International Journal of Molecular and Clinical Microbiology 11(1) (2021) 1471-1478

International Journal of
Molecular and Clinical Microbiology

Isolation and Molecular Identification of Potential Probiotic Yeasts from the gastrointestinal tract of Beluga Fish

M. Fami Zaghrami[1], S. M. Hashemi Karouei[1*], T. Shokohi [2, 3]

1 Department of Microbiology, Faculty of Veterinary Medicine, Islamic Azad University, Babol, Iran.
2 Invasive Fungi Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
3 Department of Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

ARTICLE INFO

Article history:
Received 14 Jaunuary 2021
Accepted 27 March 2021
Available online 30 June 2021

eywords:
Yeast; Probiotic;
Beluga (Huso huso);
Identification;
Internal transcribed spacer;
Sequencing

ABSTRACT

The colonized microorganisms in the fish gastrointestinal (GI) tract have recently received particular attention as a probiotic. Therefore, we aimed to identify potential probiotic yeasts from the GI tract of the beluga (*Huso huso*). The fish were randomly caught from beluga-breeding ponds. The isolated yeast strains were investigated for some properties of probiotic potentials, including tolerance to acidity and bile salt (Oxgall). Yeast strains with probiotic potentials were identified to species level using polymerase chain reaction and sequencing the internal transcribed spacer regions of ribosomal DNA. Seventeen different yeast strains were isolated from the beluga's GI tract based on macroscopic and microscopic characteristics. Nine yeasts (52.9%) could tolerate acid (pH = 2) for up to 3 h. Among acid-resistant yeasts, four yeasts could tolerate bile salts for up to 8 h. By comparing the obtained sequences with reference sequences in GenBank, all strains were identified as *Candida parapsilosis.* Sequence data of each strain were assigned in GenBank under the accession numbers. Identification of the fungal microbiota in healthy fish and determiniation their probiotic aspect may applicable in manage fish's health and nutrition. Further extensive studies are recommended to identify fungal-biota with probiotic potential in more fish species.

1. Introduction

Fish provides at least a fifth of the total animal protein intake of the world's population (Hoseinifar et al., 2018). According to Food and Agriculture Organization (FAO) studies, there was a 14% increase in global marine resource exploitation, 527% global aquaculture production, and 122% of total fish consumption in the human diet between 1990 and 2018 (FAO, 2020).

It has been recognized that some factors, such as fish nutrition, growth, the interaction of microorganisms within the gastrointestinal (GI) tract, and environmental conditions, play a peculiar role in the aquaculture industry. There is a negative interaction between microorganisms and the fish gastrointestinal tract leading to various infectious diseases and, eventually, fish's death. On the contrary, in positive interaction between beneficial microorganisms, the GI tract of fish prevents pathogens and emerging infectious diseases**.** Today, these beneficial microorganisms (probiotics), including bacteria, yeasts, and other fungi, are used as alternatives to antibiotics. They increase their host fish's growth

*Corresponding author: Seyed Masoud Hashemi Karouei
Tel: +98 911 215 9634
E-mail address: mssepid4977@gmail.com

and health by producing various beneficial chemicals (Rima et al., 2012).

According to the Food and Agriculture Organization of the United Nations and the World Health Organization, probiotics refer to live microorganisms that benefit the host when administered in appropriate amounts. Probiotics maintain the beneficial microflora population, neutralize toxins, enhance the immune system, improve absorb nutrients, boost the bioavailability of minerals, bio-enrich folate, and disease control (Bajagai et al., 2016).

In the GI tract of marine and freshwater fish, yeasts often belong to the *Rhodotorula* species. In contrast, the predominant yeast species in marine fish include *Metschnikowia zobelii*, *Trichosporon cutaneum*, and *Candida tropicalis* (Andlid et al., 1998). Commercial probiotic yeasts recorded to date include *Candida pintolopesii* (Daşkıran et al., 2012), *Saccharomyces bourlardii* (Rahman et al., 2013), and *Saccharomyces cerevisiae* (Bai et al., 2013). Given the probiotic effects of yeast species on fish's health and nutrition, it is necessary to determine the fungal biota with probiotic potential in the fish gastrointestinal tract. Interacting with the gastrointestinal normal microbial symbiotic population, *Candida* species possesses strategies for iron acquisition within the host and capable of maintaining intracellular iron homeostasis. They can also produce vitamins such as riboflavin (B2), pantothenic acid (B5), and folate (B9). These vitamins are involved in essential metabolic processes as cofactors in hundreds of enzymes (Kavanagh, 2017). The knowledge can help us manage fish's health and nutrition by manipulating gut microbiota with helpful, beneficial microbes through prebiotic and symbiotic (Nayak, 2010). Therefore, the current study aimed to isolate and identify yeast strains of beluga (Huso huso) GI tract strains using molecular methods and determine some probiotic properties.

