BIODIVERSITAS
Volume 19, Number 2, March 2018
Pages: 480-488

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190215

Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia

ESTI HANDAYANI HARDI[1,♥], RUDY AGUNG NUGROHO[2], GINA SAPTIANI[1], RIA SARINAH[1], MAULINA AGRIANDINI[1], MIRA MAWARDI[3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Mulawarman. Jl. Gunung Tabur, Gunung Kelua, Samarinda Ulu, Samarinda 75123, East Kalimantan, Indonesia. Tel./fax: +62-541-749159, ♥email: estieriyadi2011@gmail.com

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. Jl. Barong Tongkok No 4. Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia

[3]Main Center for Freshwater Aquaculture Fisheries. Ministry of Marine Affairs and Fisheries. Jl. Selabintana No. 37, Sukabumi 43114, West Java, Indonesia

Manuscript received: 21 August 2017. Revision accepted: 23 February 2018.

Abstract. *Hardi EH, Nugroho RA, Saptiani G, Sarinah R, Anggraidini M, Mawardi M. 2018. Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia. Biodiversitas 19: 480-488.* This research was conducted to isolate, identify, and characterize pathogenic bacteria from feces and water of tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture from two selected locations in Samarinda, East Kalimantan, Indonesia. Bacteria were cultured and isolated on NA, TSA, and BHIA media at 30ºC for 24 h. Colonies of the isolated bacteria were characterized morphologically in terms of their shape, chromogenesis, edge, and size. Antibiotic sensitivity test on each bacterial isolate was completed using inhibition zone tests. Commercial antibiotics used in this research were nitrofurantoin, ciprofloxacin, oxytetracycline, chloramphenicol, nalidixic acid, gentamicin, and norfloxacin. Koch's postulates test was done by intraperitoneal injection of bacterial suspension to tilapia (15 g weight) at 10^3-10^9 CFU mL^{-1} in triplicates to determine the pathogenicity of each bacterium. Overall, there were 37 isolates obtained from different sources and growth media that belonged to 14 species: *Acinetobacter calcoaceticus* (1 isolate), *Aerococcus urinae* (2 isolates), *Aerococcus viridans* (1 isolate), *Aeromonas hydrophila* (1 isolate), *Citrobacter freundii* (5 isolates), *Enterobacter amnigenus* (2 isolate), *Enterobacter cloacae* (4 isolates), *Escherichia coli* (3 isolates), *Listeria* sp. (1 isolate), *Niseria* sp. (4 isolates), *Pantoea* spp. (1 isolate), *Pseudomonas aeruginosa* (1 isolate), *Staphylococcus aureus* (9 isolates), and *Streptococcus iniae* (2 isolates). Sixteen of the isolates were grown in BHIA medium, 12 isolates in TSA medium and 9 isolates in NA medium. The highest mortality was found in tilapia injected with *Enterobacter* sp., *Listeria* sp. and *Streptococcus* sp. at a density of 10^9 CFU mL^{-1}. However, the number of bacteria causing mortality in fish was approximately 10^4-10^8 CFU mL^{-1}. All bacteria detected in the tilapia and channel catfish cultures were also known as putative pathogens in human.

Keywords: Bacteria colony, contaminant, channel catfish, pathogen bacteria, tilapia

INTRODUCTION

Tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) are two of the most widely distributed aquaculture commodities worldwide. There has been a large increase in demand for tilapia and catfish, resulting in the increased production of both fishes through the method of high stocking density. However, the high stocking density method leads to rapid deterioration of water quality and increased fish stress (Sebastião et al. 2015), resulting in disease and pathogenic epizootics.

The existing pathogens in fish represent a serious problem and major concern for aquaculturists due to loss of income, reducing global aquaculture development (Lafferty et al. 2015). Some of the pathogenic bacteria infecting tilapia include *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp., *Enterococcus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Plesiomonas* spp., *Moraxellaceae*, and *Enterobacteriaceae*. Furthermore, *Streptococcus* spp. is a bacteria that causes high mortality in fish cultured in Asia. Meanwhile, pathogenic bacteria were found in catfish including *Escherichia coli* (Zahran et al. 2016), *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholera*, and *Shigella dysenteriae*.

Gram-negative bacteria from the genera *Aeromonas*, *Flavobacterium*, *Pseudomonas*, *Francisella*, and gram-positive bacteria from the genera *Streptococcus* and *Lactococcus* cause rapid high mortality in fish as soon as 72 h after infection. Recently, there was an outbreak of fish disease in Thailand, causing a mortality rate of almost 90% (Dong et al. 2017a). Over 2015-2016, mass mortality in tilapia and hybrid red tilapia (*Oreochromis* spp.) has been investigated without determining the cause of death. However, histopathology analysis revealed that liver damage had been found in the dead tilapia. Moreover, Tilapia lake virus (TiLV) has also been detected in samples by using a scanning electron microscope (Dong et al. 2017b).

Some of pathogenic bacteria in fish are facultative (Nayak 2010) and able to survive in water for long periods

of time, thus making their presence difficult to prevent. Examination of pathogenic bacteria should be completed regularly to anticipate the occurrence of a pathogen attack. The success of disease control, including prevention and treatment, is strongly influenced by the accuracy of disease diagnosis in fish.

The present research aims to isolate, identify, and characterize pathogenic bacteria in tilapia and channel catfish culture, providing updated information on the development of disease-causing bacteria on both tilapia and channel catfish cultures. Our results also provide an early warning against the occurrence of disease outbreaks informing an anticipated disease control and management on the freshwater fish culture.

MATERIALS AND METHODS

Sample preparation

Samples of water and fish feces were collected from culture ponds containing tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*) in Samarinda City, East Kalimantan, Indonesia. Before being used, samples were cooled to 4^{o}C.

Isolation and bacteria identification

All samples were cultured on nutrient agar media (NA, Oxoid), trypticase soy agar (TSA, Oxoid), and brain heart infusion agar (BHIA, Oxoid). A pure isolate was obtained by reisolating a single bacterial colony onto each respected new media. Pure isolates were then used for colony morphology and biochemistry identification and tested for their level of pathogenicity in tilapia.

Colony identification

In bacterial colony identification, the colony was classified based on the colony color, form, and margin. According to Reynolds (2011), a bacterial colony has different morphological properties. Colony forms include irregular, filamentous, rhizoid, and curled. Meanwhile, colony margin can be entire, filamentous, undulate, and lobate. Colony elevation can be categorized as raised, flat, convex, umbonate, and crateriform. The size of a bacterial colony can be described as small, moderate, and large. Furthermore, the opacity of bacterial colony can be stated as clear, opaque, translucent, and iridescent.

