Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 359 – 369 (2022)
www.ejabf.journals.ekb.eg

Isolation and identification of potential probiotics bacteria from milkfish (*Chanos chanos* Forskal) gut in Gresik Regency, East Java, Indonesia

Ummul Firmani[1,2], Rahmi Nurdiani[3], Arning Wilujeng Ekawati[3] and Happy Nursyam[3,*]

1. Doctoral Program, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang 65145, East Java, Indonesia.
2. Department of Aquaculture, Faculty of Agriculture, University of Muhammadiyah Gresik, Gresik 61161, East Java, Indonesia.
3. Faculty of Fisheries and Marine Science, University of Brawijaya, Malang 65145, Indonesia.

[*]Corresponding Author: happy_nsy@ub.ac.id

ARTICLE INFO

Article History:
Received: Dec. 14, 2021
Accepted: March 19, 2022
Online: May 28, 2022

Keywords:
Gut bacteria,
Milkfish,
Polyculture,
Probiotic

ABSTRACT

The present study was conducted to isolate and identify probiotic bacteria from the gut of milkfish (*Chanos chanos* Forskal). Six milkfish (65-70 g) were collected from 2 traditional freshwater ponds in Ujung Pangkah, Gresik Regency, East Java, Indonesia. Isolates of bacteria were collected and purified. The morphological characteristics of bacteria were observed, including gram reaction, cell shape, colony shape, elevation, color, optic and motility. Microbact identification kits 24A and 24B were used to identify the biochemical characteristics of bacteria. The enzymatic activity of bacteria was assessed to determine the ability of bacteria to produce amylase, cellulase, protease and lipase enzymes. Results showed that 14 isolate bacteria encoded BL1.1, BL1.2, BL1.3, BL1.4, BL1.5, BL1.6, BL2.1, BL2.2, BL2.3, BL2.4, BL2. .5, BL2.6, BL2.7 and BL2.8 were obtained from the milkfish gut. These isolates had similarities with 3 genera of bacteria, such as *Bacillus* sp., *Pseudomonas* sp., and *Enterobacter* sp. BL2.6 isolates have similar characteristics to *Bacillus*, producing high amylase and cellulase enzymes, with a high amylolytic and cellulolytic index of 6.39 and 0.06, respectively. The bacterial isolates detected in this research could be used as potential probiotics bacteria.

INTRODUCTION

The Milkfish (*Chanos chanos* Forskal) is a high-value commodity in Gresik Regency, East Java, Indonesia. In 2019, milkfish production ranked higher than other fishery commodities in Indonesia, especially in Gresik Regency **(Central Bureau of Statistics, 2019)**. The milkfish cultivation technology faces several problems with respect to ponds. According to interviews with milkfish farmers in Gresik Regency, the main

problems of milkfish cultivation includes slow growth, low survival, reduced land carrying capacity and decreased water quality.

Milkfish gut bacteria had amylolytic, proteolytic, lipolytic and cellulolytic activity **(Alamsyah, 2006)**. Colonization and bacterial diversity in the digestive tract of fish usually depend on environmental conditions **(Nayak, 2010)**. In addition to the ecological conditions, the type of fish feed has a great impact on the diversity of bacteria in the digestive tract. In addition, host-related factors can influence fish gut microbial communities in addition to their local habitat **(Roeselers *et al*., 2011; Larsen *et al*., 2014; Ye *et al*., 2014; Li *et al*., 2015).**

Microorganisms found in the digestive tract of fish play an important role in the digestion of food. The role of bacterial communities in the digestive tract of fish on metabolism, immunity, and health is still rarely studied **(Ni *et al*., 2014; Pérez *et al*., 2010)**. Isolation of bacteria from the digestive tract of milkfish has been carried out in several studies, including those conducted in West Java and Central Java, while in East Java, it hasn't not yet been recognized. Some of the same types of bacteria are present in the digestive tract of fish from different populations and geographic locations **(Roeselers *et al*., 2011)**. Phylogenetic factors such as host physiology and gut anatomy interact with environmental and ecological factors such as host fish biogeography to support the interaction between microbiota composition and host biology **(Ghanbari *et al*., 2015)**.

