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INHIBITORY ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM TILAPIA GUT AGAINST *STREPTOCOCCUS AGALACTIAE*

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ABSTRACT

Tilapia culture offers cheap high-quality protein for the human population demand. However, with intensification, the cultured tilapia become threatened by Streptococcus infection, and antibiotic resistance emergence in aquaculture. Therefore, probiotics were investigated to be used as an alternative for antibiotics to treat Streptococcus infections. In the current study, probiotic lactic acid bacteria count ranged from 5.67 to 5.78 $\log_{10}$ CFU/g of tilapia gut microflora. The conventional biochemical tests were carried out for the lactic acid bacteria isolates identification. Only seven native isolates which exhibited inhibitory properties against *Streptococcus agalactiae* with an inhibition zone 26-50 mm in diameter were selected for characterization. All probiotic isolates were negative for hemolysis and produced both amylase and lipase enzymes. The selected strains tolerated the fish's gastrointestinal acidic and bile conditions. The promising features of the isolated strains indicate that probiotic bacteria of aquatic origin can be considered a safe alternative for pathogen control.

Keywords: Probiotics, Nile tilapia, lactic acid bacteria, inhibitory activity, enzyme production

INTRODUCTION

Tilapia culture is the second most important finfish culture industry in the world (Cai *et al.*, 2019). Nowadays, tilapia culture is expanding all over the world, cultured in more than 170 countries worldwide (FAO, 2020). Tilapia has been engaged in the development of many rural locations, poverty improvement, malnutrition alleviation, and help in the improvement of the human health population. Altogether, tilapia directly plays a critical role in the World Sustainable Development Goals achievement (El-Sayed and Fitzsimmons, 2023). The global tilapia production was estimated at almost 7 million tons in 2020 and is expected to reach 7.3 million tons by 2030, providing a low-cost, highly nutritious protein source (FAO, 2020). Approximately, 70 species of tilapia have been identified in the world, however, Nile tilapia is the most cultured tilapia fish (Abdel-Ghany *et al.*, 2019). Due to its amazing features including its improved growth rate, and tolerance to extreme environmental circumstances (El-Sayed, 2006), fish farmers tend to culture this fish in intensified systems, to benefit from high marketability and consumer preference (Abd-allah and Ismail, 2016).

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Despite such benefits, bacterial infection of tilapia culture is the main hurdle against the sustainability of this industry and is considered a major public health significance for consumers (Mohamed and Saleh, 2010, Elkamel *et al.,* 2011). *Streptococcus species is* considered one of the main pathogenic agents causing disease in cultured tilapia called "Streptococcosis" (Taukhid *et al.,* 2021). Streptococci are Gram-positive cocci, catalase-negative non motile organisms. Several species have been identified to cause Streptococcosis in tilapia, but *Streptococcus agalactiae* is the biggest threat to tilapia culture (Zhang *et al.,* 2022). *Streptococcus agalactiae* disease of tilapia is highly infectious, and can be horizontally spread through food and infected fish, causing high mortality and wide prevalence (Eissa *et al.,* 2021). So, it was considered to be one of the most dangerous pathogens threatening tilapia culture in the world (Shoemaker *et al.,* 2017).

Antibiotics are the first prime choice in combating this disease. The repeated use of these drugs leads to antibiotic-resistant development (Fang *et al.,* 2018). Aquatic *Streptococcus agalactiae* bacteria can acquire antibiotic resistance. Recently, two *Streptococcus agalactiae isolates* in tilapia aquaculture were identified (Kelany *et al.,* 2024), these pathogens were detected in ready-for-marketing tilapia fish, exhibited multiple antibiotic-resistance for most of the evaluated antibiotics and one of them was resistant to amoxicillin which considered drug of choice, with a consequence of human risk. So, there was a critical need to search for non-antibiotic antimicrobial agents to control these resistant pathogens.

Probiotics have been recommended as a safe new alternative method in aquaculture, and several beneficial probiotic microorganisms have been approved to be used as probiotics in aquaculture (Ringø, 2020, Ringø *et al.,* 2022). Probiotics are non-pathogenic microbial cells or microbial cell products that, when administered, the farmed species` survival and growth rates are enhanced (Jahangiri and Esteban 2018). The currently used terrestrial probiotics in the market have limited application effects in aquaculture (Wanka *et al.,* 2018). Because host-derived probiotics are more promising (Lara-Flores and Olvera-Novoa, 2013), Therefore, indigenous probiotics are more advantageous (Husain *et al.,* 2022). Lactic acid bacteria are the most common probiotic bacteria used in aquaculture (Dawood *et al.,* 2019). The natural presence of lactic acid bacteria in the fish gut makes them a suitable probiotic candidate for aquaculture (Muthukumar and Kandeepan, 2015, Reda *et al.,* 2018). The selection of probiotic bacteria with extracellular enzyme production ability was considered the main principle for the candidate probiotics because of improving nutrient utilization to support the host growth. The effective probiotic candidates should survive the high bile salt concentration and acidic pH of the fish's gastrointestinal tract conditions (de Melo Pereira *et al.,* 2018). So, the present study aimed to identify autochthonous lactic acid bacteria from Nile tilapia gut and used as potential probiotics with antimicrobial activity against *Streptococcus agalactiae*.