Biochemical identification

Biochemical identification of bacteria was performed following the method of Indonesian National Standard, also known as Standar Nasional Indonesia (SNI) No. 7303.1:2015, SNI No. 7545.3:2009 and SNI No. 8096.1:2015. The identification test includes gram staining, a motility test using semi-solid medium, an oxidative-fermentative test, a catalase test, and a growth test on D-mannitol medium.

API test

Bacteria identification using API 20 Strep and API 20 E using the methods of Poutrel and Ryniewicz (1984), Sheehan et al. (2009), and Hardi et al. (2011). All bacterial isolates were cultured in a phosphate-buffered broth base with glucose, streaked on a blood agar plate, and examined for their purity. Briefly, a suspension was made from the cultures grown on blood agar plates by transferring a heavy inoculum of the culture to a tube containing 2 ml of distilled water. Three drops of the suspension were placed into a microcapsule using a Pasteur pipette. Strips were incubated at 37°C in a normal atmosphere. After 4 h of incubation, reagents were added and strips were exposed to a strong light (100W for 10 s) for enzymatic activities reading. Test results were then recorded, and the strips were incubated again for 20 h. For the identification of *Streptococcus*, the interpretation of the test results obtained at 4 and 24h after incubation species was based on the profile index and table issued by the manufacturer.

Antibiotic sensitivity

Bacterial sensitivity test against antibiotics was completed using seven types of commercial antibiotic, namely*:* nitrofurantoin (Nitro), ciprofloxacin (Cipro), oxytetracycline (Oxy), chloramphenicol (Chlo), nalidixic acid (Na), gentamicin (Gent), and norfloxacin (Nor) using the inhibition zone method. The diameter of the zone of inhibition appearing on the agar plate was measured to determine antibiotic resistance. Using this method, bacteria were inoculated in a liquid medium of trypticase soy broth (TSB, Oxoid) followed by reading on solid medium TSA. After 5 min, antibiotic paper was placed on the medium and incubated at 30^{o}C for 24 h.

Koch's postulates test

The Koch postulates test was performed according to Jordan (1941), Kamiso et al. (2005), and Hardi et al. (2012), using tilapia. Tilapia is a common fish used for bacterial pathogenicity testing, because tilapia has a constant health condition, a differential leukocyte consisting of neutrophils, monocytes, and lymphocytes of large size, making it easier for observation immune system of fish.

Bacteria were cultured in TSB medium at 30^{o}C for 24h. After a total plate count (TPC) was performed to measure the microbial density, bacteria were administered intraperitoneally to tilapia with a dose of $10^{3,5,7,9}$ bacterial cells per fish in triplicates. Control fish was injected with 0.1 mL sterile Phosphate Buffer Saline (PBS) per fish. All fish were then reared in an aquarium with aeration and fed with pellet at a rate of 3-5% of body weight, twice a day. After 7 days of rearing, cumulative mortality was calculated until day 7.

RESULTS AND DISCUSSION

Bacteria colony

Bacterial isolates grown in TSA, NA, and BHIA medium in the first isolation and re-isolation are shown in Figure 1. There were 37 bacteria colonies discovered in the tilapia and catfish pond cultures (Table 1).

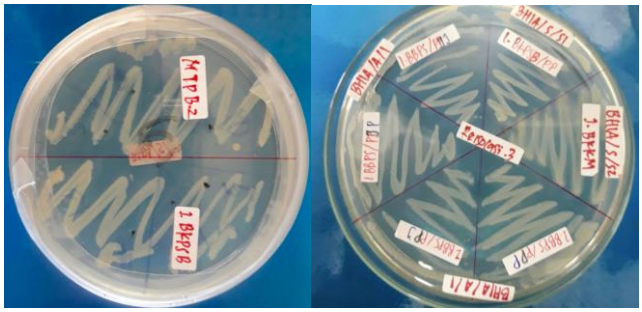
BHIA is a rich medium, likely resulting in a higher number of colonies successfully growing on it. From BHIA

culture, we obtained 16 bacterial isolates. A total of 12 isolates grew in TSA medium and only nine isolates grew in NA medium.

As seen from Table 1, the number of bacterial colonies isolated from tilapia and catfish ponds was 18 and 19 isolates, respectively. From the nine isolates obtained from the water in tilapia's pond, four isolates grew on BHIA, three isolates on NA, and two on TSA. Meanwhile, from fish feces, there were nine isolates: five grew on BHIA, three on TSA, and only one on NA.

Bacteria identification

Using identification methods according to SNI 2009 and 2015, which includes gram, catalase, motility, mannitol, fermentative test, O-F test, and growth in NaCl medium, there were 37 bacteria isolates identified, of which 22 isolates were gram-negative bacteria, and 15 isolates were gram-positive. Similar to the previous research by Marcel et al. (2013) in Malaysia, gram-negative is the most predominant bacteria found in tilapia cultured. Generally, bacteria such as *Aeromonas* sp., *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp. are obligate or facultative bacteria that can survive for long periods in ponds if they found suitable hosts (Austin and Austin 2007).



A **B**

Figure 1. Bacterial isolates. A. First isolation in artificial NA medium, B. Re-isolated bacteria

Table 1. Characterization of bacterial colonies isolated from tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*) ponds based on their form, color, and margin/edge of the colony

Isolate code	Source of sample		Medium	Morphology of bacterial colony		
	Tilapia pond	Catfish pond		Form	Color/pigmentation	Margin /edge
RA1	Water	-	TSA	Irregular	White	Erose
RA2	Water	-	TSA	Irregular	White milk	Erose
RA3	Water	-	NA	Circular	Translucent	Entire
RA18	Water	-	NA	Circular	White milk	Entire
RA14	Water	-	NA	Circular	White milk	Entire
RA22	Water	-	BHIA	Circular	Creamy yellow	Erose
RA23	Water	-	BHIA	Circular	Yellow	Erose
RA24	Water	-	BHIA	Irregular	White	Erose
RA30	Water	-	BHIA	Circular	White	Erose
RA9	Feces	-	TSA	Irregular	Translucent	Erose
RA10	Feces	-	TSA	Circular	White milk	Erose
RA11	Feces	-	TSA	Irregular	White milk	Erose
RA21	Feces	-	NA	Circular	Yellow	Erose
RA28	Feces	-	BHIA	Circular	White milk	Erose
RA29	Feces	-	BHIA	Circular	Translucent	Erose
RA31	Feces	-	BHIA	Irregular	White milk	Erose
RA32	Feces	-	BHIA	Irregular	White	Erose
RA33	Feces	-	BHIA	Irregular	Translucent	Erose
RA4	-	Water	TSA	Circular	Translucent	Undulated
RA5	-	Water	TSA	Circular	Translucent	Erose
RA6	-	Water	TSA	Irregular	Creamy yellow	Erose
RA7	-	Water	TSA	Circular	White milk	Undulated
RA8	-	Water	TSA	Irregular	Yellow	Erose
RA15	-	Water	NA	Circular	White milk	Erose
RA16	-	Water	NA	Circular	White milk	Erose
RA17	-	Water	NA	Circular	White milk	Erose
RA19	-	Water	NA	Circular	White milk	Erose
RA25	-	Water	BHIA	Circular	White milk	Erose
RA26	-	Water	BHIA	Circular	Creamy yellow	Erose
RA27	-	Water	BHIA	Circular	Yellow	Erose
RA12	-	Feces	TSA	Irregular	White milk	Erose
RA13	-	Feces	TSA	Irregular	Translucent	Erose
RA20	-	Feces	NA	Circular	Yellow	Erose
RA34	-	Feces	BHIA	Circular	Yellow	Entire
RA35	-	Feces	BHIA	Circular	White milk	Erose
RA36	-	Feces	BHIA	Circular	White milk	Erose
RA37	-	Feces	BHIA	Circular	Translucent	Erose