To the best of our knowledge, studies on exploring new probiotic bacteria from milkfish are currently limited in East Java Province. In this study, bacteria were isolated and identified from the digestive tract of milkfish in Gresik Regency, East Java, Indonesia. Additionally, the current work investigated the bacterial community in fish digestive tract to develop new probiotics from gut microorganisms in order to overcome the problems in milkfish cultivation.

MATERIALS AND METHODS

Sampling

Six milkfish (65-70 g) were obtained from 2 semi-traditional ponds in a polyculture system of Ujung Pangkah District, Gresik Regency, East Java, Indonesia (Fig. 1). Fish specimens were immediately transferred in a polyethylene bag containing oxygenated water for about 1h and 30min to the Laboratory of Fisheries, Fisheries Government Institution, Gresik Regency.

Gut Bacteria Isolation

The bacterial isolation dan purification protocol was performed following the method of **Cappuccino and Sherman (2014)**. Fish gut was isolated using a sectio set under sterile conditions. The nutrient agar (28 g/L) and nutrient broth (g/L) were sterilized at 15lbs pressure at 121^{o}C for 15min. The sterile nutrient broth and nutrient agar were then

cooled up to 50^{o}C and aseptically poured into the Petri dish under hygienic conditions. The gut homogenate was mixed in 0.9% of 9ml natrium chloride and serially diluted until 10^{-5}. About 0.1ml homogenate was spread to a plate containing sterile nutrient agar medium petri dish and incubated for 48h at 37^{o}C. Individual and different colonies were purified using the streak plate technique into nutrient agar slant in a tube. All pure isolates were refrigerated until used for a characterization assay.

Fig. 1. The milkfish was collected from a freshwater pond in Ujung Pangkah District, Gresik Regency

Morphological and Biochemical Characterization

The morphological bacteria were observed following the method of **Claus and Berkeley (1986)**. The morphological characteristics of bacteria were observed, including gram reaction, cell shape, colony shape, elevation, color, optic and motility. Bacterial colony morphology was observed by culturing one pure loop isolate on nutrient agar media in Petri dishes at 37^{o}C for 24h. Bacterial cell morphology was observed by preparing pure isolates and then staining them with crystal violet and safranin solution. Furthermore, the stained bacteria were examined using an eclipse microscope at 1000x magnification.

The biochemical identification was analyzed using Microbact Identification Kits 24A and 24B based on **Holt *et al*. (1994)** methods. Each bacterial isolate was re-cultured on NA skewed media and incubated for 24h at 37^{o}C. One loop of bacterial cultures was suspended in 10ml of 0.9% physiological NaCl. About 200µl of bacterial suspension was inserted into the 24A and 24B Microbact Identification Kits and incubated at 35^{o}C for 24h. The interpretation of color changes on each indicator media was performed in accordance with the protocol of the manufacturer.

Enzymatic Screening

The bacterial enzymatic activity screening was carried out to determine the ability of bacteria to produce amylase, cellulase, protease and lipase enzymes. A bacterial enzymatic activity test was achieved using specific growth media; namely,

Carboxymethyl cellulose agar (CMC agar), Starch agar, Skim milk agar, Tween-80 and Pepton bacto agar. CMC agar was used to test the cellulolytic ability of bacteria; Starch agar was utilized to test the amylolytic ability; Skim milk agar (SMA) was used to test the proteolytic ability, while Tween-80 was employed to test the lipolytic ability. The enzymatic activity test was used based on the method of **Teather and Wood (1982)**. The culture of each bacterium was determined on a specific medium by growing as much as 1 loop in the center of the cup, and incubated for 7-10 days for cellulolytic bacteria, 48 hours for amylolytic and proteolytic bacteria and 5-7 days for lipolytic bacteria at 37^{o}C. The clear zones or inhibition zones were observed around the colonies using 0.1% Congo red indicator solution, 1% iodine solution and iodine solution. The hydrolysis index (HI) was obtained using the following formula **(Kasana *et al*., 2008)**:

$$HI = \frac{DZ - DC}{DC}$$

Where,
DZ = Diameter of clear zone (mm)
DC = Colony diameter (mm)

RESULTS

Morphological and Biochemical Characterization

A total of 17 species of bacteria were isolated from the milkfish digestive tract. The codes of bacterial isolates were BL1.1, BL1.2, BL1.3, BL1.4, BL1.5, BL1.6, BL2.1, BL2.2, BL2.3, BL2.4, BL2.5, BL2.6, BL2.7 and BL2.8 (Table 1).

