BIODIVERSITAS
Volume 23, Number 10, October 2022
Pages: 5423-5430

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231053

Isolation and identification of potential host probiotic bacteria from Malaysian mahseer, *Tor tambroides* for aquaculture practices

MOHAMMOD KAMRUZZAMAN HOSSAIN[1,2♥], SHUMPEI IEHATA[3,♥♥], NOORDIYANA MAT NOORDIN[3], MD. ABDUL KADER[4], SHARIFAH NOOR EMILIA[3], YEONG YIK SUNG[5], AMBOK BOLONG ABOL-MUNAFI[1]

[1]Higher Institution Centre for Excellence, Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu. 21030 Kuala Nerus, Terengganu, Malaysia. Tel./fax.: +60-9-6683561, ♥email: p3321@pps.umt.edu.my

[2]Department of Fisheries, Bangladesh. Matshya Bhabon, Ramna, Dhaka 1000, Bangladesh

[3]Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu. 21030 Kuala Nerus, Terengganu, Malaysia. Tel.: +60-9-6684930, Fax.: +60-9-6684949, ♥♥email: shumpei@umt.edu.my

[4]Alternative Aquatech. Victoria 3029, Australia

[5]Institute of Marine Biotechnology, Universiti Malaysia Terengganu. 21030 Kuala Nerus, Terengganu, Malaysia

Manuscript received: 24 November 2021. Revision accepted: 21 October 2022.

Abstract. *Hossain MK, Iehata S , Noordin NM, Kader MA, Emilia SN, Sung YY, Abol-Munafi AB. 2022. Isolation and identification of potential host probiotic bacteria from Malaysian mahseer,* Tor tambroides *for aquaculture practices. Biodiversitas 23: 5423-5430.* The digestive tract has been revealed as a reservoir of potential probiotics derived from the host. The goal of this study was to isolate, identify, and characterize putative host-associated probiotic bacteria (HAPs) from the digestive tract of Malaysian mahseer *Tor tambroides*. To evaluate potential *T. tambroides* HAPs, in-vitro digestive enzyme activity (such as a cellulolytic, proteolytic, and lipolytic activity) and antibacterial activity against two fish pathogens (*Vibrio parahaemolyticus* and *Aeromonas hydrophila*) were utilized. Thirty-seven isolates with digestive enzyme activities were tested for their ability to inhibit pathogenic bacteria growth using an agar well-diffusion assay. Three isolates displayed in-vitro suppression of pathogenic bacteria, with two strains (KT03 and KM07) inhibiting the growth of *V. parahaemolyticus* and one strain (KT27) suppressing the proliferation of both harmful bacteria (*V. parahaemolyticus* and *A. hydrophila*). Strains KT03 and KM07 exhibited the most resemblance to *Enterococcus faecalis* (strains 2674 and FC11682, respectively) based on 16S rRNA sequences, whereas KT27 had a 97% similarity to *Aeromonas* sp. A8-29. The study's findings provide valuable data on the prospective use of these three isolates (KT03, KT27, and KM07) as potential HAPs for better understanding their physiological activities, such as growth and disease resistance on *T. tambroides*.

Keywords: *Aeromonas*, antibacterial activity, *Enterococcus*, probiotics, *Tor tambroides*

INTRODUCTION

Disease outbreaks reduce aquaculture production, leading in significant financial losses (Martínez et al. 2012; van Doan et al. 2018). Antimicrobial compounds like antibiotics and chemotherapeutants have been employed in this context to prevent diseases in aquatic species (Serrano 2005; Fekaninová et al. 2017). Antibiotics and chemotherapeutants are effective, however, they have a severe impact on the environment and promote the emergence of antibiotic-resistant bacteria, which might pose a health risk (Romero et al. 2012; Langdon et al. 2016; Al-Asif et al. 2021). As a result, many countries have enacted legislation prohibiting the use of antibiotics and chemotherapeutants in aquaculture (Dawood and Koshio 2016; Van et al. 2020). Alternatively, several biological control approaches, including probiotics and vaccination, have been developed as disease control strategies through research and clinical practice to replace antibiotics (Hossain et al. 2009; Hossain et al. 2011; Lazado et al. 2015).

Aeromonas hydrophila and *Vibrio parahaemolyticus* are common bacterial fish pathogens. Fish become infected when the host immune system become weak or compromised (Dawood et al. 2017; Soto Dávila et al. 2020). *Aeromonas hydrophila* has been identified as the causative pathogen for the epizootic ulcerative syndrome (EUS), fin rot disease, tail rot, and hemorrhagic septicemia in the Indian major carps, such as *Labeo rohita, Catla catla* and *Labeo calbasu*, and motile *Aeromonas* septicemia in tilapia fish (Kozińska et al. 2002; Pridgeon and Klesius 2012; Saikot et al. 2013; Ina-Salwany et al. 2019). *Vibrio parahaemolyticus*, along with *Vibrio harveyi* and *V. fluvialis*, causes vibriosis in aquatic organisms and is often found in marine and freshwater fish (Lee et al. 2018). Vibriosis can be found in a number of body parts, including the gastrointestinal (GI) tract (Mishra et al. 2009; Tey et al. 2015; Yan et al. 2019). The most prevalent clinical indications of vibriosis include lethargy, aberrant swimming patterns, skin ulcers, exophthalmia, and gill necrosis, which result in 30-80% mortality (Ina-Salwany et al. 2019). The economic consequences of this disease have been estimated to be approximately US$9 billion per year (Novriadi 2016).

Many studies on aquaculture probiotics have documented the use of non-host associated probiotics (non-HAPs) originating from the terrestrial environment to suppress pathogenic bacteria in aquaculture (Lazado et al. 2015; van Doan et al. 2018). These non-HAPs were either added to feed as a nutritional supplement to promote

