

Physical and chemical environmental factors' influence on the proteolytic potential of probiotic microorganisms' individual representatives

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Abstract. This work is devoted to the study of sanitary and hygienic monitoring of fishery water use objects in Murmansk region. The paper presents assessment of the ecological and sanitary conditions of fishery reservoirs in the Arctic region of the Kola and Tuloma rivers for the period 2016-2018 based on the use of microbiological, hydrochemical and parasitological indicators, as well as the integral index of surface water pollution. Hydrochemical studies consisted of determining the biochemical oxygen consumption, the mass content of NH_4^+ , NO_2^- , NO_3^- , iron, and dissolved O_2 . The assessment of the river waters sanitary state was carried out by means of microbiological and parasitological methods. The microbiological parameters were used to determine the number of common and thermotolerant coliform bacteria, coliphages, and the presence of intestinal infections pathogens. The water of rivers does not meet the hygienic standards in terms of hydrochemical indicators in seventeen percent and in terms of microbiological indicators in five percent of cases. According to parasitological indicators, hygienic standards meet the requirements. In five points of water sampling, the water of the Tuloma and Kola rivers was evaluated as pure according to the integral hydrochemical index value and belongs to the second category of quality. In general, the high-water quality of Murmansk region rivers was noted. In conclusion, it should be emphasized that systematic monitoring of water supply sources is necessary to obtain an idea of the indicators' variability and to assess the rivers pollution degree.

1 Introduction

Protease activity, along with other technologically significant properties of probiotic bacteria, is of considerable interest. First of all, from the point of view of evaluating the potential use of these bacteria in the technology of processing protein-containing raw materials, including hydrobionts. This fact allows not only to reduce the duration of protein-containing raw materials maturation due to the acceleration of physical and biochemical processes, but also to obtain a finished product with functional properties [1,4]. In addition, the resulting free amino acids serve as a source of other nitrogenous compounds that contribute to improving the quality of organoleptic parameters of finished products [2, 3].

The aim of our study was to assess the physical and chemical factors' influence on the proteins hydrolysis intensity of the hydrobionts' muscle tissue sarcoplasmic fraction. Another aim was to study the dynamics of probiotic microorganisms' cultures' proteolytic activity.

The proteolytic potential of the selected research objects (*Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Propionibacterium freudenreichii*), which have high biological and multifunctional activity, was considered as a possibility of using these bacteria to

obtain protein-containing products with increased biological value.

2 Materials and methods

Studying the protease activity of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*, the protein sarcoplasmic fraction of cod (*Gadus morhua*) muscle tissue was used as a protein source for cultivation. In the extract containing the sarcoplasmic fraction, the optical density of the solutions was measured on the SF-2000 spectrophotometer at wavelengths of 260 and 280 nm in three sequences. The concentration of tyrosine formed during the substrate protein components' enzymatic hydrolysis after the addition of a suspension of microorganisms to them was determined spectrophotometrically [5].

During the study, changes in tyrosine levels in culture fluids were determined at different incubation temperatures, pH, and NaCl concentrations after 3, 6, 9, 12, and 24 hours. The amount of tyrosine in the extract was previously measured without exposure or the addition of microorganisms. Further, by comparing the results of bacterial suspensions' enzymatic activity in substrates with different physical and chemical

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characteristics, the influence of incubation conditions and duration on the intensity and direction of metabolic processes was evaluated.

3 Results and discussion

The change in tyrosine concentration occurs as a result of two processes — protein proteolysis, and its assimilation by the bacterial community. The prevalence of the former can be judged by an increase in the concentration of tyrosine in comparison with its initial level. The decrease in the saturation of the culture fluid with tyrosine indicated its withdrawal due to the intensification of assimilation processes. Thus, to assess the enzymatic activity of the culture of living microorganisms, we used the relative change in the level of tyrosine in comparison with its primary values. This

method of expressing the results allows us to assess the predominance of processes (dissimilation/assimilation) rather than to measure a specific enzymatic activity [5]. From the point of view of assessing the practically significant properties of bacteria, this methodological approach is informative.

To assess the effect of the fermentation temperature on the change in the tyrosine concentration in the culture fluids, three temperature conditions were selected: 10, 20 and 37°C. These incubation modes correspond to the optimal, minimum and average growth temperatures of the selected cultures of microorganisms. The results of optical density measurements in samples after incubation of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* cultures for 24 hours at three temperatures are shown in Figure 1.