2. Materials and Methods

2.1. Sampling and yeast isolation from fish

In the present research, four Beluga-breeding ponds (coded as T, TB, M, and D) from Mazandaran province, northern Iran, were selected, and five healthy fish were randomly caught from each of them. The beluga fishes were dissected to remove the GI tract. After washing the inner surface with sterile distilled water, they transferred to the laboratory under sterile conditions, maintaining the cold chain. Each specimen was poured separately into a sterile electric blender to obtain a homogeneous mixture. To isolate the yeasts, the homogeneous mixtures of all five fish in each pool were mixed, and serially diluted samples were inoculated with Sabouraud Dextrose broth supplemented with 50 µg/ml chloramphenicol and incubated at 37ºC for 24-48 h. Then, using a loop, the grown broth specimens were inoculated onto Sabouraud Dextrose Agar to pick a single colony according to the above mentioned temperature-time conditions. The yeast colonies were isolated and identified in case of growth (Abareethan and Amsath, 2015).

2.2 Determination of probiotic potentials of isolated yeast strains

2.2.1 Determination acid tolerance of the isolates

The isolate's acid tolerance was determined as per methods described previously (Liong and Shah, 2005; Rushdy and Gomaa, 2013). Briefly, a fresh single colony of each yeast strain grown in SD broth at 37°C for 48 hr. 10µL of culture suspension with 0.5 McFarland turbidity standard inoculated into the tube containing 2 mL PBS acidified to pH 2 with 8N hydrochloride acid. Survival of the tested strain was assessed by sampling of the medium at time interval of 0, 2, and 3 h. Tenfold serial dilutions were made from each 1 mL sample, pour plated on SDA and counted growth monitoring using the plate count method. Acid tolerance was determined by comparing the final plate count after 3 h with the initial plate count at 0 h. The experiments were repeated twice. For each strain, 2 independent tests, each in duplicate were carried out and data was presented as mean.

2.2.2 Determination bile tolerance of the isolates

The tested strains were checked for bile salt tolerance. 100 µl of culture suspension was inoculated into 9mL of SDB with or without 0.3 % (W/V) Oxgall (Sigma Chemical Co., St. Louis, MO, USA), the latter was considered as control. The inoculated tubes were incubated at 37°C for 24 h in an anaerobic jar (5% CO2 in a candle jar). Growth was monitored at time interval of 0, and 8 h by measuring optical

density at 600 nm (OD600) using a spectrophotometer. The bile tolerance of each strain was defined as the difference in the time required for the absorbance value to increase by 0.3 units between strains containing Oxgall and the control (Bacha et al., 2009; Liong and Shah, 2005). Coefficient of inhibition (Cinh) was calculated using the method described by (Gopal et al., 2001): Cinh = (ΔT_8-T_0 Control - ΔT_8-T_0 Treatment) (ΔT_8-T_0 Control) Where, Δ represented the differences in absorbance between T_0 (zero hours reading) and T_8 (reading on the 8th hour). The experiment was done twice to ensure the reproducibility of the result. Based on calculated coefficient of inhibition (Cinh), isolates were classified into non-sensitive (resistant) to 0.3% bile salt (Cinh ≈ 0), with retarded growth (0.2<Cinh< 0.4), and poorly tolerant (Cinh > 0.4). The tested isolate with Cinh ≤ 0.4 was considered significant for the isolates to be probiotic

Data were entered in to computer and analyzed using SPSS for Windows version 10.0. The significance of differences (P<0.05) among isolates concerning the degree of tolerance to acidity and bile salt was compared using one-way ANOVA.

