Research Article **Open Access**

Functional Foods in Health and Disease

Biofilm formation and auto-aggregation abilities of novel targeted aqua-probiotics

Anahit Manvelyan[1,2], Marine Balayan[1,2], Shakhlo Miralimova[3], Vladimir Chistyakov[4] and Astghik Pepoyan[1,2*]

[1]Department of Food Safety and Biotechnology, Armenian National Agrarian University, 0009, Yerevan, Armenia; [2]International Association for Human and Animals Health Improvement, 0037, Yerevan, Armenia; [3]Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent 100125, Uzbekistan; [4]Center for Agrobiotechnology, Don State Technical University, 344002 Rostov-on-Don, Russia

***Corresponding author:** Astghik Pepoyan, D.Sc., Department of food safety and biotechnology, Armenian National Agrarian University; 0009, Yerevan, Armenia

Submission Date: March 2nd, 2023; **Acceptance Date:** April 3rd, 2023; **Publication Date:** April 4th, 2023

Please cite this article as: Manvelyan A., Balayan M., Miralimova S., Chistyakov V., Pepoyan A. Biofilm formation and auto-aggregation abilities of novel targeted aqua-probiotics. *Functional Foods in Health and Disease* 2023; 13(4): 179-190. DOI: https://www.doi.org/10.31989/ffhd.v13i4.1093

ABSTRACT

Background: The probiotics' auto-aggregation and biofilm formation abilities have a significant role in the development of biotechnological processes.

Objective: The aim of this study was to evaluate the biofilm formation and auto-aggregation abilities of novel, targeted aqua-probiotics isolated from aquatic organisms.

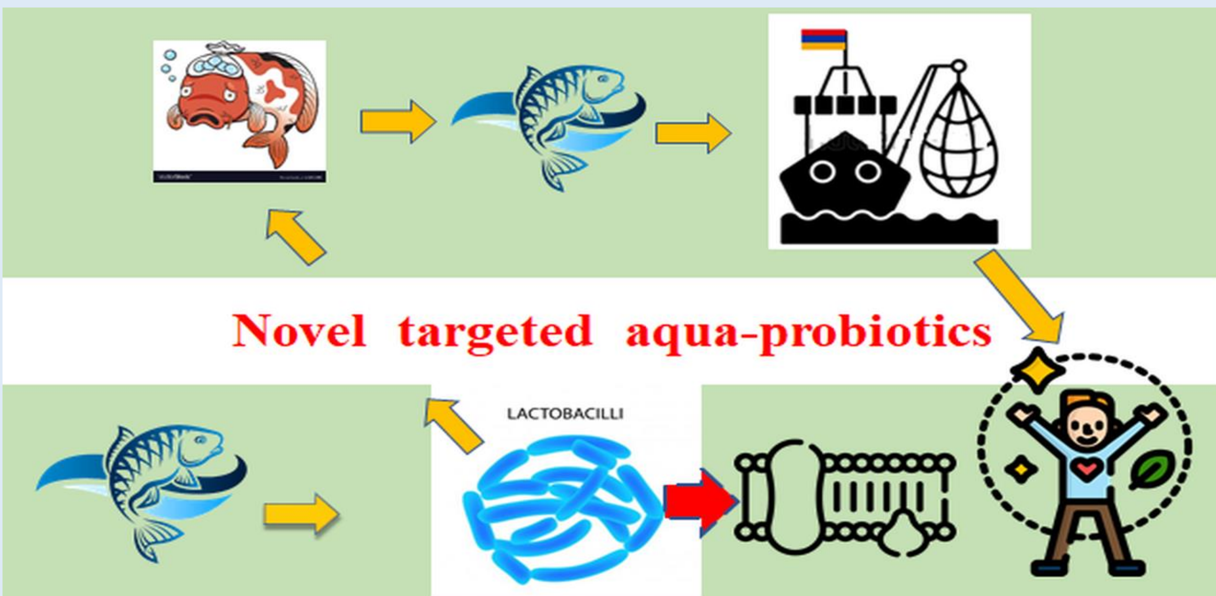
Methods: The biofilm formation abilities of *Lactobacillus delbrueckii str.* UZ-1*, Lactiplantibacillus plantarum* str. R3, *Lactococcus* str. UZ-2*, Enterococcus faecium* str. R2*, Pediococcus acidilactici* str. N from the culture collection of the Microbiology of the Academy of Sciences of the Republic of Uzbekistan, *Bacillus subtilis* str. 1R*, Bacillus amyloliquefaciens* str. 4R and from the culture collection of the Southern Federal University of Russa and *Lacticaseibacillus rhamnosus* str. 1A and *Enterococcus* str. 9-3 from the culture collection of the Armenian National Agrarian University were assessed.

Results: According to the investigations, the biofilm formation abilities of *Lactobacillus delbrueckii* str. UZ-1*, Lactiplantibacillus plantarum* str. R3, *Lactococcus* str. UZ-2*, Enterococcus faecium* str. R2*, Pediococcus acidilactici* str.

N, *Bacillus subtilis* str. 1R, *Bacillus amyloliquefaciens* str. 4R, *Bacillus amyloliquefaciens* str. 5R, *Lacticaseibacillus rhamnosus* str. 1A and *Enterococcus* str. 9-3 were 0.119 ± 0.05D, 0.113 ± 0.065D, 0.196 ± 0.04D, 0.116 ± 0.01D, 0.152 ± 0.05D, 0.74 ± 0.15D, 2.621± 0.55D, 1.831 ± 0.45D, and 0.227 ± 0.04D and 0.483 ± 0.15D respectively. The highest rate of auto-aggregation was shown by *Bacillus amyloliquefaciens* str. 5R, and *Bacillus amyloliquefaciens* str. 4R was the strain with the highest ability to form biofilm. These two *Bacillus* strains are also distinguished by the highest DNA protective properties and relatively low antioxidant activity. Despite the fact that *Bacillus amyloliquefaciens* str. 5R showed the highest rate of auto-aggregation after 2 hours, this strain showed the lowest level of auto-aggregation among the studied strains after 24 hours. The *Enterococcus* str. 9-3 strain with the highest antioxidant activity showed 0.483 ± 0.15D biofilm formation ability.

Conclusion: The novel targeted aquaprobiotics have distinct biofilm formation and aggregation properties, which are important to consider when planning appropriate biotechnological processes, requiring specific membrane properties of probiotics.

Keywords: Lactobacilli, aqua-probiotic, antioxidant activity, biofilm formation, aggregation, *Enterococcus* str. 9-3



Graphical Abstract: Membrane properties of novel targeted aquaprobiotics.

