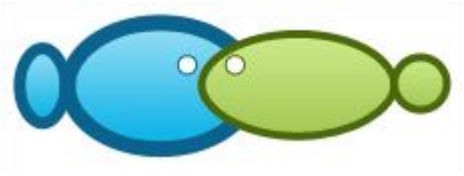

Screening of probiotics from the digestive tract of gouramy (*Osphronemus goramy*) and their potency to enhance the growth of tilapia (*Oreochromis niloticus*)

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Abstract. The aims of this study were to select probiotic candidates from the digestive tract of gouramy (*Osphronemus goramy*) and to evaluate their potency in improving the growth of tilapia (*Oreochromis niloticus*). Bacteria were isolated from the digestive tract of gouramy and were selected based on proteolytic, amylolytic and cellulolytic activity, sensitivity to antibiotics, resistance to pH 2.5 and 7.5, antagonistic activity, adhesion ability and pathogenicity test to the host. Further evaluation of probiotics ability in increasing the growth of tilapia had been conducted. The results showed that from 10 isolates obtained, only three isolates were qualified as probiotics (UG3, UG7 and UG8). These probiotic candidates had values of proteolytic index (0.41, 0.24 and 0.43), cellulolytic index (3.4, 4.0 and 3.5) and amylolytic index (0.58, 0.57 and 0.47). Those isolates were sensitive to ampicillin, tetracycline and chloramphenicol, had ability to survive at pH 2.5 and 7.5, and had antagonistic activity against *Aeromonas hydrophila* with the inhibition zone range around 8-9 mm. These isolates could adhere on a stainless steel plate and were not pathogenic to the fish. In addition, these isolates significantly increased feed efficiency and daily growth rate of tilapia.
Key Words: digestive enzymes, growth performance, feeding trial, isolates.

Introduction. Aquaculture is known as one of food-producing sectors which can provide nutrients and safety food for humans. However, it is currently experiencing severe challenges such as the emergence of fish disease outbreaks and the high price of feed. The cost for feed in aquaculture business can reach more than 50% of total operational costs (Rana et al 2009). Therefore, some efforts are needed to improve the quality of feed, so the growth performance and feed efficiency of the farmed fish can be improved. One of efforts to solve this problem is by applying probiotics as the feed supplement. Probiotics are live microbes which give some beneficial effects to the host by stimulating the growth, improving digestibility and resistance to disease (Tuan et al 2013). The probiotic application in aquaculture, not only reduces the use of antibiotics, but also improves the growth performance of the farmed fish. Some studies reported that probiotics reduce the cost in aquaculture business by improving growth and feed fish efficiency (El-Dakar et al 2007; Mohapatra et al 2012; Noveirian & Nasrollahzadeh 2012). Moreover, probiotics also have a role as the source of nutrients, such as vitamins (Sánchez-Ortiz et al 2015) and amino acids, and play an important role in the nutrient decomposition process by providing digestive enzymes such as protease, amylase, lipase and cellulase (Maity et al 2011; Sahu et al 2008).

Probiotics are commonly presented in the digestive tract of healthy fish, so this part is commonly selected to be a site for the probiotic isolation. Some studies have shown that probiotics can be isolated from the digestive tract of fish such as gouramy -

Osphronemus goramy (Ghosh et al 2007), tilapia - *Oreochromis niloticus* (Lara-Flores & Olvera-Novoa 2013; Putra & Widanarni 2015), snakehead fish - *Channa striatus* (Allameh et al 2014), other freshwater fish (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Cyprinus carpio*) (Muthukumar & Kandeepan 2015), the digestive tract of shrimp - *Litopenaeus vannamei* (Widanarni et al 2015) and molluscs - *Anadara tuberculosa* (Sánchez-Ortiz et al 2015). The bacterial strains originated from the fish intestines are the potential probiotic candidates, because these bacteria have an ability to attach on intestinal wall and have adapted to the environmental condition in the intestines. These bacteria also used to compete with pathogenic bacteria to get nutrients. Liu et al (2016) stated that the feeding habit and the trophic level of fish is the factors influencing microbiota composition, which are alive in the fish digestive tract.

