Vol. 18, No. 3, Autumn, 2022, 74-86
DOI: 10.22055/IVJ.2022.353171.2482
Journal Homepage: http://www.ivj.ir/

Original Article

Isolation and identification of lactic acid bacteria and yeasts with probiotic ability from the intestine of gilthead seabream (*Sparus aurata*)

Mehdi Kamali Kolochani[1], Seyed Mohammad Mousavi[2], Isaac Zamani[3*], Vahid Yavari[2] and Mohammad Zakeri[2]

[1] PhD Graduated in fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
[2] Professor, Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
[3] Assistant Professor, Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

Received: 24.07.2022 Accepted: 12.09.2022

Abstract

Lactic acid bacteria are the most common bacteria used as probiotics in aquaculture. This study aimed to isolate and identify lactic acid bacteria and yeasts with probiotic potential from the intestine of gilthead seabream. Five fish were randomly selected (mean weight: 279.88±17.67 gr) from Nixa Design and Development Farm located in Charak port and 25 fish fries (mean weight: 39.43±9.67 gr) from Tiab Pran Qeshm farm in Qeshm Island. The selected fish had a healthy appearance and were also chosen randomly. Lactic acid bacteria and yeasts were isolated and purified from the intestines of the specimens and identified based on morphological characteristics and molecular sequences. Then these isolates were evaluated based on fundamental probiotic indicators, including acid resistance, bile salts, antagonistic properties, and Haemolytic activity for fish. 12 isolates were purified based on color, shape, and colony size. Then, two yeasts and five bacteria with different morphology were identified using gram staining and microscopic examinations. All lactobacillus isolates had antagonistic properties against the pathogenic bacterium *Vibrio harveyi*. Two strains of yeast; *Rhodotorula mucilaginosa* CBS 316 and *Wickerhamiella infanticola* CBS 7922, were isolated. The lactic acid bacterium isolated from the intestine of the gilthead seabream included two genera of *Enterococcus* and *Bacillus* respectively. The results of probiotic potency tests showed that isolates 1 (bacteria), 3, and 6 (yeast) had the best performance. From the obtained data, it was concluded that the use of a combination of bacteria and yeasts as probiotics in aquaculture has higher efficiency.

Keywords: Aquaculture, Probiotic, Bacteria, 16S rDNA, ITS

Introduction

Nowadays, the global population will rise to nearly 10 billion by 2050, with growing pressures on resources, such as energy and food. However, food sources and proteins are not unlimited, as hunger remains a crisis that propagates due to potential competition, conflict, and climate change. Aquaculture is a promising source of quality and healthy proteins for humankind (Duarte et al., 2009; Thilsted et al., 2016; FAO, 2016), in which strategies to encourage the development of sustainable

[*] **Corresponding Author**: Isaac Zamani, Assistant Professor, Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
E-mail: zamani@kmsu.ac.ir

fish farming industries have been advocated (Carbone and Faggio, 2016). Modern aquaculture has two main challenges: one is reducing water consumption for cultivation and the second is increasing production efficiency per unit. To meet market needs, farmers may increase stock densities, which can lead to stress for creatures (Van Doan et al., 2017; Hoseinifar et al., 2020). These stressful conditions lead to a weakening of the immune system of farmed aquatic species, which has been well documented by some authors (Vatsos et al. 2010; Roosta and Hoseinifar 2016; Hoseinifar et al., 2020). The growing risk of antimicrobial resistance in animal products warrants increased attention to organic aquaculture, which avoids the use of antibiotics and chemotherapeutic agents to control emerging or emerging pathogens. Given the difficulties of treating the disease in the so-called "green aquaculture" (without antibiotics), strengthening the innate immune system is of great importance (Ringø et al. 2016; Hoseinifar et al., 2020; Keyshams et al., 2021).

The spread of the disease is one of the major problems of aquaculture, which has hurt the economic growth of this industry. In the fish production industry, larval losses are the most important problem in dense breeding systems (Lauzon et al., 2008). Bacterial diseases are the leading cause of death in shrimp and fish farms (Gomez-Gil et al., 2000; Cao et al., 2015; Wang et al., 2018). Disease control in the aquaculture industry is done by traditional methods and the use of synthetic chemicals and antibiotics (Sahu et al., 2008; Izadpanah et al., 2022). Antibiotics are now widely used in aquaculture centers to fight bacterial diseases (Lauzon et al., 2008, Wang et al., 2018). In addition to high prices, the use of antibiotics has other problems, such as accumulation in waste, the incidence of drug resistance, stopping the immune response, environmental problems, etc., which eager aquatic production centers to use medications (Sahu et al., 2008). Therefore, microbial control activities using probiotics may have a positive effect on the performance of fish culture and breeding centers (Verschuere et al., 2000; Cao et al., 2015).

The most important microbial probiotics are lactic acid bacteria (Gatesoup, 2008) and yeast (Wang et al., 2018). Probiotics produce siderophores, bacteriocins, proteases, lysozymes, and hydrogen peroxides, and inhibit the growth of harmful pathogens. Such beneficial bacteria also produce many enzymes such as amylase by *Aeromonas* spp., *Bacillus subtilis*, *Bacteridaceae*, *Clostridium* spp., *Lactobacillus plantarum*, and *Staphylococcus* sp., and protease and cellulase by *B. subtilis*, *L. plantarum*, and *Staphylococcus* sp. In aquaculture, probiotics have several benefits and play an essential role in improving growth performance, disease resistance, safety, health status, intestinal epithelial barrier integrity, intestinal microbiome, and water quality. In addition, the practical application of probiotics in aquaculture diets can minimize the side effects of that biota (Khademzade et al., 2020; El-Saadony et al., 2021).

Given the importance of probiotics in the aquaculture industry that can significantly contribute to the health, survival, and increase of aquatic production, the aim of this study was to isolate and to identify lactic acid bacteria with probiotic potential from the gilthead seabream (*Sparus aurata*).

Material and Method

Sampling

To isolate lactic acid bacteria from the intestine of gilthead seabream (Sparus aurata), five fish (mean weight: 279.88±17.67 gr) of Nixa design and development farm, Charak port, Iran, and 25 fish fry (mean weight: 39.43±9.67 gr) of Tiab Pran Qeshm farm, Qeshm Island, Iran were accidentally caught with a healthy appearance. The fish was quickly

euthanized in MS222 solution (1 ppt), and their weight and length were measured and recorded. The abdominal area of the fish was dissected and their intestines were separated under sterile conditions. The contents of the intestine were discarded and rinsed inside with sterile saline. 1 g of homogenized intestinal tissue was added to 9 ml of sterile PBS to obtain a 1:10 suspension. Accordingly, dilutions were prepared based on ten samples. After determining the best dilution, the sample was cultured on MRS agar and the plates were incubated for 24 to 72 hours under anaerobic conditions., the colonies were isolated based on color, shape, and size and coded after purification. Gram staining was performed for initial identification. The purified reserves of these bacteria were stored on an MRS broth at a temperature of -80ºC using sterile glycerol (Vine et al., 2004).