INTRODUCTION

Food-related problems span the environmental, social and economic sectors, with resource scarcity, ecosystem degradation and climate change at the center of these challenges [1-7]. At present, despite the rapid development of biotechnologies, "negative" processes in ecology are deepening [8-9]. Among them, the issues of degradation of terrestrial-aquatic ecosystems, loss of biodiversity, and excess emissions of greenhouse gases exist, and, as a result, malnutrition and hunger are especially acute [10]. These problems are exacerbated in extreme conditions [11]. The problem of the spreading of antibiotic resistance is also to some extent related to food safety [12-16]. However, the key issue of food safety has been and remains the fight against foodborne pathogens [17-20].

Water bodies are an important source of food for people and animals around the world [21-23]. Even the diet of some vegetarians (pescatarian) contains fish [24]. In recent years, a lot of research on aquatic organisms and their pathogens has been carried out [25-30]. Phage and probiotic therapies are considered as alternatives to antibiotics in combating fish pathogens [31-39]. It is known that probiotics are live micro-organisms that have a beneficial effect on the host organism, which can be a human [40-44], an animal, a plant or a soil [45-47]. Currently, when isolating or obtaining probiotics, the features of the host-microbe interaction are considered.

Although the benefits of *Escherichia coli* probiotics have also been historically proven [48–52], lactic acid bacteria remain the most widely used probiotics [53–58]. In addition to antagonistic activity against pathogens, the probiotic potential of bacterial strains is largely determined by the physicochemical properties of the surface of bacterial cells, such as the ability to auto-aggregate (the first stage of adhesion) and the ability to form biofilm [59-60]. It has recently been found that the membrane characteristics of fish lactobacilli and *E. coli* may differ from those of non-aquatic organisms, which may affect the effectiveness of the use of non-fish probiotics in aquaculture and fish production [61].

The aim of this study was the assessment of biofilm formation and auto-aggregation abilities of the novel targeted aqua-probiotics from the microbial collections of the Armenian National Agrarian University (Armenia), Southern Federal University of Russia (Russia) and Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan (Uzbekistan).

METHODS

Probiotic strains and culture media: The probiotic bacterial strains of fish or shrimp originate from the microbial collections of the Armenian National Agrarian University, Southern Federal University of Russia, and the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan [61]. *Lacticaseibacillus rhamnosus* str. 1A, *Enterococcus* str. 9-3, *Bacillus subtilis* str. 1R, *Bacillus amyloliquefaciens* str. 4R, *Bacillus amyloliquefaciens* str. 5R, *Lactobacillus delbrueckii* str. UZ-1, *Lactiplantibacillus plantarum* str. R3, *Lactococcus* str. UZ-2, *Enterococcus faecium* str. R2, and *Pediococcus acidilactici* str. N were used in this study (Table 1).

Table 1. Sources of the probiotic strains*

Strains	Sources
Lactobacillus delbrueckii str. UZ-1	Intestinal microbiota of carp^
Lactiplantibacillus plantarum str. R3	Intestinal microbiota of carp^
Lactococcus str. UZ-2	Shrimp intestinal microbiota^
Enterococcus faecium str. R2	Intestinal microbiota of carp^
Pediococcus acidilactici str. N	Intestinal microbiota of carp^
Bacillus subtilis str. 1R	Intestinal microbiota of carp^^
Bacillus amyloliquefaciens str. 4R	Intestinal microbiota of carp^^
Bacillus amyloliquefaciens str. 5R	Intestinal microbiota of carp^^
Lacticaseibacillus rhamnosus str. 1A	Intestinal microbiota (*Salmo ischchan*) ^^^
Enterococcus str. 9-3	Intestinal microbiota (*Salmo ischchan*)^^^

*- The strains were kindly provided by:
^ Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan
^^ Don State Technical University
^^^ Armenian National Agrarian University

DeMan Rogosa and Sharpe (MRS) broth were used to grow the probiotic strains. After incubation (at 37 °C for 48 h), bacterial cultures were centrifuged (1165× *g* for 15 min), washed twice, and resuspended in sterile phosphate-buffered saline (PBS, pH 7) to an optical density of 0.5 McFarland standard (OD_{600}), which corresponded to the bacterial density of 10^8 CFU/ml. The OD_{600} was measured using a spectrophotometer (Stat Fax 3300, Awareness Technology, Palm City, USA).

Biofilm formation assessment: The ability to form a biofilm was evaluated by a qualitative analysis that was based on the attachment of bacteria to the surface of polystyrene using coloring with crystal violet [61]. In particular, 200 µl of overnight bacterial suspensions (OD600 = 0.5) were transferred to polystyrene 96-well plates (Biomat, Ala, Italy) and incubated for 48 h at 37°C. Next, 25 µl of 0.5% crystal violet was added to each well and the plates were left for 15 minutes at room temperature. After aspiration of the contents, the wells were washed 3 times with PBS. Extraction of crystal violet was carried out with 96% ethanol. The optical density was then measured photometrically at 540 nm (Stat Fax 2100, Awareness Technology, Perchtoldsdorf, Austria).

Auto-aggregation assessment: The ability to auto-aggregate was studied according to Collado et al. [62]. The optical density (OD600) of the homogenized bacterial suspension was measured. Measurements were repeated after 2 and 24 hours of incubation at 37°C under static conditions. The percentage of auto-aggregation was calculated by the formula:

$$A = \left(1 - \frac{A_{time}}{A0}\right) * 100\ \%$$

where A_{time} is the absorbance of the mixture at 2 and 24 h, and A_0 is the absorbance at the starting point.

The experiments were repeated five times and the data was expressed as the mean ± standard deviation. A t-test (excel 2016) was performed to determine the statistical significance ($p < 0.05$).

RESULTS

Biofilm formation ability: The results of current investigations on bacterial biofilm formation abilities are given in Table 1. According to the data, the biofilm formation abilities of *Lactobacillus delbrueckii* str. UZ-1, *Lactiplantibacillus plantarum* str. R3, *Lactococcus* str. UZ-2, *Enterococcus faecium* str. R2, *Pediococcus acidilactici* str. N, *Bacillus subtilis* str. 1R, *Bacillus amyloliquefaciens* str. 4R, *Bacillus amyloliquefaciens* str. 5R, *Lacticaseibacillus rhamnosus* str. 1A and *Enterococcus* str. 9-3 were 0.119 ± 0.05D, 0.113 ± 0.065D, 0.196 ± 0.04D, 0.116 ± 0.01D, 0.152 ± 0.05D, 0.74 ± 0.15D, 2.621± 0.55D, 1.831 ± 0.45D, 0.227 ± 0.04D and 0.483 ± 0.15D respectively (Table 1).

