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INVESTIGATION OF THE POTENTIAL UTILIZATIONS OF HUMAN MILK ORIGINATED *Lactobacillus gasseri* STRAINS IN AQUACULTURE AND SEAFOOD PRODUCTION

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ABSTRACT

In the current study, five *Lactobacillus gasseri* MA strains from human breast milk were investigated for their usage potentials in aquaculture and seafood products as probiotic and bio-protective. The strains were tested for their susceptibilities to various clinical antibiotics used in fish cultivation, antimicrobial activities against seven fish pathogens, alpha-amylase enzyme activities, and susceptibilities to sodium benzoate which used as a food preservative. The strains were susceptible to Ampicillin, Amoxicillin and Erythromycin antibiotics. Antimicrobial activity test results indicated that the inhibition zone diameters were ranged between 1.66 mm and 11.22 mm against various pathogens originated fish. The highest antimicrobial activity was recorded against the *Aeromonas hydrophila* ATCC 19570 (11.22 mm) for the MA-2 strain, while the lowest antimicrobial activity was determined against *Vibrio anguillarum* A4 (1.66 mm) for the MA-6 strain. *L. gasseri* MA-5, MA-3 and MA-6 showed alpha-amylase activity. The spectrophotometric and live-cell count data showed that all the strains exhibited resistance to sodium benzoate. The results suggest that *L. gasseri* MA strains could be good sources of probiotic for aquaculture and bio-protectives in seafood products.

Keywords: Alpha-amylase, Antibiotic, Antimicrobial, Fish pathogen, Sodium benzoate

INTRODUCTION

Recently, marine organisms are cultivated in very high populations, beyond the capacities of growing facilities (ponds, lakes etc.) to meet the increasing nutritional demands of growing human population and for more economic gain. However, this situation causes the cultured fish to be affected by many factors such as stress, water pollution, temperature, infectious diseases caused by microorganisms **(Lakra and Ayyappan, 2003)**. These problems cause massive fish mortality and major economic losses. Antibiotics, various chemotherapeutics and vaccines are mainly used for coping with bacterial infections in aquaculture **(Vaseeharan and Thaya, 2014)**. In particular, this widespread use of antibiotics leads to resistance in pathogenic strains, as well as the accumulation of antibiotic residues in fish. Ultimately, the consumption of these aquatic products caused the transmission of antibiotic residues to consumers. This can cause many health problems including *antibiotic*-resistant infections and, allergic reactions in humans **(Cháfer-Pericás *et al.*, 2010)**. For this reason, in aquaculture, the utilization of alternative natural preservatives such as plant fatty acids and extracts **(Gunyakti *et al.*, 2017)**, enzymes and probiotic cultures has recently gained attention. Among others, probiotic microorganisms are more environmentally friendly due to their non-toxic and non-pathogenic nature and are more preferred due to their contribution to growth and development by supporting the immune system of fish **(Aly *et al.*, 2008)**. Also, applying microbial enzymes in feed formulations not only improves digestion and feed utilization but also develops environmentally friendly and sustainable aquaculture **(Ghosh and Mukhopadhyay, 2006)**.

According to the definition of Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are living microorganisms that have therapeutic effects on host health when taken in sufficient quantities **(Grajek *et al.*, 2005)**. Many *Lactobacillus* strains have been characterized as probiotics and the metabolites of these strains with antimicrobial properties are utilized as bio-control agents for balancing aquatic habitats and the microbial flora of fish gastrointestinal tract **(Ayoola *et al.*, 2013)**. Fish microflora depends on the habitat, but the gastrointestinal microbiota of fish larvae can change very rapidly with the nutrition of beneficial microorganisms **(Ng *et al.*, 2000)**. For this reason, functional feeds that are enriched with probiotic microorganisms are very important **(Dagá *et al.*, 2013)**. Besides, lactic acid bacteria and various microbial enzymes such as phytase, amylase, lipase, glucanase and protease are also used as single or in combinations as the fish feed additives. Probiotic bacteria that produce enzymes such as amylase, lipase and protease help to regulate the digestive system of fish and produce various vitamins **(De *et al.*, 2014; Ghosh *et al.*, 2007)**. Also, various lactic acid bacteria have been used as an alternative to artificial preservatives to obtain and store fermented fish products **(Kopermsub and Yunchalard, 2010)**. However, some chemical additives are widely used to protect frozen seafood products from microbial spoilage. The most commonly used among these synthetic preservatives is sodium benzoate (SB, Food Additive European Code E211) **(Hussain *et al.*, 2011)**. SB is the salt of the benzoic acid and is used as a preservative (0.1-0.2%) to inhibit the growth of some bacterial strains, mould and yeast in food industries.