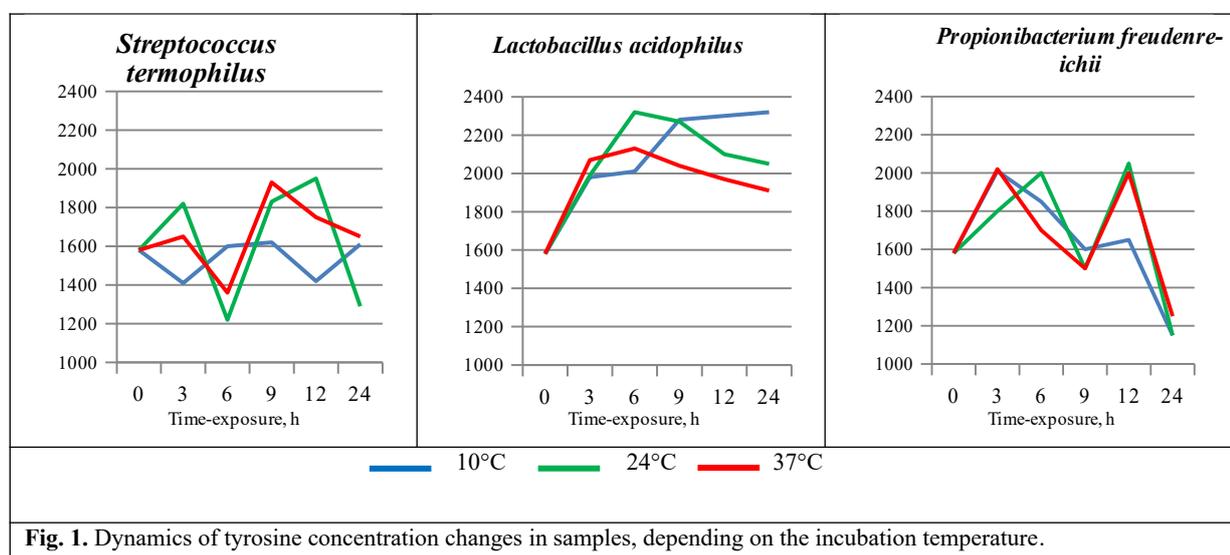


Fig. 1. Dynamics of tyrosine concentration changes in samples, depending on the incubation temperature.

On average, the amount of tyrosine in the substrate containing *Streptococcus thermophilus* culture during incubation ranged from 20–35 %. The predominance of assimilation processes of nitrogen-containing substances was observed at a temperature of 10°C, as evidenced by minimal changes in the tyrosine level. Its level in this case did not exceed the initial value for the entire period of exposure. The minimum marker index was observed at a temperature of 24°C by the 24th hour of the experiment, the concentration of the indicator amino acid decreased by 24%, and the concentration fluctuations ranged from 26 to 35 %.

During the *Lactobacillus acidophilus* cultivation, it was noted that the measured indicator varied in the range of 21–43 %, while the processes of tyrosine release into the environment prevailed over the processes of its consumption. The maximum was recorded by the end of incubation at a temperature of 10°C. The total level of tyrosine increased by 45–52%, depending on the cultivation conditions.

For the *Propionibacterium freudenreichii* culture, the highest levels were observed after 3 and 12 hours (20% higher than the initial concentration). A decrease in the tyrosine concentration was observed by 9 and 24

hours of exposure (25–30 %, depending on temperature conditions).

Temperature conditions can affect the enzymatic reactions, slowing or accelerating them; such a temperature effect is observed in all physico-chemical reactions. It is known that there is a correlation between the rates of enzymatic reactions and temperature [6].

In general, the experiment showed that at a temperature of 10°C, there were less pronounced fluctuations in the tyrosine level over time than at other temperature conditions. This can be attributed to a slowdown in the total enzymatic processes, a decrease in the negative influence of their own metabolites on bacterial community development, and, as a result, the predominance of assimilation processes. With an increase in temperature conditions, significant changes in the concentration of tyrosine in culture fluids were observed due to an increase in the rate of enzymatic multidirectional reactions. A comparative analysis of changes in tyrosine levels in the samples showed that the *Lactobacillus acidophilus* culture had a more pronounced hydrolytic activity against sarcoplasmic fraction proteins at all incubation temperature conditions than other studied objects. *Streptococcus thermophilus*

bacteria were characterized by minimal proteolytic activity when incubated at 10°C.