2.3 *Molecular Identification of the isolates*

Yeast strains isolated from the gastrointestinal tract of Beluga with probiotic potential were identified using amplification (of internal transcribed spacer (ITS-rDNA) region and sequencing of PCR amplicons.

2.3.1. DNA extraction

Genomic DNA was extracted according to our previously described method with some modifications (Silva et al., 2012). The isolates were identified by means of ITS1-5.8S-ITS2 gene amplification. Briefly, a loopful of 48-hour grown colonies was suspended in 300 µl of lysis buffer (200 mmol-l Tris-HCl [pH: 7.5], 25 mmol-l EDTA, 0.5% [w/v] SDS, 250 $mmol^{-1}$ NaCl). The mixture incubated at 100°C in a boiling water bath for 20 min and centrifuged. The supernatant was added with 300 µl of 3.0 mol^{-1} sodium acetate and incubated at -20°C for 30min. The mixture centrifuged at 12,000× *g* for 10 min. The upper aqueous layer was transferred to a clean microtube precipitated with an equal volume of cold isopropanol and kept at -20°C for 30 min, and then centrifuged at 12,000× *g* for 10 min. The resulting DNA pellets were washed with ice-cold absolute ethanol, and DNA pellet was air dried and dissolved in 50 µL of TE (Tris-HCl 10 mM pH 7.6, EDTA 1 mM pH 8.0). The DNA sample was stored -20°C until needed.

2.3.2 ITS PCR sequencing

The universal fungal primer IT1 (5′-TCC GTA GGT GAA CCT GCG -3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC–3′) targeting the conserved ITS1-5.8S-ITS2 region were used to amplify (Shokohi et al., 2010). PCR amplification was performed at final volume 25 µL. Reactions consisted of 12.5 µL Master mix 2×, 0.5 µL of each primer (25 pmol), 2 µL DNA template. Thermal cycling programme was an initial denaturation at 94°C for 5 min, followed by 30 seconds at 94°C, 45 seconds at 56°C, and 1 minute at 72°C for 25 cycles and a final extension of 72°C for 7 min. The PCR products were electrophoresed on 1.5% agarose gel in TBE buffer (90 mmol/L Tris-HCL, 90 mmol/L boric acid, 2 mmol/L EDTA) at 90V/cm for 45 min and visualized under a UV transilluminator.

Sanger sequencing of The PCR products with forward primer (ITS1) was performed using the BigDye® Terminator V3.1 sequencing kit (Applied Biosystems) on an ABI3730XL DNA Analyzer (Applied Biosystems). The FASTA formatted sequence aligned and compared with the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for final identification at species level. All sequences obtained in this study were submitted to the NCBI GenBank and their received accession numbers.

3. Results

3.1. yeast isolation

In the current study, a total of 17 types of yeast were separated from the GI tract of the fish in Sabouraud Dextrose Agar with microscopic and macroscopic differences. They included four types of yeast (coded as T1, T2, T3, T4) from T pool, five yeast strains (code as TB1, TB2, TB3, TB4, TB5) from TB pool, three yeast strains (coded as M1, M2, M3) from M pool, and five yeast strains (coded as D1, D2, D3, D4, D5) from D pool.

3.2. Result of bile salts and acid resistance test in isolated yeasts

In the present study, out of 17 isolated yeasts, 9 yeasts (52.9%), namely T1, T4, TB2, TB4, M2, M3, D1, D4, and D5, were able to survive at pH 2 for up to 3 h of incubation (Table 1). Among acid-resistant yeasts, T4, TB2, M3, and D5 yeasts have been able to tolerate bile salts for up to 8 h. They were selected and sequenced as T, TB, M, and D yeasts with probiotic potential.

The results of electrophoresis of PCR products of internal transcribed spacer (ITS) regions on yeasts isolated from the GI tract of Beluga on 1% agarose gel showed 480 to 500 base pairs, compared to the molecular marker (Figure 1). By comparing its sequence with the GenBank, four species of *Candida parapsilosis* were identified and obtained sequences deposited on GenBank with accession numbers (MT102868 to MT102871).