Auto-aggregation ability: The auto-aggregation abilities of *Lactobacillus delbrueckii* str. UZ-1, *Lactiplantibacillus plantarum* R3, *Lactococcus* str. UZ-2, *Enterococcus faecium* str. R2, *Pediococcus acidilactici* str. N, *Bacillus subtilis* str. 1R, *Bacillus amyloliquefaciens* str. 4R, *Bacillus amyloliquefaciens* str. 5R, *Lacticaseibacillus rhamnosus* str. 1A and *Enterococcus* str. 9-3 after 24 hours were 88. 917 ± 3.05 %, 97.604 ± 2.98 %, 96.336 ± 4.12 %, 93.726 ± 3.87 %, 93.82 ± 2.67 %, 96.336 ± 2.45 %, 95.194 ± 2.17 %, 79.782 ± 3.87 %, 94.34 ± 2.98 % and 95.622± 2.99 % respectively (Table 2). In addition, the results show that the investigated probiotics differ from each other in the rate of auto-aggregation; differences were shown both after 2 and 24 hours of incubation (Figure 1). The highest rate of auto-aggregation was detected for the strain of *Bacillus amyloliquefaciens* str. 5R. After 2 hours, the strain showed 42.77 ± 0.57 % of auto-aggregation ability, while the percentage of auto-aggregation for the *Lactiplantibacillus plantarum* str. R3 was 8.21 ± 0.11 % only.

Table 2. Biofilm formation and auto-aggregation abilities of novel targeted aqua-probiotics, average ± standard deviation

Strains	Biofilm formation ability; D	Auto-aggregation, 24 hours; %
Lactobacillus delbrueckii str. UZ-1	0.119 ± 0.05	88.917 ± 3.05
***Lactiplantibacillus plantarum* str. R3**	0.113 ± 0.065	97.604 ± 2.98
***Lactococcus* str. UZ-2**	0.196 ± 0.04	96.336 ± 4.12
***Enterococcus faecium* str. R2**	0.116 ± 0.01	93.726 ± 3.87
***Pediococcus acidilactici* str. N**	0.152 ± 0.05	93.82 ± 2.67
***Bacillus subtilis* str. 1R**	0.74 ± 0.15	96.336 ± 2.45
***Bacillus amyloliquefaciens* str. 4R**	2.621± 0.55	95.194 ± 2.17
***Bacillus amyloliquefaciens* str. 5R**	1.831 ± 0.45	79.78227
***Lacticaseibacillus rhamnosus* str. 1A**	0.227 ± 0.04	94.34 ± 2.98
***Enterococcus* str. 9-3**	0.483 ± 0.15	95.622± 2.99

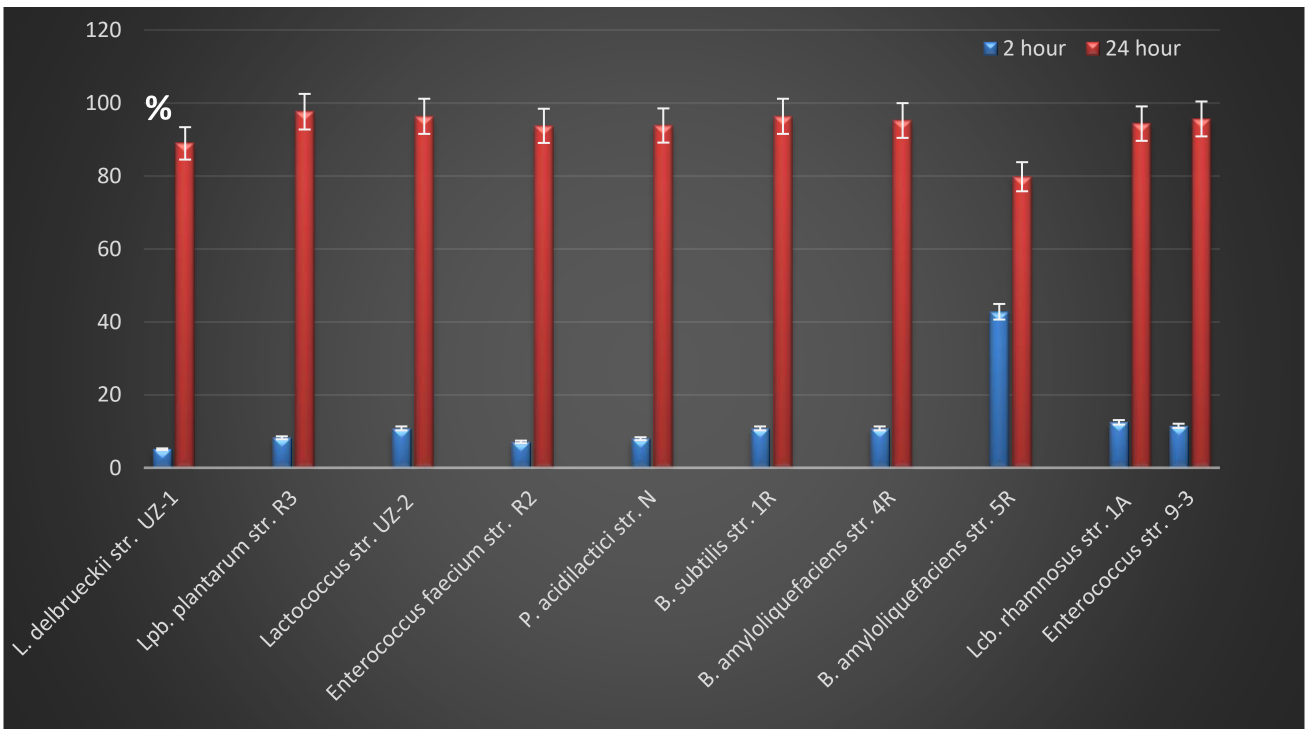


Figure 1. Auto-aggregation abilities of novel targeted aqua-probiotics: *Lactobacillus delbrueckii* str. UZ-1 (isolated from the intestinal microbiota of a carp), *Lactiplantibacillus plantarum* str. R3 (isolated from the intestinal microbiota of a carp), *Lactococcus str.* UZ-2 (isolated from a shrimp intestinal microbiota), *Enterococcus faecium* str. R2 (isolated from the intestinal microbiota of a carp), *Pediococcus acidilactici* str. N (isolated from the intestinal microbiota of a carp), *Bacillus subtilis* str. 1R (isolated from the intestinal microbiota of a carp), *Bacillus amyloliquefaciens* str. 4R (isolated from the intestinal microbiota of a carp), *Bacillus amyloliquefaciens* str. 5R (isolated from the intestinal microbiota of a carp), *Lacticaseibacillus rhamnosus* str. 1A (isolated from the intestinal microbiota of *Salmo ischchan*) and *Enterococcus* str. 9-3 (isolated from the intestinal microbiota of *Salmo ischchan*).