Gouramy (*Osphronemus goramy*) is a herbivorous fish which is commonly fed plants. Tanu et al (2012) reported that the enzymes activity in herbivorous fish dominated by the protease and cellulase activity. Based on this finding, the digestive tract of herbivorous fish is expected to be one of probiotic source sites. Some studies have reported that the beneficial effects of probiotics, not only occur in an origin host species of probiotic isolates, but also occur in other species, such as *Bacillus* sp. NP5 isolated from the digestive tract of tilapia (*Oreochromis niloticus*) has been proven to have positive effects on the growth and health status of tilapia (Agung et al 2015; Putra & Widanarni 2015; Utami et al 2015; Widanarni & Tanbiyaskur 2015), catfish (*Pangasianodon hypophthalmus*) (Tamamdusturi et al 2016) and common carp (*Cyprinus carpio*) (Djauhari et al 2016). In the present study, the probiotic candidates were isolated from the digestive tract of gouramy (herbivorous fish) and were tested in tilapia (omnivorous fish). To get good probiotic candidates, the selected probiotics have to meet the following criteria which have to be considered in the probiotic selection process: able to colonize, able to live and multiply in the digestive tract of the host, able to produce extracellular digestive enzymes such as amylase, protease, lipase and cellulase (Pundir et al 2013), non pathogenic, able to compete with pathogenic bacteria (Verschuere et al 2000) and sensitive to antibiotics (Loh et al 2014). This study aimed to get probiotic candidates from the digestive tract of gouramy and evaluate its potency to improve the growth of tilapia.

Material and Method. This study was conducted in Research and Development Center of Freshwater Fisheries, Bogor, West Java, Indonesia. This study was performed through three steps, including isolation of probiotic candidates from digestive tract of gouramy, selection of probiotic candidates, and feeding trial using selected probiotic candidates on tilapia. Isolation of probiotic candidates from digestive tract of gouramy was held on May 2012. Selection of probiotic candidates was held on July-September 2014. Feeding trial was held on January-February 2015.

Isolation of probiotic from digestive tract of gouramy. Probiotic bacteria were isolated aseptically from the digestive tract of gouramy (which had an average weight of 100 grams) obtained from private ponds in Tasikmalaya, West Java, Indonesia. One gram of the digestive tract was carefully taken from the abdomen of gouramy and was added to 0.85% NaCl solution (9 mL), it was crushed using a mortar and was serially diluted from 10^{-2} up to 10^{-8}. Sample was spread onto the plate agar containing 1% Carboxy Methyl Cellulose (CMC) with the following composition (per 100 mL of medium): 1.0 g CMC; 0.02 g $MgSO_4.7H_2O$; 0.075 g KNO_3; 0.05 g K_2HPO_4; 0.002 g $FeSO_4.7H_2O$; 0.004 g $CaCl_2.2H_2O$; 0.2 g yeast extract; 1.5 g bacto agar and 0.1 g glucose, it was then incubated at 28°C for 24-48 hours. The grown bacterial colonies were purified by repeated streaking in the same medium to obtain a single colony.

Selection of probiotic candidates

Proteolytic, amylolytic and cellulolytic activity test. This analysis was conducted to measure the ability of bacteria to hydrolyze protein, carbohydrate and cellulose. Pure bacterial isolates were grown on the plate containing Tryptic Soy Agar (TSA) medium + 2% skim milk for proteolytic activity test, TSA medium + 2% starch for amylolytic

activity test and TSA medium + 1% CMC for cellulolytic activity test. The plates were then incubated for 24-48 hours at 28^{o}C. Protein hydrolysis was characterized by the formation of clear zone around bacterial colonies, while carbohydrate and cellulose hydrolysis were characterized by the formation of clear zone around bacterial colonies after added with 1% potassium iodide (KI) solution. Proteolytic, amylolytic and cellulolytic index were calculated as the difference between the clear zone diameter with the colony diameter, divided by the colony diameter (Lim et al 1987).

Resistance to antibiotics test. The method used in this test referred to Pundir et al (2013) with several modifications. Antibiotics used were ampicillin, tetracycline and chloramphenicol in the form of paper disk. Standard concentrations of ampicillin was 10 mg, while tetracyclin and chloramphenicol were 30 g. The test was conducted by spreading probiotic on the plate containing TSA medium. A paper disk containing each antibiotic was placed onto TSA medium and the plate was incubated for 24 hours at 28°C. The inhibition zone diameter around the paper disc was then measured.

