

Determination of the antimicrobial activity of lactic acid bacteria isolated from the Black sea mussel *Mytilus galloprovincialis* Lamarck, 1819

Tsveteslava Ignatova-Ivanova¹, Sevginar Ibryamova¹, Darina Bachvarova¹, Seniha Salim¹, Simona Valkova¹, Yoanna Simeonova¹, Dimitar Dimitrov¹, Radoslav Ivanov¹, Nesho Chipev², Nikolay Natchev^{1,3}

¹ Shumen university, Shumen, Bulgaria

² Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, Sofia, Bulgaria

³ University of Vienna, Vienna, Austria

Corresponding author: Tsveteslava Ignatova-Ivanova (tsignatovaivanova@shu.bg)

Received 4 April 2022 ♦ Accepted 10 May 2022 ♦ Published 19 July 2022

Citation: Ignatova-Ivanova T, Ibryamova S, Bachvarova D, Salim S, Valkova S, Simeonova Y, Dimitrov D, Ivanov R, Chipev N, Natchev N (2022) Determination of the antimicrobial activity of lactic acid bacteria isolated from the Black sea mussel *Mytilus galloprovincialis* Lamarck, 1819. Pharmacia 69(3): 637–644. <https://doi.org/10.3897/pharmacia.69.e84850>

Abstract

The present study reports on the determination of the antimicrobial activity of lactic acid bacteria (LAB) isolated from the Black sea mussel *Mytilus galloprovincialis* Lamarck, 1819. The samples were collected in the period of August 2018 until March 2021. The BIOLOG system was used for microbiological determination. From the mussel *M. galloprovincialis* Lam. four species of LAB were isolated - *Sporolactobacillus kofuensis*, *Lactobacillus sakei*, *Streptococcus gallolyticus ss gallolyticus* and *Lactibacillus brevis*. The activity of the strains was determined against test cultures (*Escherichia coli* 3398, *Staphylococcus aureus* 745, *Bacillus subtilis* 6633, *Salmonella typhimurium* 3591, *Listeria monocytogens* 863 *Enterobacter aerogenes* 3691, *Aspergillus niger*, *Penicillium claviforme*, *Saccharomyces cerevisiae*, *Candida albicans* 8673 and *Candida glabrata* 72). Before the analysis for antimicrobial activity, the LAB were cultured in media with different concentrations of sugars - 2, 5 and 10%. The results showed that 4 strains *S. kofuensis*, *L. sakei*, *S. gallolyticus ss gallolyticus* and *L. brevis* cultured on glucose and oligosaccharides completely lost their activity in all studied variants. Therefore, some carbohydrates (glucose) and oligosaccharides induce the synthesis outside the cell of biologically active molecules, which can probably be attributed to peptides/proteins.

Keywords

antimicrobial activity, bacteriocins, Black Sea, lactic acid bacteria, *Mytilus galloprovincialis* Lam

Introduction

The consumption of safe food is a significant social problem in the modern world. Worldwide bivalve production has consistently increased over the years from 7.1 million

in 1995 to 16.1 million in 2014, and the consumer demand is expected to further increase in the next future FAO (2016) and (Prato et al. 2019). In the Bulgarian waters of the Black Sea, the cultivation of the black mussel *Mytilus galloprovincialis* Lam is a very important part of the lo-

cal industry. Consumption of seafood is an integral part of the diet not only of people living along the coast, but all over the country. There is a lot of discussion concerning the benefits and risks of eating seafood. The benefits of consuming black mussels outweigh with the fact that they contain important human vitamins D, A and B, minerals and trace elements and very little fat. However, the nutritive value of shellfish is to a large extent associated with the presence of polyunsaturated fatty acids (PUFAs) especially omega 3 (n^{-3}) such as eicosapentaenoic acid (EPA, $20:5n^{-3}$) and docosahexaenoic acid (DHA, $22:6n^{-3}$). They play an important role in the prevention of human cardiovascular diseases and cancers, lowering of incidents of diabetes and in the development and function of nervous system. Regular intake of EPA and DHA prevents cardiovascular disease, neural and inflammatory disorders (Casula et al. 2013). In most cases, the consumer is informed about the benefits of eating mussels, but not the risks. The mussels are filtering the water and as a result, large amounts of bacteria can get into their bodies. The microbiota found in shellfishes can be divided into autochthonous and allochthonous microorganisms, and reflects the microbial population of the water in which they grow (Vernocchi et al. 2007). One potential risk of eating mussels is the infections with various pathogenic bacteria that infect the seawater as a result of anthropogenic pollution. On the other hand, it has been found that seafood is a source of lactic acid bacteria (LAB), which synthesize substances with antimicrobial activity against many pathogenic microorganisms (Khouadja 2017). Consuming foods containing high quantities of chemical conservants provoked the interest in the use of natural and minimally processed foods, with a focus on the use of naturally produced antimicrobial agents - bioconservants such as bacteriocins (Françoise 2010). Bacteriocin producing LABs can improve the aquatic environment of the shrimp and fish aqua-cultures. The development of bacteriocinogenic strains and the isolation of bacteriocins from seafood and products may result in valuable strains that are used both as bioconservants and as food (and feed) additives in aqua-culture animals (Falanga et al. 2016). There are no studies in Bulgaria on the microbiology of lactic acid bacteria isolated from black mussels and the ability of these bacteria to synthesize molecules with antimicrobial activity such as bacteriocins.