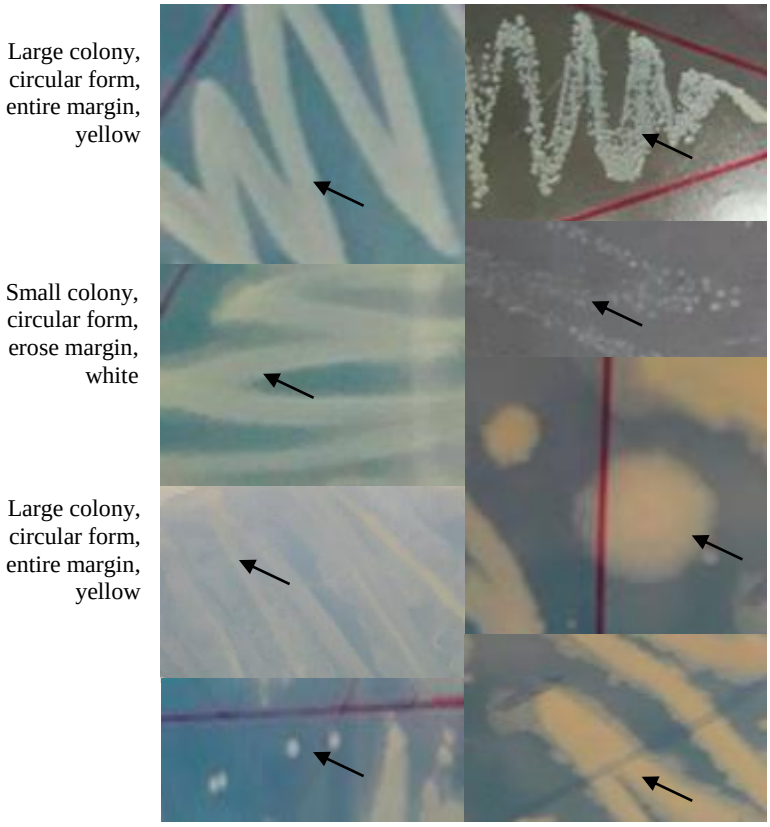


Figure 2. Bacterial colony forms in a variety of media

Based on the data presented in Table 2, there were seven genera of bacteria isolated from tilapia and catfish ponds: *Streptococcus* sp. (13.5%), *Staphylococcus* sp. (24.32%), *Pseudomonas* sp. (10.81%), *Enterobacter* sp. (43.24%), *Aeromonas* sp. (10.81%), *Neisseria* sp. (10.81%), and *Listeria* sp. (10.81%). Marcel et al. (2013) also mentioned that *Aeromonas hydrophila*, *Staphylococcus* spp., *P. aeruginosa*, and *Enterobacter cloacae* also existed in Kenyir Lake, Malaysia and found *A. hydrophila* and *Staphylococcus* sp. from Semantan River, where red tilapia is cultured in *karamba* floating nets. A total of 19 isolate bacterial isolates were found in catfish ponds, dominated by *Staphylococcus* sp. and *Enterobacter* sp. However, *Pseudomonas* sp. (5%) and *Listeria* sp. (5%) were not found both in water or feces sample from tilapia pond. According to Gauthier (2014) and Boylan (2011), bacterial groups Enterobacteriaceae, *Streptococcus*, *Staphylococcus*, *Mycobacterium*, *Nocardia*, *Aeromonas*, *Edwardsiella*, *Francisella*, *Pseudomonas*, *Vibrio*, and *Yersinia* are zoonotic bacteria in fish for human.

To confirm the type of bacteria that have been isolated and identified, a follow-up test was conducted using API 20E, API 20 Strep. The results indicate that some bacteria were able to hydrolyze some sugar (Table 3). Furthermore, the bacterial group included in Enterobacteriaceae was identified by using API 20 E, while *Streptococcus*, *Aeromonas*, *Pseudomonas* groups were tested using API 20 Strep (Tables 3 and 4).

All isolates from the *Streptococcus* group had the capability to degrade sodium pyruvate, hippuric acid, escullin, pyrrolidonyl arylamidase, α-galactosidase, β-glucuronidase, β-galactosidase but not L-arabinose and inulin (Table 3). Meanwhile, Enterobacter groups which were further tested using API 20E possessed two types of glucose that cannot be degraded by all isolate, namely trisodium citrate and urea (Table 4).

The present results of bacterial identification using biochemistry tests followed by API 20 Strep and API 20 E tests is summarized in Table 5.

Identification results of all the bacterial isolates using biochemistry tests, including API 20 E and API 20 Strep, revealed there were 14 bacterial species (Table 5) including: *Acinetobacter calcoaceticus* (1 isolates), *Aerococcus urinae* (2 isolates), *A. hydrophila* (1 isolate), *Aerococcus viridans* (1 isolate), *Citrobacter freundii* (5 isolates), *Enterobacter amnigenus* (2 isolates), *E. cloacae* (4 isolates), *E. coli* (3 isolates), *Listeria* sp. (1 isolate), *Neisseria* sp. (4 isolates), *Pantoea* spp. (1 isolate), *P. aeruginosa* (1 isolates), *S. aureus* (9 isolates), and *Streptococcus iniae* (2 isolates). All 14 bacteria are not only pathogens to fish but have also been reported causing zoonosis in human (Boylan 2011 and Gauthier 2015). According to Haenen et al. (2013), *S. iniae* is a zoonotic pathogen in freshwater and marine fish aquaculture. In addition, this bacterial invasive disease is carried by human and can cause diseases in aquaculture, resulting in economic losses in the tilapia aquaculture industry.