Table 1. Morphological characteristics of bacterial colonies from the digestive tract of milkfish in freshwater ponds, Gresik

Isolate Code	Form	Color	Round	Elevation	Optic
BL1.1	Jagged round	White	Jagged	Flat	Unshiny
BL1.2	Jagged round	Orange	Jagged	Flat	Shiny
BL1.3	Jagged round	White	Jagged	Flat	Unshiny
BL1.4	Wavy round	Yellow	Wavy	Flat	Unshiny
BL1.5	Jagged round	Pink	Jagged	Flat	Unshiny
BL1.6	Round	Orange	Align	Convex	Shiny
BL2.1	Jagged round	Yellow	Jagged	Flat	Shiny
BL2.2	Jagged round	Yellowish white	Jagged	Flat	Shiny
BL2.3	Round	Yellowish white	Align	Convex	Shiny
BL2.4	Wavy round	Orange	Wavy	Convex	Shiny
BL2.5	Wavy round	Yellow	Wavy	Convex	Shiny
BL2.6	Wavy round	Pink and red layered	Wavy	Convex	Shiny
BL2.7	Jagged round	White	Jagged	Flat	Unshiny
BL2.8	Wavy round	White	Wavy	Flat	Unshiny

Table 2. Biochemical bacterial from the gut of milkfish

Parameter	Isolates code					
	BL1.1	**BL1.2**	**BL1.3**	**BL1.4**	**BL1.5**	**BL1.6**
Colony color in N.A.	Cream	Cream	Greenish	Greenish	Cream	Cream
Gram reaction	+	+	-	-	+	+
Cell shaped	Rod	Rod	Rod	Rod	Rod	Rod
Motility	Non Motil	Non Motil	Motil	Motil	Motil	Non-Motil
Oxidase	-	-	+	+	-	-
Catalase	+	+	-	-	+	+
Indol Production	-	+	-	-	-	-
The use-C from citrate	-	-	+	+	-	-
TSIA test	As/As[b], G-H_2S^+	As/As[b], G-H_2S^-	Alk/Alk[a], G-H_2S^-	Alk/Alk[a], G-H_2S^-	Alk/Alk[a]G-H_2S^-	As/As[b], G-H_2S^+
V.P.	-	+	-	-	-	+

Note:
[a]Alk/Alk (nonfermented sugar)
[b]As/As (glucose and lactose or sucrose fermentation)
V.P (Voges-Proskauer); Triple Sugar Iron agar (TSIA)

Tables (2, 3 and 4) show the bacterial cell and biochemical tests. The morphological characteristics of the isolates (BL1.1 and BL1.6) were similar, including jagged round shape, white color, flat surface and not shiny. The cell characteristics of isolate BL1.1 were non-motile, rod, gram-negative and spores. The BL1.6 isolate had the characteristics of rod-shaped cells, gram-positive, spore-forming and non-motile. The biochemical characteristics of the two isolates were the same, such as producing nitrate, lysine, glucose, ONPG, gelatin, lactose, catalase, hydrolyzing starch and casein. Isolate BL1.2 colonies were round, with a flat and shiny surface with round, jagged edges and an orange color. The cells were rod-shaped, gram-negative, with spores and were non-motile. The biochemical characteristics have similarities with isolate BL2.6, such as producing lysine, glucose, Xylose, ONP, indole, gelatin, positive VP, rhamnose, arabinose, positive catalase, capable of hydrolyzing starch and casein. The hemolytic test is beta-hemolytic.

Isolate BL1.3 had jagged spherical colonies, white in color, had flat surface that was not shiny, with rod-shaped cells, gram-negative, no spores, and were non-motile. Isolate B1.4 colonies were spherical, whitish-yellow in color, flat surface, not shiny, rod-shaped cells, gram-positive, they did not have spores, and were non-motile. The biochemical characteristics of the two isolates were the same, such as being oxidase-positive, producing lysine, xylose, citrate, gelatin, sorbitol, sucrose, arabinose, and arginine, but unable to hydrolyze starch or casein. The hemolytic test of both isolates (BL1.3 and BL1.4) was alpha-hemolytic. Isolate BL1.5 had the characteristics of colonies

in the form of round, jagged, pink color, flat and not shiny surface, rod-shaped cells, gram-negative, spores and motile. The biochemical characteristics include producing nitrate, positive ONPG, having gelatin, being catalase-positive, being able to hydrolyze starch and casein and being beta-hemolytic.