Resistance to acid and alkali test. The aim of this test was to evaluate the ability of probiotic candidates to survive in the stomach that has a low pH and in the proximal intestine that contains bile salt that has an alkaline pH. One (1.0) mL bacterial suspension was inoculated into serial dilution tubes containing 9 mL sterile broth medium with pH 2.5 and pH 7.5, the samples were then incubated at 28°C. pH adjustment was done using 0.1 N HCl solution and NaOH 0.1 N solution. Observations were conducted on 2, 4, 6, and 8 hours after inoculation and the number of bacteria was counted by spread plate technique (Madigan et al 2003).

Antagonistic test. Antagonistic test referred to Kirby-Bauer method that has been modified, using *Aeromonas hydrophila* as a pathogenic bacteria (Madigan et al 2003). Each probiotic culture and *A. hydrophila* were incubated at 28°C for 24 hours, they were then diluted to 10^{7} CFU mL^{-1}. Probiotic culture was placed into micro tube, which has been filled with a 6 mm paper disk. The paper disk was then placed into the plate containing TSA medium + 0.1 mL of *A. hydrophila*, the plate was incubated at 28°C for 24-48 hours. Physiological solution was used as a control or substitute for probiotic candidates. The inhibition activity was demonstrated by the existence of an inhibition zone around a paper disk.

Adhesion test. Adhesion test was done using a 2 x 10 cm stainless steel plate. The test was performed by placing the plate into an Erlenmeyer flask containing 250 mL Tryptic Soy Broth (TSB), which has been inoculated with 1.0 mL bacterial culture, the sample was then incubated at 28°C for 24 hours. The density of biofilm was analyzed after 24 hours by washing the plate using phosphate buffer solution. The plate was swabbed thoroughly, it was then placed in a test tube containing 10 mL phosphate buffer solution and was stirred for 1 minute. The density of bacteria in the liquid medium was measured by taking 1.0 mL culture suspension and it was diluted with 9 mL phosphate buffer solution. The bacteria on the swab and in the liquid medium was calculated through total plate count (TPC) using TSA medium, those were then incubated at 28°C for 24 hours (Dewanti & Wong 1995).

Pathogenicity test. This test was conducted to know whether the probiotic bacteria candidates were pathogenic for the host or not. One (1.0) mL probiotic at a concentration of 10^{8} CFU mL^{-1} previously cultured for 24 hours at 28°C, was injected via intramuscular route to tilapia (10±0.53 g). As a control, the healthy tilapia was injected with 1.0 mL phosphate buffer solution pH 7. Observations were conducted during two weeks in the aquarium (60 x 50 x 40 cm) with a stocking density of 10 fish per aquarium.

Preparation of experimental feed. To study the effect of probiotic on the growth performance of tilapia obtained from Installation of Freshwater Fisheries Germplasm Research, Cijeruk, West Java, Indonesia, the feeding trial was conducted using four experimental feed group: one group was the control diet without probiotic and three groups were probiotic groups. The feed used was a commercial feed sized 1.3 mm (PF

1000 manufactured by CV. Matahari Sakti Indonesia). Each probiotic group was added with different bacteria (UG3, UG7 and UG8), which had previously cultured for 48 hours. One percent (1%) bacterial suspension with a density of 10^{11} CFU mL^{-1} was mixed with 2% egg white, the mixture was then sprayed onto the feed using a syringe and was stirred until equally spread, 2% egg white was also added to the control feed without adding bacterial suspension (Wang 2007).

Experimental design. The experimental design used was completely randomized design with four treatments and three replications. Tilapia fingerlings used (0.85±0.15 g) were randomly stocked in 12 aquariums (60 x 50 x 40 cm) at a density of 25 fish per aquarium. The fish were fed three times a day (08:00, 12:00 and 15:00 Western Indonesia Time) with a feeding rate of 5% (w w^{-1}) of the total fish biomass for 40 days of feeding trial. Parameters measured were biomass (B; g), daily growth rate (DGR; %), feed efficiency (FE; %), protein efficiency ratio (PER; %), and survival rate (SR; %). These parameters are calculated by the following formula:

Biomass (B; g) = final weight of biomass (g) - initial weight of biomass (g)

Daily growth rate (DGR; %) = [ln (final body weight - initial body weight)/feeding trial duration] x 100

Feed efficiency (FE; %) = [(weight of biomass + weight of dead fish during feeding trial) – initial weight of biomass)/feed intake during the feeding trial] x 100

Protein Efficiency Ratio (PER; %) = body weight (g)/protein consumed (g)

Survival rate (SR; %) = total number of fish alive in the end of feeding trial/total number of fish alive in the beginning of feeding trial x 100

Statistical analysis. Data were tabulated and were statistically analyzed using SPSS 20.0. Data collected from isolation and selection of probiotic candidates were described qualitatively and were analyzed by descriptive statistics. The data collected from feeding trial were analyzed by analysis of variance (ANOVA) and were continued by Duncan's test.