Molecular identification

DNA was extracted using DNA extraction kit (Pishgaman Enteghal Gene company, Iran) to identify the molecule. For PCR, a standard 25 µl reaction mix was prepared to contain 12.5 µl of master mix (ID: 5200300-1250), 2 µl of template DNA, 2 µl of both primers (10 pm), and 8.5 µl of double-distilled water. the set used for 16S rRNA (5'-CCGAATTCGTCGACAACAGAGGTTGATCCTGGCTCAG-3'/5' -CCCGGGATCCAAGCTTACGGCTACCTTGTTACGGACTT-3') and Fragments of ITS1/ITS4 were amplified using the primer pair (5'-CTTGGTCATTTAGAGGAAGTAA-3'/ 5'-TCCTCCGCTTATTGATATGC-3') for rRNA ITS regions. The PCR products were obtained by the following PCR protocol: initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for the 60s, 55 °C for 60 s, and 72 °C for 90s, and a final extension of 10 min at 72 °C.

PCR products were sequenced. The sequences were corrected and assembled manually with Chromas version 2.6.5 (http://technelysium.com.au/).

The sequences were aligned using Bio Edit software (v. 7, Hall et al. 2011). Sequences were submitted to GenBank. We also used 11 sequences belonging to closely related species from the GenBank. The best evolutionary model was selected using JModeltest (V. 2.1.4, Posada and Crandall, 1998), under the Akaike Information Criterion (AIC) for 16S and ITS. Maximum likelihood (ML) analysis was conducted using RaxMlGUI (Stamatakis, 2006; Stamatakis et al., 2008). A rapid bootstrap (BS) analysis was performed with 1000 replications to search for the best-scoring ML tree.

Investigation of probiotic power

Tolerance to pH

To investigate the tolerance to different pH conditions, first, prepare Isolates equivalent to McFarland's No. 4 tube from each of the isolates and add 10 µl to 990 µl of PBS with different pH (1.5, 3, 6, 7.5, and 9) was added and incubated for 1.5 hours at 22 °C and then 10 µl of 10^{-3} dilution was cultured and counted with three replications in MRS agar (Grzeskowiak et al, 2011).

Bile Tolerance

To measure the tolerance of isolates to bile salts (Sigma), a suspension equivalent to McFarland's No. 4 tube was prepared from each isolate and ten microliters were added to 990 microliters of phosphate buffer containing concentrations (2000, 3000, and 4000 ppm) of extra Bile salt was added. It was then incubated for 1 hour at 22 °C. In the next step, ten microliters were cultured and counted in an MRS agar with three repetitions of 10^{-3}. Finally, the number of bacteria was compared with the control sample (without bile salts) (Mohamadian et al., 2014; Arihara et al., 1998).

Evaluation of antagonistic activity

To evaluate the antagonistic activity of isolates from the intestine gilthead seabream (*Sparus aurata*), the *Vibrio*

harveyi (PTCC 1755) bacterial suspension-cultured in the TSB medium (McFarland standard 0.5) was cultured with a sterile swab on the Mueller-Hinton Agar medium, then with the help of a sterile pasteurized pipette created wells on the medium and 0.1 ml of supernatants were poured into the wells after the 24-hour culturing of Isolates in the MRS broth medium, and the plates were incubated at 37 °C for 24 hours. Then a millimeter ruler measured the diameter of growth inhibition by Isolates against each pathogenic bacterium (Mohammaddoost et al., 2015).

Hemolytic activity

The hemolytic activity was determined by inoculating the culture on blood agar plates and hemolysis zones were observed (Igarashi et al., 1999).

Statistical analysis

Data were described as means ± standard deviation (SD) of three replicates. Statistical analysis of data was performed using SPSS 19.0. Data in tests were analyzed by the one-way analysis of variance (ANOVA) and Tukey test. Significant was accepted at $p< 0.05$.

Result

Intestinal samples were taken from 30 gilthead seabream fish and 12 isolates were purified based on color, shape, and colony size. Then, using gram staining and microscopic examinations, two yeasts and five bacteria with morphologically different were identified (Table 1). Therefore, these samples were selected for molecular identification (Figure 1 of the observed agarose gel corresponds to two markers, 16S and ITS.) and evaluation of probiotic potency.

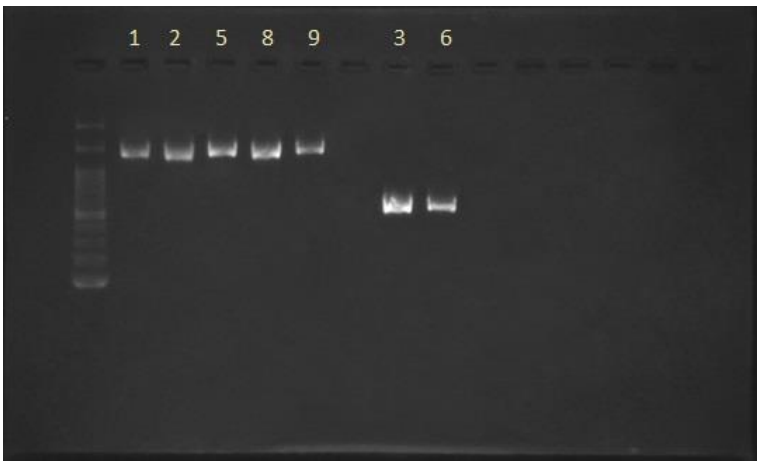

Figure 1. The observed agarose gel image related to 16S and ITS markers

Table 1: Bacterial and yeast strains isolated and identified from intestine of gilthead seabream

Isolated	Groups	Strain identification or close to it	GenBank accession no	
			ITS	16S
1	Bacteria	*Bacillus cereus*	-	Pending
2	Bacteria	*Enterococcus faecalis*	-	Pending
3	Yeast	*Rhodotorula mucilaginosa*	Pending	-
5	Bacteria	*Enterococcus faecalis*	-	Pending
6	Yeast	*Wickerhamiella infanticola*	Pending	-
8	Bacteria	*Bacillus cereus*	-	Pending
9	Bacteria	*Enterococcus faecalis*	-	Pending

Molecular account

Isolates 1 and 8 demonstrated 98% sequence similarities with two *Bacillus cereus* (WHX1, FORT 113.1), and also 99% similarities were recorded between (JCM5803, ATCC19433, and LMG7937)

and isolate five. On the other hand, 100 percentage similarity was observed between isolates 2 and 9 with *Enterococcus faecalis* JCM5803 RCB984. Also about yeasts, Ninety-eight percentage similarities were documented between isolates three and *Rhodotorula mucilaginosa* CBS316 and KC113310. Isolate 6 showed 98–99% sequence similarity with *Wickerhamiella infanticola* CBS316C, CBS11938. All sequences were submitted to the Gene bank. The phylogenetic tree constructed with the strains showing high similarity percentages is shown in Fig. 2 and 3.