DISCUSSION

Generally, probiotic therapy is also proposed as an alternative to antibiotics in the fight against fish pathogens [37-38]. At the same time, for the first time, probiotics were used to stimulate the growth of aquatic organisms back in 1986 [63]. Later, probiotics of various origins were used for this purpose, which had a significant effect on the growth of pathogens in fish [64-69]. Among the pathogens of hydrobionts, represent-tatives of the genus *Vibrio* deserve close attention, since some halophilic vibrios are the causative agents of vibriosis [70]. Fish vibriosis is widespread in the seas and brackish waters, affecting both marine and coastal freshwater fish of various species, including salmon, cod, eel, herring, perch, and flounder [71]. Along with fish vibriosis, Aeromonas septicemia, edwardsiellosis, colum-naris and streptococcus, as well as a number of other diseases, are largely responsible for economic losses in aquaculture production [72-84].

To stay "alive" under various stress conditions, bacteria also use different means, including the properties of their membranes: auto-aggregation, cell surface hydrophobicity, and the ability to form biofilm, which are largely interconnected. Interestingly, the results of recent studies show that the membranes of intestinal bacteria have their own characteristics in aquatic and terrestrial animals [61]. The species specificity of cell surface hydrophobicity of fish intestinal bacterial isolates has been described in relation to the bacterial growth medium. A relationship has also been shown between membrane auto-aggregation and biofilm formation [61]. Given this circumstance, the use of probiotics isolated from fish as probiotics (targeted probiotics) for fish seems to be relevant [61]. It is also known that the ability of pathogens to form biofilms can lead to the infections [85]. On the other hand, biofilm formation ability of lactobacilli protects the host from the infections [86-87]. In addition, constitutive or stress-induced bacterial aggregation has been shown to play an important role in bacteria-host interactions [88-89]. The candidates for targeted probiotics with lactic acid origin, including *Lactiplantibacillus plantarum*, *Lactiplanti-bacillus pentosus*, *Lactobacillus acidophilus*, *Levilactobac-illus brevis*, *Pediococcus pentosaceus*, and *Pedio-coccus acidilactici* were also isolated and characterized by Mazlumi and coauthors [90]. Auto-aggregations of these bacteria was in the range from 01.3 ± 0.5 to 82.6 ± 1.4% [90].

In the presented study, the membrane properties, such as biofilm formation and an auto-aggregation abilities of a number of fish/shrimp targeted probiotics were studied. Previously, it has been shown that all the probiotics described above (with the exception of *Bacillus* spp.) not only can inhibit the growth of *Vibrio* sp.129 by 100% within 16 hours, but also have a high antagonistic activity against major fish pathogens [61].

According to the presented study, no correlation was found between the ability to form a biofilm and auto-aggregation in the studied probiotic strains (Table 2). The highest rate of auto-aggregation was shown by *Bacillus amyloliquefaciens* str. 5R (Figure 1) and *Bacillus amylo-liquefaciens* str. 4R was the strain with the highest ability to form biofilm (Table 2). These two *Bacillus* strains are also distinguished by the highest DNA protective properties and relatively low antioxidant activity. Despite the fact that the strain 5R showed the highest auto-aggregation percentage after 2 hours of incubation (highest aggregation rate), after 24 hours this strain showed the lowest level of auto-aggregation among the studied strains. Auto-aggregation abilities of *Lacto-bacillus delbrueckii* str. UZ-1, *Lactiplantibacillus plant-arum* str. R3, *Lactococcus* str. UZ-2, *Enterococcus faecium* str. R2, *Pediococcus acidilactici* str. N, *Bacillus subtilis* str. 1R, *Bacillus amyloliquefaciens* str. 4R, *Lacticaseibacillus rhamnosus* str. 1A and *Enterococcus* str. 9-3 after 24 hours were 79.782 ± 3.87 % vs. 88.917 ± 3.05 %, 97.604 ± 2.98 %, 96.336 ± 4.12 %, 93.726 ± 3.87 %, 93.82 ± 2.67 %, 96.336 ± 2.45 %, 95.194 ± 2.17 %, 94.34 ± 2.98 % and 95.622 ± 2.99%, respectively (Table 2). The *Enterococcus* str. 9-3, strain with the highest antioxidant activity, showed 0.483 ± 0.15D biofilm formation ability. According to the presented study, the probiotics studied by us have more pronounced auto-aggregation properties compared to the strains described by Mazlumi and coauthors [91]. However, the results of our studies and the studies of these authors (unfortunately, we were unable to find scientific investigations by other authors in this direction) are difficult to compare, since, despite the same described conditions, the studies were carried out non-simultaneously. A complete assessment of auto-aggregation properties can only be given based on comparable experiments.

CONCLUSION

Various biotechnological processes, including fish production, require probiotics with different membrane properties. According to the present studies, probiotics isolated from hydrobionts, in addition to high antagonistic activities against fish pathogens, also have pronounced/specific biofilm formation and aggregation characteristics, which seems important for biotechnological processes requiring specific membrane properties. Further comparative studies are needed to assess the probiotic potential of probiotic strains obtained by us and other authors for various hydrobionts, including different fish species. However, the probiotic bacteria used in the presented study have already been tested on a number of commercial fish species in Armenia, Uzbekistan and Russia; they are already ready for use in fish farming/industry in these countries, where special properties of membrane biofilm formation and autoaggregation are needed.

Conflicts of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions: AP and SM contributed to the conception, AM, VC and MB designed the study and contributed experimental data. AP wrote the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the State Committee of Science, Armenia (project 21AG-4D065), by the Ministry of Innovative Development of the Republic of Uzbekistan (project EAPI-2021-51) and by the Russian Foundation for Basic Research, project no. 20-516-81004\20.

Acknowledgments: The authors would like to thank Drs V. Mamikonyan, S. Mirzabekyan and Ms L. Malkhasyan for their assistance in validation of the protocols.