MATERIALS AND METHODS

1. Bacterial strains:

Two *Streptococcus agalactiae* strains were previously isolated from tilapia aquaculture (Kelany *et al.,* 2024).

2. Tilapia Fish collection and preparation

Forty-eight healthy Nile tilapia were collected from cultured tilapia through 4 visits to three tilapia aquacultures in Assiut and Minia Governorates (Table 1). The visited farms were subjected to a questionnaire on some management data and the owners agreed to share the information orally. Each fish was cleaned from dirt with sterile distilled water, their body scales` were removed, the skin was disinfected by alcohol (70%), the fish body was aseptically opened with sterile scissors, and one gram of intestine was homogenized with homogenizer and suspended in 9 mL of sterile 0.85% Na Cl

then diluted in tenfold serial dilution (Saikot, 2016).

3. Enumeration of lactic acid bacteria

The probiotic bacteria were counted according to Reda *et al.* (2018). Briefly, one hundred microliters of $1/10^2$, $1/10^3$, and $1/10^4$ suspensions were poured into and dispersed over de Man, Rogosa and Sharpe (MRS) agar medium plate in pH 5.5, each dilution was represented by two plates. The inoculated volume was evenly spread using a sterile bent glass rod then a layer of soft agar (40ºC) was added above the inoculated sample (agar overlay method) and cultured anaerobically at 37°C for 48 hours. All the steps were done under sterile conditions.

2.4. Probiotic lactic acid bacteria isolation

Single round convex and creamy colonies on MRS agar were considered to be lactic acid bacteria (Kaktcham *et al.,* 2017), and were picked up to trypticase agar slants for further identification. The selected isolates were confirmed as illustrated by Bergey's manual® of Systematic Bacteriology (Vos *et al.,* 2011).

2.5. Phenotypic identification

Provisional or tentative identification of lactic acid bacteria was achieved by Gram staining, cell morphology under the microscope, catalase reaction, and gas production from glucose.

I. Gram Staining

Gram staining was done (Collins *et al.,* 2004). The purple cell color indicates a positive Gram reaction.

II. Catalase test

For the catalase reaction, each isolate inoculum was transferred and mixed with the previously added two drops of hydrogen peroxide (3% H_2O_2) on a clean glass slide. The formation of bubbles indicates a positive reaction, proving that the organism produced the catalase enzyme that converts hydrogen peroxide into water and oxygen (Collins *et al.,* 2004).

III. Glucose Fermentation test

The ability of isolates to ferment glucose and produce co٢ gas and organic acids end product was determined. Inoculate the glucose purple broth with isolated organisms, then incubate inoculated tubes in addition to control non-inoculated tube aerobically at 37ºC, for one day anaerobically. Observe for appearance of a yellow color in the medium (Razin and Cirillo, 2012).

Only pure Gram-positive catalase-negative, glucose fermentative isolates were considered potential probiotic lactic acid bacteria. All bacterial isolates were stored at − 20 °C using MRS broth supplemented with 25% glycerol.

6. Testing of Antimicrobial Activity

The antagonistic activities of the probiotic lactic acid bacteria strains against multiple antibiotic-resistant two *Streptococcus agalactiae* isolates were assessed using the agar spot method as Shokryazdan *et al.* (2014).

7. Hemolytic activity

Hemolytic action was determined according to **Semedo *et al.*, (2003),** through streaking the isolates on blood agar medium with 5% (v/v) sheep blood. After incubation for 48 hours at 37◦C, the presence or absence of zones of clearing around the colonies was observed.

8. Enzymatic activity

I. Lipase production

Lipase activity was assessed using nutrient agar with 1% tween 80 (v/v) (Husain *et al.,* 2022).

II. Amylase Assay

The isolated bacteria were inoculated on starch-agar plates, which were incubated at 37°C for 24 hours. Extracellular amylase activities were evaluated by clear zones that appeared around the colonies (Poletto *et al.,* 2018).

