

Conference Paper

Assessment of Propionibacterium Metabolic Activity on Protein Substrate of Sarcoplasmic Fraction in Hydrobionts' Muscle Tissue

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Abstract

As a result of the conducted scientific researches, the authors analyzed methodical aspects of studying the proteolytic properties of *Propionibacterium freudenreichii subsp. shermanii*-KM 186 -- the representatives of the probiotic group of microorganisms, which allowed not only to evaluate the enzymatic activity of the objects of study, but also to discover how physical and chemical factors influence the proteins hydrolysis intensity in sarcoplasmic and myofibrillary fractions of hydrobionts' muscle tissue. During the experiment which aimed at assessment the possibility of using individual protein fractions of fish muscle tissue as a substrate for proteolysis by *Propionibacterium* bacteria for the spectrophotometry analysis of their enzymatic activity, it was found that the sarcoplasmic fraction can be used as a substrate for studying the microorganisms' proteolytic activity. Determination of the information content of tyrosine concentration measuring by spectrophotometry to characterize the proteolytic activity of bacterial suspensions showed that to assess the enzymatic activity of living microorganisms culture it is advisable to use not the absolute values of the bacterial suspension activity, expressed in $\mu\text{molTYR}/\text{ml}\times\text{min}$ or the tyrosine concentration in the sample ($\mu\text{mol}/\text{L}$), but the change in the tyrosine level in comparison with its primary values. This way of expressing the results will allow us to assess the prevalence of processes (dissimilation/assimilation). In addition, the experiment revealed characteristic response of the *Propionibacterium* bacteria's metabolic activity to changes in the physical and chemical conditions.

Keywords: probiotic group of microorganisms, *Propionibacterium freudenreichii subsp. shermanii*, sarcoplasmic and myofibrillary fractions of hydrobionts' muscle tissue, proteolytic activity, Atlantic cod, physicochemical factors.

1. Introduction

Proteolytic activity can be attributed to significant technological properties inherent in certain types of lactic acid bacteria, which is determined by filtering cell proteases and intracellular enzymes released during bacteria autolysis during cultivation. Bacterial proteases involved in the proteins breakdown can change the structure of muscle fibers, improving the histological properties of the finished product.

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The resulting free amino acids are a source of other nitrogenous compounds that improve the quality of organoleptic characteristics of finished products [1, 2]. The selected research subjects, *Propionibacterium freudenreichii subsp. shermanii*, have probiotic properties. Probiotic microorganisms take root in technological environments and produce biologically active substances, allowing to produce a new generation of products possessing physiologically useful properties, with improved organoleptic characteristics, containing antioxidant and antimutagenic components. These qualities of probiotic microorganisms determine broad prospects for their use [3--5]. The study of their metabolic activity, which can affect both the intensification of technological processes and the quality of finished foods, seems to be really relevant [6].

2. Methods and Equipment

The subject of the study was the proteolytic activity of probiotic microorganisms *Propionibacterium freudenreichii subsp. shermanii*-KM 186. The study of proteolytic activity was carried out using separate fractions of muscle tissue of Atlantic cod (*Gadus morhua*). After obtaining the extracts containing sarcoplasmic or myofibrillar fractions, the optical densities of solutions were measured using the SF-2000 spectrophotometer at wavelengths of 260 and 280 nm in three sequences. The method is based on spectrophotometric determination of the concentration of dissolved aromatic amino acids and peptides formed during enzymatic hydrolysis of protein components of muscle tissue fractions after adding a suspension of microorganisms to them [7, 8].

The study was based on tracking changes in tyrosine levels in the culture fluid at different incubation temperatures, pH and NaCl concentration, after a certain period of time.

Thus, by comparing the results of the enzymatic activity of bacterial suspension in substrates with different physical and chemical characteristics, the influence of incubation conditions and duration on the intensity and direction of studied objects' metabolic processes was evaluated.