The aim of the present study was to determine the antimicrobial activity of lactic acid bacteria (LAB) isolated from the Black Sea mussel *Mytilus galloprovincialis* Lam. with a view to their use either as safe bioconservatives for seafood in food storage or as a way to protect black mussels from various pathogens present in their habitat.

Materials and methods

Place and duration of the study

The study was conducted at the Department of Biology, University of Shumen, Bulgaria, from August 2018 until March 2021. The samples were collected from the regions

of Mussel farm – Kavarna 43.4108°N, 28.3566°E; Port Varna 43.1880°N, 27.9113°E; Sozopol; 42.4005°N, 27.7202°E; Tyulenovo 43.4836°N, 28.5810°E; Constanta 44.1747°N, 28.6583°E (Fig. 1).



Figure 1. Sampling locations at the Black sea coast.

Collection of samples

After collection, the samples (about 10 kg) were immediately refrigerated (4 °C) and transported to the laboratory for the analyses.

Microbiological analyses

Three subsamples (each of about 1 kg of mussels) were used for the microbiological analyses. The mussels were scrubbed free of dirt, washed in hypochlorite solution (20 mg l^{-1}), rinsed with sterile distilled water, and shucked with a sterile knife. The whole soft tissue of the mussel's liquor samples (about 100 g) were homogenized. Lactic acid bacteria (LAB) were isolated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia). The strains were cultured overnight (16–18 h) on MRS at 37 °C and in limitation of oxygen (tubes or Petri dishes with the strains were incubated in plastic bags, which limited the oxygen content).

Carbohydrates used in this study

Since high concentrations of sugars and oligosaccharides stimulate the synthesis of bacteriocins and increase the

antimicrobial activity of ICD (Ignatova et al. 2009), before the analysis for antimicrobial activity, ICDs were cultured in media with different concentrations of sugars - 2, 5 and 10%. glucose, FOS, and GOS were used as sugars. Three types of commercially available carbohydrates presenting different degree of polymerization (DP) were studied. FOS (fructooligosaccharides) (Raftilose P95 from Orafiti, Tienen, Belgium) contained 5% glucose, fructose, and sucrose; and 12% DP2, 48% DP4 and 35% DP7. GOS (glucooligosaccharides) (BioEcolians from Solabia, Pantin, France) contained 6% glucose and leucrose, and 24% DP4, 56% DP5, 7% DP6 and 7% DP7. The concentration of each carbohydrate was set to 2, 5 and 10% to an MRS broth. Glucose (purity 99%, Merck) were used as controls. Each carbohydrate was sterilized on 0.2 µm sterile filter (Sartorius, Labsystems, Sofia, Bulgaria), and pH was not adjusted. All measurements were performed at least twice.

Fermentation

LAB were grown in MRS broth (Merck). Overnight grown cells were washed twice in saline (0.85% NaCl solution), and 10% of the bacterial suspension (10^7 cfu ml⁻¹) was used to inoculate modified MRS broth and agar medium (pH 6.8) containing either 2,5 and 10% glucose, 2,5 and 10% GOS (glucooligosaccharides), 2,5 and 10% FOS (fructooligosaccharides). The anaerobic fermentations were performed in 100 ml glass bottles at 37 °C for 48 h (BBL Gas Pak anaerobic system envelopes).

Microbial identification databases for the "BIOLOG" system

The microbial identification was performed by the BIOLOG Microbial Identification System VIO45101AM. The isolated strains were screened on BL4021502 Tryptic Soy Agar (TCA), cultured for 24 hours at 37 °C and then subjected to Gen III plaque identification to identify Gram positive and Gram negative aerobic bacteria. The microscopic pictures were performed using stereomicroscope OPTIKA (Italy) with a DinoEye, Eyepiece camera with 5 megapixels. The photographs were performed by using a Canon EOS 60D camera. The GEN III MicroPlate test panel provides a standardized micromethod using 94 biochemical tests to profile and identify a broad range of Gram-negative and Gram-positive bacteria. BIOLOG's Microbial Identification Systems software (e.g. OmniLog Data Collection) is used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate. The BIOLOGsystem allows to quickly and accurately identify more than 2900 species of aerobic and anaerobic bacteria, yeasts, and fungi. BIOLOG's advanced phenotypic technology provides valuable information on the properties of the strains, in addition to species-level identification. BIOLOG's carbon technology identifies the environment and pathogenic microorganisms by producing a characteristic pattern or "metabolic fingerprint" of discrete test

reactions performed in a 96-well microplate. The culture suspensions are tested with a panel of pre-selected assays, then incubated, read and compared with extensive data-bases. <https://www.biolog.com/products-portfolio-overview/microbial-identification>.