Results and Discussion

Isolation of probiotic candidates. Isolation of probiotic candidates from the digestive tract of gouramy founded 10 isolates that have an ability to grow on 1% CMC agar medium (Table 1). Almost all of isolates have a round shape, white, milky white, cream and light brown, except UG5 (irregular shape) and UG10 (yellowish).

Table 1

The morphology of bacterial isolates isolated from the digestive tract of gouramy

Isolate name	*Morphology*
UG1	Cream, round
UG2	Cream, round, unjagged edges, flat
UG3	Cream, big round, serrated edges, light brown, flat
UG4	Light brown, big round, serrated edges, yellow nucleated
UG5	Milky white, irregular, serrated edges, flat
UG6	Cream, round, unjagged edges
UG7	Light cream, round, unjagged edges
UG8	White, round, smooth edges, flat
UG9	White, small round, fibrous
UG10	Yellow, fibrous

There were 10 isolates grown on 1% CMC agar medium, but there were only 4 isolates, which had the highest proteolytic, amylolytic and cellulolytic index (UG3, UG6, UG7 and UG8). UG3, UG7 and UG8 had higher values of proteolytic index, while UG6 has the

lowest proteolytic index (Figure 1). UG7 and UG3 had the highest amylolytic index, while UG6 had the lowest amylolytic index (Figure 2). Cellulolytic index of UG7 was the highest, while the lowest were UG8 and UG3 (Figure 3)

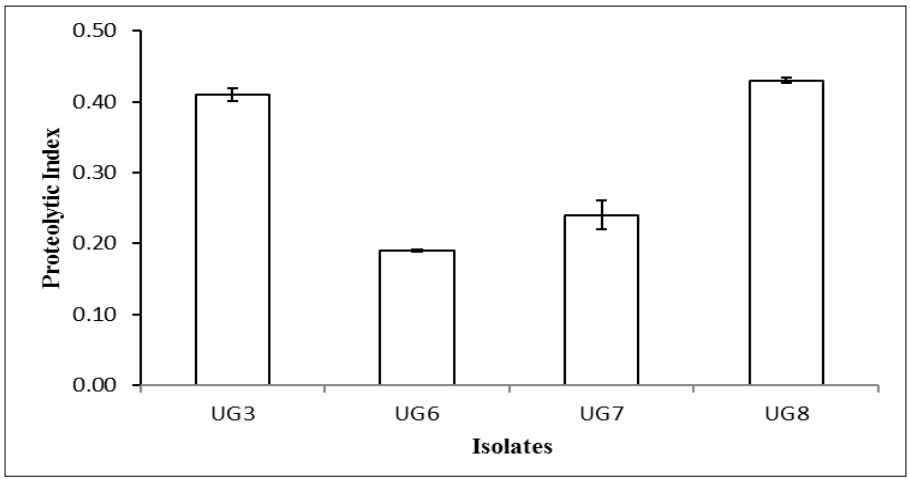

Figure 1. Proteolytic index of UG3, UG6, UG7 and UG8 isolate isolated from the digestive tract of gouramy.