Table 1. Acid tolerance pattern (log CFU/mL) of 17 strains isolated from gastrointestinal tract of beluga after 0, 2 and 3 hours' exposure in relation to the different fish ponds

Fish ponds	**(CFU*/ml)**																
Hours	T1	T2	T3	T4	TB1	TB2	TB3	TB4	TB5	M1	M2	M3	D1	D2	D3	D4	D5
0	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6
2	10^6	10^3	10^2	10^6	10^2	10^7	0	10^7	10	10^2	10^6	10^7	10^6	10	10^3	10^6	10^6
3	10^7	0	0	10^6	0	10^7	0	10^7	0	0	10^7	10^7	10^6	0	0	10^7	10^6

*CFU; Colony Forming Unit

Table 2. Studies focused on biological effects of *Candida parapsilosis* isolated from fish

Fish species	**Yeast**	**Biological effects**	**References**
Zebra fish	*Candida parapsilosis*	↓Invasion, damage, and virulence functions of C. albicans	(Gonia et al., 2017)
Whiteleg shrimp + Nile tilapia	*Candida parapsilosis* + *Pediococcus parvulus*	↑Final weight, ↓feed conversion ratio & ↓Blood cholesterol with increase Probiotic supplementation	(Apún-Molina et al., 2015)
juvenile Goldfish	*Candida parapsilosis*	Immunostimulatory activity, ↓ bacterial load i.e. total pseudomonas count	(Anusha et al., 2014)
Tilapia sp.	*Candida parapsilosis* + *Pediococcus parvulus*	↑Growth rate	(Luna-González et al., 2013)

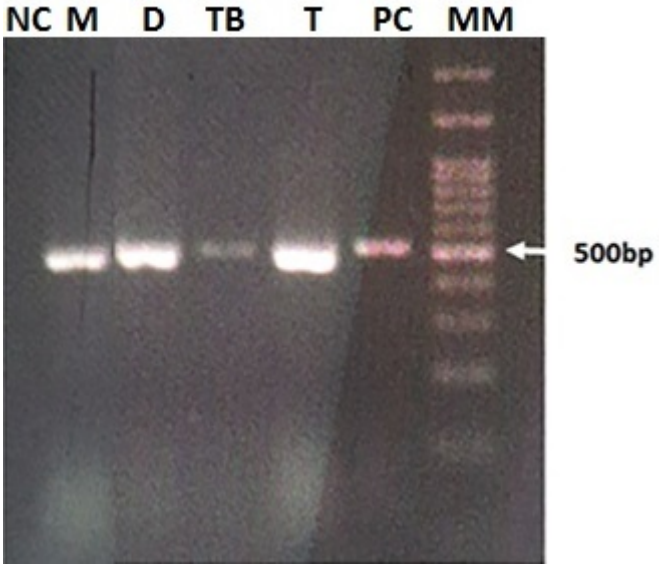

Figure 1 Agarose gel electrophoresis of amplified products of ITS rDNA(ITS1-5.8S-ITS2) region of yeast isolates with probiotic potential; left to right M, D, TB, T: *Candida parapsilosis;* PC: Positive Control; NC. Negative Control; MM: Molecular Marker (100bp)

4. Discussion

Fish microbiota is beneficial to the host and affects essential biological processes, such as nutrient uptake and processing, development of the mucosal immune system, and angiogenesis. It has been shown that even if yeast is less than 1% of the total microbial isolation in the host, given that the size of yeast cells may be more than 100 times the size of bacterial cells, their significant physiological contribution is far more outstanding than that of probiotic bacteria (Gatesoupe, 2007). Unlike bacteria, there exists a wide range of yeast cells from simple to highly complex. This phenomenon results from yeast's extensive metabolic potential, which is reflected by the production of diverse enzymes. Yeast-secreted polyamines contribute significantly to digestive tract maturation in fish larvae (Tovar-Ramírez et al., 2002). Some yeast species and their compounds, such as β-glucans and mannoproteins, can also stimulate the host's immunity and antioxidant defenses.