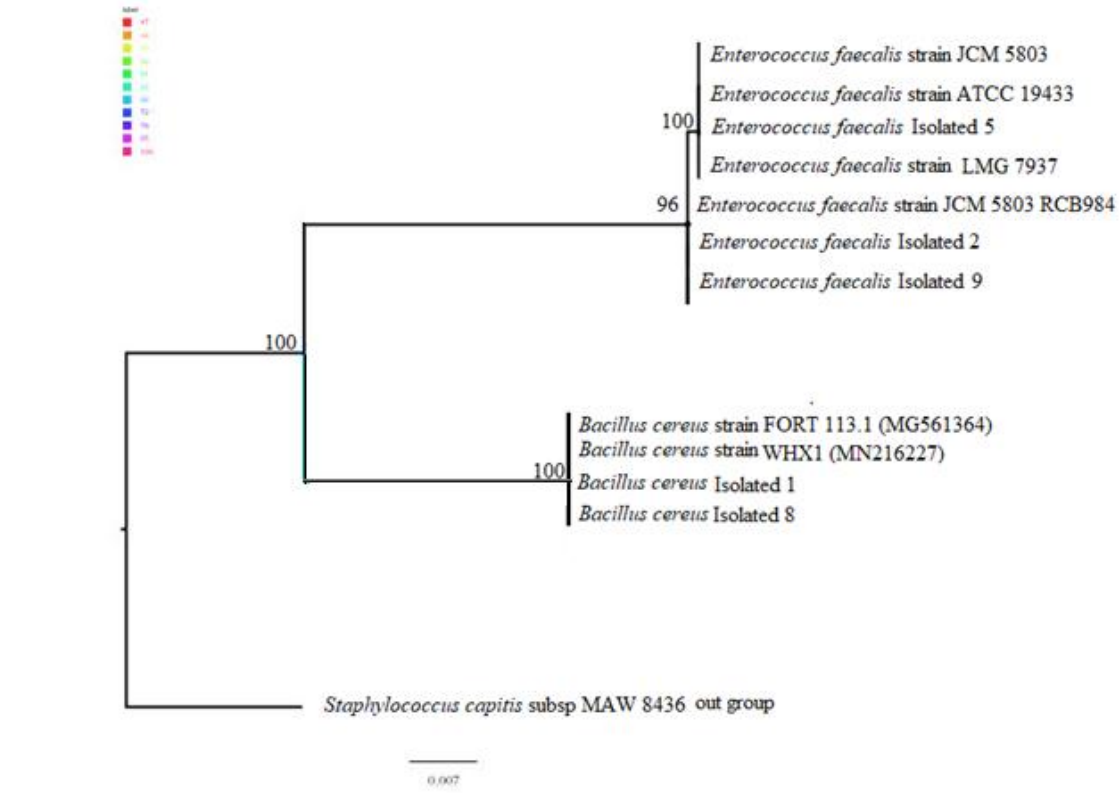

Figure 2. Phylogenetic tree inferred from maximum likelihood (ML) for Bacteria species based on 16S rRNA gene. Probability values at nodes represent support values maximum likelihood (ML).

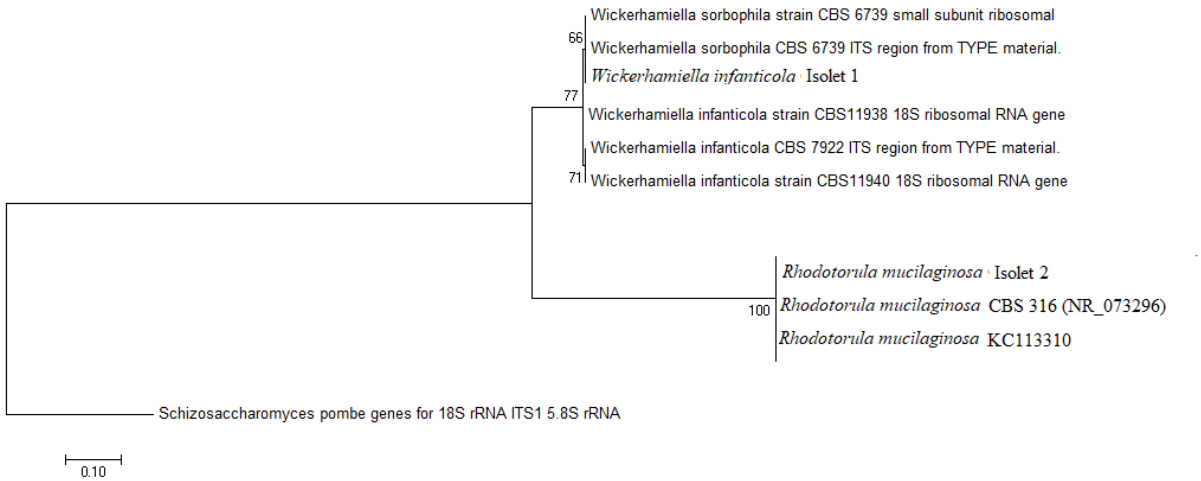

Figure 3. Phylogenetic tree inferred from maximum likelihood (ML) for yeast species based on ITS gene. Probability values at nodes represent support values maximum likelihood (ML).

Investigation of probiotic power

Tolerance pH

At pH 1.5, none of the bacterial strains showed growth, while the yeasts had good growth, But there is no significant difference between the growth of yeasts (P>0.05). Isolates 1 and 2 showed the best performance against different pHs and isolate 5 and 8 had the lowest tolerance to different acids so they had significant differences from other isolates at different pHs (P<0.05) (Table 2). The results of the pH tolerance test are shown in Table 2.

Table 2: Tolerance of lactic acid and yeast isolates (CUF/ml) at different pH concentrations for 1.5 hours (Means±STDV). (Heterogeneous letters indicate a significant difference.)