On the other hand, *Lactobacillus gasseri* is a homofermentative and thermophilic probiotic bacteria and is one of the best known 50 *Lactobacillus* species **(Kim and Rajagopal, 2001;Kawase *et al.*, 2011)**. Previous studies have generally investigated the potential use of fish gut originated lactic acid bacteria in aquaculture. Up to now, several studies have reported the antimicrobial activities and bacteriocin production from *L. gasseri* strains of different origins **(Gunyakti and Asan-Ozusaglam, 2018; Phukan *et al.*, 2018; Quigley *et al.*, 2019; Zhu *et al.*, 2000)**. However, few published data focusing on the potential usage of these strains as probiotic agents in aquaculture.

For this reason, the current study was aimed to determine the potential usage of *L. gasseri* MA strains isolated from human milk (a safety source) as probiotic in fish culture and as bio preservatives in seafood products. The antimicrobial activity of the selected strains was investigated against seven fish pathogens such as *Vibrio alginolyticus*, *Vibrio anguillarum* M1, *Vibrio anguillarum* A4, *Aeromonas hydrophila* ATCC 19570, *Lactococcus garvieae*, *Yersinia ruckeri*, *Streptococcus agalactiae* Pas. Inst. 55118. Also, the antibiotic resistances, alpha-amylase enzyme activities and sodium benzoate (used in various fish and other canned products) resistances of five *L. gasseri* MA strains were measured.

MATERIAL AND METHODS

Bacterial strains and culture conditions

Five *L. gasseri* MA strains from human milk were genetically identified by PCR-based molecular identification method (NCBI strain number:ATCC 33323 = JCM 1131). *L. gasseri* MA strains were cultured in MRS liquid/solid media and stored at 4°C in glycerol stocks for further use.

Determination of antibiotic sensitivity of MA strains

Antibiotic resistance of the strains was determined by a previously reported method by **Sharma *et al.* (2016)**. The resistance of *L. gasseri* MA strains to commercial antibiotics used in the treatment of fish diseases i.e., Erythromycin (E, 15 µg), Gentamycin (CN, 10 µg), Kanamycin (K, 30 µg), Ampicillin (AMP, 10 µg), Amoxicillin (AMC, 30 µg) was determined by disc diffusion method. Briefly, the cell densities of active *L. gasseri* MA strains were adjusted to Mc Farland 0.5 in a physiological saline solution (PSS: 0.865 g NaCl). *L. gasseri* strains were then inoculated on MRS agar medium (100 µL) and spread with the sterile drigalski spatula. Each antibiotic disc was placed on the MRS agar with three replicates and then left to incubate for 24 h at 37°C. At the end of the incubation period, the inhibition zone diameters around the antibiotic discs were measured with Vernier calliper and the results were evaluated according to CLSI (Clinical and Laboratory Standards Institute) 2012 standards.

Determination of antimicrobial activity

The antimicrobial activity of the strains against various fish pathogens was determined according to the method described by **Drago *et al.* (1997)**.

Preparation of test microorganisms

For the current study, seven fish pathogens (*Vibrio alginolyticus, Vibrio anguillarum* M1, *Vibrio anguillarum* A4, *Aeromonas hydrophila* ATCC 19570, *Lactococcus garvieae, Yersinia ruckeri, Streptococcus agalactiae* Pas. Inst. 55118) were used as test microorganisms. Test microorganisms were activated twice and adjusted to McFarland 0.5 standard.

Preparation of L. gasseri MA supernatant

For the determination of antimicrobial activity, *L. gasseri* MA strains were centrifuged at 5000 rpm using a Universal 320 R centrifuge for 15 minutes and the supernatants were sterilized with 0.2 µm pore size microfilters. These sterilized supernatants were stored at 4 °C.

Agar well diffusion assay

In well diffusion assay, 100 µL of pathogen test microorganisms were inoculated onto the specific soft agar mediums and wells (7 mm) were made in triplicates. 100 µL of *L. gasseri* MA supernatants were loaded into the wells and then allowed to incubate for 24 h at appropriate temperatures for growth of each pathogenic microorganism. At the end of the incubation period, the inhibition zone values around the wells were measured with Vernier calliper and the results are given as averages of triplicates.