9. Acid and bile tolerance

Isolate pH and bile tolerance were performed according to Taj *et al.* (2022). An in-vitro acidic condition of the fish gastrointestinal tract was created with MRS broth pH 2. The absorbance measured at 0, 2, 3 hours. Cultures grown in MRS broth pH 07 served as control. The tolerance was calculated using the following formula:

Survival rate (%) = ((O. D. (pH7) − O. D. (pH2)/ O.D. (pH7)) × 100

For bile tolerance, bile salt was added to MRS broth at 0.3% (w/v). The growth was verified by measuring the optical density (OD 600) at 0, 2, and 4 hours. The negative control was plain MRS broth without bile. The isolate bile tolerance percentage was determined using the following formula:

Survival rate (%) = ((O. D. (0%bile) − O.D. (0.3% bile)/ O.D. (0%bile)) × 100

10. Statistical Analyses

IBM SPSS 22 software was utilized for lactic acid bacteria count data. One-way analysis of variance (ANOVA) and Duncun's test were used to compare the differences between the means. Data was expressed as the mean ± standard deviation (SD).

RESULTS

The total number of probiotic lactic acid bacteria of tilapia gut was counted using serial dilution in MRS agar medium. The log10 value of the mean count of CFU/gm of lactic acid bacteria from the tilapia gut was presented in Table 2. The mean count of CFU/gm was 5.67 ± 4.3 in farm A, 4.83 ± 4.59 in farm B, 5.43 ± 4.71 and 5.78 ± 4.3 in farm C. Statistically there were significant differences in the mean count of lactic acid bacteria between farm visits.

Typical lactic acid bacteria colonies in MRS agar were creamy white, small convex colonies. Out of eighty isolates, only seven isolates met the lactic acid bacteria phenotypic properties. These colonies were Gram-positive cocci-bacilli, catalase-negative, glucose fermentative, and non-motile.

Antimicrobial activity is one of the potential probiotic properties. In this study, the isolated lactic acid bacteria strains showed different degrees of inhibitory activity against the tested strains (Table 3). All the evaluated seven lactic acid bacteria strains exerted antagonistic activity against the two *Streptococcus agalactiae* strains with a zone of inhibition ranging from 26-50 mm in diameter. Furthermore, all isolates showed no zone around the colonies on the blood agar medium, so they were considered safe.

All the tested seven lactic acid bacteria strains have the power to secrete lipase and amylase enzymes.

As presented in Table 4, the tested strains showed variable tolerance to the acidic pH. Six strains adapted to the acidic pH, while after 3 hours only five strains survived. The survival rate in pH 2 ranged from 11- 39% and 12-35% after 2 and 3 hours, respectively. As shown in Table 4 four strains were able to tolerate bile for 2 hours, and after 4 hours only 2 strains could adopt the bile stress. Moreover, the tested strains tolerated 0.3% bile with a survival rate of 3-45% after 2 hours and 4 - 37% after 4 hours.

Table 1: The three tilapia aquacultures investigated in the current study.

	Farm A	Farm B	Farm C
Type of production and fish stocking density/pond	Closed and semi-intensive	Integrated with ducks semi-intensive	Integrated with cattle and buffaloes also with agriculture semi-intensive
Number of ponds/farms	2 ponds	1 pond	2 ponds
Construction of the pond	Concrete floor	Brick blocks	muddy
Area of the pond	100 m^2	1500 m^2	4200 m^2
Water source	Groundwater with a separate drain for the spent water.	Surface water from a nearby pond branched from the River Nile The spent water pumped to agricultural fields	Groundwater for the cultured pond The spent water pumped to agricultural fields
Fish stocking management	Partial water changes every day*;* water exchange*;* aerator*;* fish fed 2-3 times per day diets with 32% protein	Partial water changes every two days- pelleted ration with 23% protein –bran –bread- rest of grinders – rest of duck feed	Water changed every long period – fish fed ration with 30% protein*;* bread and bran
Fertilizer	No	Yes - duck manure*;* duck slaughter wastes	Yes- cattle and buffalo's manure
Current mortality rate/pond/day	No	No	No
Therapeutic and prophylactic treatment	No	No	No
Disinfectants used	No	No	No
Veterinary supervision	Yes	No	No

Table 2: Probiotic lactic acid bacteria count (log10 CFU/ml intestinal content).

Farm	Minimum count	Maximum count	Mean ± SD
Farm A	5.30	5.85	5.67 ± 4.3^{c}
Farm B	4.34	5.17	4.83 ± 4.59^{a}
Farm C	5.29	5.57	5.43 ± 4.71^{b}
	5.47	5.97	5.78 ± 4.34^{d}

a*;* b*;* c*;* d: means with different letters are significantly differ.

Table 3: Antimicrobial activity of the isolated Probiotic lactic acid bacteria against *Streptococcus agalactiae* (2 isolates)

Strain	Inhibition zone (mm) against S*treptococcus agalactiae*	
	***Amoxicillin susceptible* strain**	***Amoxicillin resistant* strain**
1	40	30
2	30	33
3	30	35
4	45	50
5	32	30
6	40	26
7	33	38

Table 4: Probiotic lactic acid bacteria tolerance.