REFERENCES

1. Pörtner LM, von Philipsborn P, Fesenfeld L: Food security and sustainability in times of multiple crises. Ann Nutr Metab 2023, 79:1-2. DOI: https://www.doi.org/10.1159/000527743
2. Ohana-Levi N, Netzer Y: Long-term trends of global wine market. Agriculture 2023, 13:224. DOI: https://doi.org/10.3390/agriculture13010224
3. Habiba L, Belahsen R: Health problems associated to nutrition and lifestyle changes in the COVID-19 era. Bioactive Compounds in Health and Disease 2023, 6:26-37. DOI: https://www.doi.org/10.31989/bchd.v6i3.1038
4. Raposo A, Ramos F, Raheem D, Saraiva A, Carrascosa C: Food safety, security, sustainability and nutrition as priority objectives of the food sector. Int J Environ Res Public Health 2021, 18:8073. DOI: https://doi.org/10.3390/ijerph18158073
5. Pepoyan AZ, Chikindas ML: Plant-associated and soil microbiota composition as a novel criterion for the environmental risk assessment of genetically modified plants. GM Crops Food 2020, 11:47-53. DOI: https://www.doi.org/10.1080/21645698.2019.1703447
6. Clark MA, Springmann M, Hill J, Tilman D: Multiple health and environmental impacts of foods. Proc Natl Acad Sci 2019, 116:23357-23362. DOI: https://www.doi.org/10.1073/pnas.1906908116
7. Mirón IJ, Linares C, Díaz J: The influence of climate change on food production and food safety. Environmental Research 2023, 216:114674. DOI:

https://doi.org/10.1016/j.envres.2022.114674

8. Balayan M, Pepoyan A, Manvelyan A, Tsaturyan V, Grigoryan B, Abrahamyan A, Chikindas M: Combined use of eBeam irradiation and the potential probiotic *Lactobacillus rhamnosus* Vahe for control of foodborne pathogen *Klebsiella pneumoniae*. Ann Microbiol 2019, 69:1579-1582. DOI: https://www.doi.org/10.1007/s13213-019-01522-2
9. Guleria P, Kumar V, Mo B: Editorial: Biotechnology for agricultural sustainability. Front Sustain Food Syst 2023, 7:1128411. DOI: https://www.doi.org/10.3389/fsufs.2023.1128411
10. Muluneh MG: Impact of climate change on biodiversity and food security: a global perspective-a review article. Agric Food Secur 2021, 10:36. DOI: https://doi.org/10.1186/s40066-021-00318-5
11. Williams K, Fielding L, Davis J, Martirosyan D: The blockade of Artsakh causing long-term food, nutrition shortage and starvation: How functional food education can help resolve health related conditions. Foods in Health and Disease 2023, 13:97-116. DOI: https://www.doi.org/10.31989/ffhd.v13i3.1081
12. Lambraki IA, Cousins M, Graells T, Léger A, Henriksson P, Harbarth S, Troell M, et al: Factors influencing antimicrobial resistance in the European food system and potential leverage points for intervention: A participatory, One Health study. PLoS ONE 2022, 17:e0263914. DOI: https://doi.org/10.1371/journal.pone.0263914
13. Samtiya M, Matthews KR, Dhewa T, Puniya AK: Antimicrobial resistance in the food chain: trends, mechanisms, pathways, and possible regulation strategies. *Foods* 2022, 11:2966. DOI: https://doi.org/10.3390/foods11192966
14. Larsson DGJ, Flach CF: Antibiotic resistance in the environment. Nat Rev Microbiol 2022, 20:257-269. DOI: https://doi.org/10.1038/s41579-021-00649-x
15. Nelson DW, Moore JE, Rao JR: Antimicrobial resistance (AMR): significance to food quality and safety. *Food Quality and Safety 2019*, 3:15-22. DOI: https://doi.org/10.1093/fqsafe/fyz003
16. Kim J, Ahn J: Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table. Food Sci Biotechnol 2022, 31:1481-1499. DOI: https://doi.org/10.1007/s10068-022-01157-1
17. Csadek I, Vankat U, Schrei J, Graf M, Bauer S, Pilz B, Schwaiger K, et al: Treatment of ready-to-eat cooked meat products with cold atmospheric plasma to inactivate *Listeria* and *Escherichia coli*. *Foods* 2023, 12:685. DOI: https://doi.org/10.3390/foods12040685
18. Jacobs P, Berends B, Lipman L: The value of current *Ante Mortem* meat inspection and food chain information of dairy cows in relation to *Post Mortem* findings and the protection of public health: A case for a more risk-based meat inspection. *Foods* 2023, 12:616. DOI: https://doi.org/10.3390/foods12030616
19. Martirosyan D: The emerging potential of functional foods in viral disease prevention. Functional Foods in Health and Disease 2020, 6:95-99. DOI: https://www.doi.org/10.31989/bchd.v3i6.726
20. Fredriksson-Ahomaa M, Sauvala M, Kurittu P, Heljanko V, Heikinheimo A, Paulsen P: Characterisation of *Listeria monocytogenes* isolates from hunted game and game meat from Finland. *Foods* 2022, 11:3679. DOI: https://doi.org/10.3390/foods11223679
21. Browning H: Improving welfare assessment in aquaculture. Front Vet Sci 2023, 10: 1060720. DOI: https://doi.org/10.3389/fvets.2023.1060720
22. Davis CM, Gupta RS, Aktas ON, Diaz V, Kamath SD, Lopata AL: Clinical management of seafood allergy. J Allergy Clin Immunol Pract 2020, 8:37-44. DOI: https://doi.org/10.1016/j.jaip.2019.10.019
23. Guha S, Sharangi AB, Debnath S: Phenology and green leaf yield of coriander at different sowing dates and harvesting times. J Food Agric Environ 2014, 12:251-254. DOI: https://doi.org/10.1234/4.2014.5393
24. Kayode A, Okumede G, Alabi G, Onajobi F: Is vegan diet advisable for children? Bioactive Compounds in Health and Disease 2022, 5:33-52. DOI: https://www.doi.org/10.31989/bchd.v5i2.892
25. Miserendino ML, Epele LB, Brand C, Uyua N, Santinelli N, Sastre V: Uncovering aquatic diversity patterns in two Patagonian glacial lakes: does habitat heterogeneity matter? Aquat Sci 2023, 85:52. DOI: https://doi.org/10.1007/s00027-023-00949-9
26. Brooks MR, Medley S, Ponder M, Alexander KA: Campylobacter in aquatic and terrestrial mammals is driven by life traits: A systematic review and meta-analysis. Frontiers in Ecology and Evolution 2023, 11. DOI: https://doi.org/10.3389/fevo.2023.1070519
27. Yofukuji KY, Cardozo ALP, Schmitz MH, Fugi R: Effects of the intensity of land-use changes on taxonomic and functional diversity of fish in a Neotropical floodplain. Aquat Sci 2023, 85:48. DOI: https://doi.org/10.1007/s00027-023-00945-z