3. Results

To determine the proteolytic activity of *Propionibacterium freudenreichii subsp. shermanii* at the initial stage of the research, sarcoplasmic and myofibrillar protein fractions were selected as a substrate. Tyrosine concentration was determined in the samples after fractionation. Control samples and inoculated samples were incubated for 24

hours at three temperatures [9]. The results of optical density measurements in the samples containing myofibrillar fraction were negative. The obtained negative results of optical density measurement did not allow to use myofibrillary fraction as a substrate for evaluation of enzymatic activity of bacteria by spectrophotometric method. Later on, we used only the sarcoplasmic fraction.

During the experiment, it was noted that at the first stage of incubation, accumulation of tyrosine in the sarcoplasmic fraction filtrate was observed, and then non-protein nitrogen compounds were assimilated by bacteria, as evidenced by a decrease in the level of tyrosine in the medium.

Successive alternation of tyrosine levels during the cultivation of bacterial suspension in the medium containing sarcoplasmic fraction of proteins rather indicates not the proteolysis intensity but its predominance over biosynthetic processes.

Given that the suspension of bacterial cells is a system of living organisms, the quantitative characteristics of which are not constant, it is not correct to calculate the proteolytic activity of the culture without taking into account changes in the number of cells over time.

Thus, to assess the enzymatic activity of the living microorganisms' culture, it seems appropriate to use not the absolute values of the activity of the bacterial suspension expressed in $\mu\text{molTYR}/\text{ml}\times\text{min}$ or the tyrosine concentration in the sample ($\mu\text{mol}/\text{L}$), but the change in the level of tyrosine in comparison with its initial values. This way of expressing the results will allow to estimate the predominance of processes (disimilation/assimilation) rather than to measure the specific enzymatic activity [10]. This methodical approach is informative from the point of view of estimation of practically significant properties of propionic acid bacteria.

Since the proteolytic activity of *Propionibacterium* was considered from the point of view of the possible use of this group of microorganisms for the production of protein-containing fermented products, including hydrobionts, we chose the conditions of proteolysis corresponding to the expected modes of technological operations of the corresponding products' production.

We have determined three incubation temperatures of 10, 20 and 30 °C corresponding to the minimum and optimal temperatures of growth and development of microorganisms of *Propionibacterium* genus (Figure 1).

The concentration of tyrosine in the *Propionibacterium* incubation process in the substrate containing the sarcoplasmic fraction was changed from 1121.4 to 2054.9 $\mu\text{mol}/\text{L}$. The oscillation was about 50 %. The highest levels were observed after 3 and 12 hours, but their fluctuations did not exceed 20% of the initial tyrosine concentration.

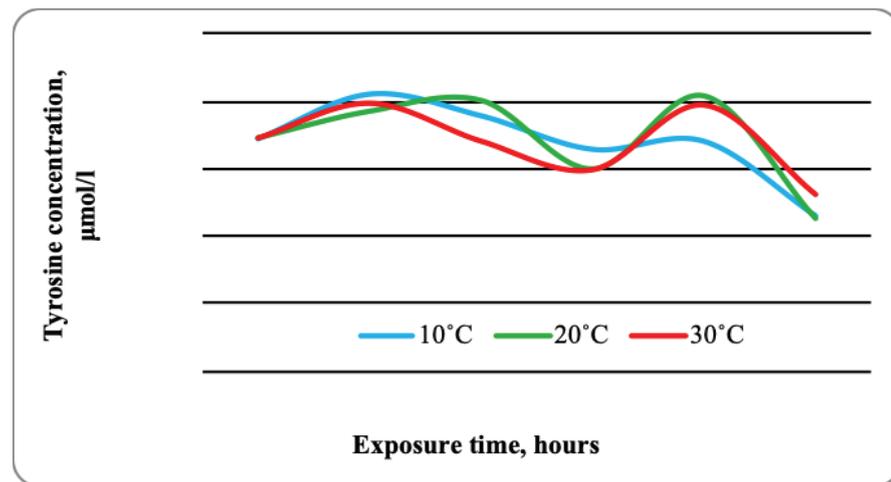


Figure 1: Changes in the amount of tyrosine 1 ml culture of *Propionibacterium* bacteria concentration of 10^9 cells/ml, depending on the fermentation temperature.