Strain	Acid		Bile	
	2 hours	3 hours	2 hours	4 hours
1	Tolerant	Tolerant	Non-tolerant	Non-tolerant
2	Tolerant	Tolerant	Non-tolerant	Tolerant
3	Tolerant	Tolerant	Tolerant	Tolerant
4	Tolerant	Non-tolerant	Non-tolerant	Tolerant
5	Tolerant	Tolerant	Tolerant	Tolerant
6	Non-tolerant	Non-tolerant	Tolerant	Tolerant
7	Tolerant	Tolerant	Tolerant	Tolerant

DISCUSSION

Despite the progress in industrial aquaculture, there are some problems such as increasing incidence and prevalence of diseases that might lead to excessive use of antibiotics which lead to the occurrence of antibiotic resistance in aquatics (Larsson and Flach, 2022). Therefore, the search for new alternatives is now recommended. Due to the host-specific application effect of probiotics, the commonly used probiotics in the market are not recommended for aquatic use (Wanka *et al.*, 2018). Therefore, native probiotics identified from the Nile tilapia intestine are greatly recommended.

1. Probiotic lactic acid bacteria Count

The total lactic acid bacteria in the tilapia intestine using MRS agar was counted. The difference in the mean count of CFU/gm may be due to the level of protein diet offered the fish (Yang *et al.,* 2021). As fish

in farm C received diets with 32% protein while farm B fish received diets with 23% protein. Our lactic acid count from the tilapia intestine is near that counted by Rifat-Al-Naser *et al.* (2016), from the intestine of *Channa punctate* (2.1×10^{10} and 1.9×10^{9} CFU/gm). While our lactic acid bacteria count from the tilapia intestinal tract was higher than the count of Govindaraj *et al.* (2021), who, recorded from five freshwater fish species a bacterial count ranged from 2.1×10^{3} to 2.7×10^{4} CFU/gm. In addition, Muthukumar and Kandeepan, (2015), recorded total probiotic counts of 2.72×106 CFU/gm from *Catla catla* gut,1.87×10^{6} CFU/gm from *Labeo rohita gut,* 1.91×10^{6} CFU/gm from *Cirrhinus mirigala* gut and 2.19×10^{6} CFU/gm from *Cyprinus carpio* gut. On the contrary, Bhatnagar and Dhillon, (2019), recorded a low mean population of intestinal microflora of lactic acid bacteria from *Labeo calbasu* gut which was 2.12×10^{5} CFU/gm. While, the total intestinal microbial flora of *Sperata seenghala* and *Lactic acid bacteria eo bata* counted by Saikot, (2016), was $2.1x10^{6}$ and $1.8x10^{5}$ CFU/gm, respectively. Vlková *et al.* (2012), counted the total probiotic count in nine freshwater fish species the counts were 4.06 - 8.23 log CFU/gm.

The alimentary tract of fish is highly populated by microbes which play a vital role in the fish's immune function (Denev *et al.*, 2009).

2. Probiotic lactobacillus bacteria isolation

We identified seven lactic acid bacteria isolates for potential probiotic characterization.

Seven lactic acid bacteria isolates were selected based on the characteristics of Dowarah *et al.* (2018) and Fečkaninová *et al.* (2019). Lactic acid strains in our study is nearly similar to that selected by Maji *et al.* (2016) from the intestine of five freshwater fish, and much lower than Govindaraj *et al.*, (2021), who selected 33 lactic acid bacteria isolates from 120 colonies on the basis of acid production during fermentation.

3. Antimicrobial activity

All the lactic acid bacteria strains (100%) inhibited the growth of the two *Streptococcus agalactiae* strains with zones of inhibition ranging from 20-50 mm.

Antibacterial activity against pathogens is one of the significant important characteristics for probiotic strain selection (Chauhan and Singh, 2019). Probiotic antagonistic activity of lactic acid bacteria mainly attributed to the antimicrobial compounds they secret including bacteriocins, hydrogen peroxide, organic acids, short-chain fatty acids which are responsible for their antimicrobial activity (Thirunavukkarasu *et al.*, 2022). Compared to our result, 935 of the lactic acid bacteria isolated from fish and fish products exhibited antimicrobial activity against *Streptococcus agalactiae* using qualitative stab-on-agar test as assayed by Muñoz-Atienza *et al.* (2013), with an inhibition zones of >10 mm. Also, 54% of lactic acid bacteria strains identified by Siangpro *et al.* (2023) from the gastrointestinal tract of fifteen *Climbing perch*, five Nile tilapia, three Asian sea bass, two Striped snake-head, one Soldier river barb, and one Common carp exhibited inhibitory effects against *Streptococcus agalactiae* with inhibition zone ranging from7-9 mm.