Isolate / pH	1.5	3	4.5	6	7.5	9
1 (CFU/ml)	0	87333 ± 18414^{Cb}	88333 ± 5607^{Db}	112666 ± 8006^{Dbc}	124000 ± 10115^{Cc}	89333 ± 7838^{Cb}
2 (CFU/ml)	0	66000 ± 4725^{BCb}	110000 ± 7094^{Ed}	118000 ± 6082^{Dde}	127666 ± 6565^{Ce}	86666 ± 3480^{Cc}
3 (CFU/ml)	25000 ± 5507^{Ba}	48000 ± 6244^{ABb}	66666 ± 2603^{Cc}	91333 ± 2027^{Cd}	97000 ± 2886^{Bd}	57333 ± 2027^{Bbc}
5 (CFU/ml)	0	22666 ± 3282^{Ab}	37000 ± 3785^{Ac}	71666 ± 1763^{Bd}	66666 ± 7218^{Ad}	59333 ± 5238^{Bd}
6 (CFU/ml)	32000 ± 2645^{Ba}	37333 ± 3282^{Aa}	53333 ± 2728^{BCb}	70666 ± 4910^{Bc}	74000 ± 3605^{ABc}	53666 ± 2185^{Bb}
8 (CFU/ml)	0	26000 ± 4582^{Ab}	41000 ± 3605^{ABc}	54666 ± 2333^{Ad}	56000 ± 3785^{Ad}	38333 ± 1763^{Ac}
9 (CFU/ml)	0	22666 ± 2728^{Ab}	38666 ± 3480^{Ab}	62333 ± 5238^{ABc}	97000 ± 14011^{Bd}	65666 ± 3711^{Bc}

Bile Tolerance

In the bile test, survival in isolates 3 and 6 was higher than in other isolates and had a significant difference compared to the control group (PBS) (P<0.05). Among the different concentrations of bile, a significant difference was observed between some strains (P<0.05) and there was no significant difference between others (P>0.05) (Table 3). The results of the bile tolerance test are shown in Table 3.

Table 3: Tolerance of lactic acid bacteria and yeast isolates (CUF/ml) at different bile concentrations (Means±STDV). (Heterogeneous letters indicate a significant difference.)

Isolate / Bile	2000 ppm	3000 ppm	4000 ppm	PBS
1 (CFU/ml)	70333 ± 1763^{Ba}	57666 ± 2333^{Cab}	46333 ± 1763^{Ca}	124000 ± 10115^{Cc}
2 (CFU/ml)	93333 ± 3179^{Cb}	82333 ± 2027^{Dab}	70333 ± 2333^{Da}	127666 ± 6565^{Cc}
3 (CFU/ml)	133000 ± 3464^{Da}	92000 ± 5567^{Dab}	81666 ± 4333^{Ea}	97000 ± 2886^{Bb}
5 (CFU/ml)	36333 ± 3282^{Ab}	18666 ± 2027^{Aa}	13000 ± 3214^{Aa}	66666 ± 7218^{Ac}
6 (CFU/ml)	125333 ± 3480^{Dc}	90666 ± 4666^{Db}	75000 ± 4358^{DEa}	74000 ± 3605^{Aba}
8 (CFU/ml)	72666 ± 4484^{Ba}	36666 ± 3179^{Bb}	18000 ± 3214^{Aa}	56000 ± 3785^{Ac}
9 (CFU/ml)	68000 ± 4618^{Bb}	43666 ± 3527^{Bab}	31000 ± 3464^{Ba}	97000 ± 14011^{Bc}

Evaluation of antagonistic activity

All lactobacillus isolates had antagonistic properties against the pathogenic bacterium *Vibrio harveyi*, while yeasts lacked this ability. The highest and lowest antagonistic activities were observed for isolates 8 and 1, respectively (Table 4). Also, there was a significant difference between some isolates and other isolates (P<0.05). The results of the antagonistic activity are shown in Table 4.

Table 4: Evaluation of antagonistic activity (Means±STDV). (Heterogeneous letters indicate a significant difference.).

Isolate	1	2	3	5	6	8	9
Antagonistic activity (mm)	1 ± 0.03^{a}	5 ± 1.5^{b}	-	5 ± 1.75^{b}	-	10 ± 3.6^{c}	3 ± 0.6^{d}

Hemolytic activity

The results of Haemolytic activity showed that except for isolate 9, which seems to have pathogenic properties, other isolates do not cause any specific tissue damage, And they lack the ability to cause disease (Table 5). There was also a significant difference between hemolysis diameter in isolate 8 and other isolates ($P<0.05$). The results of the Haemolytic activity are shown in table 5.

Table 5: Haemolytic activity (Means±STDV). (Heterogeneous letters indicate a significant difference.).

Isolate / Hemolysis	Alpha	Beta	Gamma	Hemolysis diameter (mm)
1			*	-
2	*			1 ± 0.2^{a}
3			*	-
5	*			1 ± 0.08^{a}
6			*	-
8	*			5 ± 0.23^{b}
9		*		1 ± 0.4^{a}

Discussion

Nowadays, the use of probiotics in aquaculture is significantly increasing and a growing number of studies show their positive effects on the most economically important fish species (Varela et al., 2010; Mahdhi et al., 2012; Chauhan and Singh, 2019; Moroni et al., 2021). By changing the contents of the food, the intestinal microbial flora of the fish can be changed. Probiotics are microbes when used as food additives, affect the health and growth of the host (Vijavabaskar and Somasundaram, 2008; Mohammadian et al., 2014). The probiotic must be compatible with the target species because it will better compete with the native gut microbes and settle in the new host. The antimicrobial effect of probiotics is related to the production of antibiotics, bacteriocin, siderophore, lysozyme, protease, and pH change with the production of organic acids (Bucia et al., 2006). In recent years, to increase the efficiency of aquatic production, researchers have sought to introduce better probiotics (Mohammadian et al., 2014). Fish intestines are composed of a variety of unknown microorganisms and dynamic ecosystems that play an essential role in digestion, nutrient uptake, and survival in addition to the general health of host aquatic animals (Nayak, 2010). Accordingly, the host fish's innate growth, development, and immunity most likely depend on these intestinal microbes. To take advantage of the beneficial aspects of these probiotic microbes, it is essential to isolate and identify them. Many commercial probiotics currently on the market for use in aquaculture are often relatively ineffective because many of them are separated from non-fish sources (Ghosh et al., 2007). Prescribing sufficient probiotics improves host health by controlling and stimulating the fish's immune system (Verschuere et al., 2000). Therefore, the use of probiotics instead of antibiotic therapy is an excellent approach to controlling the disease and improving the health and safety of the host.