understanding the participation of yeast microbiota in fish health and nutrition can improve both the hygiene or health conditions and fish production performance (Navarrete and Tovar-Ramírez, 2014). *Candida* species, the ascomycetous yeasts, were isolated from the fish gut of freshwater Teleost (Das and Ghosh, 2014), Zebra fish (Caruffo et al., 2015), *Tilapia oreochromis*, African catfish, and Grey mullet (Tartor et al., 2018). *Candida parapsilosis* was isolated for the first time from GI of healthy fish in eastern Iceland in 1965 (Ross and Morris, 1965). Afterward, it was isolated from young Goldfish and oral cavity and skin of rainbow trout (*Oncorhynchus mykiss*) (Anusha et al., 2014) and Nile tilapia (*Oreochromis niloticus*) (Junior et al., 2016). Consistent with other studies, in the present study, *Candida parapsilosis* was identified in gastrointestinal microbiota (Kandasamy et al., 2012; Kutty and Philip, 2008). These studies noted that *Candida* species are common and ubiquitous in the oceans and other aquatic environments. In these studies, the yeasts were isolated from gills, skin, mouth, feces, and various fish species' intestines. Most of the studies on the fungal microbiota of fish are based on the identification of cultivable yeasts. *Candida parapsilosis* produce numerous enzymes with biocatalytic properties such as aspartic proteases (Hrušková-Heidingsfeldová et al., 2009), lipases (Csutak et al., 2016), and phytase (Raghavendra and Halami, 2009), and able to degrade various phenolic derivatives (Middelhoven et al., 1992), which makes it suitable for use in various food industries. Phytase phosphorylates the phytate, an anti-nutritional factor in cereals (Raghavendra and Halami, 2009). Mandal and Ghosh demonstrated that Candida parapsilosis as the herbivorous fish gut tannase-producing microbiota might

improve tannin-rich feeds' nutrition value (Mandal and Ghosh, 2013).

The optimum temperature for most yeasts is 28-30º C. A strain with probiotic potential must maintain its cellular viability and metabolic function at a temperature of 37ºC (Sealey et al., 2009; Walker, 2009). In the present study, the isolated yeasts had good growth at 37ºC.

Yeasts can survive in a wide range of pHs, even in extremely acidic environments (pH=1.5). This is because they maintain their metabolic function in the gastrointestinal tract, where the pH varies from 2.5-3.5 (Czerucka et al., 2007; Hellström et al., 2012).

Table 2 summarized studies focused on the biological effects of *Candida parapsilosis* isolated from fish and the impact of probiotics on stimulating the fish immune system. However, the current study was limited by the small size of samples and one fish species (beluga). In addition, due to lack of facilities and budget, we only evaluated acid and bile tolerance in probiotic properties. Therefore, it should be better to screen the studied strains for the additional probiotic criteria includes carbohydrate and protein assimilation patterns; and intestinal epithelial adhesion properties.

Conclusion

Our results show that *Candida parapsilosis* strains isolated from the gastrointestinal GI tract of beluga have promising *in-vitro* probiotic potential. Further studies are recommended to identify fungal-biota with probiotic potentials in more fish species inhabiting different geographical areas.

Refereces

Abareethan, M., Amsath, A. (2015). Characterization and evaluation of probiotic fish feed. Int. J. Pure Appl. Zool. 3: 148-153.

Andlid, T., Vazquez-Juarez, R., Gustafsson, L. (1998). Yeasts isolated from the intestine of Rainbow trout adhere to and grow in intestinal mucus. Mol. Mar. Biol. Biotechnol. 7: 115-126. doi: 10.1007/BF00171938

Anusha, S.U., Sundar, S.K., Williams, P.G. (2014). Studies on the isolation and characterisation of marine yeast, glucan production and immunostimulatory activity on Carassius auratus. Int. J. Curr. Microbiol. Appl. Sci. 3: 230-240.

Apún-Molina, J.P., Santamaría-Miranda, A., Luna-González, A., et al. (2015). Growth and metabolic responses of whiteleg shrimp Litopenaeus vannamei and Nile tilapia Oreochromis niloticus in polyculture fed with potential probiotic microorganisms on different schedules. Lat. Am. J. Aquat. Res. 43: 435-445.

Bacha, K., Mehari, T., Ashenafi, M. (2009). In-vitro probiotic potential of lactic acid bacteria isolated from 'Wakalim', a traditional Ethiopian fermented beef sausage. Ethiop. J. Health Sci., 19: 121-123.