growth performance or used in culture water to improve water quality in aquaculture systems. Through food and the aquatic environment, they may colonize the digestive systems of fish and other aquaculture species. As a result, the use of host-associated probiotics (HAPs) in aquaculture has been promoted (van Doan et al. 2020), and they are thought to be safe for the host and the surrounding environment (Iehata et al. 2010; Interaminense et al. 2018; van Doan et al. 2018; Tarkhani et al. 2020). Due to the presence of digestive enzymes such as amylase, proteases, and lipases produced by probiotics during digestion and absorption, dietary supplementation with HAPs has been proven to improve growth performance (Ray et al. 2012; van Doan et al. 2020; Zaineldin et al. 2021). The host probiotics served to promote disease resistance through immune activation, as well as to maintain the interlinkage between immune responses and intestinal bacteria in the host gut (van Doan et al. 2020), and to improve the host's response to stressful conditions and to stabilize water quality parameters by removing nitrogen from wastes in water (Giraffa et al. 2010; Abdelkhalek et al. 2017). HAPs act as an effective growth promoter and antibiotic substitute to improve aquatic animal disease resistance (Nath et al. 2019). For example, in vitro and ex vivo studies demonstrated lactic acid bacteria (LAB) colonization in sturgeon, *Acipenser persicus* fish, and their adhesion capability varies depending on their isolation habitat (Lazado et al. 2015; van Doan et al. 2020), and their adhesion capability varies based on their isolation habitat (Askarian et al. 2011; Buntin et al. 2017). Potential probiotic bacteria in the host gut may offer health benefits (Adnan et al. 2017; Alshammari et al. 2019), however, the gut bacterial composition may be altered by genetic, dietary, and aquatic environmental factors (Abomughaid 2020; Mohammadi et al. 2022). Thus, screening of HAPs is focused primarily on their antibacterial inhibitory action against pathogens, as well as digesting enzyme activity, which plays an important role in the development of species and environment-specific probiotics (Hai 2015).

The Malaysian mahseer *Tor tambroides* is a valuable cyprinid fish species in Malaysia and other Asian countries, both as a food and ornamental fish in great demand among anglers and hobbyists (Azfar et al. 2019). Despite the fact that this piscine species' breeding is effective, slow growth in culture systems remains a major concern for commercial farming (Lee et al. 2014). *Tor tambroides* growth performance has been accelerated by supplying good nutrition, such as protein, fat, carbohydrate, and vitamins (Ng et al. 2008; Ng and Andin 2011; Kamarudin et al. 2012; Azfar-Ismail et al. 2020). Probiotic bacteria obtained from the ideal fish host are expected to perform better in their native habitat than those derived from terrestrial hosts. Thus, in recent years, studies focused on the application of host probiotic supplements for nutrition and health management of juvenile *T. tambroides* where feeds were supplemented with gut-derived probiotic bacteria (Asaduzzaman et al. 2018a; Asaduzzaman et al. 2018b). The effects on growth performance, muscle fiber, growth-related gene expressions, intestinal morphology, and formation of volatile short-chain fatty acids in the gut were determined for *T. tambroides* (Asaduzzaman et al. 2018a; Asaduzzaman et al. 2018b). Therefore, the study's objective was to isolate, screen, and identify suitable host gut-derived probiotic bacteria from *T. tambroides*. In this study, the isolates were screened in vitro to determine their nutritional enzyme activity and disease resistance capability through antibacterial activity against *A. hydrophila* and *V. parahaemolyticus*.

MATERIALS AND METHODS

Ethical statement

Tor tambroides is frequently and openly marketed for human consumption in Malaysian markets. They were purchased from the farm with the intention of isolating a probiotic strain from their intestines. Throughout the course of this study, no live *T. tambroides* fish were harmed or utilized in any other experiments. All of the experiments in this study were carried out in line with Universiti Malaysia Terengganu and European regulations on laboratory animal welfare, as well as the guiding principles for laboratory animal use and care.

Fish collection

Three healthy *T. tambroides* (average weight 75.18 ± 6.00g and age 24 weeks) were transported from a local aquaculture farm (Ikan Kelah Aquafarm Selangor, Malaysia) to the hatchery of the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Malaysia. The fish were acclimatized for 3 hours, anesthetized using clove oil (50 mg/L), and their skin wiped with 70% alcohol to minimize surface contamination.

Isolation of candidate probiotic bacteria

For isolation of candidate probiotic bacteria, fish were dissected in aseptic conditions (Zhou et al. 2009), and the whole digestive tract was removed prior to washing three times with sterile 0.01M phosphate buffer saline (PBS; Sigma-Aldrich Cheme GmbH, Munich, Germany). Then, individual digestive tract samples were aseptically removed, homogenized, and suspended with a sterile 0.01M PBS solution. The homogenized samples were diluted using sterile PBS and plated on TSA agar or poured on de Man, Rogosa, and Sharpe (MRS) agar containing 1.0% $CaCO_3$. TSA plates were incubated at room temperature (28-30°C) for 24h and inoculated MRS plates were incubated at room temperature (28-30°C) for 48-72h. Bacterial colonies with different shapes, characteristics and colors were selected from TSA agar, while colonies with clear zones on MRS agar were selected as candidate probiotics bacteria (Garcés et al. 2020).

In-vitro digestive enzyme activity analysis

Isolated candidate probiotic bacteria were subcultured for 24-48h at room temperature and spotted on each selective media to observe the cellulolytic, lipolytic, and proteolytic activity. We used carboxymethyl cellulose agar (CMCA) with 1% congo red for cellulolytic activity, TSA

or MRS medium consisting of 1% Tween-80 and 0.001% $CaCl_2.2H_2O$ for lipolytic activity, and TSA or MRS medium consisting of 1% skim milk for proteolytic activity. Each media was subsequently incubated for 72-90h at room temperature (28-30°C) (Asaduzzaman et al. 2018b; Selim et al. 2019). After incubation, clear zones surrounding colonies in the selective media were an indicator for the presence of cellulolytic, lipolytic, and proteolytic activity, whereas the presence of white particles surrounding the colonies were identified as positive of lipolytic activity.

In-vitro antibacterial analysis

Pathogenic bacteria *V. parahaemolyticus* (Deris et al. 2020) and *A. hydrophila* ATCC19570 were cultured at 35°C for 24 hours in agar plates containing tryptone soya agar (TSA) media. Isolates with positive nutritional enzyme activity were further selected to be analyzed for antibacterial activity analysis against two pathogens (*A. hydrophila* and *V. parahaemolyticus*) by the agar well diffusion method (Amin et al. 2017; Azahar et al. 2018) with some modification. Briefly, each pathogen was incubated in trypticase soya broth (TSB) broth for 24h and spread on TSA plate. After the spread of each pathogen on TSA plate, five (5) wells measuring 5mm in diameter were punched using sterile micropipette tips. After all candidate bacteria were incubated in broth media for 24 to 48 hours at room temperature (28-30°C), centrifuged at 3000 rpm for 10 minutes with 5 mL of broth culture and 50 uL of the supernatant put into the well of TSA plate. After incubation at 37°C 24h, the plates were observed and measured clear zone around the well. Positive results were evaluated when the clear zone exceeded 1 mm.