28. Hutson KS, Davidson IC, Bennett J, Poulin R, Cahill PL: Assigning cause for emerging diseases of aquatic organisms. Trends in Microbiology 2023, Advance online publication. DOI: https://doi.org/10.1016/j.tim.2023.01.012
29. Huyben D, Jarau M, MacInnes J, Stevenson R, Lumsden J: Impact of infection with *Flavobacterium psychrophilum* and antimicrobial treatment on the intestinal microbiota of Rainbow Trout. *Pathogens* 2023, 12:454. DOI: https://doi.org/10.3390/pathogens12030454
30. Algammal AM, Ibrahim RA, Alfifi KJ, Ghabban H, Alghamdi S, Kabrah A, Khafagy AR, et al: A first report of molecular typing, virulence traits, and phenotypic and genotypic resistance patterns of newly emerging XDR and MDR *Aeromonas veronii* in *Mugil seheli*. *Pathogens* 2022, 11:1262. DOI: https://doi.org/10.3390/pathogens11111262
31. Pereira C, Duarte J, Costa P, Braz M, Almeida A: Bacteriophages in the control of *Aeromonas* spp. in aquaculture systems: An integrative view. Antibiotics 2022, 11:163. DOI: https://doi.org/10.3390/antibiotics11020163
32. Nyotohadi D, Kok T. Potential of multi-strain probiotics extract as an anti-inflammatory agent through inhibition of macrophage migration inhibitory factor activity. Functional Foods in Health and Disease 2023, 13:1.
33. Leem C and Martirosyan DM: The bioactive compounds of probiotic foods/supplements and their application in managing mental disorders. Bioactive Compounds in Health and Disease 2019, 2(10):206-220. DOI: https://doi.org/10.31989/bchd.v2i10.431
34. Thakuria A, Sheth M, Patel S, Sriram: An in vitro study of the prebiotic properties of Xylooligosaccharide (XOS) and organoleptic evaluation of XOS added Prawn patia and Black rice kheer. Bioactive Compounds in Health and Disease 2020, 3(1):1-14. DOI: https://doi.org/10.31989/bchd.v3i1.682
35. Kussmann M, Cunha DHA: Nature has the answers: Discovering and validating natural bioactives for human health. Bioactive Compounds in Health and Disease 2022, 5(10):222-234. DOI: https://www.doi.org/10.31989/bchd.v5i10.1000
36. Amenyogbe E, Chen G, Wang Z, Huang J, Huang B, Li H: The exploitation of probiotics, prebiotics and synbiotics in aquaculture: present study, limitations and future directions: a review. Aquacult Int 2020, 28:1017-1041. DOI: https://www.doi.org/10.1007/s10499-020-00509-0
37. Ben Hamed S, Tavares Ranzani-Paiva MJ, Tachibana L, de Carla Dias D, Ishikawa CM, Esteban MA. Fish pathogen bacteria: Adhesion, parameters influencing virulence and interaction with host cells. Fish Shellfish Immunol 2018, 80:550-562. DOI: https://doi.org/10.1016/j.fsi.2018.06.053
38. Abdel-Latif HMR, Yilmaz E, Dawood MAO, Ringø E, Ahmadifar E, Yilmaz S: Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. Aquaculture 2022, 551:737951. DOI: https://www.doi.org/10.1016/j.aquaculture.2022.737951
39. Yilmaz S, Yilmaz E, Dawood MAO, Ringø E, Ahmadifar E, Abdel-Latif HMR: Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. Aquaculture 2022, 547:737514. DOI: https://www.doi.org/1016/j.aquaculture.2021.737514
40. Nazir Y, Hussain SA, Abdul Hamid A, Song Y. Probiotics and Their Potential Preventive and Therapeutic Role for Cancer, High Serum Cholesterol, and Allergic and HIV Diseases. Biomed Res Int 2018, 29, 2018:3428437 DOI: https://doi.org/10.1155/2018/3428437
41. Santonicola A, Molinari R, Piccinocchi G, Salvetti A, Natale F, Cimmino G. Role of a novel nutraceutical composition for irritable bowel syndrome management: symptoms relief and unexpected triglycerides- lowering effect. Functional Foods in Health and Disease. 2023, 13:2. DOI: http://dx.doi.org/10.31989/ffhd.v13i2.1068
42. Martirosyan DM, Leem C: The bioactive compounds of probiotic foods/supplements and their application in managing mental disorders. Bioactive Compounds in Health and Disease 2019, 2:206-220. DOI: https://www.doi.org/10.31989/bchd.v2i10.431
43. Pepoyan A, Manvelyan A, Balayan M, McCabe K, Tsaturyan V, Melnikov, V., et al: The effectiveness of potential probiotics *Lactobacillus rhamnosus* Vahe and *Lactobacillus delbrueckii* IAHAHI in irradiated rats depends on the nutritional stage of the host. Probiotics Antimicrob Proteins 2020, 12:1439-1450. DOI: https://www.doi.org/10.1007/s12602-020-09662-7
44. Laosee W, Kantachote D, Chansuwan W, Thongraung Ch, Sirinuipong N. Anti-salmonella potential and antioxidant activity of fermented fruit-based juice by lactic acid bacteria and its biotransformation. Functional Foods in Health and Disease 2021, 11:8. DOI: https://www.doi.org/10.31989/ffhd.v11i8.813
45. Pepoyan A, Tsaturyan V, Badalyan M, Weeks R, Kamiya S, Chikindas M: Impact of probiotic *Lactobacillus acidophilus* Narine on Salmonella carriage in sheep. Benef Microbes 2020, 11:183-189. DOI: https://www.doi.org/10.3920/BM2019.0138