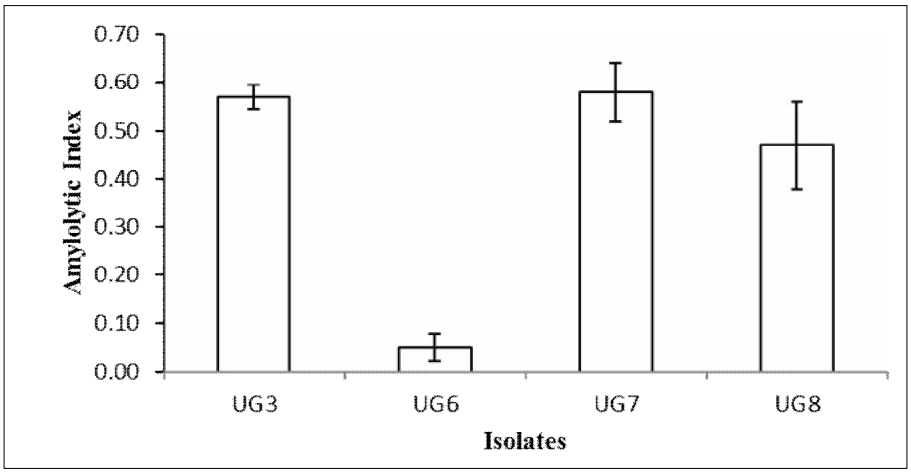

Figure 2. Amylolytic index of UG3, UG6, UG7 and UG8 isolate isolated from the digestive tract of gouramy.

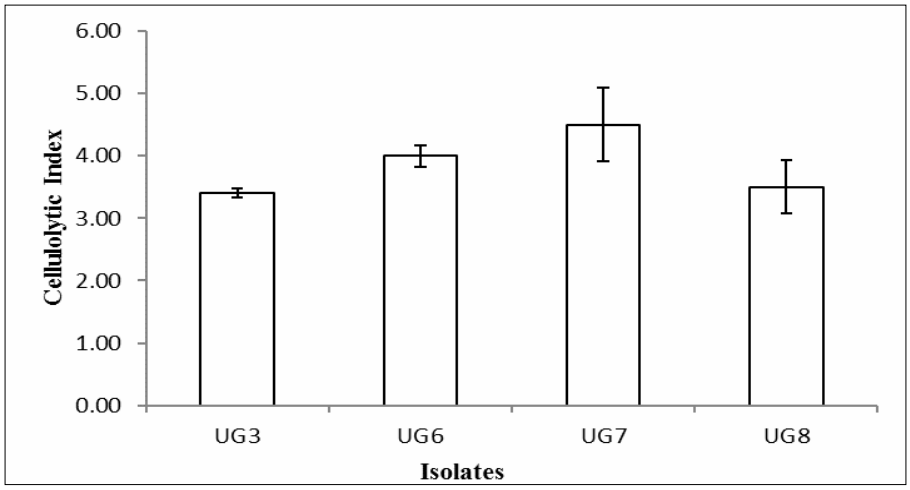

Figure 3. Cellulolytic index of UG3, UG6, UG7 and UG8 isolate isolated from the digestive tract of gouramy.

The presence of clear zone on the proteolytic, amylolytic and cellulolytic test showed that UG3, UG6, UG7 and UG8 were able to degrade protein from skim milk, carbohydrate from starch and cellulose from CMC. The existence of extracellular enzymes, such as protease, amylase and cellulase, is a criteria that have to be had by probiotics to improve feed digestibility. The enzymes produced by probiotics play an important role in the degradation of various feed materials that are difficult to be digested by the fish. According to Balcázar et al (2006), the ability of probiotics to produce extracellular enzymes affects on the improvement of the host digestibility. Cellulolytic activity values of probiotic candidates isolated in this study were higher than their amylolytic and proteolytic activity, it indicated that the bacteria have a tendency as cellulolytic bacteria that can degrade cellulose. According to Ganguly & Prasad (2012), the fish gastrointestinal tract collects microbial populations (microflora) from aquatic environment (water or food). Probiotics in this study were isolated from the digestive tract of gouramy (herbivorous fish). This fish has a habit to eat plants that generally have a high fiber content.

Antibiotic resistance. Based on antibiotic resistance test, UG3, UG6, UG7, and UG8 showed negative results in antibiotic resistance (sensitive). This indicates that all isolates tested were not resistant to ampicilin, tetracycline and chloramphenicol (Table 2).

Table 2

Result of the bacterial resistance to antibiotics

Isolate	*Antibiotic*		
	Ampicilin	*Tetracyclin*	*Chloramphenicol*
UG3	-	-	-
UG6	-	-	-
UG7	-	-	-
UG8	-	-	-

Notes: - = sensitive to antibiotics; + = resistant to antibiotics.

As a feed supplement, the safety of probiotics is needed, especially related to the resistance of probiotics to commercial antibiotics. Probiotics which are resistant to a particular antibiotic, are feared being able to transfer their genes to the pathogenic bacteria in the digestive tract of the fish (Loh et al 2014). This condition is harmful to the fish and humans consuming them. In this study, the probiotic candidates (UG3, UG6, UG7 and UG8) were sensitive to antibiotics, such as ampicillin, tetracycline and chloramphenicol. This occurred because the gouramy used for this study were obtained from traditional ponds that did not use drugs or antibiotics in the cultivation practice.