Bai S., Wu, A., Ding, X.M., et al. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poult. Sci. 92: 663-670. doi: 10.3382/ps.2012-02813

Bajagai, Y.S., Klieve, A.V., Dart, P.J., Bryden, W.L. (2016). Probiotics in animal nutrition: production, impact and regulation (H. S. P. Makkar Ed.). FAO Animal Production and Health Paper: FAO.

Caruffo, M., Navarrete, N., Salgado, O., et al. (2015). Potential probiotic yeasts isolated from the fish gut protect zebrafish (Danio rerio) from a Vibrio anguillarum challenge. Front. microbiol. 6: 1093. doi:10.3389/fmicb.2015.01093

Csutak, O., Sabău, E., Pelinescu, D., et al. (2016). Molecular identification and metabolic screening of some yeast strains from foods. AgroLife Sci. J. 5: 51-58.

Czerucka, D., Piche, T., Rampal, P. (2007). Yeast as probiotics–Saccharomyces boulardii. Aliment. Pharmacol. Ther. 26: 767-778.doi:10.1111/j.1365-2036. 2007. 03442.x

Das, P., Ghosh, K. (2014). Evaluation of phytase production by Candida tropicalis isolated from fish gut and subsequent bio processing of groundnut oil cake under solid state fermentation. J. microbiol. biotechnol. food sci. 3: 470-476.

Daşkıran, M., Önol, A.G., Cengiz, Ö., et al. (2012). Influence of dietary probiotic inclusion on growth performance, blood parameters, and intestinal microflora of male broiler chickens exposed to posthatch holding time. J. Appl. Poult. Res. 21: 612-622. doi:10.3382/japr. 2011-00512

FAO. (2020). The state of world fisheries and aquaculture. Retrieved from http://www.fao.org/state-of-fisheries-aquaculture

Gatesoupe, F.J. (2007). Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. Aquac. 267: 20-30. doi:10.1016/j.aquaculture.2007.01.005

Gonia, S., Archambault, L., Shevik, M., et al. (2017). Candida parapsilosis protects premature intestinal epithelial cells from invasion and damage by Candida albicans. Front. Pediatr. 5: 1-10. doi:10.3389/fped.2017.00054

Gopal, P.K., Prasad, J., Smart, J., Gill, H.S. (2001). In vitro adherence properties of Lactobacillus rhamnosus DR20 and Bifidobacterium lactis DR10 strains and their antagonistic activity against an enterotoxigenic Escherichia coli. Int. J. Food Microbiol. 67: 207-216. doi:10.1016/S0168-1605(01)00440-8

Hellström, A.M., Almgren, A., Carlsson, N.G., Svanberg, U. and Andlid, T., et al. (2012). Degradation of phytate by Pichia kudriavzevii TY13 and Hanseniaspora guilliermondii TY14 in Tanzanian togwa. Int. J. Food Microbiol. 153: 73-77. doi:10.1016/ j.ijfoodmicro. 2011.10.018

Hoseinifar, S.H., Sun, Y.Z., Wang, A., Zhou, Z. (2018). Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front. Microbiol. 9: 2429. doi:10.3389/fmicb.2018.02429

Hrušková-Heidingsfeldová, O., Dostál, J., Majer, F., et al. (2009). Two aspartic proteinases secreted by the pathogenic yeast Candida parapsilosis differ in expression pattern and catalytic properties. Biol. Chem. 390: 259-268. doi:10.1515/BC.2009.034

Junior, E.M.L., Bandeira, T.D.J.P.G., De Miranda, M.J.B., et al. (2016). Characterization of the microbiota of the skin and oral cavity of Oreochromis niloticus. J. Health Biol. Sci. 4: 193-197. doi:10.12662/2317-3076jhbs.v4i3. 767.p193-197.2016

Kandasamy, K., Alikunhi, N.M., Subramanian, M. (2012). Yeasts in marine and estuarine environments. J. Yeast Fungal Res. 3: 74-82. doi:10.5897/JYFR12.003

Kavanagh, K. (2017). Fungi: Biology and Applications (Third ed.): Wiley-Blackwell.