Table 2. Characteristics of bacteria isolated from tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*) ponds using biochemical methods

Isolate code	Gram	Catalase	Motil	Mannitol fermentative	O/F	Growth in NaCl	Bacterial group
RA1	+	-	-	-	F +	+	*Streptococcus*
RA2	+	-	-	-	F +	+	*Streptococcus*
RA3	+	-	-	-	F +	+	*Streptococcus*
RA4	-	+	+	+	F+	+	*Enterobacter*
RA5	+	-	-	-	F +	+	*Streptococcus*
RA6	-	+	+	+	F+	+	*Enterobacter*
RA7	+	+	-	+	O+	+	*Staphylococcus*
RA8	-	+	+	+	O-	-	*Enterobacter*
RA9	-	+	+	+	F+	+	*Enterobacter*
RA10	-	+	+	+	F+	+	*Enterobacter*
RA11	-	+	+	+	F+	+	*Enterobacter*
RA12	-	+	-	-	O-	+	*Pseudomonas*
RA13	-	+	+	+	O-		*Enterobacter*
RA14	+	+	-	+	O+	+	*Staphylococcus*
RA15	+	+	-	+	O+	+	*Staphylococcus*
RA16	-	+	+	+	O-	-	*Enterobacter*
RA17	-	+	+	+	O-		*Enterobacter*
RA18	+	-	-	-	F +	+	*Streptococcus*
RA19	-	+	+	+	O-	-	*Enterobacter*
RA20	+	+	-	+	O+	+	*Staphylococcus*
RA21	-	+	+	+	F+	+	*Enterobacter*
RA22	-	+	+	-	O+	-	*Neisseria*
RA23	-	+	+	-	O+	-	*Neisseria*
RA24	-	+	+	+	F+	+	*Enterobacter*
RA25	+	+	-	+	O+	+	*Staphylococcus*
RA26	-	+	+	-	O+	-	*Neisseria*
RA27	+	+	-	+	O+	+	*Staphylococcus*
RA28	-	+	+	+	O-	-	*Enterobacter*
RA29	+	+	-	+	O+	+	*Staphylococcus*
RA30	+	+	-	+	O+	+	*Staphylococcus*
RA31	-	+	+	+	O-	-	*Enterobacter*
RA32	-	+	+	+	F+	+	*Aeromonas*
RA33	-	+	+	+	F+	+	*Enterobacter*
RA34	-	+	+	-	O+	-	*Neisseria*
RA35	-	+	+	+	O-	-	*Enterobacter*
RA36	+	+	+	-	O-	-	*Listeria*
RA37	+	+	+	-	O-	-	*Staphylococcus*

Further identification of commercial antibiotic sensitivity suggests that bacteria can be inhibited by antibiotics such as Nitro = nitrofurantoin, Cipro: ciprofloxacin, Oxy: oxytetracycline, Chlo: chloramphenicol, NA: nalidixic acid, Gent: gentamicin, and Nor: norfloxacin (Table 6).

The present study found that the antibiotic nitrofurantoin effectively inhibited all the isolated bacteria except *S. aureus* isolates RA 7 and RA 29. Meanwhile, *A. viridans* was only sensitive to nitrofurantoin but not to other commercial antibiotics. According to Mohan et al. (2017), the two of isolates *A. viridans* were resistant to nitrofurantoin, which is considered to be a first line drug in patients.

Table 3. Overview of *Streptococcus* bacteria characteristics using the API 20 strep test

Active ingredients	Isolate code				
	RA1	RA2	RA3	RA5	RA18
Sodium pyruvate	+	+	+	+	+
Hippuric acid	+	+	+	+	+
Escullin	+	+	+	+	+
Pyrrolidonyl arylamidase	+	+	+	+	+
α-galactosidase	+	+	+	+	+
β-glucuronidase	+	+	+	+	+
β-galactosidase	+	+	+	+	+
Alkaline phosphatase	+	+	+	-	-
L-leucine aminopeptidase	+	+	+	-	-
L-arginine	+	-	+	+	+
D-ribose	-	+	+	+	-
L-arabinose	-	-	-	-	-
D-mannitol	-	+	-	-	-
D-sorbitol	-	+	-	-	-
D-lactose	-	+	-	-	-
D-trehalose	-	+	-	+	+
Inulin	-	-	-	-	-
D-raffinose	-	-	+	-	-
Starch	-	+	+	+	-
Glycogen	-	+	-	-	-

Streptococcus sp. is a pathogenic bacterium in freshwater fish. There are two main groups of *Streptococcus* sp. which have pathogenic properties in fish, namely *S. iniae* and *S. agalactiae* (Hardi et al. 2011). The infection of *S. agalactiae* has caused neonatal meningitis in human, and mastitis in cows (Elliott et al. 1990; Bohnsack et al. 2004; Lindah et al. 2005). In tilapia, these bacteria have caused whirling disease, exophthalmia, opacity, and purulence (Hardi et al. 2011). Besides fish, marine mammals, cows, horses, cats, and humans can host these bacteria. *S. agalactiae* outbreaks are acute and have previously caused 100% mortality in fish cultured by 14 days post-infection (Evans et al. 2006).

Furthermore, *Staphylococcus* sp. is a gram-positive, non-spore, non-motile, facultative anaerobic chemoorganotrophic bacterium with a fermentative metabolism (Holt et al. 1994). *Staphylococcus* sp. is a fish pathogen that can be found in the intestine and feces of fish (Caretto et al. 2005). In England, European sea brass (*Dicentrarchus labrax*) has been infected by *S. xylosus, S. chromogens, S. warneri*, which was marked by a dark body, ulceration, and necrosis either in fins or skin (Nizan and Hammerschlag, 1993). *Staphylococcus* sp. is a dominant bacteria also found in tilapia from Kenyir Lake, Malaysia (Zahra et al. 2004; Zahra et al. 2008; Marcel et al. 2013).

According to Pirie (1940), *Listeria* sp. belongs to the order *Bacillales*, family of *Listeriaseae* and the *Listeria* genus, which can be found in both water and soil (Esteben et al. 2009). *Listeria monocytogenes*, found in freshwater, brackish, and marine fish (Novotny et al. 2004), can also be found in harvested marine fish, presumably as a result of contamination during the handling process (Huss et al. 2000; Ariyanti 2010).