Identification of probiotic bacterial strains

Bacterial genomic DNA was extracted with a commercial genomic DNA purification kit, NucleoSpinR (Macherey-Nagel GmbH and Co.KG, Duren, Germany), following the manufacturer's protocol. The extracted DNA was subjected to polymerase chain reaction (PCR) amplification in PCR tubes that contained: 2.5uL of 10X PCR buffer with $MgCl_2$, 0.5µL of dNTP, 0.5 uL of each primer, 1µL of DNA extract supernatant, 19.5 uL sterile double distilled water with 0.5 uL master mix (MyTaq Mix; Bioline, London, UK), and 0.2mM of universal primers for bacterial PCR amplification (8F: 5'-AGAGTTTGATCATGGCTCAG-3' and 1492R: 5'-GGCTACCTT TTACGACTT-3') (Asaduzzaman et al. 2018b). PCR reaction samples were run for 30 cycles: 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1.5 minutes, followed by an extension of 72°C for 7 minutes (SuperCycler Thermal Cycler; Kyratec, Queensland, Australia). After PCR amplification, the PCR products were analyzed on 1.5% agarose gel at 100 volts for 30 minutes (Mohd-Nosi et al. 2018). Thereafter, the PCR products were purified and sequenced at First BASE Laboratories Sdn. Bhd. in Selangor, Malaysia.

Data analysis

After sequencing, the results of chromatograms were examined using chromas version 2.6.2 (Technelysium, Queensland, Australia). Then, all sequences were checked with DECIPHER (http: //decipher.cee.wisc.edu/Find Chimeras.html) for chimeric sequences (Wright et al. 2012). Homology searches were performed for culturable bacterial isolates, and sequences of approximately ~600 nucleotides were used. The close relatives were assigned by the GenBank database of the National Centre for Biotechnology (NCBI) through the BLAST program (http: //blast.ncbi.nlm.nih.gov./Blast.cgi). Multiple alignments were performed by MEGA version7 software (Kumar et al. 2016).

RESULTS AND DISCUSSION

Isolation and selection of bacteria for nutritional enzyme activity analysis

In the present study, a total of 37 bacterial isolates were obtained (30 isolates marked as 'KT' from TSA media and 7 isolates from 'MRS' media) from the digestive tracts of *Tor tambroides* (Table 1). Nine of 37 isolates (6 isolates from TSA and 3 isolates from MRS media) exhibited all three nutritional enzyme activities (lipolytic, proteolytic, and cellulolytic activity) and were selected to use the next process.

Antibacterial activity analysis

Antibacterial activity of nine isolates with positive nutritional enzyme activities was tested against two pathogens (*A. hydrophila* and *V. parahaemolyticus*) by well diffusion method. Three isolates displayed inhibitory activity (Figure 1 and Table 2). Isolates KT03 and KM07 showed growth suppression against fish pathogen *V. parahaemolyticus*, whereas KT27 exhibited growth suppression against both pathogens, *A. hydrophila* and *V. parahaemolyticus*, in well diffusion assay.

Identification of the selected bacterial isolates

Three isolates with nutritional and antibacterial activities were identified. Strain KT03 had 100% similarity with *Enterococcus* sp. strain 2674, while strain KM07 showed 93% similarity with *Enterococcus* sp. strain FC11682. Strain KT27 was 97% similar with *Aeromonas* sp. strain A8-29. The phylogenetic analysis demonstrated that isolates KT03 and KM07 composed the same cluster with *Enterococcus* sp., whereas isolate KT27 was in the same branch as the genus *Aeromonas* (Figure 1).

Discussion

Applications of probiotic bacteria have drawn a lot of interest and offer an alternate strategy for managing diseases in aquaculture (Van Doan et al. 2018). Moreover, attention has been given to host-associated microorganisms for investigation of beneficial probiotic bacteria because such bacteria are assumed to play a key function in increasing resistance against pathogenic bacteria and being well-habituated to the targeted host gut environment (Mukherjee et al. 2016).

Table 1. Bacterial nutritional enzyme activity of isolates from *Tor tambroides* digestive tract 'KT' where letter 'K' for Kelah-local name of *T. tambroides* and letter 'T' from the word 'TSA' agar medium 'KM' where 'K' stands for Kelah and 'M' stand for MRS medium

Isolates number*	Cellulolytic**	Proteolytic**	Lipolytic**
KM01	+	+	+
KM02	-	-	+
KM03	-	-	+
KM04	-	-	+
KM05	-	-	+
KM06	+	+	+
KM07	+	+	+
KT01	-	-	+
KT02	-	+	-
KT03	+	+	+
KT04	-	+	+
KT05	-	+	+
KT06	+	-	-
KT07	+	-	+
KT08	-	-	-
KT09	+	+	+
KT10	-	-	+
KT11	-	-	-
KT12	+	+	+
KT13	+	-	-
KT14	-	+	+
KT15	-	-	-
KT16	+	-	+
KT17	+	+	+
KT18	-	-	-
KT19	+	-	+
KT20	-	-	-
KT21	+	+	+
KT22	+	+	+
KT23	+	+	+
KT24	-	+	-
KT25	+	+	+
KT26	+	-	-
KT27	+	+	+
KT28	+	+	-
KT29	+	+	-
KT30	-	-	+

Note: *KT represents isolation using TSA medium; *KM represents isolation using MRS medium; **+ represents presence of nutritional enzyme activity; **- represents absence of nutritional enzymes activity

Table 2 Antibacterial activities of isolates from *Tor tambroides* digestive tract

Strain	Growth inhibition of pathogen in well diffusion assay			
	A. hydrophila	Diameter of inhibition zones (mm)	*V. parahaemolyticus*	Diameter of inhibition zones (mm)
KM01	-	-	-	-
KM06	-	-	-	-
KM07	-	-	+	12
KT03	-	-	+	14
KT17	-	-	-	-
KT22	-	-	-	-
KT23	-	-	-	-
KT25	-	-	-	-
KT27	+	15	+	16

Note: "+" represents presence of clear zone and "-'' represents absence of clear zone in antibacterial activity analysis