Table 3. Biochemical bacterial of the gut of milkfish

Parameter	**Isolate codes**							
	BL2.1	**BL2.2**	**BL2.3**	**BL2.4**	**BL2.5**	**BL2.6**	**BL2.7**	**BL2.8**
Colony color in NA	Bluish-green	Bluish-green	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	-	-	-	-	-	+	-	-
Cell shaped	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	Motil	Motil	Motil	Motil	Motil	Non Motil	Motil	Motil
Oxidase	+	+	+	+	-	-	-	-
Catalase	+	+	-	-	-	+	-	-
Indol Production	-	-	-	-	-	+	-	-
The use-C from citrate	-	-	+	+	+	-	+	+
TSIA test	Alk/Alk[a], G-H_2S^-	Alk/Alk[a], G-H_2S^-	Alk/Alk[a], G-H_2S^-	Alk/Alk[a], G-H_2S^-	As/As[b], G-H_2S^-	As/As[b], G-H_2S^-	As/As[b] G-H_2S^-	As/As[b] G-H_2S^-
VP	-	-	-	-	+	+	+	+

Note:
[a]Alk/Alk (nonfermented sugar)
[b]As/As (glucose and lactose or sucrose fermentation)
V.P (Voges-Proskauer); Triple Sugar Iron agar (TSIA)

The morphological characteristics of the isolates (BL2.1 and BL2.2) were round, jagged edges, yellowish-white color, and the surface of the colonies was convex and shiny. The characteristics of BL2.1 isolate cells were cocci, gram-negative, without spores and were motile, while isolate BL2.2 cells were rod-shaped, gram-negative, with no spores and were motile. Isolates (BL2.1 and BL2.2) had similar biochemical characteristics, such as positive oxidase, producing nitrate, lysine, ornithine, glucose, mannitol, gelatin, arabinose, salicyne, arginine, but unable to hydrolyze casein and starch, and they use xylose and citrate as carbon source.

Table 4. Bacterial biochemical tests of the bacterial isolates from the digestive tract of milkfish

Biochemical test	Isolate codes													
	BL1.1	**BL1.2**	**BL1.3**	**BL1.4**	**BL1.5**	**BL1.6**	**BL2.1**	**BL2.2**	**BL2.3**	**BL2.4**	**BL2.5**	**BL2.6**	**BL2.7**	**BL2.8**
Spore	+	+	-	-	+	+	-	-	-	-	-	+	-	-
Oxidase	-	-	+	+	-	-	+	+	+	+	-	-	-	-
Motility	-	-	-	-	+	-	+	+	+	+	+	-	+	+
Nitrate	+	-	-	-	+	+	+	+	+	+	-	-	-	-
Lysine	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Ornithine	-	-	-	-	-	-	+	+	+	+	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	-	-	-	-	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Xylose	-	+	+	+	-	-	+	+	-	-	+	+	+	+
ONPG	+	+	-	-	+	+	-	-	-	-	+	+	+	+
Indole	-	+	-	-	-	-	-	-	-	-	-	+	-	-
Urease	-	-	-	-	-	-	-	-	+	+	+	-	+	+
V-P	-	+	-	-	-	-	-	-	-	-	+	+	+	+
Citrate	-	-	+	+	-	-	+	+	+	+	+	-	+	+
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin	+	+	+	+	+	+	+	+	+	+	-	+		-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	+	+	-	-	-	-	-	-	+	-	+	+
Rhamnose	-	+	-	-	-	-	-	-	-	-	-	+	-	-
Sucrose	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Lactose	+	-	-	-	-	+	-	-	-	-	+	-	+	+
Arabinose	-	+	+	+	-	-	+	+	-	-	-	+	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Arginine	-	-	+	+	-	-	+	+	-	-	-	-	-	-
Catalase	+	+	-	-	+	+	-	-	-	-	-	+	-	-
Coagulase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemolysis	alpha	beta	alpha	alpha	beta	Beta	beta	beta	alpha	alpha	No	alpha	No	No
Sensitivity test of Novobiocin	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Starch hydrolysis	+	+	No	No	+	+	No	No	No	No	No	+	No	No
Casein hydrolysis	+	+	No	No	+	+	No	No	No	No	No	+	No	No[a]