In this study, five bacterial strains and two yeast strains were isolated from the gilthead seabream intestine. They were identified based on morphological

characteristics and molecular sequences as shown in Table 1. Bacteria that produce lactic acid include various genera such as *Lactobacillus*, *Enterococcus*, *Bacillus*, *Pediococcus*, *Aerococcus*, *Staphylococcus*, etc (Axelsson 1998; Wang et al., 2019). In the present study, the lactic acid bacterium isolated from the intestine of the gilthead seabream included two genera of *Enterococcus* and *Bacillus*, of which three isolates were identified from *Enterococcus* and two isolates for *Bacillus*. *Enterococcus* spp. was isolated from the intestine of *Pagellus bogarareo* (Sarra et al. 2013), gilthead seabream (*S. aurata*) (Makridis et al., 2005; Chabrillon et al., 2005; Suzer et a., 2008; Bourouni et al., 2012; Román et al., 2015; Moroni et al., 2021), sea bass (Bourouni et al., 2012) and crucian carp (*Carassius auratus*) (Mao et al., 2020). Kavitha et al. (2018) evaluated the probiotic potential of *Bacillus* spp. isolated from the gastrointestinal tract of freshwater fish *Labeo calbasu* (Hamilton, 1822) and stated that the selective isolate of *Bacillus amyloliquefaciens* FC6 could be a promising probiotic to control *A.hydrophila* in *L. calbasu*. In addition, in vivo evaluation studies are needed to determine its applications in aquaculture, and evidence supports the use of probiotics as a helpful approach to increasing resistance to infections. Also, these bacteria were isolated from the intestine of Japanese coastal fish (Sugita et al. 1998), Indian Major Carp *(Labeo rohita*) (Giri et al. 2011; Thankappan et al., 2015; Ramesh et al., 2015), Indian major carp (*Catla catla*) (Mukherjee and Ghosh, 2016), catfish (Meidong et al., 2018).

In this study, two isolates 3 and 6 were identified by morphological and molecular studies, which are two types of yeast *Rhodotorula mucilaginosa* CBS 316 and *Wickerhamiella infanticola* CBS 7922, respectively. Yeasts have been identified as an important part of the natural microbiota of wild and farmed fish, and their role in health and nutrition has been studied in the literature because yeast is used live to feed live or post-processed food organisms (Navarrete and Tovar-Ramírez, 2014). Even when it accounts for less than 1% of all microbial isolates in the host, yeast can show a significant physiological contribution beyond that observed for probiotic bacteria. The volume of yeast cells maybe 100 times larger than the volume of cells (Gatesoupe, 2007). Yeast cells use a range of simpler and more complex organic compounds. This phenomenon is due to the extensive metabolic potential of yeast, which is reflected in the production of various enzymes. Yeasts secreted by yeasts are also involved in the maturation of fish larvae. In addition, some yeast species and their components, such as β-glucans and mannoproteins, can stimulate the host's immune and antioxidant systems. Understanding the involvement of yeast microbiota in fish health and nutrition may improve health conditions and fish production performance (Bagni et al., 2005; Selvaraj et al., 2006; Kim et a., 2013; Navarrete and Tovar-Ramírez, 2014).

Tests pH, bile, antagonistic activity, and hemolytic activity were evaluated to determine whether the isolates from the gilthead seabream intestine were probiotic or not and probiotic potency. The tolerant ability against acid and bile is important for a probiotic strain to survive and colonize the fish gastrointestinal tract (Pérez-Sánchez et al., 2011; Sica et al., 2012). Regardless, there is still no agreement on the exact concentrations that the selected strains should tolerate (Nikoskelainen et al., 2001). According to a previous report on the physiological concentration of bile in the intestines of fish (Buntin et al., 2008), bile salt concentrations of 2000, 3000, and 4000 ppm were selected to evaluate the tolerance of bile to isolated strains in this study. When the fish's stomach is full of food, chyme pH values can reach 3.0-4.0 (Sugiura et al., 2006; Lavelle and Harris 1997). Here, low pH values from 1.5 to 9.0 were selected to

evaluate the ability to tolerate strong acid. None of the bacterial strains grew at 1.5 acidities, while the yeast strains did grow well at this pH. By increasing the pH of the isolated strains in both bacterial and yeast groups, they showed an increasing growth pattern and showed the highest efficiency in the neutral pH range, and their growth rate decreased by entering the alkaline range. Many previous studies confirm the results obtained in this study (Schleifer and Kilpper-Balz 1984; Tallapragada et al., 2018; Mao et al., 2020). Yeast strains showed the best performance against low pH, and among bacterial strains, isolates 1 and 2 had the best resistance against low pH. Isolates 3 and 6 showed the best performance against different concentrations of bile. Also, with increasing bile concentration, the performance of all isolated strains decreased. Many researchers have reported decreased performance of strains with probiotic potential due to increased bile concentrations (Zhang et al., 2013; Ramesh et al., 2015; Tallapragada et al., 2018).

Examining of pathogenicity of a candidate probiotic strain is one of the essential criteria before its application (Mao et al., 2020). The hemolysis test can be used to filter strains safely, quickly, and efficiently (Schulze et al., 2006; Mao et al., 2020), while the challenging test with tested bacterial isolates will provide a more accurate assessment of its pathogenicity. In this study, all lactobacillus isolates had antagonistic properties against the pathogenic bacterium *Vibrio harveyi*, while yeasts lacked this ability. The best antagonistic activities were observed in isolate 8 (*Bacillus cereus* strain IAM 12605). Previous studies have shown that *Bacillus* spp. exhibited the antagonism due to its sticking to the intestinal sites, subsequently interfering with the colonization and growth of pathogenic microorganisms (Thankappan et al., 2015; Ramesh et al., 2015; Wanka et al., 2018; Wang et al., 2019). Also, negative results in the hemolysis test and no death in the challenge test were observed only in strains 1, 3, and 6, indicating that these strains had no pathogenicity for gilthead seabream fish.

In general, it can be concluded from the obtained data that both isolates (bacteria and yeast) passed the probiotic potency tests well. However, the yeasts lacked antagonistic ability, in which case they also multiply rapidly and can inhibit the growth in the fish gut by limiting the space for pathogens. Among the bacterial isolates, isolate number 1 showed the best performance against probiotic tests. It seems that using a combination of yeasts and bacteria as probiotics in aquaculture ecosystems is very effective for specific purposes.

Acknowledgments

The authors wish to express their appreciation to the research council of the Khorramshahr University of Marine Science and Technology for their financial support of this research project.

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

This research project has been funded by the Khorramshahr University of Marine Science and Technology under grant no.: 1397

Reference

Arihara, K., Ota, H., Itoh, M., Kondo, Y., Sameshima, T., Yamanaka, H., Akimoto, M., Kanai, S., and Miki, T. (1998). Lactobacillus acidophilus group lactic acid bacteria applied to meat fermentation. Journal of Food Science 63, 544-547.

Axelsson, L. (1998). Lactic acid bacteria: Classification and physiology. In, S. Salminen and A. Von Wright (eds). Lactic Acid Bacteria: Microbiology and Functional Aspects. Inc, New York.

Bagni, M., Romano, N., Finoia, M., Abelli, L., Scapigliati, G., Tiscar, P. G., Sarti, M., and Marino, G. (2005). Short-and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (Dicentrarchus labrax). *Fish & Shellfish Immunology* 18, 311-325.