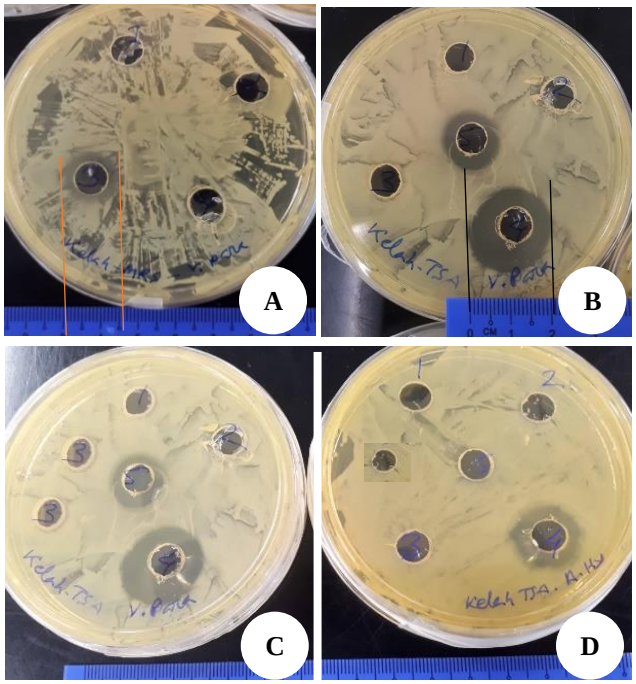

Figure 1. Antibacterial activity analysis of isolates against *Aeromonas hydrophila* and *Vibrio parahaemolyticus*; well diffusion method: A. Isolate KM01, KM06, KM07: against *A. hydrophila;* B. Against *V. parahaemolyticus;* C. For isolates KT03, KT17, KT22, KT23, KT25, KT27 against *A. hydrophila;* D. *V. parahaemolyticus*

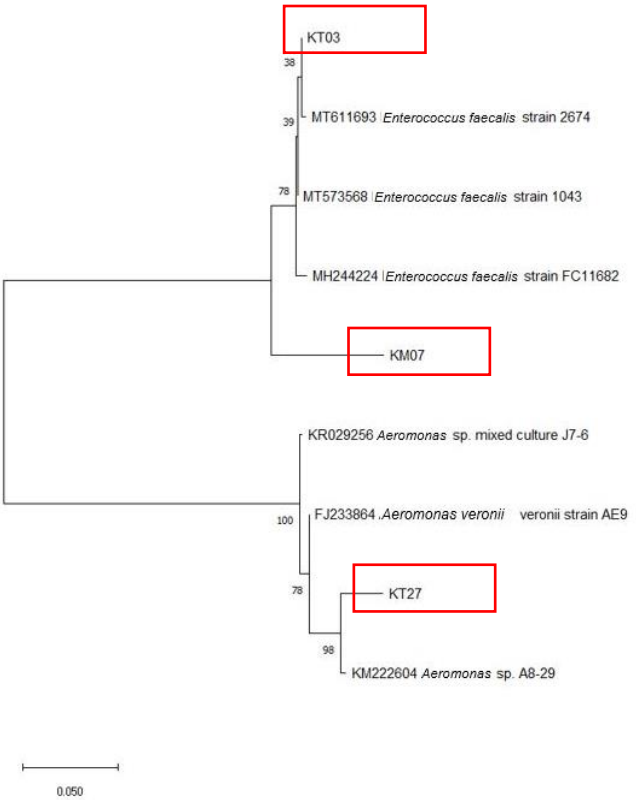

Figure 2. Phylogenetic relationship among the sequences of 16S rRNA gene of potential probiotics candidate (KT03, KM07 and KT27) derived from *Tor tambroides* digestive tract

A series of sequential screening processes for both biosafety and functional properties were required to find well-suited probiotics for use in aquaculture. The use of an in vitro screening process for selecting probiotic bacteria with the highest potential is essential as a means for reducing the number of potential strains. Thus, the screening methods play a vital role in reducing time and resources required for final selection of probiotics (Hai 2015). Digestive enzymatic activity and antibacterial activity are two important recommended methods for probiotic bacteria screening (Ray et al. 2010; Ray et al. 2012). Digestive enzymes enhance the efficiency of feed utilization and assimilation during the digestion process. Consequently, amino acids, fatty acids, and vitamins are produced, which improve general health condition of the host (Ray et al. 2012; Dawood et al. 2019; Ashraf et al. 2020; Patel et al. 2020). Digestive enzyme activity analysis was used to isolate and to prove the efficacy of probiotics (Kuebutornye et al. 2020). Similarly, antibacterial activity analysis was used in the probiotic screening process (Patel et al. 2020) for the selection of potential probiotics (Gomez-Gil et al. 2000; Messaoudi et al. 2013; Liu et al. 2014), which was considered for reduction, or removal of the abundance aquatic pathogens in the intestine of fish (Merrifield et al. 2010; Etyemez and Balcazar 2016; Kong et al. 2021). Probiotic bacteria candidates must be tested against pathogenic bacteria for the effectiveness of inhibitory function to demonstrate their abilities to protect host fish and to increase the survival rate. Furthermore, isolates with antibacterial properties can survive and colonize the digestive system of fish (Christensen 1989; Kavitha et al. 2018). Several studies reported the isolation of candidate probiotic bacteria based mainly on in vitro inhibitory activity against some common fish pathogens (Hai 2015; Nandi et al. 2017; Abomughaid 2020). Thus, this study was conducted in vitro on digestive enzyme activity and antibacterial activity analysis to isolate probiotics from Malaysian mahseer *T. tambroides.*

In the present study, all the selected isolates (KT03, KM07 and KT27) through nutritional and antibacterial activity were examined and their identity was determined; isolate KT03 was identified as *Enterococcus* sp. strain 1043 displaying the highest similarity (100%), isolate KM07 displayed 93% similarity with *Enterococcus* sp. strain FC11682, and isolate KT27 was identified as *Aeromonas* sp. strain A8-29 showing 97% match with the genus *Aeromonas*. It has been reported that *E. faecalis* is an intestinal bacterium capable of producing amylase, lipase, and protease (Kong et al. 2021), and displayed robust antagonistic activity against many pathogenic bacteria, such as *A. hydrophila, A. veronii, A. salmonicida,* and *A caviae* (Kong et al. 2020). Despite the fact that studies on using bacteria from this species as probiotics have been conducted, such as *Enterococcus hirae* (Adnan et al. 2017), and *E. durans* F3 was isolated from freshwater fish *Catla catla* (Alshammari et al. 2019). Protease and lipase activity significantly increased when the probiotic *E. faecalis* was applied to the culture of Javanese carp, *Puntius gonionotus* (Allameh et al. 2017) reported and *Channa argus* (Kong et al. 2021). In our present study, isolates KT03 and KM07 demonstrated in vitro digestive enzyme activity for all three digestive enzymes, such as cellulolytic, proteolytic, and lipolytic activity.