Determination of alpha-amylase enzyme activity

The alpha-amylase enzyme activity of the MA strains was determined using the method reported by **Gupta *et al.* (2003)** with some slight modifications. Alpha-amylase enzyme activity was determined using supernatants of *L. gasseri* MA strains on nutrient agar medium containing 0.5% soluble starch (w/v) with spot culture method. For this purpose, the twice-activated cultures were centrifuged at 9000 rpm for 15 minutes. The supernatants were dropped on a nutrient agar medium at 20 and 50 µL in two different concentrations and allowed to incubate at 37 °C for 30 min. At the end of the incubation period, the media was stained with iodine and clear zones were appeared around positive colonies.

Sodium benzoate resistance

The sodium benzoate resistance of *L. gasseri* MA strains was determined using the method reported by **Gunyakti and Asan-Ozusaglam (2019)**. The MA strains cultures adjusted to Mc Farland 0.5 standard were inoculated as 1% to experimental and control groups with ten different concentrations (0.015-1%) of sodium benzoate. The tubes were then allowed to incubate at 37 °C for 24 h. At the end of the incubation period, optical densities (OD) were measured spectrophotometrically at 600 nm. Besides, acid production abilities of strains were determined on sodium benzoate containing (at ten different concentrations) media. MRS containing sodium benzoate at a concentration of 0.1% was used to determine live cell count. After dilution, the culture was inoculated on the MRS-agar medium in two replicates and then incubated at 37° C for 24 h under anaerobic conditions.

Statistical analysis

Statistical analysis was performed with the Mann-Whitney U-test to identify significant differences in antimicrobial activity and sodium benzoate resistance assay results. The differences were considered significant at a p-value of <0.05. The statistical analyses were conducted using SPSS version 22 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Determination of antibiotic sensitivity of MA strains

As previously mentioned, resistance characteristics of MA strains to various commercial antibiotics were determined according to the disk diffusion method. Lactic acid bacteria with inhibition zone diameter as greater than 20 mm, 14-19 mm and below 14 mm were considered as sensitive, moderately resistant and fully resistant, respectively according to CLSI 2012 standards (Table 1). All the *L. gasseri* MA strains were observed resistant to Gentamycin, Kanamycin and sensitive against Ampicillin, Amoxicillin, Erythromycin antibiotics. Among the strains, the highest sensitivity was observed for MA-3 strains against erythromycin antibiotics (30.60 mm). The highest resistance was observed for all the tested lactic acid bacteria against Kanamycin (no inhibition) and for MA-3 and MA-6 strains against Gentamicin (no inhibition) antibiotics.

Table 1 Antibiotic susceptibilities of *L. gasseri*MA strains

***L. gasseri* strains**	**Antibiotics[a]**				
	AM (10 µg)	**AMC (30 µg)**	**CN (10 µg)**	**E (15 µg)**	**K (30 µg)**
MA-1	S	S	R	S	R
MA-2	S	S	R	S	R
MA-3	S	S	R	S	R
MA-5	S	S	R	S	R
MA-6	S	S	R	S	R

[a]CLSI, Clinical and Laboratory Standards Institute. The inhibition zones are evaluated according to the standard values given by CLSI. Susceptible >20, Intermediate % 15–19, Resistant < 14 (CLSI, 2012)
AM: Ampicillin, AMC: Amoxicillin, CN: Gentamicin, E: Erythromycin, K: Kanamycin, R: Resistant, S: Sensitive

In a previous study, the antibiotic resistance properties of two *L. gasseri* strains from human breast milk were observed as resistant to Gentamycin and Kanamycin while susceptible to Ampicillin and Erythromycin antibiotics **(Martín *et al.*, 2005)**. In another study, *L. gasseri* NLRI-312 strain isolated from feces of newborn infants was determined as resistant to Kanamycin while susceptible to Erythromycin antibiotics **(Kim *et al.*, 2006)**. Some sources have been reported trouble that lactic acid bacteria used as probiotic or starter cultures can transfer antibiotic resistance genes to other lactic acid bacteria or pathogenic microorganisms. The results of the present study are similar to the above reports which cleared that *L. gasseri* MA strains exhibit resistance profiles to some clinically tested antibiotics. However, it has been also reported that probiotic microorganisms contain antibiotic resistance genes in genomic structures, structural resistance (chromosomal resistance) do not constitute a safety concern on their own as long as these genes are not transferred by horizontal transfer **(Darsanaki *et al.*, 2013)**.