To assess the hydrogen index effect on the metabolic processes of the studied objects, the most significant, from a technological point of view, pH levels were selected – 4.5; 7 and 8. The assessment of the medium hydrogen index influence on the change in the

concentration of tyrosine during fermentation is shown in Figures 2–4.

Enzymes, like any protein substances, are sensitive to the hydrogen index of the medium. The pH level determines the ionization of the functional groups in the enzyme's protein molecule. Thus, hydrogen ions affect the active center of the protein molecule [7, 8].

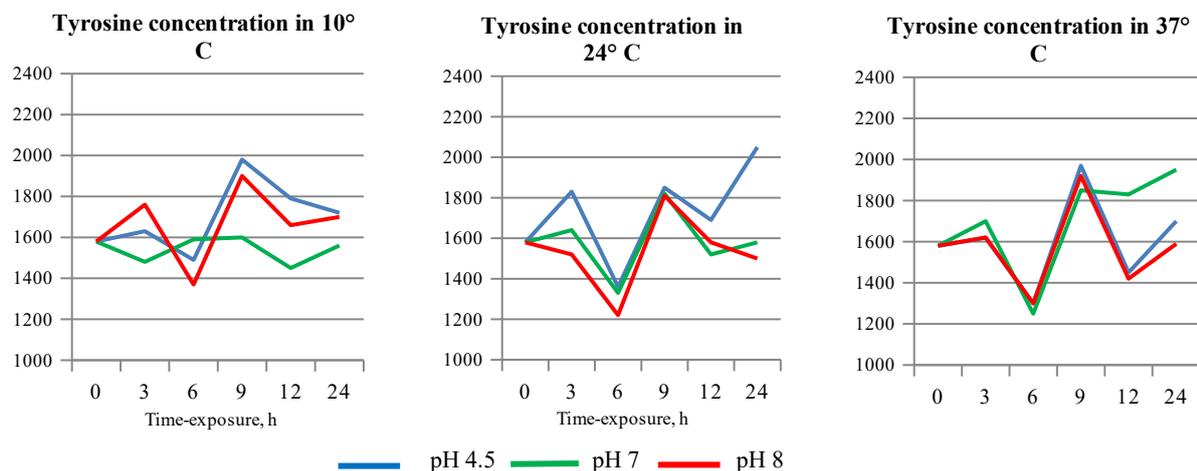


Fig. 2. Dynamics of changes in the tyrosine concentration in samples during incubation of *Streptococcus thermophilus* culture depending on the pH value.

When assessing the pH effect on the enzymatic activity of *Streptococcus thermophilus* culture, it was shown that changes in the tyrosine concentration in the substrate were more significant in a medium with a hydrogen ions concentration, other than neutral. The maximum level of tyrosine was observed in an acidic environment at the 9th hour of sample exposure at temperatures of 10°C and

37°C, at the 24th hour – at 24°C. The concentration in comparison with the initial value increased by 25 and 30%, respectively. In the neutral environment, the predominance of hydrolytic processes reached a maximum after 24 hours of incubation at 37°C. The difference between the maximum and the initial level was 35%.

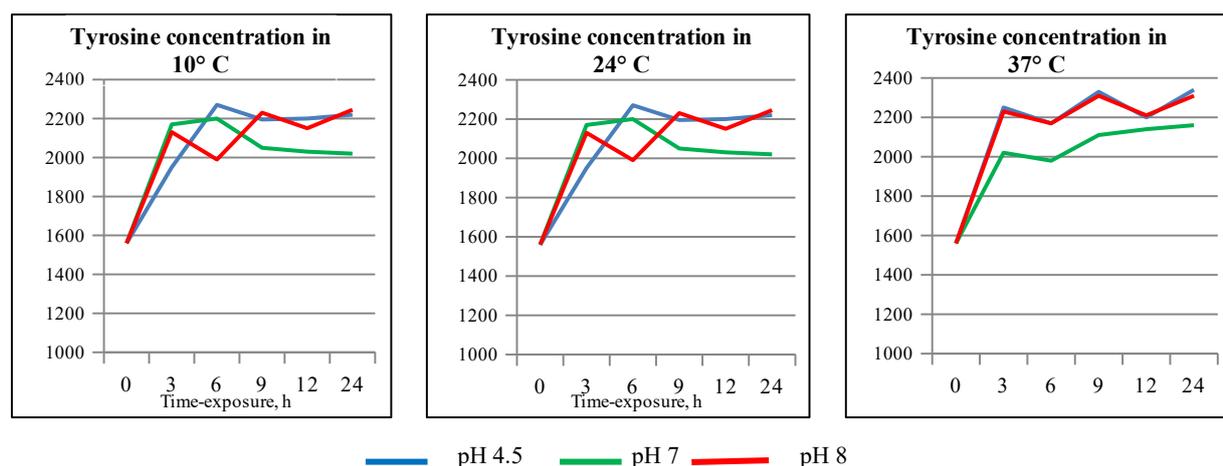


Fig. 3. Dynamics of changes in the tyrosine concentration in samples during incubation of *Lactobacillus acidophilus* culture, depending on the pH value.