The antibacterial activity of *E. faecalis* against common fish pathogens such as *Aeromonas* spp. *Flavobacterium* spp., *Pseudomonas* spp. infection in fish has also been investigated in several studies (Pękala-Safińskz 2018; Zaheen et al 2022). Shahid et al. (2017) identified *E. faecalis* from Indian major carps, such as *Labeo rohita* and *Cirrhinus mrigala*, and investigated its antibacterial activity against some pathogenic bacteria, and found that they exhibited potential antibacterial activity against common fish pathogens, such as *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli.* Moreover, Touraki et al. (2012) observed the inhibitory activity of *E. faecalis* against pathogenic *Vibrio anguillarum* and *Photobacterium damselae*. Similar results were obtained in this work, where isolates KT03 and KM07 demonstrated in vitro inhibitory action against *V. parahaemolyticus.*

Aeromonas sp. strain A8-29 was identified in isolate KT27. It has been reported that the genus *Aeromonas* is a fish pathogen; nevertheless, it has also been stated that *Aeromonas* may be used as probiotic bacteria in both fin and shellfish aquaculture (Ringo 2020). In our study, *Aeromonas* sp. KT27 exhibited both nutritional enzyme activity and growth inhibition of pathogens (Table 1 and Table 2). Several studies revealed that the application of live *Aeromonas* spp. during culture decreased the mortality of rainbow trout *Oncorhynchus mykiss* against *Aeromonas salmonicida* infection (Irianto and Austin 2002), while formalin-inactivated cells improved disease resistance in goldfish, *Carassius auratus* (Irianto et al. 2003). In another study, *Aeromonas veronii* enhanced common carp, *Cyprinus carpio*, tolerance to *A. hydrophila* infection (Chi et al. 2014). The probiotic effects of live *A. hydrophila* on brine shrimp *Artemia* were also demonstrated, increasing total biomass output from nauplii through more effective digestive enzymes secretion (Gunasekara et al. 2010). The exact mechanism by which *Aeromonas* spp. contribute to pathogen defense is unknown, but they have been shown to aid in the production of cellulase, protease, and lipase, all of which are essential enzymes that help in digestion (Ray et al. 2012). In our study, the isolates KT03, KM07, and KT27 from the *T. tambroides* exhibited nutritional enzyme activity and suppressed the growth of two fish pathogens (*A. hydrophila* and *V. parahaemolyticus*).

The study identified three possible probiotic strains from *T. tambroides*, namely *E. faecalis* strains (KT03 and KM07) and *Aeromonas* sp. strain (KT27). These probiotics candidates produced digestive enzymes and displayed growth suppression of *A. hydrophila* and *V. parahaemolyticus*, suggesting the implementation their possible use in aquaculture, although further research is needed to investigate how these bacteria influence nutritional adsorption and immune status, which is the first step in developing strategies to promote the general health and well-being of fish during aquaculture.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Higher Education (MOHE) of Malaysia under the grant of the Fundamental Research Grant Scheme (FRGS) (Vot 59370).

REFERENCES

Abdelkhalek NKM, Eissa IAM, Ahmed E, Kilany OE, El-Adl M, Dawood MAO, Abdel-Daim MM. 2017. Protective role of dietary *Spirulina platensis* against diazinon-induced Oxidative damage in Nile tilapia; *Oreochromis niloticus*. Environ Toxicol Pharmacol 54: 99-104. DOI: 10.1016/j.etap.2017.07.002.

Abomughaid MM. 2020. Isolation and identification of some probiotic bacteria and their potential role in improving immune response and resistance of Nile Tilapia (*Oreochromis niloticus*) in comparison with a commercial product. Intl J Microbiol 2020: 1-9. DOI: 10.1155/2020/8865456.

Adnan M, Patel M, Hadi S. 2017. Functional and health promoting inherent attributes of *Enterococcus hirae* F2 as a novel probiotic isolated from the digestive tract of the freshwater fish *Catla catla*. PeerJ 5: e3085. DOI: 10.7717/peerj.3085.

Allameh SK, Ringø E, Yusoff FM, Daud HM, Ideris A. 2017. Dietary supplement of *Enterococcus faecalis* on digestive enzyme activities, short-chain fatty acid production, immune system response and disease resistance of Javanese carp (*Puntius gonionotus*, Bleeker 1850). Aquac Nutr 23 (2): 331-338. DOI: 10.1111/anu.12397.

Alshammari E, Patel M, Sachidanandan M, Kumar P, Adnan M. 2019. Potential evaluation and health fostering intrinsic traits of novel probiotic strain *Enterococcus dur*ans F3 isolated from the gut of fresh water fish *Catla catla*. Food Sci Anim Resour 19 (5): 844-861. DOI: 10.5851/kosfa.2019.e57.

Amin M, Adams M, Bolch CJS, Burke CM. 2017. In vitro screening of lactic acid bacteria isolated from gastrointestinal tract of Atlantic Salmon (*Salmo salar*) as probiont candidates. Aquac Intl 25 (1): 485-498. DOI: 10.1007/s10499-016-0045-6.

Asaduzzaman M, Iehata S, Akter S, Kader MA, Ghosh SK, Khan MNA, Abol-Munafi AB. 2018a. Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer *Tor tambroides*. Aquac Rep 9: 53-61. DOI: 10.1016/j.aqrep.2017.12.003.

Asaduzzaman M, Sofia E, Shakil A, Haque NF, Khan MNA, Ikeda D, Abol-Munafi AB. 2018b. Host gut-derived probiotic bacteria promote hypertrophic muscle progression and upregulate growth-related gene expression of slow-growing Malaysian Mahseer *Tor tambroides*. Aquac Rep 9: 37-45. DOI: 10.1016/j.aqrep.2017. 12.001.

Ashraf SA, Adnan M, Patel M, Siddiqui AJ, Sachidanandan M, Snoussi M, Hadi S. 2020. Fish-based bioactives as potent nutraceuticals: Exploring the therapeutic perspective of sustainable food from the sea. Mar Drugs 18: 65. DOI: 10.3390/md18050265.

Al-Asif A, Hossain A, Hamli H, Islam S, Kabir, SL. 2021. Research trends of aqua medicines, drugs and chemicals (AMDC) in Bangladesh: The last decade's (2011-2020) story to tell. Asian J Med Biol Res 7 (2): 90-106. DOI: 10.3329/ajmbr.v7i2.54987.

Askarian F, Kousha A, Salma W, Ringø E. 2011. The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry. Aquac Nutr 17: 488-97. DOI: 10.1111/j.1365-2095.2010.00826.x.