Bakke, I., Coward, E., Andersen, T., and Vadstein, O. (2015). Selection in the host structures the microbiota associated with developing cod larvae (G adus morhua). *Environmental microbiology* 17, 3914-3924.

Bourouni, O. C., El Bour, M., Calo-Mata, P., and Barros-Velàzquez, J. (2012). "Antimicrobial resistance and potential probiotic application of Enterococcus spp. in sea bass and sea bream aquaculture," Citeseer.

Bucio, A., Hartemink, R., Schrama, J. W., Verreth, J., and Rombouts, F. M. (2006). Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. Food microbiology 23, 476-482.

Buntin, N., Chanthachum, S., and Hongpattarakere, T. (2008). Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin Journal of Science & Technology 30.

Cao, L., Wang, L., Yang, L., Tao, S., Xia, R., and Fan, W. (2015). Long-term effect of early-life supplementation with probiotics on preventing atopic dermatitis: A meta-analysis. *Journal of Dermatological Treatment* 26, 537-540.

Carbone, D., and Faggio, C. (2016). Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of Sparus aurata and Dicentrarchus labrax. Fish & Shellfish Immunology 54, 172-178.

Chabrillón, M., Rico, R., Arijo, S., Diaz-Rosales, P., Balebona, M., and Moriñigo, M. (2005). Interactions of microorganisms isolated from gilthead sea bream, Sparus aurata L., on Vibrio harveyi, a pathogen of farmed Senegalese sole, Solea senegalensis (Kaup). *Journal of fish diseases* 28, 531-537.

Chauhan, A., and Singh, R. (2019). Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis* 77, 99-113.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., and Karakassis, I. (2009). Will the oceans help feed humanity? BioScience 59, 967-976.

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., Dhama, K., and Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish & Shellfish Immunology* 117, 36-52.

FAO Food and Agricultural Organization, (2016). The State of World Fisheries and Aquaculture 2016. FAO, Rome.

Forberg, T., Sjulstad, E. B., Bakke, I., Olsen, Y., Hagiwara, A., Sakakura, Y., and Vadstein, O. (2016). Correlation between microbiota and growth in Mangrove Killifish (Kryptolebias marmoratus) and Atlantic cod (Gadus morhua). *Scientific reports* 6, 1-9.

Gatesoupe, F. (2007). Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture* 267, 20-30.

Gatesoupe, F. J. (1999). The use of probiotics in aquaculture. *Aquaculture* 180, 147-165.

Gatesoupe, F.-J. (2008). Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *Journal of molecular microbiology and biotechnology* 14, 107-114.

Ghosh, S., Sinha, A., and Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research 38, 518-526.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., and Verdegem, M. (2015). The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Scientific reports* 5, 1-15.

Gomez-Gil, B., Roque, A., and Turnbull, J. F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* 191, 259-270.

Grześkowiak, Ł., Isolauri, E., Salminen, S., and Gueimonde, M. (2011). Manufacturing process influences properties of probiotic bacteria. *British Journal of Nutrition* 105, 887-894.

Hall, T., Biosciences, I., & Carlsbad, C. (2011). BioEdit: an important software for molecular biology. GERF Bull Biosci, 2(1), 60-61.

Hoseinifar, S. H., Sun, Y.-Z., Zhou, Z., Van Doan, H., Davies, S. J., and Harikrishnan, R. (2020). Boosting immune function and disease bio-control through environment-friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. *Reviews in Fisheries Science & Aquaculture* 28, 303-321.

Igarashi, T., Aritake, S., and Yasumoto, T. (1999). Mechanisms underlying the hemolytic and ichthyotoxic activities of maitotoxin. *Natural toxins* 7, 71-79.

Izadpanah, E., Saffari, S., Torfi Mozanzadeh, M., Mousavi, S.M., Pasha-Zanoosi, H. 2022. Nano-selenium supplementation in plant protein-based diets changed thyroid hormones status and hepatic enzymes activity in *Acanthopagrus arabicus* female broodfish and their offspring. Aquaculture Report, 24, https://doi.org/10.1016/j.aqrep.2022.101134

Kavitha, M., Raja, M., and Perumal, P. (2018). Evaluation of probiotic potential of Bacillus spp. isolated from the digestive tract of freshwater fish Labeo calbasu (Hamilton, 1822). *Aquaculture Reports* 11, 59-69.

Keyshams, P., Mousavi, S.M., Zakeri, M., Kochanian, P., Souri, M. 2021. Effects of different levels of commercial food supplement, Biotronic® Top3 on biochemical and immune parameters of *Litopenaeus vannamei*, Iranian Veterinary Journal, 17(1): 33-45. doi: 10.22055/ivj.2021.263466.2334

Khademzade, O., Zakeri, M., Haghi, M., Mousavi, S.M. 2020. The effects of water additive *Bacillus cereus* and *Pediococcus acidilactici* on water quality, growth performances, economic benefits, immunohematology, and bacterial flora of white leg shrimp (*Penaeus vannamei* Boone, 1931) reared in earthen ponds. Aquaculture Research, 51: 1759-1770. doi: https://doi.org/10.1111/are.14525

Kim, J. Y., Hahn, H. J., Choe, Y. B., Lee, Y. W., Ahn, K. J., and Moon, K. C. (2013). Molecular biological identification of Malassezia yeasts using pyrosequencing. *Annals of dermatology* 25, 73-79.

Koščová, J., Nemcová, R., Gancarčíková, S., Jonecová, Z., Bomba, A., and Buleca, V. (2006). Effect of two plant extracts and Lactobacillus fermentum on colonization of gastrointestinal tract by Salmonella enterica var. Düsseldorf in chicks. *Biologia* 61, 775-778.

Lauzon, H., Gudmundsdottir, S., Pedersen, M., Budde, B., and Gudmundsdottir, B. (2008). Isolation of putative probionts from cod rearing environment. *Veterinary microbiology* 132, 328-339.

Lavelle, E., and Harris, J. (1997). The processing of an orally administered protein antigen in the digestive tract of rainbow trout, Oncorhynchus mykiss. Comparative Biochemistry and Physiology Part A: Physiology 117, 263-275.

Mahdhi, A., Kamoun, F., Messina, C., and Bakhrouf, A. (2012). Probiotic properties of Brevibacillus brevis and its influence on sea bass (Dicentrarchus labrax) larval rearing. *African Journal of Microbiology Research* 6, 6487-6495.

Makridis, P., Martins, S., Vercauteren, T., Van Driessche, K., Decamp, O., and Dinis, M. T. (2005). Evaluation of candidate probiotic strains for gilthead sea bream larvae (Sparus aurata) using an in vivo approach. *Letters in applied microbiology* 40, 274-277.