Table 4. Overview of Enterobacter bacteria characteristics using API 20E

Active ingredients	Isolate code																
	RA4	RA6	RA8	RA 9	RA 10	RA11	RA13	RA16	RA17	RA19	RA21	RA24	RA28	RA31	RA32	RA33	RA 35
2-nitrophenyl-ßD-galactopyranoside	-	+	+	+	+	-	-	+	-	+	+	+	-	+	-	+	+
L-arginine	+	-	-	+	+	-	+	+	-	-	+	-	+	+	+	+	-
L-lysine	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+
L-ornithine	-	+	+	+	+	-	-	+	-	-	+	+	-	+	-	+	+
Trisodium citrate	-	-	-	+	+	-	+	+	-	-	+	-	-	+	-	+	-
Sodium thiosulfate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-tryptophane	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-tryptophane	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Sodium pyruvate	-	-	+	+	+	-	-	-	+	-	-	-	+	-	+	+	-
Gelatin	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-
D-glucose	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+
D-mannitol	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+
Inositol	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	+	-
D-sorbitol	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+
L-rhamnose	-	+	+	-	-	-	-	+	-	-	+	+	+	+	-	-	+
D-sucrose	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+
D-melibiose	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+
Amygdalin	-	-	-	+	+	-	-	+	+	-	+	-	+	+	+	+	-
L-arabinose	+	+	+	+	+	+	-	+	-	-	+	+	-	+	-	+	+
Oxidase	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-

Table 5. Bacteria identification from tilapia and catfish culture ponds in Samarinda, East Kalimantan, Indonesia

Isolate code	Bacteria
RA 1	*Aerococcus urinae*
RA 2	*Aerococcus viridans*
RA 3	*Aerococcus urinae*
RA 4	*Citrobacter freundii*
RA 5	*Streptococcus iniae*
RA 6	*Escherichia coli*
RA 7	*Staphylococcus aureus*
RA 8	*Escherichia coli*
RA 9	*Citrobacter freundii*
RA 10	*Enterobacter cloacae*
RA 11	*Acinetobacter calcoaceticus*
RA 12	*Pseudomonas aeruginosa*
RA 13	*Citrobacter freundii*
RA 14	*Staphylococcus aureus*
RA 15	*Staphylococcus aureus*
RA 16	*Enterobacter amnigenus*
RA 17	*Enterobacter amnigenus*
RA 18	*Streptococcus iniae*
RA 19	*Escherichia coli*
RA 20	*Staphylococcus aureus*
RA 21	*Citrobacter freundii*
RA 22	*Niseria* sp.
RA 23	*Niseria* sp.
RA 24	*Citrobacter freundii*
RA 25	*Staphylococcus aureus*
RA 26	*Neisseria* sp.
RA 27	*Staphylococcus aureus*
RA 28	*Pantoea* spp.
RA 29	*Staphylococcus aureus*
RA 30	*Staphylococcus aureus*
RA 31	*Enterobacter cloacae*
RA 32	*Aeromonas hydrophila*
RA 33	*Enterobacter cloacae*
RA 34	*Neisseria* sp.
RA 35	*Enterobacter cloacae*
RA 36	*Listeria* sp.
RA 37	*Staphylococcus aureus*

Table 6. Bacterial sensitivity to various antibiotics (mm)

Isolate code	Nitro	Cipro	Oxy	Chlor	Na	Gent	Nor
RA 1	19	28	31	16	23	26	28
RA 2	16	0	0	0	0	7	0
RA 3	17	16	18	15	8	10	8
RA 4	10	16	17	15	8	11	0
RA 5	15	17	9	10	8	10	12
RA 6	12	25	17	16	18	13	26
RA 7	8	22	21	19	17	18	19
RA 8	13	27	22	20	16	13	25
RA 9	11	22	12	11	10	13	10
RA 10	12	26	17	10	20	11	16
RA 11	10	18	13	19	20	13	15
RA 12	12	12	11	14	16	15	17
RA 13	11	10	17	17	14	12	21
RA 14	17	8	8	20	15	7	7
RA 15	17	9	24	23	16	10	13
RA 16	13	21	12	17	17	12	23
RA 17	14	12	11	13	12	9	8
RA 18	15	15	13	0	0	7	20
RA 19	17	17	20	19	15	10	22
RA 20	19	22	9	21	13	14	13
RA 21	18	24	7	19	25	12	25
RA 22	12	14	17	15	15	13	12
RA 23	12	16	21	15	15	13	14
RA 24	10	17	20	16	16	12	13
RA 25	18	17	27	16	24	16	20
RA 26	16	16	14	14	7	11	11
RA 27	16	16	21	16	14	11	18
RA 28	12	15	16	10	16	13	15
RA 29	9	14	19	20	15	15	16
RA 30	13	15	15	19	17	12	15
RA 31	12	25	22	18	19	14	23
RA 32	14	15	17	17	17	15	17
RA 33	10	16	13	16	17	12	20
RA 34	11	22	21	16	10	10	14
RA 35	10	24	19	16	16	12	19
RA 36	14	17	7	19	20	15	18
RA 37	14	15	12	16	24	16	17

Note: Nitro = nitrofurantoin, Cipro: ciprofloxacin, Oxy: oxytetracycline, Chlo: chloramphenicol, NA: nalidixic acid, Gent: gentamicin, Nor: norfloxacin

Enterobacter sp. belongs to order *Enterobacteriales*, the family *Enterobacteriaceae*, and genus *Enterobacter*. Besides living as opportunistic pathogens in fish (Rajasekaran 2008), *Enterobacter* sp. can be used as indicator of waste pollution because of their presence in humans, animals, water, soils, plants, insects, and processed products (Davidson et al. 2000; Neto et al. 2003). Previous research performed in Egypt by Hasan et al. (2012) reported that 9 species of bacteria of *Enterobacteria, E. coli, Salmonella arizonae, Citrobacter braakii, Enterobacter sakazakii, C. freundii, Rautella ornithinolytica, Klebsiella ozaenae, E. cloacae*, and *Proteus vulgaris* have been found in tilapia, with an infection prevalence of 2.7-27 %. Noor El-Deen et al. (2010) and Olurin et al. (2006) revealed that a fish infected with *Enterobacter* exhibited pale internal organs such as liver and kidney, and caused anemia, intestinal congestion, ulceration of the anus accompanied by mucus and bleeding in its infected external organs.

Dharma (1982) stated that *C. freundii* disrupt the internal organs of fish, including intestines, liver, and kidneys. The intestines of an infected fish blacken and become mushy and juicy in consistency. Disruption of liver and bile functions is caused by increased liver activity from processing and neutralizing metabolites and toxic substances.

According to Novotny et al. (2004), bacteria that was found both in feces and water in tilapia and catfish ponds are categorized as pathogenic to humans. The occurrence of pathogenic bacteria such as *Mycobacterium* spp., *S. iniae, Photobacterium damselae, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholera, E. coli, Aeromonas* spp., *S. aureus, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Campylobacter jejuni* must be anticipated in order to reduced economic loss in cultured freshwater fish and to prevent their transmission to the humans consuming these fish.