Azahar NZ, Iehata S, Fadhil F, Bulbul M, Kader MA. 2018. Antimicrobial activities of lactic acid bacteria isolated from Malaysian prawn, *Macrobrachium rosenbergii*. J Environ Biol 39 (5): 821-824. DOI: 10.22438/jeb/39/5(SI)/13.

Azfar IM, Kamarudin MS, Syukri F, Latif K. 2020. Larval development of a new hybrid Malaysian mahseer (*Barbonymus gonionotus* ♀ × *Tor tambroides* ♂). Aquac Rep 18: 100416. DOI: 10.1016/j.aqrep.2020.100416.

Azfar IM, Kamarudin MS, Syukri F, Nur AS, Latif K. 2019. Changes in the mouth morpho-histology of hybrid Malaysian mahseer (*Barbonymus gonionotus* ♀ × *Tor tambroides* ♂) during the larval development. Aquac Rep 15: 100-210. DOI: 10.1016/j.aqrep.2019.100210.

Buntin N, de Vos WM, Hongpattarakere T. 2017. Variation of mucin adhesion, cell surface characteristics, and molecular mechanisms among *Lactobacillus plantarum* isolated from different habitats. Appl Microbiol Biotechnol 101: 7663-7674. DOI: 10.1007/s00253-017-8482-3.

Chi C, Jiang B, Yu XB, Liu TQ, Xia L, Wang GX. 2014. Effects of three strains of intestinal autochthonous bacteria and their extracellular products on the immune response and disease resistance of common carp, *Cyprinus carpio*. Fish Shellfish Immunol 36 (1): 9-18. DOI: 10.1016/j.fsi.2013.10.003.

Christensen BE. 1989. The role of extracellular polysaccharides in biofilms. J Biotechnol 10: 181-202. DOI: 10.1016/0168-1656(89)90064-3.

Dawood MAO, Koshio S. 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. Aquaculture 10: 181-202. DOI: 10.1016/j.aquaculture.2015.12.033.

Dawood MAO, Koshio S, Abdel-Daim MM, Van DH. 2019. Probiotic application for sustainable aquaculture. Rev Aquac 454: 243-251. DOI: 10.1111/raq.12272.

Dawood MAO, Koshio S, El-Sabagh M, Billah MM, Zaineldin AI, Zayed MM, Omar AAE. 2017. Changes in the growth, humoral and mucosal immune responses following β-glucan and vitamin C administration in red sea bream, *Pagrus major*. Aquaculture 470: 214-222. DOI: 10.1016/j.aquaculture.2016.12.036.

Deris ZM, Iehata S, Ikhwanuddin M, Sahimi MBMK, Dinh DT, Sorgeloos P, Wong LL. 2020. Immune and bacterial toxin genes expression in different giant tiger prawn, *Penaeus monodon* post-larvae stages following AHPND-causing strain of *Vibrio parahaemolyticus* challenge. Aquac Rep 16: 100248. DOI: 10.1016/j.aqrep.2019.100248.

Etyemez M, Balcazar JL. 2016. Isolation and characterization of bacteria with antibacterial properties from Nile tilapia (*Oreochromis niloticus*). Res Vet Sci 105: 62-64. DOI: 10.1016/j.rvsc.2016.01.019.

Fečkaninová A, Koščová J, Mudroňová D, Popelka P, Toropilová J. 2017. The use of probiotic bacteria against *Aeromonas* infections in salmonid aquaculture. Aquaculture 469: 1-8. DOI: 10.1016/j.aquaculture.2016.11.042.

Garcés ME, Olivera NL, Fernández M, Riva RC, Sequeiros C. 2020. Antimicrobial activity of bacteriocin-producing *Carnobacterium* spp. isolated from healthy Patagonian trout and their potential for use in aquaculture. Aquac Res 51 (11): 4602-4612. DOI: 10.1111/are.14806.

Giraffa G, Chanishvili N, Widyastuti Y. 2010. Importance of lactobacilli in food and feed biotechnology. Res Microbiol 161 (6): 480-487. DOI: 10.1016/j.resmic.2010.03.001.

Gomez-Gil B, Roque A, Turnbull JF. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 91 (1-3): 259-270. DOI: 10.1016/S0044-8486(00)00431-2.

Gunasekara R, Rekecki A, Baruah K, Bossier P, Van den BW. 2010. Evaluation of probiotic effect of *Aeromonas hydrophila* on the development of the digestive tract of germ-free *Artemia franciscana* nauplii. J Exp Mar Biol Ecol 393: 78-82. DOI: 10.1016/j.jembe.2010.07.006.

Hai NV. 2015. The use of probiotics in aquaculture. J Appl Microbiol 119: 917-935. DOI: 10.1111/jam.12886.

Hossain MM, Kawai K, Oshima S. 2011. Immunogenicity of pressure inactivated *Edwardsiella tarda* bacterin to *Anguilla japonica* (Japanese eel). Pak J Biol 14 (15): 755-757. DOI: 10.3923/pjbs.2011.755.767.

Hossain MM, Kawai K, Oshima S. 2009. An inactivation method of *Edwardsiella tarda* vaccine for fish. J Appl Anim Res 14 (15): 755-767. DOI: 10.1080/09712119.2009.9707003.

Iehata S, Inagaki T, Okunishi S, Nakano M, Tanaka R, Maeda H. 2010. Improved gut environment of abalone *Haliotis gigantea* through *Pediococcus* sp. Ab1 treatment. Aquaculture 305 (1-4): 59-65. DOI: 10.1016/j.aquaculture.2010.04.012.

Ina-Salwany MY, Al-saari N, Mohamad A, Mursidi FA, Mohd-Aris A, Amal MNA, Zamri-Saad M. 2019. Vibriosis in fish: A review on disease development and prevention. J Aquat Anim Health 31 (1): 3-22. DOI: 10.1002/aah.10045.

Interaminense JA, Vogeley JL, Gouveia CK, Portela RWS, Oliveira JP, Andrade HA, Bezerra RS. 2018. In vitro and in vivo potential probiotic activity of *Bacillus subtilis* and *Shewanella* algae for use in *Litopenaeus vannamei* rearing. Aquaculture 488: 114-122. DOI: 10.1016/j.aquaculture.2018.01.027.

Irianto A, Austin B. 2002. Probiotics in aquaculture. J Fish Dis 25: 633-642. DOI: 10.1046/j.1365-2761.2002.00422.x.

Irianto A, Robertson PAW, Austin B. 2003. Oral administration of formalin-inactivated cells of *Aeromonas hydrophila* A3-51 controls infection by atypical *A. salmonicida* in goldfish, *Carassius auratus* (L.). J Fish Dis 26 (2): 117-120. DOI: 10.1046/j.1365-2761.2003.00439.x.