4. Hemolytic activity

The Hemolytic activity is one of the virulence factors of the bacteria, facilitates the infection by microbial invasion to their host (Sperandio *et al.*, 2010). Therefore, hemolysis analysis is very important as a safety prerequisite (FAO/WHO, 2002). The seven selected lactic acid bacteria isolates

were negative for hemolytic activity (100%). Our strain˙s non hemolytic activity is similar to that obtained by Govindaraj *et al.* (2021) from freshwater fish intestine none of the lactic acid bacteria isolates exhibited hemolytic activity in blood agar (100%), but higher than that identified by Coulibaly *et al.* (2022) 7% of lactic acid bacteria isolates were non hemolytic.

5. Enzymatic activity

All lactic acid strains have the power to produce extracellular lipases and amylase enzymes. Enzyme production is a valuable character in the probiotic bacteria as this bacteria is considered as safe and their enzymes have valuable effects for the host, as it help in food digestion with improvement in feed intake and weight (Peivasteh-Roudsari *et al.,* 2020). These extracellular enzymes produced by the beneficial intestinal microflora were imperative for several natural processes such as feed digestion as well as for metabolism (Kesarcodi-Watson *et al.,* 2008). Lipases in the digestive tract act on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol (Gupta *et al.,* 2004), While, amylase is important for starch degradation (Huddy and Coyne, 2015). Compared to our results, four lactobacillus isolates from tilapia gut showed amylase activity were obtained by Reda *et al.,* (2018).

6. Acid and bile tolerance

Tolerance to acid and bile is a principal character in candidate strains to be selected as probiotics. The tested strains tolerated the acidic and bile stress with variable degrees. Compered to our results, seven lactic acid bacteria isolates evaluated by Govindaraj *et al.,* (2021) tolerated pH 2 with survival rate 45.1-84.4%. While, Muthukumar and Kandeepan, (2015) recorded that only 5 lactic acid bacteria isolates from freshwater fish gut tolerated 0.3% bile for 2 hours.

CONCLUSION

The concept of disease control using probiotics has received a widespread attention for their safety and no resistance develops. Seven lactic acid bacteria isolated from Nile tilapia gut could form a promising competent for controlling *Streptococcus agalactiae*. The present study confirms that Nile tilapia gut can be considered a source of probiotics for future use. Additionally, full identification and in vivo studies are required to determine its applications in the aquaculture environment. The obtained probiotic lactic acid bacteria can be used in the aquaculture as a feed additive in the aquaculture feed. Molecular identification of the identified isolates is required in further research to determine the type of bacteria to the species level.

ETHICAL STANDARDS

The permission (Number: 06/2023/0114) of the ethical committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, was followed in tilapia fish caught, transportation, and examination.

REFERENCES

Abd-allah, S.M.S. and Ismail, H.A.A. (2016): Quality Parameters and Nutritive Value of Wild and Cultured Nile Tilapia Sold in Assiut City, Egypt. *Assiut Veterinary Medical Journal*, *62*(151), 90–100.

Abdel-Ghany, H.M.; El-Sayed, A.F.M.; Ezzat, A.A.; Essa, M.A. and Helal, A.M. (2019): Dietary lipid sources affect cold tolerance of Nile tilapia (Oreochromis niloticus). *Journal of Thermal Biology*, *79* (May 2018), 50–55.

Beaud, D.; Tailliez, P. and Aba-Mondoloni, J. (2005): Genetic characterization of the β-glucuronidase enzyme from a

human intestinal bacterium Ruminococcus gnavus. *Microbiology*, *151*(7), 2323–2330.

Bhatnagar, A. and Dhillon, O. (2019): Characterization, screening, and application of bacteria with probiotic properties isolated from the gut of Labeo calbasu (Hamilton). *Fisheries and Aquatic Life*, *27*(4), 178–189.

Cai, J.; Zhou, X.; Xue, Y.; Lucentea, D. and Laganaa, C. (2019): Top 10 species groups in global aquaculture 2017. *Food and Agriculture Organization of the United Nations*, 12.

Chauhan, A. and Singh, R. (2019): Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis*, *77*(2), 99–113.

Collins, C.H.; Lyne, P.M.; Grange, J.M. and Falkinham III, J.O. (2004): Microbiological Methods Eight Edition. *By Arnold*, 466.

Coulibaly, W.H.; Camara, F.; Diguta, C. and Matei, F. (2022): Probiotic and functional properties potential of lactic acid bacteria isolated from Tilapia (Oreochromis niloticus) in Ivory Coast. *Research Square*, 1–23.

Dawood, M.A.O.; Koshio, S.; Abdel-Daim, M. M. and Van Doan, H. (2019): Probiotic application for sustainable aquaculture. *Reviews in Aquaculture*, *11*(3), 907–924.

De Melo Pereira, G.V.; De Oliveira Coelho, B.; Magalhães Júnior, A.I.; Thomaz-Soccol, V. and Soccol, C.R. (2018): How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*(8), 2060–2076.

Denev, S.; Beev, G.; Staykov, Y. and Moutafchieva, R. (2009): Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in Finnish aquaculture. *International Aquatic Research*, *1*(1), 1–29.