Koch's postulate test

To examine each bacterium's pathogenicity in fish, we performed Koch's postulate test following the protocol of Jordan (1941), Kamiso et al. (2005), and Hardi et al. (2012). The test revealed that *Streptococcus* sp., *C. freundii*, *Staphylococcus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Neisseria* sp. and *Listeria* sp. caused varying degrees of mortality in tilapia. The highest mortality was found in tilapia injected with *Enterobacter* sp., *Listeria* sp., and *Streptococcus* sp. at a density 10^9 CFU mL^{-1} (Table 3). However, the number of bacteria causing mortality in fish was approximately 10^4-10^8 CFU mL^{-1} bacterial density. According to Angka et al. (2002), bacteria that caused mortality at a density above 10^5 CFU mL^{-1} was categorized as being less pathogenic.

Citrobacter freundii is a bacterial pathogen that caused 60% mortality in tilapia fish sampled in the present study (Table 6). This finding is in line with a previous report by Jeremić, et al. (2003) who isolated *C. freundii* from all sick and dead *Cyprinus carpio*. Previously, Sato et al. (1982) also indicated that *C. freundii* caused mortality in fish. In 1990, Baya et al. found *C. freundii* from Atlantic salmon (*Salmo salar*) in Spain and the USA, while Sans (1991) identified the bacteria in *Cyprinus carpio* from India (Karunasagar and Pari 1992).

Moreover, the percentage of fish mortality caused by *Enterobacter* sp. was 90% in the present study (Table 6). This finding is in line with Sekar et al. (2008), who reported that *Enterobacter* sp. caused 100% mortality in *Mugil cephalus* six days after bacterial injection. The previous research also suggests that tilapia injected intraperitoneally with *Streptococcus agalactiae* (density 10^4 CFU mL^{-1}) experienced mortality of up to 48% at day 7 (Hardi et al. 2011). Similar to previous research, the current findings revealed that a bacterial density of 10^3 CFU mL^{-1} resulted in 40% mortality in tilapia sampled. Additionally, *Staphylococcus* sp. caused mortality of up to 80% at a density of 10^9 CFU mL^{-1}, with an average LD 50 at a density of $10^{4.40}$ CFU mL^{-1} of bacteria. According to Kamiso et al. (2005), bacteria with an LD50 below 10^5 CFU mL^{-1} has been categorized as being pathogenic.

Table 6. The percentage of cumulative mortality in tilapia (*Oreochromis niloticus*) injected at varying bacterial densities

Bacteria	Density (CFUmL^{-1})	Day						
		1	2	3	4	5	6	7
Citrobacter freundii	10^3	0	0	10	10	20	20	20
	10^5	0	10	10	20	30	30	30
	10^7	20	30	30	30	30	40	50
	10^9	30	30	40	40	50	60	60
Enterobacter sp.	10^3	0	0	0	10	20	20	20
	10^5	10	10	20	30	30	30	30
	10^7	20	40	50	50	50	80	80
	10^9	20	40	50	60	80	80	90
Staphylococcus sp.	10^3	0	0	0	0	20	30	30
	10^5	20	20	20	40	40	50	60
	10^7	0	20	30	40	60	60	60
	10^9	10	20	30	60	70	80	80
Listeria sp.	10^3	0	0	10	10	10	10	10
	10^5	20	30	30	30	30	30	50
	10^7	60	60	60	60	60	60	80
	10^9	50	50	60	70	70	70	90
Streptococcus iniae	10^3	10	10	20	20	20	40	40
	10^5	20	20	30	50	50	60	60
	10^7	30	30	40	50	60	70	70
	10^9	30	40	50	60	80	80	90
Pseudomonas sp.	10^3	0	10	10	10	10	10	10
	10^5	0	10	20	20	20	30	30
	10^7	0	30	40	50	50	50	50
	10^9	20	30	30	40	50	60	60
Aeromonas hydrophila	10^3	0	10	10	20	20	30	30
	10^5	0	10	20	20	20	30	40
	10^7	0	30	30	30	40	40	45
	10^9	20	30	30	40	50	60	60
Neisseria sp.	10^3	0	0	0	0	0	0	0
	10^5	0	0	0	10	10	10	10
	10^7	0	0	0	10	20	30	30
	10^9	20	20	30	30	30	30	30

Table 7. The LD50 of bacteria density in tilapia (*Oreochromis niloticus*)

Bacteria	LD50 density ($CFUmL^{-1}$)
Citrobacter freundii	$10^{8.67}$
Enterobacter sp.	$10^{6.40}$
Staphylococcus sp.	$10^{4.40}$
Listeria sp.	$10^{6.57}$
Streptococcus sp.	$10^{4.50}$
Pseudomonas sp.	$10^{8.67}$
Aeromonas hydrophila	$10^{8.67}$
Neisseria sp.	$10^{9.00}$

The present study discovered 14 bacterial isolates from the water and feces of tilapia and catfish cultivation ponds in Samarinda City, East Kalimantan, Indonesia. Eight genera of bacteria that have been tested against tilapia showed only two isolates of the bacteria (*Staphylococcus* sp. and *Streptococcus* sp.) were pathogenic. The other two of isolates, *Enterobacter* sp. and *Listeria* sp., have the potential to be pathogenic to tilapia. Four isolates, namely *C. freundii*, *A. hydrophila*, *Pseudomonas* sp., and *Neisseria* sp. are categorized as non-pathogenic to tilapia. All groups of bacteria identified in this study are a putative pathogen to humans, so further research about pathogenicity of these bacteria in humans in the context of aquaculture should be conducted.

ACKNOWLEDGEMENTS

This research was supported and funded by the Directorate General of Higher Education of the Republic of Indonesia (DIKTI) with National Strategic Research Grant (no. 370/UN17.41/KL/2017). Authors would like to thank the faculty of Fisheries and Marine Sciences, Mulawarman University and the Marine and Fisheries Office, Kutai Kartanegara District, East Kalimantan, Indonesia for all of their supports during this study.

REFERENCES

Angka SL, Sutama IKJ, Yulita I. 2002. Antibacterial and phytopharmaca activity in vitro and in vivo test against *Aeromonas hydrophila* in catfish. Jurnal Mikrobiologi Indonesia 7 (2): 47-50. [Indonesian]

Ariyanti T. 2010. *Listeria monocytogenes* bacteria as food contaminants of animal origin (Foodborne Disease). Wartazoa 20 (2): 94-102. [Indonesian]

Austin B, Austin DA. 1993. Bacterial fish pathogens: diseases in farmed and wild fish. Ellis Horwood Ltd., Chichester, England.