Decrease of tyrosine concentration in comparison with the initial level was observed after 9 and 24 hours and varied within 25--30% depending on temperature regimes.

A change in temperature within certain limits affects the rate of enzymatic reaction, similar to the effect of temperature on any chemical reaction. The rate of enzymatic reactions in total, of course, depends on the temperature. Thus, at an incubation temperature of 10 °C, we observe less pronounced fluctuations in tyrosine levels over time than in other modes.

With an increase in the cultivation temperature, more abrupt changes in the concentration of marker molecules in the medium were observed, due to the acceleration of enzymatic multidirectional reactions [11].

However, the change in the rate of different chemical reactions catalyzed by enzymes has its own temperature optimum, which is reflected in the graph in the form of predominant assimilation processes at 10 °C, and hydrolytic -- at 30 and 20 °C. Hydrolytic processes at 20 °C were observed at the 6th hour of exposure, whereas at 30 °C -- it happened 3 hours earlier. Based on this, it can be concluded that the optimal temperature for fermentation is 30 °C.

To study the effect of the hydrogen index on the course of fermentation, we have identified three pH regimes -- 4.5, 7 and 8, which are potentially significant from a technological point of view (Figures 2, 3).

Enzymes, like any proteins, are sensitive to the pH value of the medium. The ionization of functional groups in the enzyme protein molecule depends on the concentration of hydrogen ions. For each enzyme there is its optimal pH of the medium, at which its activity is maximal [12]. Thus, the total change in enzyme activity observed when the

hydrogen index of the medium deviates from the neutral to the acid or alkaline side was reflected in the curve of tyrosine concentration change.

To assess NaCl effect on the metabolic activity of bacteria in the substrate for their cultivation, salt concentrations at the levels of 2, 6 and 8% were established, which corresponded to the parameters of the proposed technological modes of application of microorganisms.

The change in tyrosine concentration during incubation of bacteria in the substrate with pH levels 4.5 and 8 varied within 60 % (from 1460 to 2356.4 $\mu\text{mol/L}$). After 9 hours of exposure, the maximum level of the marker was observed, which differed from the initial level by 30-60 %. The minimum value was observed after 12 hours of incubation and varied between 25-40 %.