Determination antimicrobial activity

Lactic acid bacteria inhibit the development of many enteric pathogens and play an important role in the treatment of gastrointestinal disorders in humans and animals **(Fernández *et al.*, 2003)**. Therefore, the inhibition of pathogenic microorganisms is expected from a probiotic strain by secreting various antimicrobial substances. In this study, antimicrobial activities of *L. gasseri* MA strains against fish pathogens were determined by well diffusion method (Table 2). The inhibition zone diameters for MA strains against the seven fish pathogens varied from 1.66 mm to 11.22 mm. The highest antimicrobial activity was determined for the MA-2 strain against *A. hydrophila* ATCC 19570 while the lowest activity was determined against the *V. anguillarum* A4 for MA-6 strain. Among the tested strains, only MA-1 strain did not show any inhibitory activity against the only *Y. ruckeri*. There are average differences between the obtained data of antimicrobial activity among *L. gasseri* MA strains. However, the statistical analysis indicated that there are no significant difference between these results (p>0.05). The probable cause is that all the bacteria tested are different strains but the same bacteria (*L. gasseri*).

Table 2 Antimicrobial activities of *L. gasseri*MA strains

Test Microorganisms	Inhibition zone diameter (mm)								
	L. gasseri strains (Mean ±standard deviation)					Antibiotics (Mean ±standard deviation)			
	MA-1	**MA-2**	**MA-3**	**MA-5**	**MA-6**	**AM (10 µg)**	**CN (10 µg)**	**E (15 µg)**	**K (30 µg)**
***A. hydrophila* ATCC 19570**	5.47±0.50[a]	11.22±1.11	10.31±1.46	7.32±0.93	6.29±0.77	17.89±1.34	13.38±0.84	26.94±2.31	13.23±0.52
V.alginolyticus	5.91±0.99	4.99±0.31	5.51±0.43	4.55±0.58	7.03±0.42	16.95±1.11	18.27±1.47	20.99±2.04	8.74±0.65
***V.anguillarum* M1**	4.18±0.00	3.51±0.25	3.07±0.20	2.67±0.49	3.05±0.22	15.9±1.17	18.30±0.30	17.97±0.31	6.91±0.20
***V.anguillarum* A4**	3.02±0.47	3.37±0.73	2.39±0.42	3.15±0.27	1.66±0.21	17.52±0.58	9.33±0.01	17.53±1.04	8.72±.28
L. garvieae	5.18±0.47	3.50±0.28	4.63±0.03	3.42±0.44	3.97±0.22	16.40±0.12	17.30±0.95	31.08±1.01	10.42±0.27
Y. ruckeri	-[b]	4.48±0.82	3.92±0.09	4.14±0.20	4.90±0.43	18.92±0.88	-	11.37±0.83	12.37±0.41
***S. agalactiae* Pas. Inst. 55118**	4.27±0.83	4.73±0.51	3.99±0.59	6.93±0.72	5.31±0.38	35.34±3.40	-	30.28±1.68	16.25±1.18

AM: Ampicillin, CN: Gentamicin, E: Erythromycin, K: Kanamycin

[a] Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of three separate experiments.

[b] Indicates no antimicrobial activity.

Until now, there are quite a limited number of studies in the literature regarding the activity of *L. gasseri* strains against fish pathogens **(Sahoo *et al.*, 2015)**. In a previous study, **Pirarat *et al.* (2009)** investigated the antimicrobial activity of human-induced *Lactobacillus rhamnosus* ATCC 53103 strain against some fish and frog pathogens by disc diffusion and agar spot methods. *L. rhamnosus* strain was found to have antimicrobial activity against all of the test pathogens (*A. hydrophila, S. agalactiae, Streptococcus iniae, Chryseobacterium indologenes* and *Edwardsiella tarda*). In another study, the antimicrobial activity of *Bacillus subtilis* UTM 126 against *V. alginolyticus* reported with 10-15 mm inhibition zone diameter **(Balcázar *et al.*, 2008;Balcázar and Rojas-Luna 2007)**. In this study, *L. gasseri* MA-2 strain showed inhibitory activity against *V. alginolyticus* with *zone diameters* of 7.03 mm. **Balcázar *et al.* (2008)** tested the antimicrobial potential of different fish originated probiotics such as *Lactococcus lactis* CLFP 101, *L. fermentum* CLFP 24 and *L. plantarum* CLFP 238 strains against *V. anguillarum, A. hydrophila, Y. ruckeri* fish pathogens. The results of the study revealed that *L. fermentum* CLFP 24 and *L. plantarum* CLFP 238 strains exhibited no antimicrobial activity while *L. lactis* CLFP 101 strain showed activity against all the tested fish pathogens. In the current study, human breast milk originated *L. gasseri* MA strains showed inhibitory activity against all the tested fish pathogens except for MA-1 against the only *Y. ruckeri* pathogen. In another report by **Zhou *et al.* (2010)**, the inhibitory effect of *L. lactis* RQ516 on *A. hydrophila* fish pathogen was recorded with 14.77 mm inhibition zone diameter. Considering this report, in our study, *L. gasseri* MA-2 strain showed a bit lower inhibitory activity (11.22 mm) against *A. hydrophila* pathogen than *L. lactis* strain RQ516.