Test microorganisms

Escherichia coli 3398 NBIMCC, *Staphylococcus aureus* 745 NBIMCC, *Bacillus subtilis* 6633 ATCC, were obtained American Type, Culture Collection (ATCC), *Salmonella typhimurium* 3591 NBIMCC, *Listeria monocytogens* 863 NBIMCC, *Enterobacter aerogenes* 3691 NBIMCC, *Aspergillus niger* NBIMCC, *Penicillium claviforme* NBIMCC, *Saccharomyces cerevisiae* NBIMCC, *Candida albicans* 8673 NBIMCC and *Candida glabrata* 72 NBIMCC were obtained from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria (NBIMCC). All the isolates were checked for purity and maintained in slants of Nutrient agar. The test organisms were propagated in appropriate media as follows: *Escherichia coli* grown on LB (Luria-Bertani) agar medium (Sigma, St. Louis, MO), *Listeria monocytogens* 863 grown on BH (Brain Heart)-agar medium (Biokar Diagnostics, Beauvais, France), *Salmonella typhimurium* grown on Elliker, and *Bacillus* sp. grown on nutrition broth and agar.

Assay for antimicrobial activity

Antimicrobial assay was performed by the well diffusion method using soft 2% agar. Agar medium was added to sterile Petri dishes seeded with 100 µl of each test bacterial strains. Wells of equal distance were dug on the seeded plates. Each well was filled up with 100 µl of exponential *Lactobacillus* cultures in mMRS broth were used as inoculum for the antifungal tests. After adjusting the pH at 6.5 by NaOH, the activity of the plant extracts was checked. The plates were incubated at 37 °C for 48 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (Bertrand-Harb et al. 2003). The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well (Nwamaioha and Ibrahim 2018). 24 hour LAB cultures grown on skim milk were mixed with PDA agar at 45 °C in equal volumes and afterwards poured into plates. Agar plates, with sterile skim milk, together with laboratory cultures were used as controls. After drying the plates, 1 ml of mold / yeast spores suspension was added dropwise to the center of the agar layer surface in each plate. The plates were incubated aerobically in an upright position at 29 °C. The diameters of the growing mold colonies were measured daily until the mold in the control sample fully filled the volume of the petri dishes. The diameter of the obtained zones is measured with a digital caliper. The diameter of the obtained zones is measured with a digital caliper. All experiments were performed in triplicate.

Effects of heat and hydrolytic enzymes (trypsin from Sigma, No. T-8253; proteinase K from Sigma, No. P-0390) on bacteriocin activity were determined according to (Pantev et al. 2002). Samples of supernatants, obtained after culturing lactic acid bacteria on media with different oligosaccharides in an amount (100 μ l) were treated for 2 h with 0.1 mg/ ml⁻¹ and 1 mg/ ml⁻¹ final concentration of trypsin or proteinase K, respectively. The obtained samples were tested for activity against *E. aerogenes* 3691 for *L. sakei* and *St. gallolyticus*, and *B. subtilis* 6633 for *L. brevis* and *Sp. kofuensis*.

Results

The microorganisms were isolated from *M. galloprovincialis* Lam collected from five localities of the north and south Bulgarian Black Sea aquatory and the region of Constanta, Romania. After 24 h of cultivation on different media, various microbial colonies were obtained. The species of microorganisms were confirmed not only on selective media, but also by the results of the BIOLOG system.

The results of the antimicrobial activity test for each LAB species are represented in Figs 2–5.

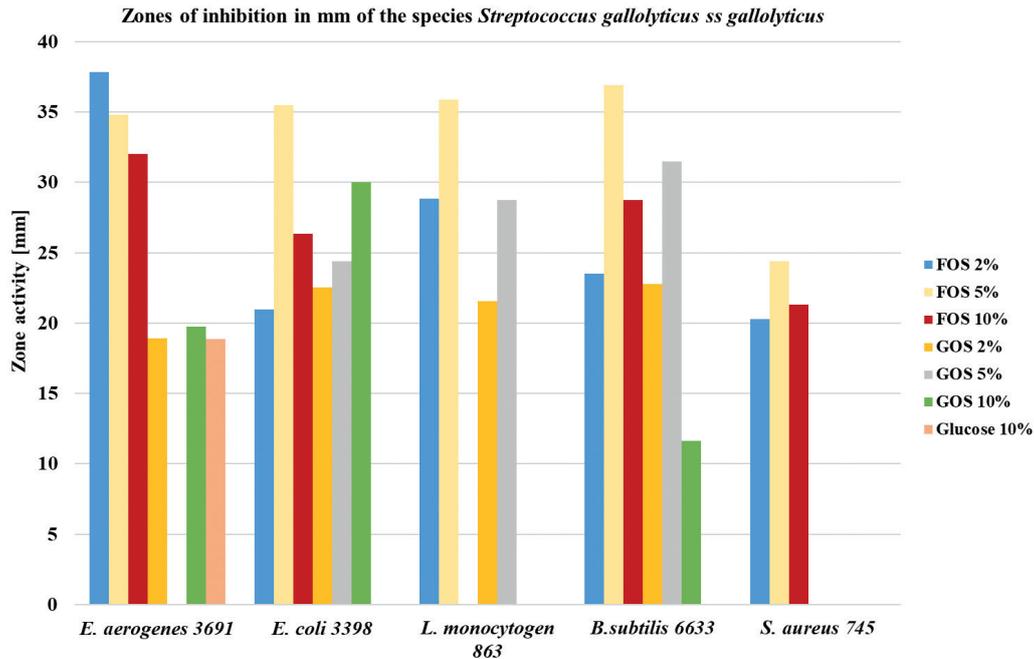


Figure 2. Zones of inhibition in mm of the species *Streptococcus gallolyticus ss gallolyticus*.