The change in the tyrosine concentration during *Lactobacillus acidophilus* cultivation was characterized by an increase in the indicator during the entire incubation period. The concentration increased in the range from 21 to 48.5 %. In acidic and alkaline media,

the tyrosine level was 5–10% higher compared to this indicator during cultivation at a pH value of 7. The maximum was at pH 4.5 after 24 hours of fermentation at 37°C (48.5% higher than the initial level).

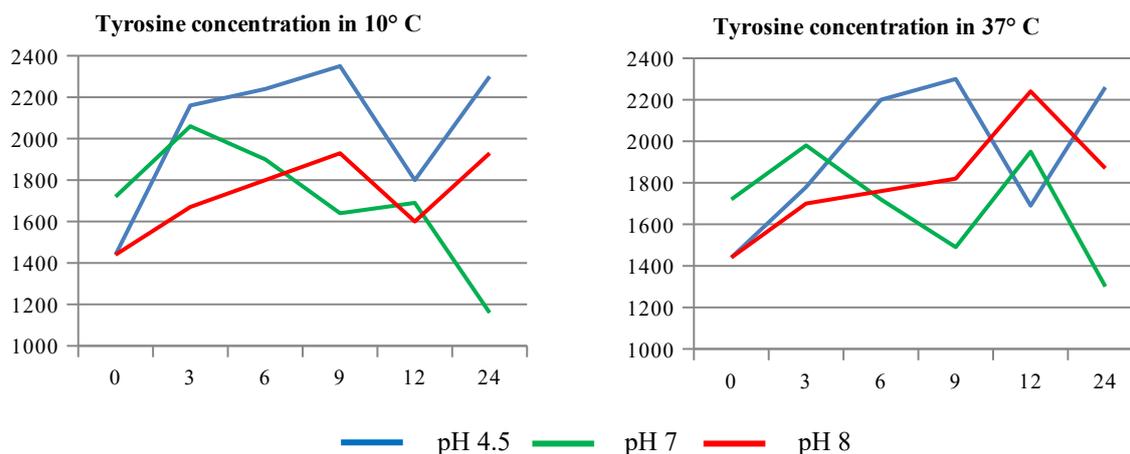


Fig. 4. Dynamics of changes in the tyrosine concentration in samples during *Propionibacterium freudenreichii* culture incubation depending on the pH value.

The tyrosine concentration during *Propionibacterium freudenreichii* cultivation varied in a wider range in an alkaline pH medium. It was noted that in a neutral environment, the difference in tyrosine concentration between the initial and maximum values did not exceed 15%. At pH 4.5, the amount of the marker after a slight increase (no more than 10%) significantly decreased (by 30-50 %).

At different levels of the hydrogen index in the reaction medium, the active center can be ionized weaker or stronger, more or less shielded by neighboring groups. The concentration of hydrogen ions affects the state of the enzyme (its protein part), determining the level of the ratio of anionic and cationic groups, which affects the tertiary structure. During the formation of the tertiary structure, the active enzyme centers are formed. In addition, the medium pH affects the degree of ionization of the reaction products, the substrate, and the enzyme-substrate complex. In general, it can be argued that for all the studied cultures at a pH of 4.5, the predominance of hydrolytic processes was observed, and for assimilation ones, a neutral medium was required.

The evaluation of the effect NaCl has on the metabolic activity of the studied objects was carried out in the culture medium, where salt concentrations were established at the levels of 2%, 4% and 8%. These incubation modes corresponded to the parameters of the expected technological modes of microorganisms' application (Figures 5–7).

NaCl has a direct effect on enzymatic processes. At certain concentrations, salt causes the destruction of the hydrate shell of protein-enzymes, which contributes to a decrease in their activity or a temporary loss of native properties. The functional properties of enzymes are due to the hydration of the protein molecule, in which the proteins are dissolved in water and aqueous solutions. The result is the formation of an electrocharged water layer around the protein, which includes water molecules oriented in relation to the protein molecule in a strictly defined way. The hydrate shell (an electrically charged water layer) prevents the aggregation of protein particles and contributes to the stability of the protein solution. The greater the probability of salting out is, the higher the mass NaCl fraction is [9].