Mao, Q., Sun, X., Sun, J., Zhang, F., Lv, A., Hu, X., and Guo, Y. (2020). A candidate probiotic strain of Enterococcus faecium from the intestine of the crucian carp Carassius auratus. *AMB Express* 10, 1-9.

Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K., and Tongpim, S. (2018). Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance, and innate immunity of Pla-mong Pangasius bocourti. *Fish & shellfish immunology* 73, 1-10.

Merrifield, D. L., and Ringo, E. (2014). "Aquaculture nutrition: gut health, probiotics, and prebiotics," John Wiley & Sons.

Mohammadian, T., Tabandeh, M., and Gharibi, D. (2014). Isolation and biochemical identification of potentially probiotic bacteria from Barbus grypus intestine. *Iranian Veterinary Journal* 10, 88-97.

Moroni, F., Naya-Català, F., Piazzon, M. C., Rimoldi, S., Calduch-Giner, J., Giardini, A., Martínez, I., Brambilla, F., Pérez-Sánchez, J., and Terova, G. (2021). The Effects of Nisin-Producing Lactococcus lactis Strain Used as Probiotic on Gilthead Sea Bream (Sparus aurata) Growth, Gut Microbiota, and Transcriptional Response. *Frontiers in Marine Science* 8, 364.

Mukherjee, A., and Ghosh, K. (2016). Antagonism against fish pathogens by cellular components and verification of probiotic properties in autochthonous bacteria isolated from the gut of an I ndian major carp, C atla catla (H amilton). *Aquaculture Research* 47, 2243-2255.

Navarrete, P., and Tovar-Ramírez, D. (2014). Use of yeasts as probiotics in fish aquaculture. *Sustainable aquaculture techniques* 1, 135-72.

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. Aquaculture Research 41, 1553-1573.

Nikoskelainen, S., Salminen, S., Bylund, G., and Ouwehand, A. C. (2001). Characterization of the properties of human-and dairy-derived probiotics for prevention of infectious diseases in fish. Applied and Environmental Microbiology 67, 2430-2435.

Olafsen, J. A. (2001). Interactions between fish larvae and bacteria in marine aquaculture. *Aquaculture* 200, 223-247.

Pérez-Sánchez, T., Balcázar, J., García, Y., Halaihel, N., Vendrell, D., De Blas, I., Merrifield, D., and Ruiz-Zarzuela, I. (2011). Identification and characterization of lactic acid bacteria isolated from rainbow trout, Oncorhynchus mykiss (Walbaum), with inhibitory activity against Lactococcus garvieae. Journal of fish diseases 34, 499-507.

Ramesh, D., Vinothkanna, A., Rai, A. K., and Vignesh, V. S. (2015). Isolation of potential probiotic Bacillus spp. and assessment of their subcellular components to induce immune responses in Labeo rohita against Aeromonas hydrophila. *Fish & shellfish immunology* 45, 268-276.

Ringø, E., Zhou, Z., Vecino, J. G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R., Dimitroglou, A., Foey, A., and Davies, S. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquaculture Nutrition* 22, 219-282.

Román, L., Padilla, D., Acosta, F., Sorroza, L., Fátima, E., Déniz, S., Grasso, V., Bravo, J., and Real, F. (2015). The effect of probiotic Enterococcus gallinarum L-1 on the innate immune parameters of outstanding species to marine aquaculture. *Journal of Applied Animal Research* 43, 177-183.

Roosta, Z., and Hoseinifar, S. H. (2016). The effects of crowding stress on some epidermal mucus immune parameters, growth performance, and survival rate of tiger barb (Pentius tetrazona). *Aquaculture Research* 47, 1682-1686.

Sahu, M. K., Swarnakumar, N., Sivakumar, K., Thangaradjou, T., and Kannan, L. (2008). Probiotics in aquaculture: importance and future perspectives. *Indian journal of microbiology* 48, 299-308.

Sarra, M., Taoufik, G., Benjamin, B., Yannick, F., and Khaled, H. (2013). Isolation and characterization of enterococci bacteriocinic strains from Tunisian fish viscera.

Schleifer, K. H., and Kilpper-Bälz, R. (1984). Transfer of Streptococcus faecalis and Streptococcus faecium to the genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. *International Journal of Systematic and Evolutionary Microbiology* 34, 31-34.

Schulze, A. D., Alabi, A. O., Tattersall-Sheldrake, A. R., and Miller, K. M. (2006). Bacterial diversity in a marine hatchery: balance between pathogenic and potentially probiotic bacterial strains. *Aquaculture* 256, 50-73.

Selvaraj, V., Sampath, K., and Sekar, V. (2006). Adjuvant and immunostimulatory effects of β-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with Aeromonas hydrophila. *Veterinary immunology and immunopathology* 114, 15-24.

Sica, M. G., Brugnoni, L. I., Marucci, P. L., and Cubitto, M. A. (2012). Characterization of probiotic properties of lactic acid bacteria isolated from an estuarine environment for application in rainbow trout (Oncorhynchus mykiss, Walbaum) farming. Antonie Van Leeuwenhoek 101, 869-879.

Stephens, W. Z., Burns, A. R., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., and Bohannan, B. J. (2016). The composition of the zebrafish intestinal microbial community varies across development. *The ISME journal* 10, 644-654.

Sugita, H., Hirose, Y., Matsuo, N., and Deguchi, Y. (1998). Production of the antibacterial substance by Bacillus sp. strain NM 12, an intestinal bacterium of Japanese coastal fish. Aquaculture 165, 269-280.

Sugiura, S. H., Roy, P. K., and Ferraris, R. P. (2006). Dietary acidification enhances phosphorus digestibility but decreases H+/K+-ATPase expression in rainbow trout. Journal of Experimental Biology 209, 3719-3728.

Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., and Küçüksari, H. (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture* 280, 140-145.

Tallapragada P, Rayavarapu B, Rao PP, Ranganath N, Veerabhadrappa P (2018) Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. J Genetic Eng Biotechnol 16:357–362

Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., and Anbarasu, K. (2015). Characterization of Bacillus spp. from the gastrointestinal tract of Labeo rohita—towards to identify novel probiotics against fish pathogens. *Applied biochemistry and biotechnology* 175, 340-353.

Thilsted, S. H., Thorne-Lyman, A., Webb, P., Bogard, J. R., Subasinghe, R., Phillips, M. J., and Allison, E. H. (2016). Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. Food Policy 61, 126-131.

Trinh, L. T., Bakke, I., and Vadstein, O. (2017). Correlations of age and growth rate with microbiota composition in Atlantic cod (Gadus morhua) larvae. *Scientific reports* 7, 1-9.

Vadstein, O. (1997). The use of immunostimulation in marine larviculture: possibilities and challenges. *Aquaculture* 155, 401-417.