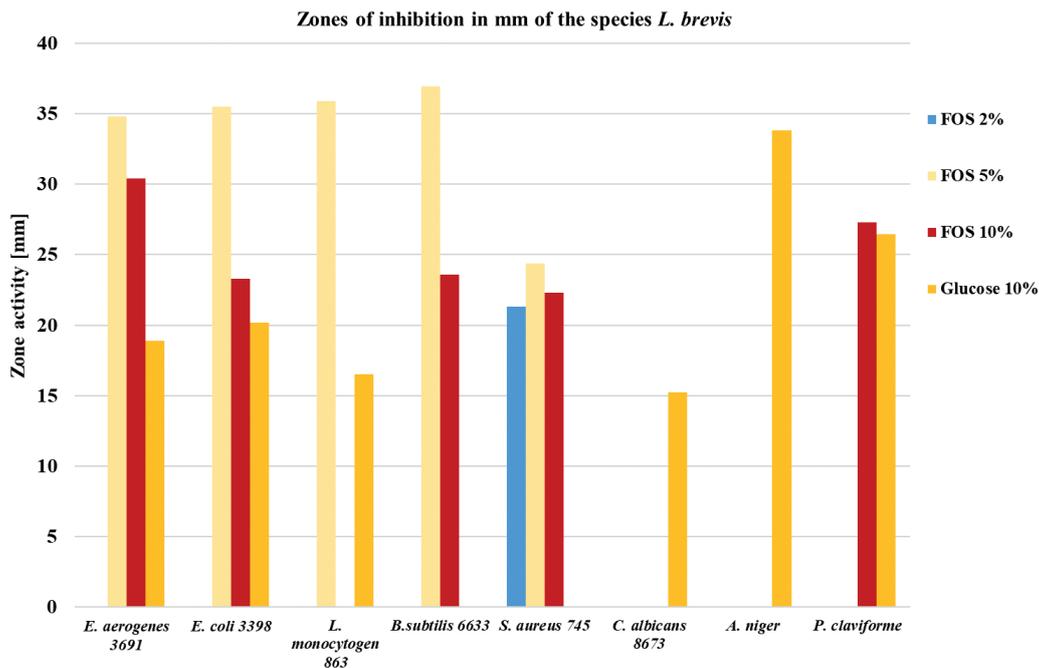


Figure 3. Zones of inhibition in mm of the species *L. brevis*.

From the data in figure 2 it is evident that the species *St. gallolyticus ss gallolyticus* has the highest zone of inhibition relative to *E. aerogenes* 3691 when culturing 2% FOSs. Relatively high zones of inhibition were also observed against *E. coli* 3398, *S. aureus* 745, *B. subtilis* 6633 and *L. monocytogens* 863 in the cultivation of 5% FOSs. In the cultivation of the strain on GOS activity was observed against *E. aerogenes* 3691, *E. coli* 3398, *S. aureus* 745, *B. subtilis* 6633 and *L. monocytogens* 863, the highest being against *B. subtilis* 6633. Compared to *S. typhimurium* 3591, molds and fungi have no areas of activity.

The data represented in Fig. 3 demonstrate that the species *L. brevis* has the highest zone of inhibition compared to *A. niger* when culturing 2% FOSs. Relatively high zones of inhibition were also observed against *E. coli* 3398, *S. aureus* 745, *B. subtilis* 6633, *P. claviforme* and *L. monocytogens* 863 in the cultivation of 5% FOSs. When culturing the strain *L. brevis* on GOS, activity was not observed. Activity against *C. albicans* 8673 occurred when cultured on 10% glucose solution. In 10% glucose solution it also had activities against *E. aerogenes* 3691, *E. coli* 3398, *L. monocytogens* 863, *A. niger* and *P. slaviforme*.