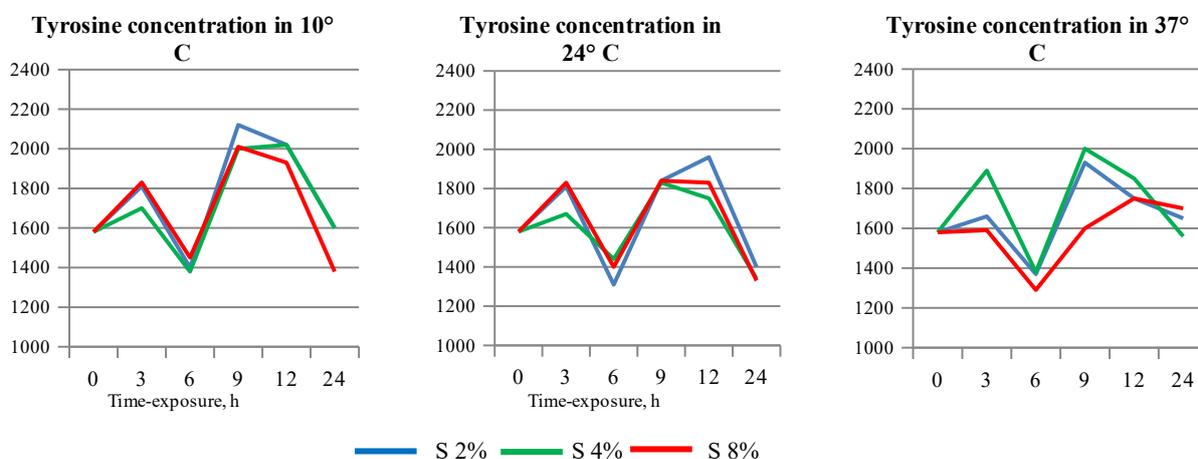


Fig. 5. Dynamics of changes in the tyrosine concentration in samples during incubation of *Streptococcus thermophilus* culture depending on the mass NaCl fraction in the substrate.

It was noted that the tyrosine content in the substrate with the NaCl addition in the selected concentrations was less affected by the direction and intensity of the metabolic processes of the studied cultures of microorganisms in comparison with other environmental factors (temperature and pH).

For *Streptococcus thermophilus* cultures, it was noted that the fluctuations in the tyrosine concentration at different NaCl concentrations in the medium had a general trend, but this indicator changed less widely during cultivation in comparison with the control sample (the difference between the initial and minimum did not exceed 20%).

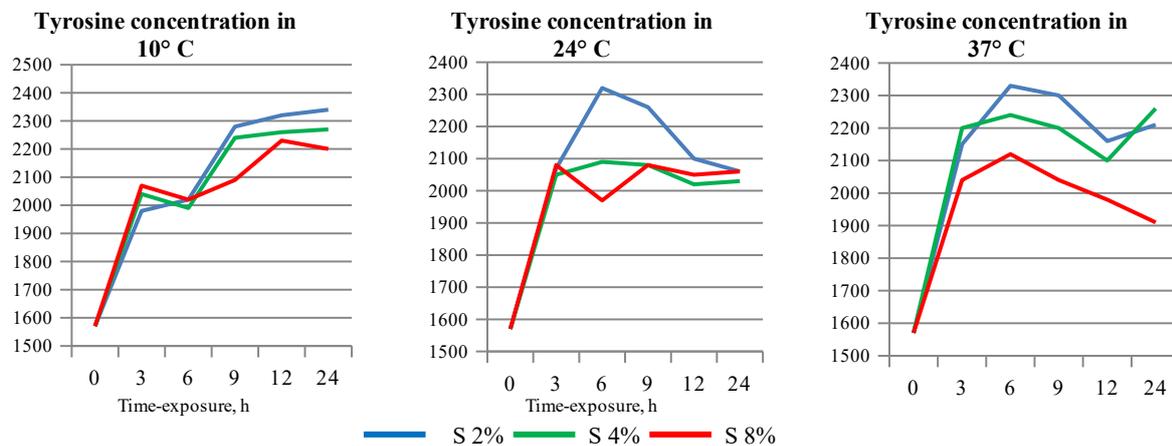


Fig. 6. Dynamics of changes in the tyrosine concentration in samples during incubation of *Lactobacillus acidophilus* culture depending on the mass NaCl fraction of in the substrate.