Note:
[a]No (not identified)

BL2.3 was round jagged colonies, yellow in color, with flat and shiny surface. The cells were rod-shaped, gram-negative, did not have spores and were motile. The morphological and cell characteristics of BL2.4 isolates were wavy round, orange color, convex and shiny surface, spiral-shaped cells, gram-negative, did not have spores and were motile. The biochemical characteristics of the isolates of BL2.3 and BL2.4 were the same, including positive oxidase, producing nitrate, lysine, ornithine, glucose, citrate, gelatin, salicyne, and alpha hemolysis, but unable to hydrolyze starch and casein.

Isolates BL2.5 and BL2.6 had the characteristics of round colonies, wavy edges, convex surface, shiny, yellow color (BL2.5) and pink coated with red at the edges (BL2.6). BL2.5 isolate cells were rod-shaped, gram-negative, with no spores and were motile, while BL2.6 isolates were rod-shaped, gram-positive, had spores and were non-motile. The biochemical characteristics of BL2.5 isolate were oxidase negative, producing lysine, glucose, xylose, positive for ONPG, urease, positive for Voges-Proskauer, citrate, sorbitol, lactose, negative for catalase, and unable to use carbon from citrate. BL2.6 isolate produced lysine, glucose, positive ONPG, produced indole, urease, gelatine, rhamnose, arabinose, negative oxidase, positive catalase and used citrate and xylose as a carbon source.

BL2.7 was formed of round colonies, jagged edges, white in color, flat surface and not shiny. The morphological characteristics of BL2.8 isolate colonies were round, wavy edges, white, flat surface and not shiny. The cell characteristics of isolates BL2.7 and BL2.8 were the same, including rods, gram-negative, no spores, and motile. The biochemical characteristics of isolates BL2.3 and BL2.4 were the same: oxidase-positive, producing nitrate, lysine, ornithine, glucose, citrate, gelatin, salicyne, alpha hemolysis, and unable to hydrolyze starch and casein.

Enzymatic screening

The enzymatic screening showed that among 17 isolates, only 4 isolates produced inhibition zones, including BL2.1, BL2.4, BL2.6 and BL2.7 (Table 5). BL2.6 isolate had a higher amylolytic and cellulolytic activity than the four isolates, with an amylolytic and cellulolytic index of 6.39 and 0.06, respectively (Table 6).

Table 5. The enzymatic activity of bacteria isolated from the digestive tract of milkfish

Bacteria isolate	Amylolytic	Cellulolytic	Proteolytic	Lipolytic
BL1.1	-	-	-	-
BL1.2	-	-	-	-
BL1.3	-	-	-	-
BL1.4	-	-	-	-
BL1.5	-	-	-	-
BL1.6	-	-	-	-
BL2.1	+	-	-	-
BL2.2	-	-	-	-
BL2.3	-	-	-	-
BL2.4	+	-	-	-
BL2.7	+	-	-	-
BL2.8	-	-	-	-

Table 6. Amylolytic and cellulolytic index of bacterial isolates from the digestive tract of milkfish

Bacteria isolate	Amylolytic index	Cellulytic index
BL2.1	3.02	-
BL2.4	0.38	-
BL2.6	6.39	0.06
BL2.7	3.62	-

DISCUSSION

The current study revealed that isolates BL1.1, BL1.2, BL1.5 and BL2.6 have similarities with *Bacillus* sp. and isolates BL1.3, BL1.4, BL1.6, BL2.1, BL2.2, BL2.3 and BL2.4 are similar to the characteristics of *Pseudomonas* sp. Isolates BL2.5, BL2.7 and BL2.8 have similar characteristics of *Enterobacter* sp. According to **Turnbull (1996)**, *Bacillus* species are rod-shaped, endospore-forming aerobic or facultatively anaerobic, and categorized as gram-positive bacteria. The BL2.6 isolates in this study were rod-shaped and gram-positive, but species BL1.1, BL1.6, BL1.2, and BL1.5 were rod-shaped, gram-negative, and all isolates contained spores. The spores are resistant to heat, cold, radiation, desiccation and disinfectants.