Resistance to acid and alkali. The resistance of the isolates to gastric acid and bile salt is an analogue with the ability to survive in a broth medium with acidic and alkaline pHs. The result of the resistance of bacterial isolates to gastric acid and bile salt is presented in a percentage of log number of bacteria that are resistant to pH 2.5 and 7.5 (Figure 4).

Each isolate had different response to acidic pH, but all isolates could survive in alkaline pH. After 8 hour of observation, all isolates were still alive, but their number tended to decrease. Based on the result, UG3 and UG8 had the highest resistance to pH 2.5 followed by UG7, and UG6 which had the lowest values. In general, the isolates tested had high resistance to pH 7.5 (UG3, UG6, and UG8), while UG7 had lower resistance values than other isolates.

Based on the results of this study, UG3, UG6, UG7 and UG8 could survive in the acidic and alkaline condition. Tolerance to acidic and alkaline pH is an important criteria needed by probiotics to survive in the fish digestive tract. When enter the fish digestive tract, probiotics must pass the stomach with a high acid level and then they must be able to grow in the environment containing bile salt to survive in the fish digestive systems (Amraii et al 2014). Tolerance to bile salt is necessary for probiotics to colonize and carry out metabolic activity in the small intestine (Havenaar & Veld 1992). Resistance to acidic

and alkaline pH will help probiotics to reach the small intestine and colon and also contribute to the balance of microflora in the fish digestive tract (Tambekar & Bhutada 2010). UG3, UG6, UG7, and UG8 were resistant to acidic and alkaline condition, because the initial environment of those isolates were the digestive tract of gouramy.

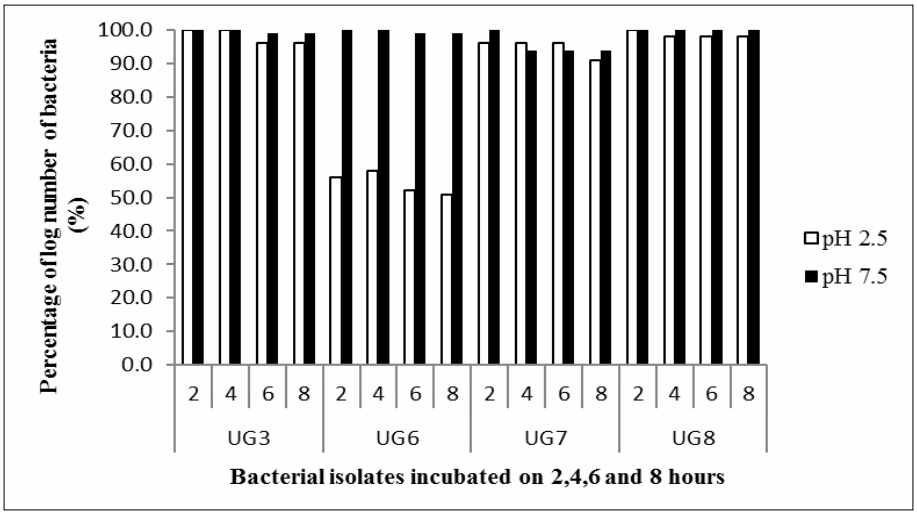

Figure 4. Percentage of log number of bacterial populations that were resistant to pH 2.5 and 7.5.

Antagonistic activity. One of the important criteria in the probiotic selection process was the ability of probiotic candidate to suppress the growth of pathogenic bacteria in the digestive tract of the host. Antagonistic activity of isolates tested against pathogenic bacteria (*A. hydrophila*), showed that of the four bacteria tested had antagonistic activity and only UG6 did not have the ability to inhibit *A. hydrophila* (Table 3). Isolates with antagonistic activity had an inhibition zone around the colony with a diameter range of 8-9 mm.

Table 3

Antagonistic activity of probiotic candidates against *A. hydrophila*

Isolate	*Inhibition ability to A. hydrophila*	
	Inhibition zone	*Diameter (mm)*
UG3	+	8
UG6	-	-
UG7	+	9
UG8	+	8

Notes: + = had antagonistic activity or showed inhibition zone; - = had no antagonistic activity or did not showed inhibition zone.