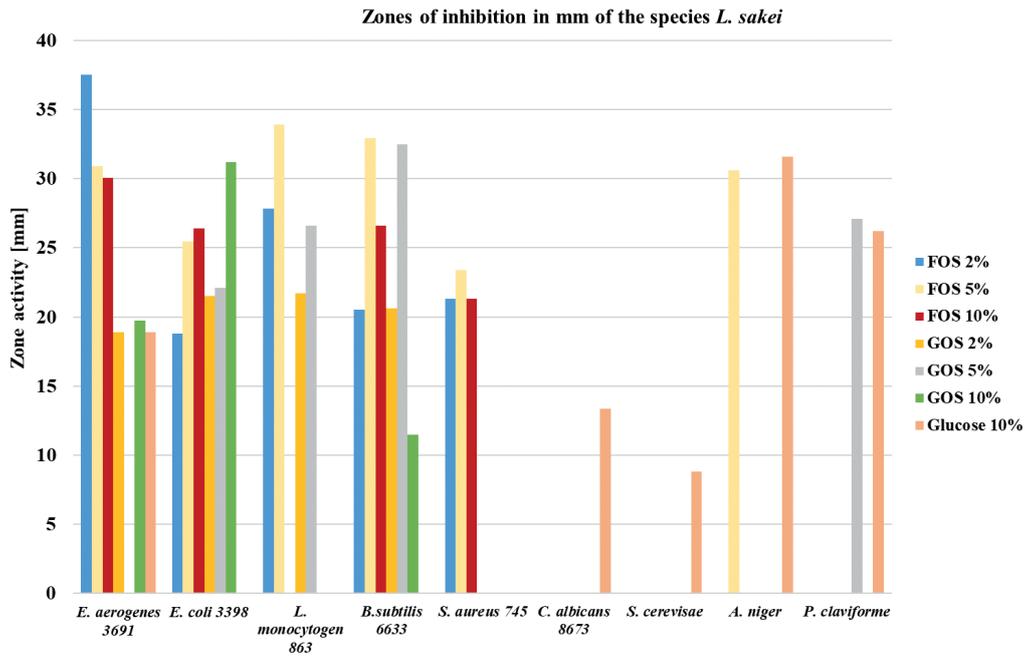


Figure 4. Zones of inhibition in mm of the species *L. sakei*.

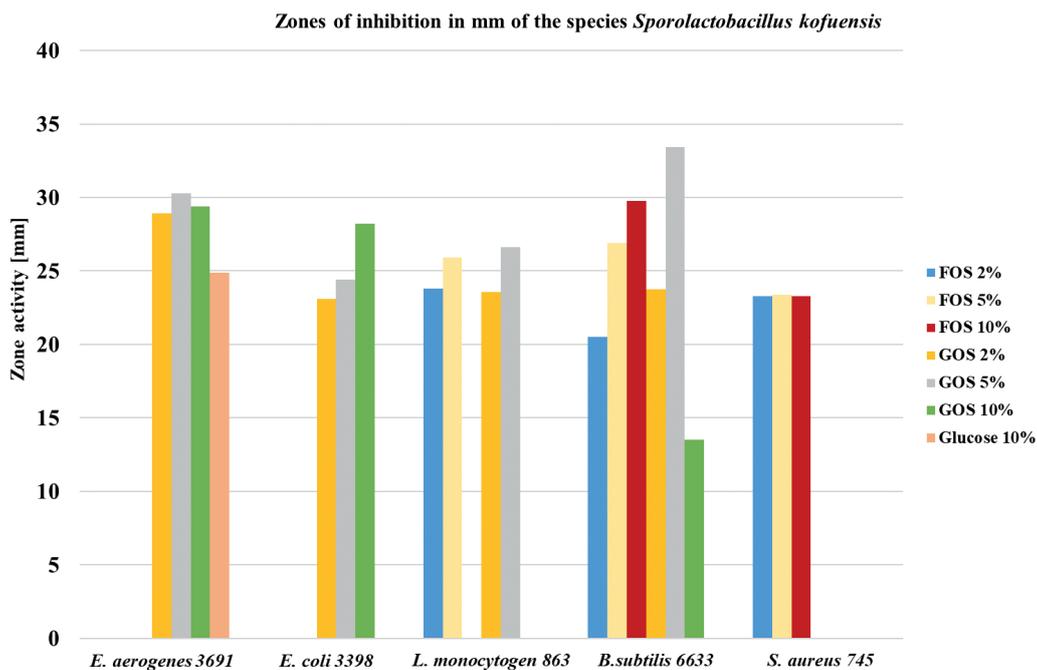


Figure 5. Zones of inhibition in mm of the species *Sporolactobacillus kofuensis*.

Table 1. Effect of enzymes and temperature on the inhibitory activity of supernatants of *L. brevis* and *L. sakei*.

Strain	After cultivation on FOS (mm sterile zone)				After cultivation on GOS (mm sterile zone)				After cultivation on glucose (mm sterile zone)			
	control	temperature	Trypsin	proteinase K	control	temperature	Trypsin	proteinase K	control	temperature	Trypsin	proteinase K
<i>L. brevis</i>	31.9	no zone	no zone	no zone	8	no zone	no zone	no zone	10	no zone	no zone	no zone
<i>L. sakei</i>	37.5	no zone	no zone	no zone	19,74	no zone	no zone	no zone	11	no zone	no zone	no zone

Samples of 100 µl were treated for 2 h with 0.1 mg ml⁻¹ and 1.0 mg ml⁻¹ final concentrations of the enzymes trypsin (Sigma, n. T-8253); proteinase K (Sigma, n. P-0390). The temperature treatment was done after boiling the samples over 60 degrees during 10 minutes. Abbreviations used: FOS - fructooligosaccharide; GOS - glycoligosaccharide.

Table 2. Effect of enzymes and temperature on the inhibitory activity of *St. gallolyticus* and *Sp. kofuensis*.

