp. bacteria. is a pathogenic bacterium that infects and causes disease when shrimp conditions are weak and environmental factors are extreme. Number of *Vibrio* sp. bacteria in intensive aquaculture ponds there is much less than extensive cultivation (Nasi et al, 2007).

*Pseudomonas* sp. Bacteria in intensive cultivation there were 14 isolates found in 4 isolates from
pond 1, 5 isolates from farm 2, and 5 isolates from ponds 3. Bacteria Pseudomonas sp. in extensive cultivation there were 13 colonies which were found there were 6 from farms 1, 3 from farms 2, and 4 from farms 3. According to Hardhianto (2010), these bacteria are types of bacteria capable of producing several enzymes such as proteases, amylase and lipase, so that they can help the digestive process that takes place in the stomach and intestines.

There were 28 isolates of *Bacillus* sp. in intensive cultivation consisting of 7 isolates from pond 1, 10 isolates from farm 2, 11 isolates from ponds 3. While in extensive cultivation there were 37 isolates consisting of 11 isolates from farm 1, 10 isolates from pond 2, 16 isolates from pond 3. The bacteria showed the characteristics of Gram positive bacteria in the form of stem cells, catalase and positive oxidase. According to Holt et al., (1994) *Bacillus* sp. grows at optimum temperature 28°C. *Bacillus* sp. Bacteria is a proteolytic bacterium that can break down proteins (Fardiaz, 1992).

| No | Parameter | Unit | Range          |
|----|-----------|------|----------------|
|    | Intensive |      | Extensive      |
| 1  | Temperature | Celsiuse | 27-30          | 28-31          |
| 2  | Salinity   | Ppt  | 23             | 30             |
| 3  | pH         | -    | 7.7-7.3        | 7.3-7.7        |
| 4  | DO         | mg/l | 4              | 4              |

*Staphylococcus* sp. bacteria in intensive cultivation contain 9 isolates consisting of 3 isolates from pond 1, 3 isolates from farm 2, and 3 isolates from pond 3. Whereas in extensive cultivation there were 17 isolates consisting of 6 isolates from pond 1, 6 isolates from pond 2, 5 isolates from ponds 3. *Staphylococcus* sp. Bacteria is a Gram positive bacterium, motile, with milky white colony (Holt et al., 1994).

Bacterial growth is influenced by various factors and each bacterium needs a different growth condition, several factors that influence the growth of bacteria, namely nutrient content, temperature, pH, DO and light. The value of the temperature range in intensive ponds is 27°C-30°C, while the temperature range in extensive ponds is 28°C-31°C. This is in accordance with Haliman and Adijaya (2005) that the optimum temperature of *vannamei* shrimp growth is between 26°C-32°C.

The results of pH measurements in intensive ponds are around 7.7-7.3, while the pH range in extensive ponds is around 7.3-7.7. The pH value of the water is still suitable for the growth of *vannamei* shrimp, this is in accordance with the opinion of Wyban and Sweeny (1991) that the pH range of water that is suitable for intensive *vannamei* shrimp cultivation is from 7.4 to 8.9 with optimum value 8.0.

The results of Dissolved Oxygen (DO) measurements from intensive and extensive ponds are 4 mg/L. This is in accordance with the opinion of Suprapto (2005), that the optimum dissolved oxygen level for *vannamei* shrimp cultivation is >3 mg/L with a tolerance of 2 mg/L. Bacteria have a minimum DO in the range of 2 mg/L, the higher the DO value the better for growth (Saoud et al., 2003). Salinity has a role in osmoregulation. The results of measurements of salinity from intensive ponds were 23 ppt, while in extensive farms it was 30 ppt. According to Saoud et al. (2003) that *vannamei* shrimp is able to tolerate a wide salinity range in the range of 0.5-60 ppt.

### 5. Conclusion

The number of types of bacteria found from intensive and extensive ponds is almost the same, namely *Plesiomonas* sp., *Clostridium* sp., *Flavobacterium* sp., *Acinetobacter* sp., *Vibrio* sp., *Pseudomonas* sp., *Bacillus* sp., and *Staphylococcus* sp. morphological test method, Gram staining test and biochemical test. The number of bacteria found in intensive ponds differed from the number of bacteria found from extensive ponds. Intensive ponds have a number of *Plesiomonas* sp. bacteria at 12 isolates, *Clostridium* sp. at 28 isolates, *Flavobacterium* sp. at 21 isolates, *Acinetobacter* sp. at 18 isolates, *Vibrio* sp. at 16 isolates, *Pseudomonas* sp. at 14 isolates, *Bacillus* sp. at 28 isolates, and
Staphylococcus sp. at 9 isolates. Meanwhile, extensive ponds have a number of Plesiomonas sp. bacteria at 10 isolates, Clostridium sp. at 12 isolates, Flavobacterium sp. at 15 isolates, Acinetobacter sp. at 18 isolates, Vibrio sp. at 17 isolates, Pseudomonas sp. at 13 isolates, Bacillus sp. at 37 isolates, and Staphylococcus sp. at 18 isolates.

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