UG3, UG7 and UG8 had antagonistic activity against pathogenic bacteria (*A. hydrophila*). This study is in line with the previous study by Pannu et al (2014) who states that commercial probiotics (Aquapro), Exide and *Lactobacillus sporogenes* can inhibit the growth of several pathogenic bacteria with an inhibition zone range between 0.667 to 2.433 cm. Antagonistic activity of probiotics on pathogenic bacteria occurred, because probiotics are capable in producing antibacterial compounds, such as bacteriocins and organic acids (Dixit et al 2013). Bacteriocins are antimicrobial compounds consisting of proteins or polypeptides and are synthesized by bacteria. Bacteriocins generally work as anticompetitor compounds secreted by bacteria to compete other microbes in the natural environment and invade the complex and stable bacterial communities (Mantovani et al 2011). Action mechanism of bacteriocins in inhibiting the growth of bacteria is by blocking the formation of cell wall and cell membrane pores of the target bacteria, so it

interferes the cell permeability and inhibits the activity of DNA and RNA (Güllüce et al 2013).

Adhesion test. The adhesion ability of probiotics is based on the ability of probiotic to form biofilm on a stainless steel plate. It is used as an approach to test the ability of isolate to adhere on the intestines. The result of adhesion test showed that the four isolates tested had the ability to adhere on the stainless steel plate (Figure 5), UG3 had the highest ability compared to other isolates.

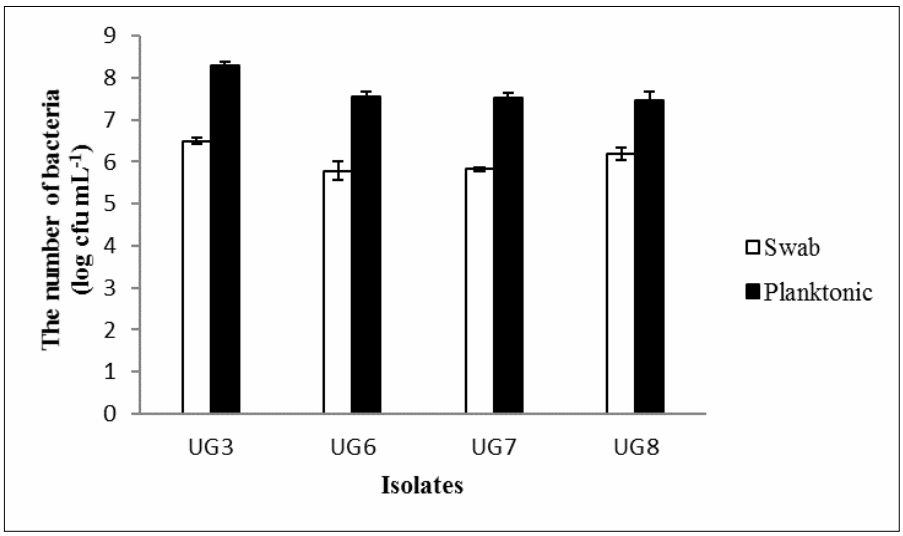

Figure 5. The adhesion ability of UG3, UG6, UG7, and UG8.

UG3, UG6, UG7 and UG8 had the adhesion ability on a stainless steel plate as an analogue of the digestive tract of the fish. The ability to adhere is essential for probiotics candidates, because this is a protective mechanism against pathogenic bacteria through the competition of attachment site and nutrient or the immune modulation (Ibrahem 2015). The probiotics that are successfull to adhere in the mucosa of the gastrointestinal tract, will form colonies and prevent the pathogen community to grow in this location by blocking the interaction between the specific cells receptor or by inhibiting pathogens to adhere through the steric interaction (Sánchez-Ortiz et al 2015). According to Ige (2013), several types of probiotics were able to attach in the intestinal mucosa to inhibit infections caused by pathogenic bacteria. The study conducted by Vine et al (2006), using five types of probiotics and two types of pathogens for adhesion test in the intestinal mucus of the fish, discovered the existence of probiotics in the mucus preventing the adhesion of the test pathogens.

Pathogenicity test. Based on the result of pathogenicity test, it was known that all bacterial isolates tested did not cause mortality on tilapia (Table 4).

Table 4

Result of pathogenicity test of probiotic candidates on tilapia for two weeks

Isolate	*Pathogenicity test (two weeks)*	
	Alive fish	*Dead fish*
UG3	10	0
UG6	10	0
UG7	10	0
UG8	10	0
Control	9	1

Note: the control fish died due to fungal infection.