Kutty, S., Philip, R. (2008). Marine yeasts—a review. Yeast. 25: 465-483. doi:10.1002/yea.1599

Liong, M.T., Shah, N.P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci. 88: 55-66. doi:10.3168/jds.S0022-0302(05) 72662-X

Luna-González, A., Quiñónez-Zúñiga, D., Fierro-Coronado J.A., et al. (2013). Effect of Pediococcus parvulus and Candida parapsilosis on growth and survival of tilapia, Oreochromis niloticus and Oreochromis sp. Afr. J. Microbiol. Res. 7: 1-7. doi:10.5897/ AJMR2012.2295

Mandal, S., Ghosh, K. (2013). Isolation of tannase producing microbiota from the gastrointestinal tracts of some freshwater fish. J. Appl. Ichthyol. 29: 145-153. doi:10.1111/j.1439-0426.2012. 02054x

Middelhoven, W.J., Coenen, A., Kraakman, B., Gelpke, M.D.S. (1992). Degradation of some phenols and hydroxybenzoates by the imperfect ascomycetous yeasts Candida parapsilosis and Arxula adeninivorans: evidence for an operative gentisate pathway. Antonie van Leeuwenhoek. 62: 181-187. doi:10. 1007/ BF00582578

Navarrete, P., Tovar-Ramírez, D. (2014). Use of yeasts as probiotics in fish aquaculture. In Hernandez-Vergara M.P. and Perez-Rostro C.I. (Eds.), Sustainable aquaculture techniques (Vol. 1, pp. 135-172). BoD - Books on Demand: InTech.

Nayak S.K. (2010). Role of gastrointestinal microbiota in fish. Aquac. Res. 41: 1553-1573. doi:10.1111/j.1365-2109. 2010.02546.x

Raghavendra, P., Halami, P. (2009). Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine. Int. J. Food Microbiol. 133: 129-134. doi:10.1016/ j.ijfoodmicro. 2009.05.006

Rahman, M.B.A., Mustari, A., Salauddin, M., Rahman M. (2013). Effects of probiotics and enzymes on growth performance and haematobiochemical parameters in broilers. J. Bangladesh Agril. Univ. 11: 111-118. doi:10.3329/jbau.v11i1.18221

Rima, H., Steve, L., Ismail, F. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Front. Microbiol. 3: 1-12. doi:10.3389/fmicb.2012.00421

Ross S.S., Morris E.O. (1965). An investigation of the yeast flora of marine fish from Scottish coastal waters and a fishing ground off Iceland. J. App. Bacteriol. 28: 224-234. doi:10.1111/j.1365-2672.1965.tb02147.x

Rushdy, A.A., Gomaa, E.Z. (2013). Antimicrobial compounds produced by probiotic Lactobacillus brevis isolated from dairy products. Ann. Microbiol. 63: 81-90. doi:10.1007/s13213-012-0447-2.

Sealey, W.M., Barrows, F.T., Smith, C.E., et al. (2009). Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout Oncorhynchus mykiss to utilize high levels of soybean meal during grow-out. Aquac. 293: 195-203. doi:10.1016/ j.aquaculture. 2009.04.013

Shokohi, T., Hashemi-Soteh, M.B., Saltanat-Pouri, Z., et al. (2010). Identification of Candida species using PCR-RFLP in cancer patients in Iran. Indian J. Med. Microbiol. 28: 147-151. doi:10.4103/ 0255-0857.62493

Tartor, Y., Taha, M., Mahboub, H., El-Ghamery, M. (2018). Yeast species associated with diseased fish: Occurrence, identification, experimental challenges and antifungal susceptibility testing. Aquac. 488: 134-144. doi:10.1016/j.aquaculture.2018.01.033

Tovar-Ramírez, D., Zambonino, J.L., Cahu, C.L., et al. (2002). Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (Dicentrarchus labrax) larvae. Aquac. 204: 113-123. doi:10.1016/S0044-8486(01)00650-0

Walker, G.M. (2009). Yeasts. In M. Schaechter (Ed.) Desk encyclopedia of microbiology (Second ed., 1: 1174-1187): Academic Press/Elsevier.