Dowarah, R.; Verma, A.K.; Agarwal, N.; Singh, P. and Singh, B.R. (2018): Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. *PLoS ONE*, *13*(3).

Eissa, A.E.; Attia, M.M.; Elgendy, M.Y.; Ismail, G.A.; Sabry, N.M.; Prince, A.; Mahmoud, M.A.; El-Demerdash, G.O.; Abdelsalam, M. and Derwa, H.I.M. (2021): Streptococcus, Centrocestus formosanus and Myxobolus tilapiae concurrent infections in farmed Nile tilapia (Oreochromis niloticus). *Microbial Pathogenesis*, *158*, 105084.

El-Sayed, A.F.M. (2006): Tilapia culture. In *Tilapia Culture*. Academic Press.

El-Sayed, A.F.M. and Fitzsimmons, K. (2023): From Africa to the world—The journey of Nile tilapia. *Reviews in Aquaculture*, *15*(S1), 6–21.

Elkamel, A.A.G. Saad El-DEEN, A.Y.A. and Afifi, S.M. (2011): Vibriosis in Nile Tilapia, Oreochromis Niloticus, and Susceptibility of Catfish, Clarias Gariepinus. *Assiut Veterinary Medical Journal*, *57*(131), 1–10.

Fang, T.; Wang, H.; Cui, Q.; Rogers, M. and Dong, P. (2018): Diversity of potential antibiotic-resistant bacterial pathogens and the effect of suspended particles on the spread of antibiotic resistance in urban recreational water. *Water Research*, *145*, 541–551.

FAO/WHO. (2002): Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. *World Health Organization and Food and Agriculture Organization of the United Nations, Rome, Italy*.

FAO. (2020): The state of world fisheries and aquaculture sustainability in action. In *FAO* (Vol. 35, Issue 3).

Fečkaninová, A.; Koščová, J.; Mudroňová,

D.; Schusterová, P.; Cingeľová Maruščáková, I. and Popelka, P. (2019): Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (Oncorhynchus mykiss, Walbaum) in Slovakia. *Aquaculture*, *506*(October 2018), 294–301.

Govindaraj, K.; Samayanpaulraj, V.; Narayanadoss, V. and Uthandakalaipandian, R. (2021): Isolation of Lactic Acid Bacteria from Intestine of Freshwater Fishes and Elucidation of Probiotic Potential for Aquaculture Application. *Probiotics and Antimicrobial Proteins*, *13*(6), 1598–1610.

Gupta, R.; Gupta, N. and Rathi, P. (2004): Bacterial lipases: An overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology*, *64*(6), 763–781.

Huddy, R.J. and Coyne, V.E. (2015): Characterisation of the role of an alkaline protease from Vibrio midae SY9 in enhancing the growth rate of cultured abalone fed a probiotic-supplemented feed. *Aquaculture*, *448*, 128–134.

Husain, F.; Duraisamy, S.; Balakrishnan, S.; Ranjith, S.; Chidambaram, P. and Kumarasamy, A. (2022): Phenotypic assessment of safety and probiotic potential of native isolates from marine fish Moolgarda seheli towards sustainable aquaculture. *Biologia*, *77*(3), 775–790.

Jahangiri, L. and Esteban, M.Á. (2018): Administration of probiotics in the water in finfish aquaculture systems: A review. *Fishes*, *3*(3), 1–13.

Kaktcham, P.M.; Temgoua, J.B.; Ngoufack Zambou, F.; Diaz-Ruiz, G.; Wacher, C. and Pérez-Chabela, M. De L. (2017): Quantitative analyses of the bacterial microbiota of rearing environment, tilapia and common carp cultured in earthen ponds and inhibitory activity of its lactic acid bacteria on fish spoilage and pathogenic bacteria. *World Journal of Microbiology and Biotechnology*, *33*(2), 1–12.

Kelany, N.; Abdel-Mohsein, H.; Kotb, S. and Ismail, A.E.M. (2024): Multiple antibiotic resistant Streptococcus agalactiae and Streptococcus iniae in Nile tilapia aquaculture. *Journal of Advanced Veterinary Research*, *14*(1), 180–186.

Kesarcodi-Watson, A.; Kaspar, H.; Lategan, M.J. and Gibson, L. (2008): Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*(1), 1–14.

Lara-Flores, M. and Olvera-Novoa, M.A. (2013): The use of lactic acid bacteria isolated from intestinal tract of Nile tilapia (Oreochromis niloticus), as growth promoters in fish fed low protein diets. *Latin American Journal of Aquatic Research*, *41*(3), 490–497.

Larsson, D.G.J. and Flach, C.F. (2022): Antibiotic resistance in the environment. *Nature Reviews Microbiology*, *20*(5), 257–269.