Vibrio species, *Aeromonas* species and *S. agalactiae* are important pathogens, responsible for causing pathologic outbreaks and high rate mortalities in aquaculture **(Wang *et al.*, 2008; Evans *et al.*, 2006)**. The consumption of *A. hydrophila* from contaminated water and food has been reported in a variety of studies which resulted in adverse effects on human health **(Swift *et al.*, 1997)**. Lactococcosis caused by *L. garviae* and yersiniosis caused by *Y. ruckeri* make a major economic loss in the fish breeding industry **(Vendrell *et al.*, 2006; Tobback *et al.*, 2007)**. In the current study, antimicrobial activity was recorded for *L. gasseri* MA strains against vital pathogens such as *Vibrio spp.* (except MA-1), *A. hydrophila, Y. ruckeri, L. garviae, S. agalactiae* Pas. Inst. 55118. For this reason, the use of *L. gasseri* MA strains in aquaculture may reduce or prevent epidemic diseases caused by pathogenic microorganisms. *L. gasseri* MA strains with antimicrobial activity may be recommended as a bio-preservative agent in aquaculture, seafood and canned fish products. The in-vitro results of the current study will also provide a basis for in-vivo studies.

Determination of alpha-amylase enzyme activity

The alpha-amylase is one of the most important industrial enzymes that cleave the alpha-1,4-glucosidic linkages of starch and produces different products such as glucose, maltose and maltotriose units **(Gupta *et al.*, 2003)**. Amylases and many other exogenous enzymes have been reported to be widely used as fish feed supplements. In the present study, alpha-amylase activity was observed for MA-5 (with 20 µL of *L. gasseri* supernatant) and MA-3, MA-5, MA-6 (with 50 µL of *L. gasseri* supernatant) (Figure 1). However, MA-1 and MA-2 strains showed no activity at both concentrations. **Carter *et al.* (1992)** observed that the alpha-amylase enzyme used as dietary supplement affects positively the growth and development of fish. Amylases from bacteria promote starch digestion in the intestine of *Cyprinus carpio* (common carp) **(Fusheng *et al.*, 1994)**. The diets contain additional exogenous α-amylase enzyme might raise the enzyme activity of the fish and improve feed digestibility **(Ji *et al.*, 2012)**. However, the purification of enzymes from the microbial sources could be expensive. Therefore, the usage of bacterial strains producing enzymes in feeding is a more economic way. Although most lactic acid bacteria do not have amylolytic enzymes, a limited number of lactic acid bacteria can produce alpha-amylase **(Asoodeh *et al.*, 2010)**. **Ijeoma *et al.* (2015)** reported amylase activity for *Lactobacillus curvatus, Lactobacillus plantarum, Lactococcus lactis* subsp. *lactis, Lactobacillus fermentum* and *Lactobacillus plantarum* subsp. *plantarum* strains, while no activity for *Weissella cibaria, Enterococcus faecalis, Enterococcus lactis, Leuconostoc pseudomesenteroides, Leuconostoc mesenteroides* and *Leuconostoc mesenteroides* subsp. *dextranicum* strains. To our knowledge, the number of *L. gasseri* strains produce amylase enzyme is very limited **(Oh *et al.*, 2015)**. This study will give a biotechnological perspective to the MA strains. Because of this property, the MA strains that produce amylase enzyme can be a new source for the feed industry and enzyme technology.