Strain	After cultivation on FOS (mm sterile zone)				After cultivation on GOS (mm sterile zone)				After cultivation on glucose (mm sterile zone)			
	control	temperature	Trypsin	proteinase K	control	temperature	Trypsin	proteinase K	control	temperature	Trypsin	proteinase K
<i>St. gallolyticus</i>	47.85	no zone	no zone	no zone	19.74	no zone	no zone	no zone	18.87	no zone	no zone	no zone
<i>Sp. kofuensis</i>	26.9	no zone	no zone	no zone	33.45	no zone	no zone	no zone	9	no zone	no zone	no zone

Samples of 100 µl were treated for 2 h with 0.1 mg ml⁻¹ and 1.0 mg ml⁻¹ final concentrations of the enzymes trypsin (Sigma, n. T-8253); proteinase K (Sigma, n. P-0390). The temperature treatment was done after boiling the samples over 60 degrees during 10 minutes. Abbreviations used: FOS - fructooligosaccharide; GOS - glucooligosaccharide

The data in Fig. 4 demonstrate that the species *L. sakei* has the highest zone of inhibition compared to *E. aerogenes* 3691 in the cultivation of 2% POPs. Relatively high zones of inhibition were also observed against *E. coli* 3398, *S. aureus* 745, *B. subtilis* 6633, *A. niger* and *L. monocytogenes* 863 in the cultivation of 5% FOSs. When cultivating the strain on GOC, the activity against *B. subtilis* 6633 is the highest when cultivating 5% GOS. Activity against *C. albicans* 8673 occurs when cultured on 10% glucose solution. In 10% glucose solution, it also has activities against *E. aerogenes* 3691, *E. coli* 3398, *A. niger* and *P. slaviforme* and *S. cerevisiae*.

From the data in Fig. 5 it is evident that the species *Sp. kofuensis* has the highest zone of inhibition relative to *E. aerogenes* 3691 when culturing 5% GOS. Relatively high zones of inhibition were also observed for *E. coli* 3398, *L. monocytogenes* 863 and *B. subtilis* 6633 in GOS culture. When culturing the strain on FOS, the activity against *B. subtilis* 6633 is the highest. Activity against *L. monocytogenes* 863 and *S. aureus* 745 was observed when culturing on FOS.

To clarify the nature of the biologically active components, neutralized cell supernatants treated with trypsin and proteinase K were used. The results obtained are shown in Tables 1, 2.

The results of Tables 1, 2 demonstrate that 4 strains cultured on glucose and oligosaccharides completely lose their activity in all studied samples variants. It should be underlined that probably some carbohydrates (glucose) and oligosaccharides induce the synthesis of biologically active molecules, which can probably be attributed to peptides / proteins.

Discussion

Mussels can filter up to 10 liters of water per hour (Powell et al. 1992). This can lead to the accumulation of pathogenic bacteria, and when consuming raw bivalve organisms can cause food poisoning in humans (Ottaviani et al. 2008). In previous publications, it has been shown that large amounts of pathogenic species such as *E. coli*, *Enterococcus* sp. and *Enterobacter* sp. at

high concentrations can cause gastrointestinal problems in humans (Ignatova-Ivanova et al. 2018). Also a previous work from (Ibryamova et al. 2020) reported that LABs with antifungal activity were isolated from black mussels. At this stage it is difficult to state what is the reason species of the genus *Lactobacillus* sp. to be isolated only during the summer season. A sample site was chosen in Constanta, Romania in order to see if there is a difference among the species of lactic acid bacteria in two nationally different water areas. The results did not show a distinction among lactic acid bacteria taxonomic affiliation in the Bulgarian and Romanian waters of the Black Sea. This suggests that these species are characteristic for black mussels and they did not develop as a result of anthropogenic pressure. The probable cause, in our opinion, on the one hand is an increase in the number of tourists, and on the other hand, during these periods the number of pathogenic species also increases significantly. Probiotic applications, in biological control of seafood associated pathogens can be an alternative solution, providing the consumer with a product of good quality owing to the use of nontoxic compounds. Most of the consumed shellfish in Bulgaria is produced on aquaculture farms. Because much of the shellfish is grown and harvested as aquacultures, this offers an opportunity to monitor and improve microbiological safety of the product both preharvest and postharvest. Several processes, including freezing, low-temperature pasteurization, high pressure processing and irradiation have been reported to be capable of reducing pathogenic bacteria. The massive use of antibiotics may lead to the emergence of resistant bacteria, which can spread in the environment and jeopardize human health (Durlu-Ozkaya 2005). Probiotics are among the most promising alternatives to antibiotics and the application in aquaculture is now widely accepted. It may be possible to reduce the load of pathogens in seafood by improving water quality and also by introducing biocontrol bacteria capable of excluding pathogens from shellfish associated microbial communities Who (2014).

A review of the available literature reveal, that several probiotics alone or in combination can increase both

systemic and local immunity in fish (Al-Dohail et al. 2009), but no data are available for the black mussel. Undoubtedly, however, proving the probiotic properties of LAB isolation from the mussels themselves can have the effect of reducing the environmental risk of consuming these foods.

There is much evidence in the literature that excessive use of antibiotics in the fight against pathogenic bacteria has led to the emergence of high levels of microbial resistance, which poses new challenges to human health (Falanga et al. 2016). In this regard, the search for new molecules with antibacterial activity such as bacteriocins is a priority. Such antimicrobial peptides (AMPs) have been isolated from various organisms (Yeaman and Yount 2003) and are known to play a key role in building their defense strategies as part of humoral natural protection against infections. According to Galdiero (2015), the number of AMPs effective against pathogenic bacteria continues to grow. They are relatively small peptides (<60 amino acids) with a broad-spectrum of activity against microorganisms (Gram-positive and Gram-negative bacteria, fungi, viruses, parasites) (Cruz et al. 2014) and a low likelihood of developing resistance (Hancock and Sahl 2006) and (Marr et al. 2006). Moreover, AMPs may play multifunctional roles which extend far beyond their ability to function as antibiotics.