Beaz-Hidalgo R, Figueras MJ. 2012. Molecular detection and characterization of furunculosis and other *Aeromonas* fish infections. In: Carvalho E (eds). Health and Environment in Aquaculture. InTech Open Access Publisher, Rijecka, Croatia.

Birkbeck TH, Feist SW, Verner-Jeffreys DW. 2011. Francisella infections in fish and shellfish. J Fish Diseases 34 (3): 173-187.

Bohnsack, John F, Whiting AA, Martinez G, Jones N, Adderson EE, Detrick S, Blaschke-Bonkowsky AJ, Bisharat N, Gottschalk M.2004. Serotype III *Streptococcus agalactiae* from bovine milk and human neonatal infections. Emerg Infect Dis 10 (8): 1412.

Burr, Ellen S, Goldschmidt-Clermont E, Kuhnert P, Frey J. 2012. Heterogeneity of Aeromonas populations in wild and farmed perch, *Perca fluviatilis* L. J Fish Dis 35 (8): 607-13.

Caretto, Sofia, Linsalata V, Colella G, Mita G, Lattanzio V. 2015. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Intl J Mol Sci 16 (11): 26378-26394.

Dash, Sucharita S, Das BK, Pattnaik P, Samal SK, Sahu S, Ghosh S. 2009. Biochemical and serological characterization of F*lavobacterium columnare* from freshwater fishes of Eastern India. J World Aquacult Soc 40 (2): 236-247.

Daskalov H. 2006. The importance of *Aeromonas hydrophila* in food safety. Food Control 17 (6): 474-83.

Davidson, Elizabeth W, Rosell RC, Hendrix DL. 2000. Culturable bacteria associated with the whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae). Florida Entomol 83 (2): 159.

Dharma A. 1982. Histology. 3rd ed. CV EGC, Jakarta. [Indonesian]

Dong HT, Siriroob S, Meemetta W, Santimanawong W, Gangnonngiw W, Pirarat N, Khunrae P. 2017a. Emergence of tilapia lake virus in thailand and an alternative semi-nested RT-PCR for detection. Aquaculture 476: 111-118.

Dong HT, Siriroob S, Meemetta W, Santimanawong W, Gangnonngiw W, Pirarat N, Khunrae P, Rattanarojpong T, Vanichviriyakit R, Senapin S. 2017b. A warning and an improved pcr detection method for tilapia lake virus (TiLV) disease in Thai Tilapia Farms. https://enaca.org/?id=858

Elliott JA, Richard RF, Conrad BR. 1990. Whole-cell protein patterns of nonhemolytic group B, type IB, Streptococci isolated from humans, mice, cattle, frogs, and fish. J Clin Microbiol 28 (3): 628-630.

Esteban-Tejeda L, Malpartida F, Esteban-Cubillo F, Pecharromán C, Moya JS. 2009. The antibacterial and antifungal activity of a soda-lime glass containing silver nanoparticles. Nanotechnology 20 (8): 085103.

Evans JJ, Klesius PH, Shoemaker CA. 2009. First isolation and characterization of *Lactococcus garvieae* from Brazilian nile tilapia, *Oreochromis niloticus* (L.), and Pintado, *Pseudoplathystoma corruscans* (Spix & Agassiz). J Fish Dis 32 (11): 943-51.

Evans JJ, David JP, Klesius PH, Al-Ablani S. 2006. First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild Bottlenose Dolphin (*Tursiops truncatus*). J Wildlife Dis 42 (3): 561-569.

Figueiredo HCP, Netto LN, Leal CAG, Ulisses PP, Mian GF. 2012. *Streptococcus iniae* outbreaks in Brazilian Nile Tilapia (*Oreochromis niloticus* L:) farms. Brazilian J Microbiol 43 (2): 576-80.

Hardi EH, Pebrianto CA, Hidayanti T, Handayani RT. 2014. Phatogenicity of *Aeromonas hydrophila* via some port entryin cultured nila tilapia (*Oreochromis niloticus)* from Loa Kulu Kutai Kartanegara Kalimantan Timur. Indonesian J Veterinary Sci 8 (2): 130-134. [Indonesian]

Hardi EH, Pebrianto CB. 2012. Isolation and postulate koch *Aeromonas* sp. and *Pseudomonas* sp. on nila tilapia (*Oreocromis niloticus)* in loa Kulu Kutai Kartanegara). J Ilmu Perikanan Tropis 16 (2): 35-39. [Indonesian]

Hardi EH, Sukenda, Harris E, Lusiastuti AM. 2011. Karakteristik dan patogenisitas *Streptococcus agalactiae* tipe β-hemolitik dan non-hemolitik pada ikan nila. Jurnal Veteriner 12 (2): 152-64. [Indonesian]

Hassan AHM, El Deen AEN, Galal HM, Dorgham SM, Bakry MA, Hakim AS. 2012. Further characterization of Enterobacteriaceae isolated from cultured freshwater fish in Kafr El Shiek Governorate: clinical, biochemical and histopathological study with emphasis on treatment trials. Global Veterinaria 9 (5): 617-629.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. Determinative Bacteriology. 9 ed. Lippincott Williams & Wilkins Company, USA.

Huss HH, Reilly A, Embarek PKB. 2000. Prevention and control of hazards in seafood. Food Control 11 (2): 149-156.

Jeremić S, Jakić-Dimić D, Veljović LJ. 2003. *Citrobacter freundii* as a cause of disease in fish. Acta Veterinaria 53 (5-6): 399-410.

Karunasagar I, Pai R. 1992. Systemic *Citrobacter freundii* infection in common carp, *Cyprinus carpio* L., fingerlings. J Fish Dis 15 (1): 95-98.

Lafferty KD, Harvell CD, Conrad JM, Friedman CS, Kent ML, Kuris AM, Powell EN, Rondeau D, Saksida SM, 2015. Infectious diseases affect marine fisheries and aquaculture economics. Ann Rev Mar Sci 7: 471-496.

Lindah G, Stålhammar-Carlemalm M, Areschoug T. 2005. Surface proteins of *Streptococcus agalactiae* and related proteins in other bacterial pathogens. Clinical Microbiology Reviews 18 (1): 102-27.

Marcel G, Sabri MY, Siti-Zahrah A. 2013. Water condition and identification of potential pathogenic bacteria from red tilapia reared in cage-cultured system in two different water bodies in Malaysia." African Journal of Microbiology Research 7 (47): 5330-5337.

Mohan BKZ, Anand N, Taneja N. 2017. *Aerococcus viridans*: a rare pathogen causing urinary tract infection. J Clin Diagnost Res 11 (1): DR01-DR03.

Muroga K. 2001. Viral and bacterial diseases of marine fish and shellfish in japanese hatcheries. Aquaculture 202 (1): 23-44.