A comparison of tyrosine levels in *Lactobacillus acidophilus* bacteria cultivation showed that an increase in the mass NaCl fraction in the substrate as a whole led

to a decrease in the intensity of multidirectional enzymatic activity of microorganisms.

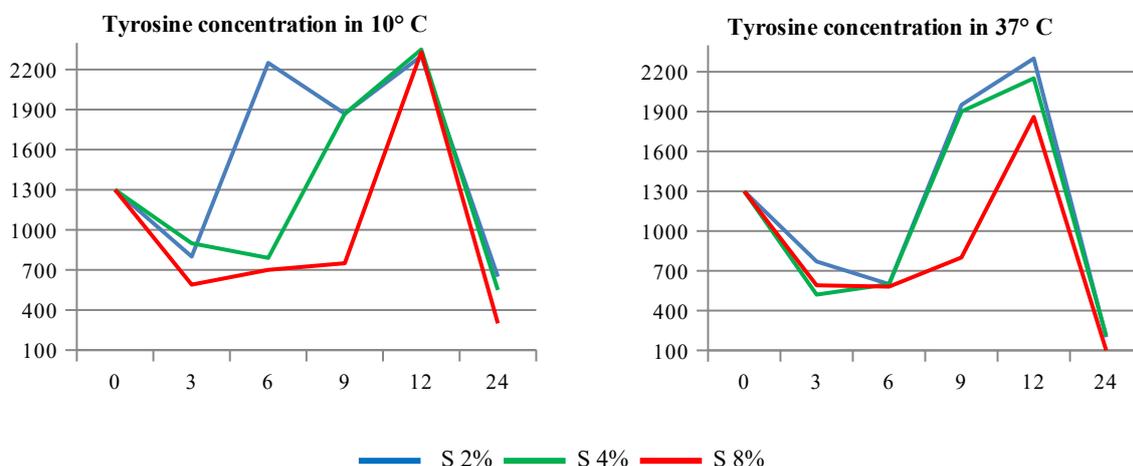


Fig. 7. Dynamics of changes in the tyrosine concentration in samples during incubation of *Propionibacterium freudenreichii* culture depending on the mass NaCl fraction.

In the presence of NaCl, the increase in the tyrosine concentration in the culture fluid of *Propionibacterium freudenreichii* occurred much later than in the control. In a medium with a mass fraction of 2% NaCl, the maximum tyrosine level was observed after 6 hours of cultivation at a temperature of 10 °C, and after 12 hours for substrates with 4 and 8% salt at 37° C. The response of the enzyme systems of the *Propionibacterium freudenreichii* bacteria was expressed in the lengthening of the period of hydrolytic processes predominance onset.

It follows from this that the less intensive course of multidirectional enzymatic processes in the presence of NaCl is determined by the influence of salts of alkaline and alkaline earth metals on both enzymes and individual subcellular structures. Thus, the experiments carried out in the framework of this work confirm the ability of the selected lactic acid cultures to proteolysis, which allows to consider them as potential probiotic cultures for the production of protein-containing products from aquatic biological resources. The introduction of proteolytic lactic acid bacteria will speed up the physico-chemical and biochemical

processes and reduce the duration of protein-containing raw materials maturation.

4 Conclusion

Lactobacillus acidophilus cultures were characterized by the predominance of proteolytic processes, regardless of the physico-chemical conditions of cultivation, for the other studied strains, the alternation of proteolysis and assimilation is inherent.

Streptococcus thermophilus bacteria had minimal proteolytic activity.

It is shown that the incubation temperature was a factor determining the direction of metabolic processes in general. At a temperature of 10° C, less pronounced fluctuations in the level of tyrosine were observed, which indicated a slowdown in the total enzymatic processes and led to the predominance of assimilation processes over hydrolytic ones.

When the pH of the medium changed to the acidic side, the predominance of hydrolytic processes was observed during the cultivation of all the objects of the study, under conditions of neutral hydrogen index of the substrate, assimilation processes prevailed, characterized by a decrease in the level of tyrosine in the samples.

The general reaction of all the studied cultures of microorganisms to an increase in the mass fraction of NaCl in the substrate was to reduce the total intensity of the multidirectional enzymatic activity of microorganisms. *Propionibacterium freudenreichii* cultures were characterized by growth in the time of tyrosine concentration increase in the medium.

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