The marine environment is extremely hydrophilic and contains not only a wide range of microorganisms but also a high salt content. The marine environment is constituted by approximately 10^6 bacteria/mL and 10^9 virus/mL of seawater and, thus, represents a rich source of pathogens (Falanga et al. 2016). Marine organisms live in close proximity with pathogenic microbes; thus, in order to survive in such a harsh environment they need to have a robust and effective immune system, and AMPs constitute the first line of defense against invading microbes. Marine AMPs have been shown to be structurally different from their analogues derived by terrestrial species and often present novel structures (Cheung et al. 2015).

The antimicrobial activity of AMPs is based on their initial electrostatic interaction with the negatively charged surface of bacteria and thus, the free ions produced by the high environmental concentrations of salt characteristic of some diseases could reduce the interaction and antimicrobial activity. Marine AMPs have evolved to adapt to high salt concentrations in seawater. Marine organisms are therefore a promising source of new bioactive substances for the development of therapeutic agents. There is evidence in the literature for the isolation of AMPs from different marine organisms

- different species of fish, crabs, shrimps, jellyfish and sea urchin (Falanga et al. 2016). For the mussel *M. galloprovincialis*, the only isolated AMPs were called Myticin A (Mitta 1999) and they showed antimicrobial activity against gram-positive bacteria and are less active against fungi and gram-negative bacteria. Marine AMPs can be expected to have increased stability and efficacy for therapeutic applications in humans. More specifically, the high salinity (up to 600 mM) of the marine environment gives marine AMPs greater salt resistance than those obtained from other sources, which may allow them to maintain their biological activity in a relatively high salt environment. For example in saliva, gastrointestinal fluid, serum or other body fluids. In this regard, the results obtained by us are very important, as they show that the synthesized peptides / proteins have a wide range of activity against a relatively large number of pathogenic microorganisms.

Conclusion

Seawater is a rich and as yet undiscovered source of bioactive molecules for the development of new drugs against microbial pathogens in the current situation of growing antimicrobial resistance. In this regard, marine microorganisms and in particular mussels live in extreme and stressful environments and can become a rich source of templates for the design of new antimicrobial peptides that could become effective drugs for human and veterinary medicine. These are the first results published in the literature on the presence of lactic acid bacteria which exhibit probiotic properties in the microflora of the black mussel in the Bulgarian Black Sea. Our results are extremely important because if these strains successfully ferment oligosaccharides and at the same time produce biologically active substances, they would be important for the functions of the intestinal microflora of seafood consumers and for the protection of mussels from other pathogenic bacteria. The isolated strains may also possess the potential also be used in cosmetics industry.

Acknowledgements

The contributors express their gratitude for the funding by fund scientific research Bulgaria programme grant KP-06-H21/7/18 Dec 2018, the project by Shumen University project 08-80/09.02.2022 Department of Biology.

References

- Al-Dohail MA, Hashim R, Aliyu-Paiko M (2009) Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquaculture Research* 40(14): 1642–52. <https://doi.org/10.1111/j.1365-2109.2009.02265.x>
- Bertrand-Harb C, Ivanova I, Dalgalarondo M, Hartle T (2003) Evolution of b-lactoglobulin and a lactalbumin content during yoghurt