All isolates in the study were gram-negative, which is indicated by the characteristics of *Pseudomonas* cells. **Tryfinopoulou *et al*. (2002**) reported that, the characteristics of *Pseudomonas* were catalase and oxidase-positive, showing oxidative metabolism on Hugh-Leifson medium, could hydrolyze arginine and grow at 4°C. Isolates BL1.3, BL1.4, BL2.1, BL2.2, BL2.3, and BL2.4 were oxidase-positive, while only BL1.6 isolate was catalase positive. Isolates BL1.3, BL1.4, BL2.1 and BL2.2 were able to hydrolyze arginine. The strains were able to assimilate arabitol, hydroxy-l-proline, d-mannitol, d-quinate and d-glucose. Proteolytic and lipolytic activities as well as acid production from maltose and assimilation of 11 carbon sources, varied among the strains. **Nursyam *et al*. (2018)** stated that *P. aeruginosa* used glucose, xylose, d-malonic, and rhamnose fermentative as carbon sources. Isolates BL2.1, BL2.2, BL2.3 and BL2.4 in this study showed the same activity using glucose, and some isolates also used xylose as a carbon source.

Isolates BL2.5, BL2.7 and BL2.8 showed rod, gram-negative and motile characteristics that matched the characteristics of *Enterobacter*. **Hormaeche and Edwards (1960**) postulated that, the *Enterobacter* genus has the characteristics of rod-shaped cells, with a length of 2µm and a diameter of 0.6-1m, gram-negative, facultative anaerobes, motile with peritrichous flagella as a means of motion. The genus *Enterobacter* produces acid on glucose fermentation, negative methyl red, positive Voges-Proskauer, produces twice or more carbon dioxide as hydrogen from glucose, using citric acid and citric acid salts as the sole carbon source and does not produce hydrogen sulfide. Isolates B2.5, BL2.7 and BL2.8 were positive Voges-Proskauer, using citric acid as the carbon source, oxidase-positive, producing nitrate, lysine, ornithine, glucose, citrate, gelatin, salicyne, alpha hemolysis, and were unable to hydrolyze starch and casein.

The highest amylolytic index was produced by isolate BL2.6 with clear zone index of 2, which is categorized in the high category according to **Choi *et al.* (2005)**. Several amylolytic, proteolytic, lipolytic, and cellulolytic bacteria were identified in the digestive tract of milkfish in West Java, including *Moraxella* sp., *Aeromonas hydrophila*, *Citrobacter* sp., *Carnobacterium* sp., *Staphylococcus* sp., *Flavobacterium* sp., *Vibrio* sp., *Vibrio algynoliticus*, *Streptococcus* sp., *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Planococcus* sp., *Plesiomonas* sp., *Kurthia* sp. and *Serratia* sp. **(Alamsyah, 2006)**. The bacteria isolates in this study with strong amylolytic characteristics belonged to the genus *Bacillus*.

CONCLUSION

The study found several bacteria isolates from milkfish gut, including BL1.1 BL1.2, BL1.3, BL1.4, BL1.5, BL1.6, BL2.1, BL2.2, BL2.3, BL2.4, BL2.5, BL2.6, BL2.7 and BL2.8. The bacterial isolates were similar to the genus *Bacillus* sp., *Pseudomonas* sp. and *Enterobacter* sp. based on morphological and biochemical characteristics. BL2.6 isolates have similar characteristics of *Bacillus*, producing high amylase and cellulase enzymes, with a high amylolytic and cellulolytic index of 6.39 and 0.06, respectively. The bacterial isolates found in this research could be used as potential probiotics bacteria.

ACKNOWLEDGEMENT

This research was funded by the Ministry of Research and Higher Education through doctoral education scholarships. The authors thank Mr. Ahmad Robah for providing fish samples, the laboratory of the Gresik Regency Fisheries for providing a place for research, and the biomedical laboratory staff at the Faculty of Medicine, Brawijaya University, for assisting bacterial biochemical testing.