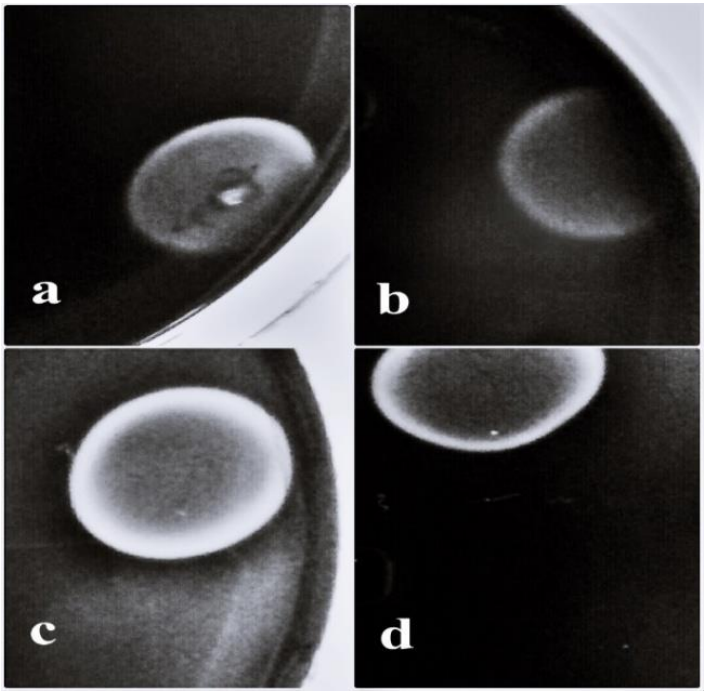

Figure 1 The alpha-amylase activity of *L. gasseri* MA strains

a: MA-3 (50 µL of supernatant)
b: MA-5 (20 µL of supernatant)
c: MA-5 (50 µL of supernatant)
d: MA-6 (50 µL of supernatant)

Determination of sodium benzoate resistance

Sodium benzoate is an artificial food additive used as a preservative in food and beverage industries against bacteria, fungi, and yeast (**Chen *et al.*, 2009)**. SB can be used in some seafood products or ice used in fish conservation **(Zhang and Ma, 2013)**. Probiotic microorganisms naturally present in or added later to food products should be tolerant to SB. For this reason, this study can be crucial in determining the effects of SB during the applications of MA strains as a natural food additive. The development of MA strains in the sodium benzoate medium (OD) was generally reduced compared to the control group (except for 0.075% and 0.1 mg/mL SB concentrations), but the strains never lost their viability (Table 3). There are mean differences between the observational data of spectrophotometric results of *L. gasseri* MA strains. The statistical analysis also indicated that there are significant differences between *L. gasseri* MA strains (p|<0.05). Even, the average differences between MA 1 and MA-4, MA-1 and MA 5, MA 2 and MA-5 and MA-5 and MA-6 at various SB concentrations are statistically different at the

significance level of 0.05 (p|<0.05). The tested MA strains originated from human milk are the same bacteria (*L. gasseri*) but different strains. Therefore, the strains showed different resistance activities against various SB concentrations.

The pH values of the MA strains after culturing in media contained different concentrations of SB (Table 4) showed decreases compared to the initial pH values. This indicates that the strains survive throughout the incubation period and that initial pH values decreased with the production of various organic acids. However, MA-2 and MA-3 strains were found to have a value close to their initial pH values in a sodium benzoate medium with a concentration of 1 mg/mL. As the application rate of sodium benzoate as a food preservative in various food industries is generally 0.1%, live-cell counts of strains have been evaluated at this concentration. The number of viable cells of MA strains was found to be in the range of 10.78-6.85 Log CFU/mL (Table 5). The viable cell counts of four *L. gasseri* strains (MA-1, MA-2, MA-3, MA-6) showed bit decreases when compared to the control group. However, it was observed that MA-5 showedincreased growth and this result was following the spectrophotometric data. Up to now, no study has been reported on the resistance properties of *L. gasseri* strains to sodium benzoate. Considering the results obtained in this study, it was determined that all MA strains were resistant to 0.1% mg/mL SB, which is generally used concentration in food products.