- fermentation. *International Dairy Journal* 13(1): 39–45. [https://doi.org/10.1016/S0958-6946\(02\)00140-1](https://doi.org/10.1016/S0958-6946(02)00140-1)
- Casula M, Soranna D, Catapano AL, Corrao G (2013) Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: a meta-analysis of randomized, double blind, placebo controlled trials. *Atherosclerosis Supplements* 14(2): 243–251. [https://doi.org/10.1016/S1567-5688\(13\)70005-9](https://doi.org/10.1016/S1567-5688(13)70005-9)
- Cheung RC, Ng TB, Wong J (2015) Marine peptides: Bioactivities and applications. *Marine Drugs* 13(7): 4006–4043. <https://doi.org/10.3390/md13074006>
- Cruz J, Ortiz C, Guzman F, Fernandez-Lafuente R, Torres R (2014) Antimicrobial peptides: Promising compounds against pathogenic microorganisms. *Current Medicinal Chemistry* 21(20): 2299–2321. <https://doi.org/10.2174/0929867321666140217110155>
- Durlu-Ozkaya F, Karabicak N, Kayali R, Essen B (2005) Inhibition of yeasts isolated from traditional Turkish cheeses by *Lactobacillus* spp. *International Journal of Dairy Technology* 58(2): 111–114. <https://doi.org/10.1111/j.1471-0307.2005.00196.x>
- Françoise L (2010) Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology* 27(6): 698–709. <http://doi.org/10.1089/ind.2019.29175.lbs>
- Falanga A, Lombardi L, Franci G, Vitiello M, Iovene MR, Morelli G, Galdiero M, Galdiero S (2016) Marine Antimicrobial Peptides: Nature Provides Templates for the Design of Novel Compounds against Pathogenic Bacteria. *International Journal of Molecular Sciences* 17(5): e785. <https://doi.org/10.3390/ijms17050785>
- FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome, 200 pp. <http://www.fao.org/3/a-i5555e/pdf>
- Galdiero S, Falanga A, Berisio R, Grieco P, Morelli G, Galdiero M (2015) Antimicrobial peptides as an opportunity against bacterial diseases. *Current Medicinal Chemistry* 22(14): 1665–1677. <https://doi.org/10.2174/0929867322666150311145632>
- Hancock REW, Sahl HG (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology* 24: 1551–1557. <https://doi.org/10.1038/nbt1267>
- Marr AK, Gooderham WJ, Hancock RE (2006) Antibacterial peptides for therapeutic use: Obstacles and realistic outlook. *Current Opinion in Pharmacology* 6(5): 468–472. <https://doi.org/10.1016/j.coph.2006.04.006>
- Ibryamova S, Arhangelova N, Koynova T, Dimitrov D, Dimitrova Z, Ivanov R, Kalchev K, Chipev N, Natchev N, Ignatova-Ivanova Ts (2020) Antifungal Activity of Lactic Acid Bacteria, Isolated from (*Mytilus galloprovincialis* Lam.) in The Bulgarian Black Sea aquatory. *Journal of IMAB - Annual Proceeding (Scientific Papers)* 26(1): 2875–2882. <https://doi.org/10.5272/jimab.2020261.2875>
- Ignatova Ts, Iliev I, Kirilov N, Vassileva T, Dalgalarondo M, Haertle T, Chobert JM, Ivanova I (2009) Effect of Oligosaccharides on the Growth of *Lactobacillus delbrueckii* Subsp. *bulgaricus* Strains Isolated from Dairy Products. *Journal Agriculturaland Food Chemistry* 57: 9496–9502. <https://doi.org/10.1021/jf901684z>
- Ignatova-Ivanova Ts, Ibrjmov S, Stanachkova E, Ivanov R (2018) Microbiological characteristic of microflora of (*Mytilus galloprovincialis* Lam.) in The Bulgarian Black Sea aquatory. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 9(1): 199–205. [ISSN 0975-8585] [https://www.rjpbcs.com/pdf/2018_9\(1\)/\[29\].pdf](https://www.rjpbcs.com/pdf/2018_9(1)/[29].pdf)
- Khouadja S, Haddaji N, Hanchi M, Bakhrouf A (2017) Selection of lactic acid bacteria as candidate probiotics for *Vibrio parahaemolyticus* depuration in pacific oysters (*Crassostrea gigas*). *Aquaculture Research* 48(4): 1885–1894. <https://doi.org/10.1111/are.13026>
- Mitta G, Hubert F, Noel T, Roch P (1999) Myticin, a novel cysteine-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis*. *European Journal of Biochemistry* 265: 71–78. <https://doi.org/10.1046/j.1432-1327.1999.00654.x>
- Nwamaioha NO, Ibrahim SA (2018) A selective medium for the enumeration and differentiation of *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Journal of Dairy Science* 101(6): 4953–4961. <https://doi.org/10.3168/jds.2017-14155>
- Ottaviani D, Leoni F, Rocchegiani E, Santarelli S, Canonico C, Masini L, Ditrani V, Carraturo A (2008) First clinical report of pandemic *Vibrio parahaemolyticus* O3:K6 infection in Italy. *Journal of Clinical Microbiology* 46(6): 2144–2145. <https://doi.org/10.1128/JCM.00683-08>
- Pantev A, Kabadjova P, Dalgalarondo M, Haertle T, Ivanova I, Dousset X, Pre'vost H, Chobert JM (2002) Isolation and partial characterization of an antibacterial substance produced by *Enterococcus faecium*. *Folia Microbiologica* 47: 391–400. <https://doi.org/10.1007/BF02818696>
- Powell EN, Hoffman EE, Klinck JN, Ray SM (1992) Modeling oyster populations: 1. A commentary on filtration rate. Is faster always better. *Journal of Shellfish Research* 11: 387–398. <http://hdl.handle.net/1969.3/25743>
- Prato E, Biandolino F, Parlapiano I, Giandomenico S, Denti G, Calò M, Spada L, Dileo A (2019) Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health. *Science of The Total Environment* 648(15): 153–163. <https://doi.org/10.1016/j.scitotenv.2018.07.382>
- Vernocchi P, Maffel M, Lanciotti R, Suzzi G, Gardini F (2007) Characterization of Mediterranean mussels (*Mytilus galloprovincialis*) harvested in Adriatic Sea (Italy). *Food Control* 18(12): 1575–1583. <https://doi.org/10.1016/j.foodcont.2006.12.009>
- Who [World health organisation] (2014) Strategic and Technical Advisory Group on Antimicrobial Resistance (STAG-AMR). http://apps.who.int/iris/bitstream/10665/128675/1/WHO_HSE_PED_AIP_2014.4_eng.pdf
- Yeaman MR, Yount NY (2003) Mechanisms of antimicrobial peptide action and resistance. *Pharmacological Reviews* 55(1): 27–55. <https://doi.org/10.1124/pr.55.1.2>