REFERENCES

Alamsyah, S. (2006). Penggunaan Mikroflora Saluran pencernaan Sebagai Probiotik untuk Meningkatkan Pertumbuhan dan kelangsungan Hidup Ikan Bandeng (*Chanos chanos* Forsskal). IPB University, Bogor.

Claus, D. and Berkeley, R.C.W. (1986) Genus Bacillus Cohn, 1872. In: "Bergey's Manual of Systematic Bacteriology." Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (Eds.). The Williams & Wilkins Co., Baltimore, pp. 1105-1139.

Cappuccino, J.G. and Sherman, N. (2014). Manual Laboratorium Mikrobiologi Edisi Kedelapan. EGC, Jakarta.

Central Bureau of Statistics. (2019). Production and Production Value of Aquaculture by Regency/Municipality and Main Commodity in Jawa Timur Province, 2019. https://jatim.bps.go.id/statictable/2021/09/07/2247/produksi-dan-nilai-produksi-perikanan-budidaya-menurut-kabupaten-kota-dan-komoditas-utama-di-provinsi-jawa-timur-2019.html

Choi, Y.W.; Hodgkiss, I.J. and Hyde, K.D. (2005). Enzyme production by endophytes of *Brucea javanica*. J. Agric. Technol., 1: 55-66.

Ghanbari, M.; Kneifel, W. and Domig, K.J. (2015). A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture, 448: 464–475.

Hormaeche, E. and Edwards, P.R. (1960). A proposed genus Enterobacter. Int. Bull. Bacteriol. Nomencl. Taxon., 10(2): 71–74.

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology. Willams and Wilkins, Baltimore, pp. 786-788.

Kasana, R.C.; Salwan, R.; Dhar, H.; Dutt, S. and Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. Curr. Microbiol. 57(5): 503–507.

Larsen, A.M.; Mohammed, H.H. and Arias, C. R. (2014). Characterization of the gut microbiota of three commercially valuable warmwater fish species. J. Appl. Microbiol., 116(6): 1396–1404.

Li, T.; Long, M.; Gatesoupe, F.J.; Zhang, Q.; Li, A. and Gong, X. (2015). Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. Microb. Ecol., 69(1): 25–36.

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. Aquac. Res., 41(11): 1553–1573

Ni, J.; Yan, Q.; Yu, Y. and Zhang, T. (2014). Fish gut micro ecosystem: a model for detecting spatial pattern of microorganisms. Chinese J. Oceanol. Limnol., 32(1): 54–57.

Nursyam, H.; Prihanto, A.A.; Warasari, N.I.; Saadah, M.; Masrifa, R.E.; Nabila, N.A.; Istiqfarin, N. and Siddiq, I.J. (2018). The isolation and identification of endophytic bacteria from mangrove (*Sonneratia alba*) that produces gelatinase. IOP Conf. Ser. Earth Environ. Sci., 137: 1-4.

Pérez, T.; Balcázar, J.L.; Ruiz-Zarzuela, I.; Halaihel, N.; Vendrell, D.; de Blas, I. and Múzquiz, J.L. (2010). Host-microbiota interactions within the fish intestinal ecosystem. Mucosal Immunol., 3(4): 355–360.

Roeselers, G.; Mittge, E.K.; Stephens, W.Z.; Parichy, D.M.; Cavanaugh, C.M.; Guillemin, K. And Rawls, J.F. (2011). Evidence for a core gut microbiota in the zebrafish. ISME J., 5(10): 1595–1608.

Teather, R.M. and Wood, P.J. (1982). Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from bovine rumen. Appl. Environ. Microbiol., 43(4): 777–780.

Turnbull, P.C.B. (1996). Bacillus. In: "Medical Microbiology, 4th ed." Baron, S. (Eds.). University of Texas Medical Branch at Galveston, Galveston (TX).

Tryfinopoulou, P.; Tsakalidou, E. and Nychas, G.J.E. (2002). Characterization of *Pseudomonas* spp. Associated with Spoilage of Gilt-Head Sea Bream Stored under Various Conditions. J. Appl. Environ. Microbiol., 68(1): 65-72.

Ye, L.; Amberg, J.; Chapman, D.; Gaikowski, M. and Liu, W.-T. (2014). Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. ISME J., 8(3): 541–551.