Vadstein, O., Attramadal, K. J., Bakke, I., and Olsen, Y. (2018). K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. *Frontiers in Microbiology* 9, 2730.

Van Doan, H., Hoseinifar, S. H., Dawood, M. A., Chitmanat, C., and Tayyamath, K. (2017). Effects of Cordyceps militaris spent mushroom substrate and Lactobacillus plantarum on mucosal, serum immunology and growth performance of Nile tilapia (Oreochromis niloticus). *Fish & shellfish immunology* 70, 87-94.

Varela, J., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arijo, S., León-Rubio, J., García-Millán, I., Del Río, M. M., Moriñigo, M., and Mancera, J. (2010). Dietary administration of probiotic Pdp11 promotes growth and improves stress tolerance to high stocking density in gilthead seabream Sparus auratus. *Aquaculture* 309, 265-271.

Vatsos, I., Kotzamanis, Y., Henry, M., Angelidis, P., and Alexis, M. (2010). Monitoring stress in fish by applying image analysis to their skin mucous cells. *European journal of histochemistry: EJH* 54.

Verschuere, L., Rombaut, G., Sorgeloos, P., and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and molecular biology reviews* 64, 655-671.

Verschuere, L., Rombaut, G., Sorgeloos, P., and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and molecular biology reviews 64, 655-671.

Vijayabaskar, P., and Somasundaram, S. (2008). Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen Aeromonas hydrophila. *Biotechnology* 7, 124-128.

Vine, N. G., Leukes, W., Kaiser, H., Daya, S., Baxter, J., and Hecht, T. (2004). Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *Journal of fish diseases* 27, 319-326.

Wang, A., Ran, C., Wang, Y., Zhang, Z., Ding, Q., Yang, Y., Olsen, R. E., Ringø, E., Bindelle, J., and Zhou, Z. (2019). Use of probiotics in aquaculture of China—a review of the past decade. *Fish & shellfish immunology* 86, 734-755.

Wang, J., Ji, H., Wang, S., Liu, H., Zhang, W., Zhang, D., and Wang, Y. (2018). Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. *Frontiers in microbiology* 9, 1953.

Wanka, K. M., Damerau, T., Costas, B., Krueger, A., Schulz, C., and Wuertz, S. (2018). Isolation and characterization of native probiotics for fish farming. *Bmc Microbiology* 18, 1-13.

Zhang, W., Liu, M., and Dai, X. (2013). Biological characteristics and probiotic effect of Leuconostoc lactis strain isolated from the intestine of black porgy fish. Brazilian journal of microbiology 44, 685-691.

Received:24.07.2022
Accepted: 12.09.2022

DOI: 10.22055/IVJ.2022.353171.2482

جداسازی و شناسایی باکتری‌های اسید لاکتیک و مخمر با توان پروبیوتیکی از روده‌ی ماهی سیم سرطلایی (*sparus aurata*)

مهدی کمالی‌کلوچانی[۱]، سید محمد موسوی[۲]، اسحاق زمانی[۳*]، وحید یاوری[۲] و محمد ذاکری[۲]

[۱] دانش‌آموخته دکتری شیلات، دانشکده منابع طبیعی دریا، دانشگاه علوم و فنون دریایی خرمشهر، خرمشهر، ایران

[۲] استاد گروه شیلات، دانشکده منابع طبیعی دریا، دانشگاه علوم و فنون دریایی خرمشهر، خرمشهر، ایران

[۳] استادیار گروه زیست شناسی دریا، دانشکده علوم دریایی و اقیانوسی، دانشگاه علوم و فنون دریایی خرمشهر، خرمشهر، ایران

دریافت: ۱۴۰۱/۵/۲ پذیرش: ۱۴۰۱/۶/۲۱

چکیده

باکتری‌های اسید لاکتیک رایج‌ترین باکتری‌هایی هستند که به عنوان پروبیوتیک در آبزی پروری استفاده می‌شوند. این مطالعه با هدف جداسازی و شناسایی باکتری‌های اسید لاکتیک و مخمرهای دارای پتانسیل پروبیوتیک از روده‌ی ماهی سیم دریایی انجام شد. پنج ماهی با میانگین وزن ۱۷/۶۷±۲۷۹/۸۸ گرم از مزرعه طراحی و توسعه‌ی نیکسا واقع در بندر چارک و ۲۵ ماهی با میانگین وزن ۹/۶۷±۳۹/۴۳ گرم از مزرعه‌ی تیاب پران قشم در جزیره‌ی قشم انتخاب شدند. ماهی‌های انتخاب شده ظاهری سالم داشتند و همچنین به صورت تصادفی انتخاب شدند. باکتری‌های اسید لاکتیک و مخمرها از روده‌ی نمونه‌ها جداسازی و خالص‌سازی شدند و بر اساس ویژگی‌های مورفولوژیکی و توالی‌های مولکولی شناسایی شدند. سپس این جدایه‌ها بر اساس شاخص‌های اساسی پروبیوتیک از جمله مقاومت اسیدی، نمک‌های صفراوی، خواص آنتاگونیستی و فعالیت همولیتیک ماهی مورد ارزیابی قرار گرفتند. ۱۲ جدایه بر اساس رنگ، شکل و اندازه‌ی کلنی خالص‌سازی شدند. سپس با استفاده از رنگ‌آمیزی گرم و بررسی میکروسکوپی دو مخمر و پنج باکتری با مورفولوژی متفاوت شناسایی شدند. تمامی جدایه‌های لاکتوباسیلوس دارای خواص آنتاگونیستی در برابر باکتری بیماری‌زای *Vibrio harveyi* بودند. دو سویه مخمر؛ *Rhodotorula mucilaginosa* CBS 316 و *Wickerhamiella infanticola* CBS 7922، جداسازی شدند. باکتری‌های اسید لاکتیک جدا شده از روده‌ی ماهی به ترتیب شامل دو جنس انتروکوک و باسیلوس بود. نتایج آزمایش‌های توان پروبیوتیکی نشان داد که جدایه‌های ۱ (باکتری)، ۳ و ۶ (مخمر) بهترین عملکرد را داشتند. از داده‌های به دست آمده، نتیجه‌گیری شد که استفاده از ترکیبی از باکتری‌ها و مخمرها به عنوان پروبیوتیک در آبزی‌پروری کارایی بالاتری دارد.

کلمات کلیدی: آبزی پروری، پروبیوتیک، باکتری، ۱۶s RNA، ITS

*نویسنده مسئول : اسحاق زمانی، استادیار گروه زیست شناسی دریا، دانشکده علوم دریایی و اقیانوسی، دانشگاه علوم و فنون دریایی خرمشهر، خرمشهر، ایران

E-mail: zamani@kmsu.ac.ir