One of important criterias for bacteria to be selected as probiotics, is not pathogenic or toxic to the host (Kesarcodi-Watson et al 2008). Probiotic candidates must be safe to be applied and do not harm to the fish health. Based on the pathogenicity test result, UG3, UG6, UG7 and UG8 did not cause infection or death in tilapia.

Feeding trial. After going through a series of selection process to obtain probiotic candidates, there were three isolates that qualified as probiotic candidates, including UG3, UG7 and UG8. Those isolates were then tested for their ability to improve the growth of tilapia. Based on the results, the fish fed probiotic candidates showed the better growth performance than control. FE and DGR of the fish fed UG3, UG7 and UG8 were higher than the fish fed the control feed ($p < 0.05$). PER on UG3 and UG8 were significantly higher than control ($p < 0.05$), while that on UG7 was not significantly different from control ($p > 0.05$). SR of the fish fed probiotic candidates was not significantly different from control ($p > 0.05$) (Table 5).

Table 5

Growth performance of tilapia fed the feed containing UG3, UG7 and UG8

Parameter	*Treatment*			
	UG3	*UG7*	*UG8*	*Control*
Biomass (g)	117.83 ± 3.67^{b}	115.60 ± 2.65^{ab}	115.50 ± 6.12^{ab}	111.37 ± 0.81^{a}
DGR (%)	3.69 ± 0.06^{b}	3.62 ± 0.04^{b}	3.67 ± 0.10^{b}	3.55 ± 0.01^{a}
FE (%)	89.83 ± 2.36^{b}	89.82 ± 1.59^{b}	88.92 ± 4.22^{b}	86.82 ± 0.50^{a}
PER	1.48 ± 0.05^{b}	1.45 ± 0.01^{ab}	1.49 ± 0.03^{b}	1.40 ± 0.03^{a}
SR (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}

DGR = Daily Growth Rate; FE = Feed Efficiency; PER = Protein Efficiency Ratio; SR = Survival Rate; different superscript letters in the same row indicate significant different results ($p < 0.05$); the values shown in the table are mean and standard deviation.

Based on the feeding trial results, the addition of UG3, UG7 and UG8 into the feed significantly affected on the increase of DGR and FE of tilapia compared to control. These results are consistent with the study by Putra & Widanarni (2015), probiotic in tilapia can improve feed efficiency and specific growth rate. Maity et al (2011) also reported that *Bacillus subtilis* and *B. licheniformis* could increase the growth rate, nutrient digestibility, digestive enzyme activity and microbial populations in the gastrointestinal tract of barramundi fingerlings (*Lates calcarifer*). Sánchez-Ortiz et al (2015) reported that the probiotic isolated from blood clams (*A. tuberculosa*) could improve growth performance and immunity of white shrimp (*L. vannamei*). According to Widanarni et al (2015), probiotics isolated from white shrimp could promote growth, protein digestibility and protein retention of white shrimp. Probiotics that enter the fish stomach, will attach to intestines and use large amounts of carbohydrates for growth and the production of digestive enzymes, so they will increase the digestibility of organic matters and protein. According to Lara-Flores & Olvera-Novoa (2013), an increase in fish growth performance may occur due to the balance of microflora in the gastrointestinal tract leading to the increase of the feed absorption quality and digestive enzymes, such as amylase, protease, lipase and cellulase. This will result in more nutrients that will converted into basal energy for growth. The presence of enzymes secreted by the probiotic will also prevent damage in the intestines and stimulates the predigest of secondary compounds in plant-based raw materials. According to Hemaiswarya et al (2013), probiotic can improve the fish growth through stimulation of appetite and increase the nutritional value by producing vitamins, detoxification compounds in the feed and simplifying those compounds, so they will be easier to be digested.

Conclusions. From 10 bacterial isolates isolated from the digestive tract of gouramy, there were three isolates that were qualified as probiotics (UG3, UG7 and UG8). These isolates had proteolytic, amylolytic and cellulolytic activity, were not pathogenic to tilapia, could survive at pH 2.5 and 7.5, had adhesion ability, were sensitive to ampicillin,

tetracycline and chloramphenicol and also had antagonistic activity against *A. hydrophila*. Application of UG3, UG7 and UG8 through the feed, increased feed efficiency and daily growth rate of tilapia.

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