Najiah M, Aqilah NI, Lee KL, Khairulbariyyah Z, Mithun S, Jalal KCA, Shaharom-Harrison F, Nadirah M. 2012. Massive mortality associated with *Streptococcus agalactiae* infection in cage-cultured red hybrid Tilapia *Oreochromis niloticus* in Como River, Kenyir Lake, Malaysia. J Biol Sci 12 (8): 438.

Neto, Rodrigues J, Yano T, Beriam LOS, Destéfano SAL, Oliveira VM, Rosato YB. 2003. Comparative RFLP-Its analysis between *Enterobacter cloacae* strains isolated from plants and clinical origin. Arq Inst Biol 70: 367-72.

Nizan S, Hammerschlag E. 1993. First report of pasteurellosis in freshwater hybrid Tilapia (*Oreochromis aureus* X O. *nilotica*) in Israel. Bull Eur Assoc Fish Pathol 13: 179-80.

Noor El-Deen AE, Atta NS, Abd El-Aziz MA. 2010. Oral vaccination of Nile Tilapia (*Orechromis niloticus*) against motile *Aeromonas septicemia*. Nat Sci 8 (2): 21-25.

Novotny L, Dvorska L, Lorencova A, Beran V, Pavlik I. 2004. Fish: a potential source of bacterial pathogens for human beings. A Review. Veterinarni Medicina-UZPI, Czech Republic.

Olivares-Fuster, Oscar, Klesius PH, Evans J, Arias CR. 2008. Molecular typing of *Streptococcus agalactiae* isolates from fish. Journal of Fish Diseases 31 (4): 277-83.

Olurin KB, Olojo EAA, Mbaka GO, Akindele AT. 2006. Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to herbicide, glyphosate. African J Biotechnol 5 (24): 2480.

Pirie JH. 1940. The Genus *Listerella* Pirie. Science 91 (2364): 383-383.

Plumb JA. 1999. Tilapia Bacterial Diseases. In: Plumb JA, Ames IA. (eds.). Health Maintenance and Principal Microbial Diseases Cultured Fishes. Iowa State University Press, USA.

Rajasekaran P. 2008. Enterobacteriaceae group of organisms in sewage-fed fishes. Adv Biotech 8: 12-14.

Reynolds J. 2011. Bacterial Colony Morphology. Richland College, Texas.

Sanz F. 1991. Rainbow trout mortalities associated with a mixed infection with *Citrobacter freundii* and IPN Virus. Bull Eur Assoc Fish Pathol 11: 222.

Sato N, Yamane N, Kawamura T. 2004. Systemic *Citrobacter freundi* infection among sun fish *Mola mola* in Matsushima Aquarium. Bull Japan Soc Sci Fish 49: 1551-1557.

Sebastião FA, Furlan LR, Hashimoto DT, Pilarski F. 2015. Identification of bacterial fish pathogens in Brazil by direct colony PCR and 16s rRNA gene sequencing. Adv Microbiol 5 (6): 409-424.

Sekar T, Santiago TC, Vijayan KK, Alavandi SV, Stalin Raj V, Rajan JJS, Sanjuktha M, Kalaimani N. 2008. Involvement of *Enterobacter cloacae* in the mortality of the fish, *Mugil capito*. Lett Appl Microbiol 46: 667-672.

Silva BC, Mouriño JLP, Vieira FN, Jatobá A, Seiffert WQ, Martins ML. 2012. Haemorrhagic septicaemia in the Hybrid Surubim (*Pseudoplatystoma corruscans* × *Pseudoplatystoma fasciatum*) caused by *Aeromonas hydrophila*. Aquacult Res 43: 908-916.

SNI. 2015a. Standar Nasional Indonesia: Deteksi bakteri *Edwardsiella ictaluri* pada ikan-Bagian 1: metode polymerase chain reaction (PCR). Badan Standardisasi Nasional 8096 (1): 13. [Indonesian]

SNI. 2015b. Standar Nasional Indonesia: Identifikasi bakteri *Aeromonas hydrophila* pada ikan bagian 1: metode konvensional. *badan standardisasi nasional* 7303 (1): 18. [Indonesian]

SNI. 2009. Standar Nasional Indonesia: Metode identifikasi bakteri pada ikan secara konvensional bagian 3: *Streptococcus iniae* dan *Streptococcus agalactiae*. Badan Standardisasi Nasional 7545 (3): 16. [Indonesian]

Soto-Rodriguez SA, Simoes N, Jones DA, Roque A, Gomez-Gil B. 2003. Assessment of fluorescent-labeled bacteria for evaluation of in vivo uptake of bacteria (*Vibrio* spp.) by crustacean larvae. J Microbiol Meth 52 (1): 101-114.

Staroscik AM, David WH, Archibald KE, Nelson DR. 2008. Development of methods for the genetic manipulation of *Flavobacterium columnare*. BMC Microbiol 8 (1): 115.

Surachetpong W, Janetanakit T, Nonthabenjawan N, Tattiyapong P, Sirikanchana K, Amonsin A. 2017. Outbreaks of Tilapia Lake Virus infection, Thailand 2015-2016. Emerg Infect Dis 23 (6): 1031.

Vendrell D, Balcázar JL, Ruiz-Zarzuela I, De Blas I, Gironés O, Múzquiz JL. 2006. *Lactococcus garvieae* in fish: A review. Comp Immunol Microbiol Infect Dis 29 (4): 177-198.

Zahra SA, Misri S, Padilah B, Zulkafli R, Kua BC, Azila A, Rimatulhana R. 2004. Predisposing factors associated with outbreak of *Streptococcal* infection in floating cage cultured red tilapia in reservoirs. Paper presented at the Proceedings of the 7th Asian Fisheries Forum, The Triennial Meeting of The Asian Fisheries Society, Penang Malaysia, 30 November-4 December 2004.

Zahrah SA, Shahidan H, Wan-Norazlan G, Amal AMN, Nur-Nazifah M, Misri S. 2008. Isolation of *Staphylococcus* spp. In cage-cultured tilapia of different water bodies. Paper presented at the Proceedings of the National Fisheries Symposium, Wisma Darul Iman, Kuala Terengganu Malaysia, 14-16 July 2008.

Zahran E, Manning B, Seo JK, Noga EJ. 2016. The effect of ochratoxin A on antimicrobial polypeptide expression and resistance to water mold infection in channel catfish (*Ictalurus punctatus*). Fish Shellfish Immunol 57: 60-67.

Zorrilla I, Chabrillon M, Arijo S, Dıaz-Rosales P, Martınez-Manzanares E, Balebona MC, Morinigo MA. 2003. Bacteria recovered from diseased cultured gilthead sea bream (*Sparus aurata* L.) in Southwestern Spain. Aquaculture 218 (1): 11-20.