Lebeer, S.; Vanderleyden, J. and De Keersmaecker, S.C.J. (2008): Genes and Molecules of Lactobacilli Supporting Probiotic Action. *Microbiology and Molecular Biology Reviews*, *72*(4), 728–764.

Legario, F.S.; Choresca, C.H.; Turnbull, J.F. and Crumlish, M. (2020): Isolation and molecular characterization of streptococcal species recovered from clinical infections in farmed Nile tilapia (Oreochromis niloticus) in the Philippines. *Journal of Fish Diseases*, *43*(11), 1431–1442.

Maji, U.J.; Mohanty; S.; Mahapatra, A.S. and Maiti, N.K. (2016): Diversity and probiotic potentials of putative lactic

acid bacteria for application in freshwater aquaculture. *Turkish Journal of Fisheries and Aquatic Sciences*, *16*(4), 805–818.

Mohamed, S.G. and Saleh, W.D. (2010): Flavobacterium Columnare Infection in Cultured Oreochromis Niloticus. *Assiut Veterinary Medical Journal*, *56*(125), 1–16.

Muñoz-Atienza, E.; Gómez-Sala, B.; Araújo, C.; Campanero, C.; Del Campo, R.; Hernández, P.E.; Herranz, C. and Cintas, L.M. (2013): Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology*, *13*(1).

Muthukumar, P. and Kandeepan, C. (2015): Isolation, Identification and Characterization of Probiotic Organisms From Intestine of Fresh Water Fishes. *International Journal of Current Microbiology and Applied Sciences*, *4*(3), 607–616.

Peivasteh-Roudsari, L.; Pirhadi, M.; Karami, H.; Tajdar-oranj, B.; Molaee-Aghaee, E. and Sadighara, P. (2020): Probiotics and food safety: an evidence-based review. *Journal of Food Safety and Hygiene, January 2020*.

Poletto, T.V.; Vieira, C.R.W.; Silva, C.P. and Fracalossi, D.M. (2018): Isolation and Identification of a Potential Amylolytic Probiotic Bacterium from the Gut of Jundiá Catfish, Rhamdia quelen. *Brazilian Archives of Biology and Technology*, *61*, 1–9.

Rahman, M.; Rahman, M.; Monir, M.; Haque, M.; Siddique, M.; Khasruzzaman, A.; Rahman, M. and Islam, M. (2021): Isolation and molecular detection of Streptococcus agalactiae from popped eye disease of cultured Tilapia and Vietnamese koi fishes in Bangladesh. *Journal of Advanced Veterinary and Animal Research*, *8*(1), 1.

Razin, S. and Cirillo, V.P. (2012): Sugar fermentation. *Methods in Mycoplasmology*, *1*, 337–343.

Reda, R.M.; Selim, K.M.; El-Sayed, H.M. and El-Hady, M.A. (2018): In vitro selection and identification of potential probiotics isolated from the gastrointestinal Tract of Nile Tilapia, Oreochromis niloticus. *Probiotics and Antimicrobial Proteins*, *10*(4), 692–703.

Rifat-Al-Naser; Saifullah, Abdullah-Al-Mamun, M.; Saddam Hossain, M.; Arifuzzaman, M.; and Saikot, F. (2016): Probiotic Properties Profiling of Isolated Lactic Acid Bacteria from the Intestine of Channa punctata. *British Microbiology Research Journal*, *17*(4), 1–8.

Ringø, E. (2020): Probiotics in shellfish aquaculture. *Aquaculture and Fisheries*, *5*(1), 1–27.

Ringø, E.; Li, X.; Doan, H. van, and Ghosh, K. (2022): Interesting Probiotic Bacteria Other Than the More Widely Used Lactic Acid Bacteria and Bacilli in Finfish. Frontiers in Marine Science, 9(June), 1–27.

Saikot, F.K. (2016): Probiotic profiling of Lactobacillus spp . isolated from the intestine of Sperata seenghala and Labeo bata. *AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH*, *4*(5), 2321–3647.

Semedo, T.; Almeida Santos, M.; Silva Lopes, M.F.; Figueiredo Marques, J.J.; Barreto Crespo, M.T. and Tenreiro, R. (2003): Virulence factors in food, clinical and reference enterococci: A common trait in the genus? *Systematic and Applied Microbiology*, *26*(1), 13–22.

Shoemaker, C.A.; Xu, D.H.; Garcia, J.C. and Lafrentz, B.R. (2017): Capsular typing of Streptococcus agalactiae

(Lancefield group B streptococci) from fish using multiplex PCR and serotyping. *Bulletin of the European Association of Fish Pathologists*, *37*(5), 190–197.

Shokryazdan, P.; Sieo, C.C.; Kalavathy, R.; Liang, J.B.; Alitheen, N.B.; Faseleh Jahromi, M. and Ho, Y.W. (2014): Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. *BioMed Research International*, *2014*.