Table 3 The resistance properties of *L. gasseri* MA strains against sodium benzoate (spectrophotometric)

Sodium benzoate concentrations (mg/mL)	*L. gasseri* strains				
	MA-1	**MA-2**	**MA-3**	**MA-5**	**MA-6**
Control	0.068±0.00	1.54±0.00	1.58±0.00	1.88±0.00	1.67±0.00
0.015	0.67±0.14	1.22±0.06	1.08±0.29	1.59±0.12	1.58±0.10
0.025	0.47±0.09	0.46±0.03	1.44±0.21	1.76±0.03	0.48±0.00
0.050	0.60±0.05	1.51±0.01	0.63±0.05	0.59±0.05	0.60±0.06
0.075	1.64±0.16	1.46±0.01	1.62±0.02	1.74±0.01	1.28±0.01
0.1	1.74±0.02	1.36±0.00	1.54±0.06	1.89±0.02	1.67±0.05
0.2	0.47±0.02	0.53±0.05	0.52±0.00	1.28±0.06	1.18±0.21
0.3	0.37±0.00	0.38±0.03	0.42±0.03	0.41±0.03	0.40±0.02
0.4	0.26±0.06	0.84±0.07	0.55±0.06	0.56±0.05	0.56±0.02
0.5	0.55±0.00	0.38±0.02	0.52±0.82	0.54±0.85	0.49±0.59
1	0.49±0.15	0.21±0.06	0.06±0.01	0.61±0.02	0.09±0.04

Sodium benzoate is used as preservatives in the food industry in a pH range below 4.5. The initial pH after the inoculation of the MA strains in the growth medium was nearly 5.6 (growth medium pH). After incubation, the pH of the growth medium was generally decreased under pH 4.5 which is below the pH limit at which sodium benzoate is active (Table 4).

Table 4 The pH value of *L. gasseri* MA strains after incubation in the medium containing sodium benzoate

% Sodium benzoate (mg/mL)	Initial pH	*L. gasseri* strains				
		MA-1	**MA-2**	**MA-3**	**MA-5**	**MA-6**
0.015	5.619	3.8	3.8	3.7	3.7	3.7
0.025	5.581	3.9	3.9	3.9	3.9	3.8
0.050	5.628	3.9	4.0	3.7	3.8	3.7
0.075	5.638	3.9	4.0	3.9	4.0	3.9
0.1	5.639	3.9	3.9	3.8	3.8	3.8
0.2	5.677	4.2	4.4	4.1	4.1	4.1
0.3	5.605	4.1	4.1	3.9	3.9	3.9
0.4	5.637	4.3	4.8	4.4	4.3	4.3
0.5	5.640	4.4	5	4.7	4.3	4.4
1	5.702	4.9	5.5	5.5	4.8	4.5

The spectrophotometric data showed that the strains did not lose their viability after incubation (below pH 4.5). Besides, the viable cell results slightly decreased as compared with the control group at the application concentration of sodium benzoate (0.1%) in the food industry, suggesting that these strains survived under pH 4.5 at which sodium benzoate was active (Table 5).

Table 5 Theviability of *L. gasseri*MA strains against sodium benzoate (Log CFU/mL)

L. gasseri strains	Control Group	0.1 mg/mL Sodium Benzoate
MA-1	9.52	7.94
MA-2	9.33	6.85
MA-3	8.52	8.12
MA-5	8.93	10.26
MA-6	8.45	8.28

As a result, when SB as a food preservative and MA strains as probiotic are used together in the food industry, these strains have a potential to be used as natural bio-preservatives against pathogen microorganisms as well as probiotic effects for the food industry (including marinated fish, fish sauces, salad dressings, etc.) since they do not lose their viability.

CONCLUSION

In this study, the usage potential of human milk origin *L. gasseri* MA strains in aquaculture and various seafood products was investigated. The results of this study demonstrate that *L. gasseri* MA strains can be good candidates for use as fish probiotics since these strains have a susceptibility to various clinical antibiotics in addition to their capacity to produce antimicrobial compounds against seven fish pathogens responsible for high morbidity and mortality in aquaculture. The results of this study also revealed that *L. gasseri* MA strains can be suggested as an alternative bio-preservative for chemical preservatives to limit the microbial-induced diseases in fish farming. It is also suggested that strains having positive alpha-amylase enzyme activity may be used as feed supplementation to enhance nutritional value in the fish feed industry and to increase fish feed utilization. Additionally, all the strains were found resistant to sodium benzoate applied at different concentrations. Since strains are resistant to sodium benzoate, which is used as a preservative for canned fish or fish products, it may be advisable to use *L. gasseri* MA strains as alternative preservatives alone or in combination with sodium benzoate in such foods materials.

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