46. Mansilla FI, Ficoseco CA, Miranda MH, Puglisi E, Nader-Macías MEF, Vignolo GM, Fontana CA: Administration of probiotic lactic acid bacteria to modulate fecal microbiome in feedlot cattle. Sci Rep 2022, 12:12957. DOI: https://www.doi.org/10.1038/s41598-022-16786-z
47. Woo SL and Pepe O. Microbial consortia: Promising probiotics as plant biostimulants for sustainable agriculture. Front. Plant Sci. 9:1801. DOI: https://doi.org/10.3389/fpls.2018.01801
48. Shahinyan A, Garibyan J, Pepoyan A, Karapetyan O: Cancerolitic action of *E. coli*. J Nat Sci 2003, 1:53-58.
49. Wassenaar TM. Insights from 100 Years of research with probiotic *E. Coli*. Eur J Microbiol Immunol 2016, 6(3):147-161. DOI: https://doi.org/10.1556/1886.2016.00029
50. Zimmer C, Dorea C. Enumeration of *Escherichia coli* in probiotic products. *Microorganisms*. 2019, 7:437. DOI: https://doi.org/10.3390/microorganisms7100437
51. Henker J, Laass M, Blokhin B.M. *et al.* The probiotic *Escherichia coli* strain Nissle 1917 (EcN) stops acute diarrhoea in infants and toddlers. Eur J Pediatr 2007, 166: 311–318. DOI: https://doi.org/10.1007/s00431-007-0419-x
52. Teng G, Liu Z, Liu Y, Wu T, Dai Y, Wang H, Wang W: Probiotic *Escherichia coli* Nissle 1917 expressing Elafin protects against inflammation and restores the gut microbiota. Front Microbiol 2022, 13:819336. DOI: https://www.doi.org/10.3389/fmicb.2022.819336
53. Kalikyan Z, Avagyan V, Abrahamyan A, Vardanyan L, Selimyan A, Avagyan M: Armenian fermented milk product Choratan and its influence on gut microbiota in health and pathology. BCHD 2018, 1:60-70. DOI: https://www.doi.org/10.31989/bchd.v1i5.562
54. Zheng J, Wittouck S, Salvetti E, Franz CH, Harris H, Mattarelli P, O'Toole P, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillace and *Leuconostocaceae*. *Int J Syst Evol Microbiol*. 2020. DOI: https://doi.org/10.1099/ijsem.0.004107
55. Pepoyan A and Trchounian A: Biophysics, molecular and cellular biology of probiotic activity of bacteria. Edited by Trchunyan AH. Kerala, India: Bacterial Membranes; 2009:275-287.
56. Pepoyan A, Balayan M, Manvelyan A, Pepoyan S, Malkhasyan L, Bezhanyan T, Paronikyan R, et al: Radioprotective effects of lactobacilli with antagonistic activities against human pathogens. Biophys J 2018, 114(3):665a. DOI: https://doi.org/10.1016/j.bpj.2017.11.3586
57. Chee W, Chew SY, Than LT. Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *Microb Cell Fact* 19, 203 (2020). DOI: https://doi.org/10.1186/s12934-020-01464-4
58. Dlamini ZC, Langa R, Aiyegoro OA, Okoh AI: Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. Probiotics Antimicrob Proteins 2019, 11:397-402. DOI: https://doi.org/10.1007/s12602-018-9430-y
59. Pepoyan A, Manvelyan A, Balayan M, Galstyan S, Tsaturyan V, Grigoryan B, Chikindas M: Low-dose electron beam irradiation for the improvement of biofilm formation by probiotic lactobacilli. Probiotics Antimicrob Proteins 2020, 12:667-671. DOI: https://doi.org/10. 1007/ s12602- 019- 09566-1
60. Tatsaporn T, Kornkanok K: Using potential lactic acid bacteria biofilms and their compounds to control biofilms of foodborne pathogens. Biotechnol Rep 2020, 26: e00477. DOI: https://doi.org/10.1016/j.btre.2020.e00477
61. Mirzabekyan S, Harutyunyan N, Manvelyan A, Malkhasyan L, Balayan M, Miralimova Sh, Chikindas M, et al: Fish probiotics: Cell surface properties of fish intestinal Lactobacilli and *Escherichia coli.* Microorganisms 2023, 11(3):595. DOI: https://doi.org/10.3390/microorganisms11030595
62. Collado M, Meriluoto J, Salminen S: Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol 2008, 226:1065-1073. DOI: https://doi.org/10.1007/s00217-007-0632-x
63. Cruz PM, Ibanez AL, Hermosillo OAM, Saad HCR: Use of probiotics in aquaculture. ISRN microbiology 2012, 2012:916845. DOI: https://doi.org/10.5402/2012/916845
64. Wuertz S, Beça F, Kreuz E, Wanka KM, Azeredo R, Machado M, Costas B: Two probiotic candidates of the genus *Psychrobacter* modulate the immune response and disease resistance after experimental infection in Turbot (*Scophthalmus maximus*, Linnaeus 1758). *Fishes* 2023, 8:144. DOI: https://doi.org/10.3390/fishes8030144
65. Amin M, Adams MB, Burke CM, Bolch CJS: Screening and activity of potential gastrointestinal probiotic lactic acid bacteria against *Yersinia ruckeri* O1b. J Fish Dis 2023, 46:369-379. DOI: https://doi.org/10.1111/jfd.13750
66. Quintanilla-Pineda M, Achou CG, Díaz J, GutiérrezFalcon A, Bravo M, HerreraMuñoz JI, Peña-Navarro N, et al: In vitro evaluation of postbiotics produced from bacterial isolates obtained from Rainbow Trout and Nile Tilapia against the pathogens *Yersinia ruckeri* and *Aeromonas salmonicida subsp. salmonicida*. Foods 2023, 12:861. DOI:

https://doi.org/ 10.3390/foods12040861

67. Niu KM, Kothari D, Lee VD, Lim JM, Khosravi S, Lee SM, Lee BJ, et al: Autochthonous *Bacillus licheniformis*: Probiotic potential and survival ability in low-fishmeal extruded pellet aquafeed.MicrobiologyOpen 2019, 8:e00767. DOI: https://doi.org/10.1002/mbo3.767
68. Bahi A, Guardiola FA, Messina C, Mahdhi A, Cerezuela R, Santulli A, Bakhrouf A, Esteban MA: Effects of dietary administration of fenugreek seeds, alone or in combination with probiotics, on growth performance parameters, humoral immune response and gene expression of gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol 2017, 60:50-58. DOI: https://doi.org/10.1016/j.fsi.2016.11.039.
69. Gao XY, Liu Y, Miao LL, Li EW, Hou TT, Liu ZP: Mechanism of anti-Vibrio activity of marine probiotic strain *Bacillus pumilus* H2, and characterization of the active substance. AMB Express 2017, 1:23. DOI: https://doi.org/10.1186/s13568-017-0323-3
70. Navaneeth KA, Bhuvaneswari T, Rajan JJS, Alavandi SV, Vijayan KK, Otta SK: Characterization of Vibrio parahaemolyticus isolates from shrimp farms of Southeast coast of India with special reference to Acute Hepatopancreatic Necrosis Disease (AHPND) status. Aquaculture 2020, 518:734813. DOI: https://doi.org/10.1016/j.aquaculture.2019.734813
71. Tilusha M, Annas S, Noor Azmai AM, Salwany Md YI, Mohd ZS: Pathology and pathogenesis of Vibrio infection in fish: A review. Aquaculture Reports, 2023, 28:101459. DOI: https://doi.org/10.1016/j.aqrep.2022.101459
72. Semwal A, Kumar A, Kumar N: A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. Heliyon 2023, 9:e14088. DOI: https://doi.org/10.1016/j.heliyon.2023.e14088
73. Pintor-Cora A, Tapia O, Elexpuru-Zabaleta M, Ruiz de Alegría C, Rodríguez-Calleja JM, Santos JA, Ramos-Vivas J: Cytotoxicity and antimicrobial resistance of Aeromonas strains isolated from fresh produce and irrigation water. Antibiotics 2023, 12:511. DOI: https://doi.org/10.3390/ antibiotics12030511
74. Dubey S, Ager-Wick E, Kumar J, Karunasagar I, Karunasagar I, Peng B, Evensen Ø, Sørum H, Munang'andu HM: *Aeromonas* species isolated from aquatic organisms, insects, chicken, and humans in India show similar antimicrobial resistance profiles. Front Microbiol 2022, 13:1008870. DOI: https://doi.org/10.3389/fmicb.2022.1008870
75. Manzoor K, Rasool F, Khan N, Anjum KM, Parveen S: Resistance patterns of frequently applied antimicrobials and occurrence of antibiotic resistance genes in *Edwardsiella Tarda* detected in Edwardsiellosis-infected Tilapia species of fish farms of Punjab in Pakistan. J Microbiol Biotechnol 2023, 33:1-10. DOI: https://doi.org/10.4014/jmb.2301.01008
76. Sai S, Mani R, Ganesan M: Isolation and identification of Edwardsiellosis-causing microorganism. Edited by Thomas J, Amaresan N. New York: Aquaculture Microbiology; 2023:11-18: Part of Springer Protocols Handbooks. DOI: http://dx.doi.org/10.1007/978-1-0716-3032-7_2
77. Sun B, Sun B, Zhang B, Sun L: Temperature induces metabolic reprogramming in fish during bacterial infection. Front Immunol 2022, 13:1010948. DOI: https://doi.org/10.3389/fimmu.2022.1010948
78. Thunes NC, Mohammed HH, Evenhuis JP, Lipscomb RS, Pérez-Pascual D, Stevick RJ, Birkett C, et al: Secreted peptidases contribute to virulence of fish pathogen *Flavobacterium columnare*. Front Cell Infect Microbiol 2023, 13:1093393. DOI: https://doi.org/ 10.3389/fcimb.2023.1093393
79. Pirollo T, Perolo A, Mantegari S, Barbieri I, Scali F, Alborali GL, Salogni S: Mortality in farmed European eel (*Anguilla anguilla*) in Italy due to *Streptococcus iniae*. Acta Vet Scand 2023, 65:5. DOI: https://doi.org/10.1186/s13028-023-00669-y
80. Kayansamruaj P, Dinh-Hung N, Srisapoome P, Na-Nakorn U, Chatchaiphan S: Genomics-driven prophylactic measures to increase streptococcosis resistance in tilapia. J Fish Dis 2023, 36708284. DOI: https://doi.org /10.1111/jfd.13763.
81. Akter T, Foysal MJ, Alam M, Ehsan R, Paul SI, Momtaz F, Siddik MAB, et al: Involvement of Enterococcus species in streptococcosis of Nile tilapia in Bangladesh. Aquaculture 2021, 531:735790. DOI: https://doi.org/10.1016/j.aquaculture.2020.735790
82. Akter T, Haque MN, Ehsan R, Paul SI, Foysal MJ, Tay A, Islam MT, et al: Virulence and antibiotic-resistance genes in *Enterococcus faecalis* associated with streptococcosis disease in fish. Sci Rep 2023, 13: 1551. DOI: https://doi.org/10.1038/s41598-022-25968-8
83. Bi B, Yuan Y, Jia D, Jiang W, Yan H, Yuan G, Gao Y: Identification and pathogenicity of emerging fish pathogen acinetobacter johnsonii from a disease outbreak in Rainbow Trout (Oncorhynchus mykiss). Aquaculture Research 2023, 2023:1995494. DOI: https://doi.org/10.1155/2023/1995494
84. Irshath AA, Rajan AP, Vimal S, Prabhakaran VS, Ganesan R: Bacterial pathogenesis in various fish diseases: Recent

advances and specific challenges in vaccine development. Vaccines 2023, 11:470. DOI: https://doi.org/10.3390/vaccines11020470

85. Zhang D, Gan R, Ge Y, Yang Q, Ge J, Li H, Corke H: Research progress on the antibacterial mechanisms of carvacrol: A Mini Review. Bioactive Compounds in Health and Disease 2018, 1(6):71-81. DOI: https://doi.org/10.31989/bchd.v1i6.551
86. Oluwole OM: Biofilm: Formation and natural products approach to control – A review. Afr J Infect Dis 2022, 16(Suppl 2):59-71. DOI: https://doi.org/10.21010/Ajid.v16i2S.7
87. Rajasekharan SK, Shemesh M: Spatiotemporal bio-shielding of bacteria through consolidated geometrical structuring. Biofilms Microbiomes 2022, 8:37. DOI: https://doi.org/10.1038/s41522-022-00302-2
88. Trunk T, Khalil HS, Leo JC: Bacterial autoaggregation. AIMS Microbiology 2018, 4(1):140-164. DOI: https://doi.org/10.3934/microbiol.2018.1.140
89 Geirnaert A, Lacroix C: Bistable auto-aggregation phenotype in *Lactiplantibacillus plantarum* emerges after cultivation in in vitro colonic microbiota. BMC Microbiol 2021, 21:268. DOI: https://doi.org/10.1186/s12866-021-02331-x
90 Mazlumi A, Panahi B, Hejazi MA, Nami Y: Probiotic potential characterization and clustering using unsupervised algorithms of lactic acid bacteria from saltwater fish samples. Sci Rep 2022, 12:11952. DOI: https://doi.org/10.1038/s41598-022-16322-z