Siangpro, N.; Chuakrut, S.; Sirimanapong, W.; Tanasupawat, S.; Phongsopitanun, W.; Meksiriporn, B.; Boonnorat, J.; Sarin, S.; Kucharoenphaibul, S. and Jutakanoke, R. (2023): Lactiplantibacillus argentoratensis and Candida tropicalis Isolated from the Gastrointestinal Tract of Fish Exhibited Inhibitory Effects against Pathogenic Bacteria of Nile Tilapia. *Veterinary Sciences*, *10*(2), 1–20.

Sperandio, D.; Rossignol, G.; Guerillon, J.; Connil, N.; Orange, N.; Feuilloley, M.G.J. and Merieau, A. (2010): Cell-associated hemolysis activity in the clinical strain of Pseudomonas fluorescens MFN1032. *BMC Microbiology*, *10*, 124.

Sun, J.; Fang, W.; Ke, B.; He, D.; Liang, Y.; Ning, D.; Tan, H.; Peng, H.; Wang, Y.; Ma, Y.; Ke, C. and Deng, X. (2016): Inapparent Streptococcus agalactiae infection in adult/commercial tilapia. *Scientific Reports*, *6*, 1–11.

Taukhid, Lusiastuti, A.M.; Andriyanto, S.; Sugiani, D.; Sumiati, T. and Suhermanto, A. (2021): Efficacy of inactive bivalent and trivalent Streptococcus agalactiae bacteria (biotype 1 and 2) vaccines on tilapia, Oreochromis niloticus. *E3S Web of Conferences*, *322*, 1–10.

Thirunavukkarasu, R.; Pandi, P.; Balaraman, D.; Albalawi, F.; Ahmad; N.; Panagal; M.; Nageswara Rao; T.; Subramanian; K.; George; E.G.J.; Rajan; M.S.A.; Renuga; P.S.; Aruni; W. and AlOmar; S.Y. (2022): Influence of extracellular protein isolated from fish gut associated bacteria as an enhancer of growth and innate immune system in Mugil cephalus. *Scientific Reports; 12*(1)*;* 1–20.

Vlková; E.; Kalous; L.; Bunešová; V.; Rylková; K.; Světlíková; R. and Rada; V. (2012): Occurrence of bifidobacteria and lactobacilli in digestive tract of some freshwater fishes. *Biologia; 67*(2), 411–416.

Vos; P.; Garrity; G.; Jones; D.; Krieg; N.R.; Ludwig; W.; Rainey; F.A.; Schleifer; K.-H. and Whitman; W.B. (2011): Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes (Vol. 3). Springer Science and Business Media.

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;* 1–14.

Yang; C.; Jiang; M.; Lu; X. and Wen; H. (2021): Effects of dietary protein level on the gut microbiome and nutrient metabolism in tilapia (Oreochromis niloticus). *Animals; 11*(4).

Zhang; Z.; Mao; L.; Qin; Y.; Zhao; L.; Huang; L.; Xu; X. and Yan; Q. (2022): Comparative transcriptome analysis revealed the role and mechanism of a FeoC-like LuxR-type regulator in intracellular survival of Aeromonas hydrophila. *Aquaculture; 556* (February), 738287.

النشاط المثبط لبكتيريا حمض اللاكتيك المعزوله من أمعاء البلطي ضد بكتريا العقدية *Streptococcus agalactiae*

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استزراع سمك البلطى يوفر للانسان مصدر بروتين رخيص و ذو قيمه عاليه. و لكن تكديس الاستزراع يعرض الاسماك للاصابه بمرض بكتريا العقديه المقيحه. ظهور مقاومه البكتريا ضد المضادات الحيويه; ادى الى الحاجه لتقييم كفاءه بكتريا البروبايوتك لعلاج هذه البكتريا. فى هذه الدراسه; تراوح متوسط عدد بكتريا البروبايوتك ما بين 5.67 و5.78 لو١٠ بكتريا لكل ا جم من امعاء سمك البلطى. استخدامت الاختيبارات المناسبه للكشف عن ببكتريا حمض اللاكتيك ذات خواص البروبايوتك; وتم التعرف على سبع عزلات لهم تاثير مثبط ضد بكتريا العنقديه *Streptococcus agalactiae* بمنطفه تثبيط تراوح قطرها من ٢٦- ٥٠ ملليمتر. كل العزلات المعرفه لم يتفاعلوا ضد كريات الدم فى اختبار التخثر; و اظهروا القدره على انتاج انزيم الاميليز و الليبيز. كذلك قاوموا ظروف حامضيه المعده و العصاره الصفراويه الموجوده فى الامعاء. ان هذه الصفات التى اظهرتها العزلات المعرفه; يعطى انطباعا عن امكانيه استخدام بكتريا البروبايوتك المعزوله من الاسماك كبديل جيد للمضادات الحيويه لمجابهة هذه العدوى.