Kamarudin MS, Ramezani-Fard E, Saad CR, Harmin SA. 2012. Effects of dietary fish oil replacement by various vegetable oils on growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer, *Tor tambroides*. Aquac Nutr 8 (5): 532-543. DOI: 10.1111/j.1365-2095.2011.00907.x.

Kavitha M, Raja M, Perumal P. 2018. Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquac Rep 11: 59-69. DOI: 10.1016/j.aqrep.2018.07.001.

Kong Y, Li M, Chu G, Liu H, Shan X, Wang G, Han G. 2021. The positive effects of single or conjoint administration of lactic acid bacteria on *Channa argus*: Digestive enzyme activity, antioxidant capacity, intestinal microbiota and morphology. Aquaculture 531: 735-852. DOI: 10.1016/j.aquaculture.2020.735852.

Kong Y, Li M, Li R, Shan X, Wang G. 2020. Evaluation of cholesterol lowering property and antibacterial activity of two potential lactic acid bacteria isolated from the intestine of snakehead fish (*Channa argus*). Aquac Rep 17: 100342. DOI: 10.1016/j.aqrep.2020.100342.

Kozińska A, Figueras, MJ, Chacon MR, Soler L. 2002. Phenotypic characteristics and pathogenicity of *Aeromonas genomospecies* isolated from common carp (*Cyprinus carpio* L.). J Appl Microbiol 93: 1034-1041. DOI: 10.1046/j.1365-2672.2002.01784.x.

Kuebutornye FKA, Wang Z, Lu Y, Abarike ED, Sakyi ME, Li Y, Hlordzi V. 2020. Effects of three host-associated *Bacillus* species on mucosal immunity and gut health of Nile tilapia, *Oreochromis niloticus* and its resistance against *Aeromonas hydrophila* infection. Fish Shellfish Immunol 97: 83-95. DOI: 10.1016/j.fsi.2019.12.04695.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33 (7): 1870-1874. DOI: 10.1093/molbev/msw054.

Langdon A, Crook N, Dantas G. 2016. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome Med 8 (39): 1-16. DOI: 10.1186/s13073-016-0294-z.

Lazado CC, Caipang CMA, Estante EG. 2015. Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. Fish Shellfish Immunol 45: 2-12. DOI: 10.1016/j.fsi.2015.02.023.

Lee-kui S, Lihan S, Farvoin F, Dashagir G, Mikal M, Collick F, Kok NH. 2014. Microbiological and physicochemical analysis of water from empurau fish (*Tor Tambroides)* farm in Kuching, Sarawak, Malaysian Borneo. Intl J Sci Tech Res 3 (6): 285-292.

Lee LH, Mutalib NSA, Law JWF, Wong SH, Letchumanan V. 2018. Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in Selangor reveals carbapenemase producing *Vibrio parahaemolyticus* in marine and freshwater fish. Front Microbiol 9: 2513. DOI: 10.3389/fmicb.2018.02513.

Liu H, Li Z, Tan B, Lao Y, Duan Z, Sun W, Dong X. 2014. Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol 41 (2): 300-307. DOI: 10.1016/j.fsi.2014.08.028.

Martínez CP, Ibáñez AL, Monroy HOA, Ramírez SHC. 2012. Use of probiotics in aquaculture. ISRN Microbiol 2012: 91684. DOI: 10.5402/2012/916845.

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RTM, Bøgwald J, Ringø E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids Aquaculture 302 (1-2): 1-18. DOI: 10.1016/j.aquaculture.2010.02.007.

Messaoudi S, Manai M, Kergourlay G, Prévost H, Connil N, Chobert JM, Dousset X. 2013. *Lactobacillus salivarius*: Bacteriocin and probiotic activity. Food Microbiol 36: 296-304. DOI: 10.1016/ j.fm.2013. 05.010.

Mishra P, Samanta M, Maiti NK, Sarangi N. 2009. Characterization of extracellular cytotoxic protein of *Vibrio* spp. isolated from freshwater carps and prawns. Indian J Fish 56 (4): 307-311.

Mohammadi G, Hafezieh M, Karimi AA, Azra MN, van Doan H, Tapingkae W, Abdelrahman HA, Dawood MAO. 2022. The synergistic effects of plant polysaccharide and *Pediococcus acidilactici* as a synbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. Fish Shellfish Immunol 120: 304-313. DOI: 10.1016/j.fsi.2021.11.028.

Mohd-Nosi MZ, Syed JFSNE, Muhammad MDD, Iehata S. 2018. Assessment of gut microbiota in different developmental stages of Malaysian Mahseer (*Tor tambroides)*. Aquac Res 49 (9): 2977-2987. DOI: 10.1111/are.13757.

Mukherjee A, Dutta D, Banerjee S, Ringø E, Breines EM, Hareide E, Ghosh K. 2016. Potential probiotics from Indian major carp, *Cirrhinus mrigala*, characterization, pathogen inhibitory activity, partial characterization of bacteriocin and production of exoenzymes. Res Vet Sci 108: 76-84. DOI: 10.1016/j.rvsc.2016.08.011.

Nandi A, Dan SK, Banerjee G, Ghosh P, Ghosh K, Ringø E, Ray AK. 2017. Probiotic Potential of autochthonous bacteria isolated from the gastrointestinal tract of four freshwater Teleosts. Probiotics Antimicrob Proteins 9 (1): 12-21. DOI: 10.1007/s12602-016-9228-8.

Nath S, Matozzo V, Bhandari D, Faggio C. 2019. Growth and liver histology of *Channa punctatus* exposed to a common biofertilizer. Nat Prod Res 33 (11): 1591-1598. DOI: 10.1080/14786419.2018.1428586.

Ng WK, Abdullah N, De Silva SS. 2008. The dietary protein requirement of the Malaysian mahseer, *Tor tambroides* (Bleeker), and the lack of protein-sparing action by dietary lipid. Aquaculture 284: 201-206. DOI: 10.1016/j.aquaculture.2008.07.051.

Ng WK, Andin VC. 2011. The Malaysian mahseer, *Tor tambroides* (Bleeker), requires low dietary lipid levels with a preference for lipid sources with high omega-6 and low omega-3 polyunsaturated fatty acids. Aquaculture 322-323: 82-90. DOI: 10.1016/j.aquaculture.2011.09.021.

Novriadi R. 2016. Vibriosis in aquaculture. Omni-Akuatika 12 (1): 1-12. DOI: 10.20884/1.oa.2016.12.1.24.

Patel M, Ashraf MS, Siddiqui AJ, Ashraf SA, Sachidanandan M, Snoussi M, Adnan, M, Hadi S. 2020. Profiling and role of bioactive molecules from *Puntius sophore* (Freshwater/brackish fish) skin mucus with its potent antibacterial, antiadhesion, and antibiofilm activities. Biomolecules 10 (6): 9-20. DOI: 10.3390/biom10060920.

Pękala-Safińska A. 2018. Contemporary threats of bacterial infections in freshwater fish. J Vet Res 62 (3): 261-267. DOI: 10.2478/jvetres-2018-0037.

Pridgeon JW, Klesius PH. 2012. Major bacterial diseases in aquaculture and their vaccine development. CAB Rev 7: 1-16. DOI: 10.1079/PAVSNNR20127048.

Ray AK, Ghosh K, Ringø E. 2012. Enzyme-producing bacteria isolated from fish gut: A review. Aquac Nutr 18: 465-492. DOI: 10.1111/j.1365-2095.2012.00943.x.

Ray AK, Roy T, Mondal S, Ringø E. 2010. Identification of gut-associated amylase, cellulase and protease-producing bacteria in three species of Indian major carps. Aquac Res 41 (10): 1462-1469. DOI: 10.1111/j.1365-2109.2009.02437.x.

Ringø E. 2020. Probiotics in shellfish aquaculture. Aquac Fish 5 (1): 1-27. DOI: 10.1016/j.aaf.2019.12.001.

Romero J, Gloria C, Navarrete P. 2012. Antibiotics in aquaculture -use, abuse and alternatives: Health and environment in aquaculture. In: Carvalho ED, David GS, Silva RJ (eds). Health and Environment in Aquaculture. IntechOpen, United Kingdom.

Saikot FK, Zaman R, Khalequzza M. 2013. Pathogenecity test of *Aeromonas* isolated from motile *Aeromonas Septicemia* (MAS) infected Nile Tilapia on some freshwater fish. Sci Intl 1 (9): 325-329. DOI: 10.17311/sciintl.2013.325.329.

Selim KM, El-Sayed HM, El-Hady MA, Reda RM. 2019. In vitro evaluation of the probiotic candidates isolated from the gut of *Clarias gariepinus* with special reference to the in vivo assessment of live and heat-inactivated *Leuconostoc mesenteroides* and *Edwardsiella* sp. Aquac Intl 27: 35-51. DOI: 10.1007/s10499-018-0297-4.

Serrano PH. 2005. Responsible Use of Antibiotics in Aquaculture. FAO Fisheries Technical Paper, Rome.

Shahid M, Hussain B, Riaz D, Khurshid M, Ismail M, Tariq M. 2017. Identification and partial characterization of potential probiotic lactic acid bacteria in freshwater *Labeo rohita* and *Cirrhinus mrigala*. Aquac Res 48 (4): 1688-1698. DOI: 10.1111/are.13006.

Soto DM, Latimer MF, Dixon B. 2020. Enhancing immune function and fish health in aquaculture. Fish Physiol 38: 123-161. DOI: 10.1016/bs.fp.2020.09.003.

Tarkhani R, Imani A, Hoseinifar SH, Ashayerizadeh O, Moghanlou KS, Manaffar R, Reverter M. 2020. Comparative study of host-associated and commercial probiotic effects on serum and mucosal immune parameters, intestinal microbiota, digestive enzymes activity and

growth performance of roach (*Rutilus rutilus caspicus*) fingerlings. Fish Shellfish Immunol 98: 661-669. DOI: 10.1016/j.fsi.2019.10.063.

Tey YH, Jong KJ, Fen SY, Wong HC. 2015. Occurrence of *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Vibrio vulnificus* in the aquacultural environments of Taiwan. J Food Prot 78 (5): 969-976. DOI: 10.4315/0362-028X.JFP-14-405.

Touraki M, Frydas I, Karamanlidou G, Mamara A. 2012. Partial purification and characterization of a bacteriocin produced by *Bacillus subtilis* NCIMB 3610 that exhibits antimicrobial activity against fish pathogens. J Biol Res 18: 310-319.

van Doan H, Hoseinifar SH, Khanongnuch C, Kanpiengjai A, Unban K, van Kim V, Srichaiyo S. 2018. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 491: 94-110. DOI: 10.1016/j.aquaculture.2018.03.019.

van Doan H, Hoseinifar SH, Ringø E, Esteban MA, Dadar M, Dawood MAO, Faggio C. 2020. Host-associated probiotics: A Key factor in sustainable aquaculture. Rev Fish Sci Aquac 28 (1): 16-42. DOI: 10.1080/23308249.2019.1643288.

Van TTH, Yidana Z, Smooker PM, Coloe PJ. 2020. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. J Glob Antimicrob Resist 20: 170-177. DOI: 10.1016/j.jgar.2019.07.031.

Wright ES, Yilmaz LS, Noguera DR. 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl Environ Microbiol 78 (3): 717-725. DOI: 10.1128/AEM.06516-11.

Yan L, Pei X, Zhang X, Guan W, Chui H, Jia H, Yang D. 2019. Occurrence of four pathogenic Vibrios in Chinese freshwater fish farms in 2016. Food Control 95: 85-89. DOI: 10.1016/j.foodcont.2018.07.043.

Zaheen Z, War AF, Ali S, Yatoo AM, Ali MN, Ahmad SB, Rehman MU, Paray BA. 2022. Common bacterial infections affecting freshwater fish fauna and impact of pollution and water quality characteristics on bacterial pathogenicity. Bact Fis Dis 2022: 133-154. DOI: 10.1016/b978-0-323-85624-9.00006-3.

Zaineldin AI, Hegazi S, Koshio S, Ishikawa M, El Basuini MF, Dossou S, Dawood MAO. 2021. The influences of *Bacillus subtilis* C-3102 inclusion in the red sea bream diet containing high levels of soybean meal on growth performance, gut morphology, blood health, immune response, digestibility, digestive enzymes, and stress resistance. Aquac Nutr 27 (6): 2612-2628. DOI: 10.1111/anu.13389.

Zhou Z, Liu Y, Shi P, He S, Yao B, Ringø E. 2009. Molecular characterization of the autochthonous microbiota in the gastrointestinal tract of adult yellow grouper (*Epinephelus awoara*) cultured in cages. Aquaculture 286 (3-4): 184-189. DOI: 10.1016/j.aquaculture.2008.10.002.