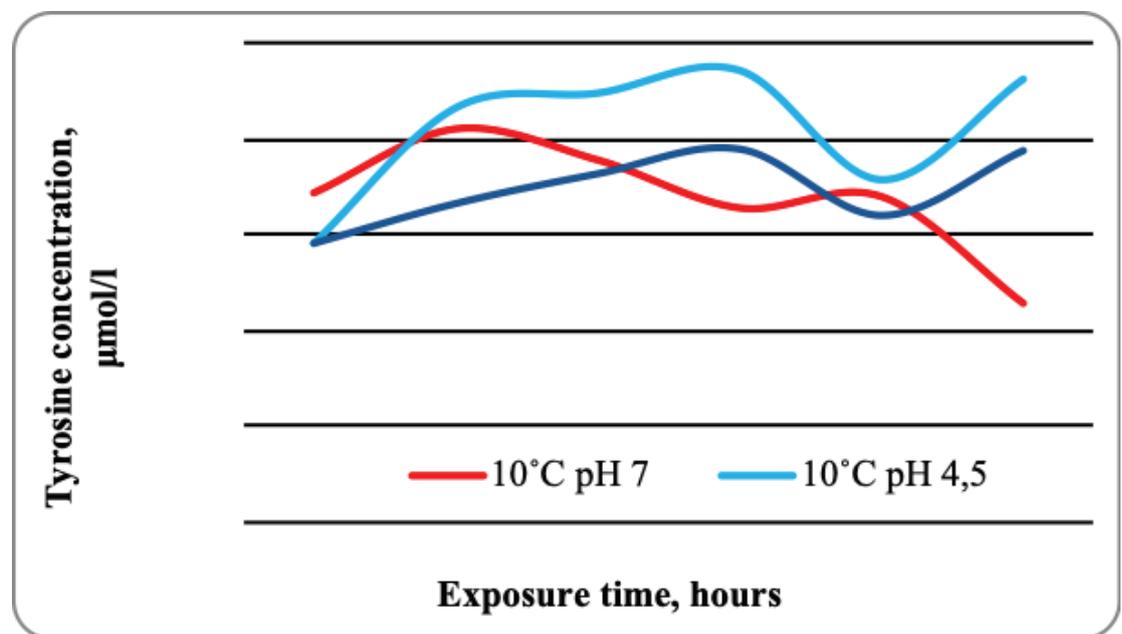


Figure 2: Change of tyrosine level in samples during incubation of *Propionibacterium* culture with concentration of 109 cells/ml at temperature of 10 °C depending on pH value.

The tyrosine concentration during the cultivation of microorganisms at a temperature of 30 °C varied from 1460 to 2323.4 $\mu\text{mol/L}$, which was 60 %, like in the previous experiment. The maximum values of the measured index were observed after 9 and 12 hours of exposure. Reduction of tyrosine concentration in the samples did not exceed 15 %.

In general, it was noted that the content of tyrosine in media with the addition of NaCl, in any of the selected concentrations, varied over a wider range compared to this indicator in the control sample (Figures 4, 5).

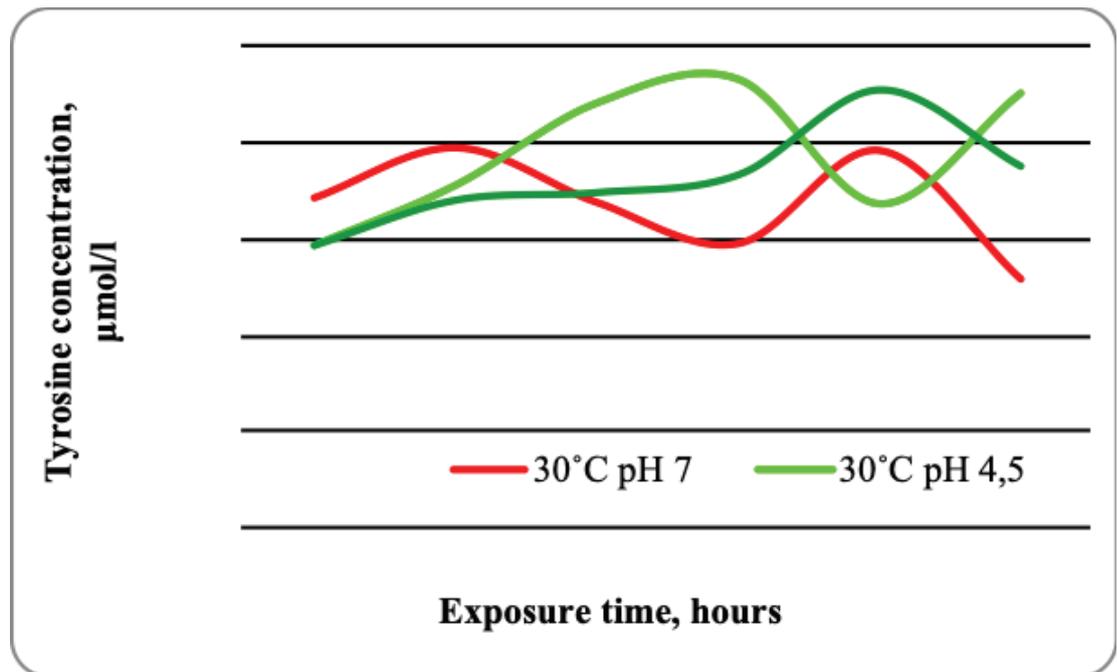


Figure 3: Changes in tyrosine levels in samples during incubation of *Propionibacterium* culture at a concentration of 109 cells/ml at a temperature of 30 °C, depending on the pH value.

In general, it was noted that in the presence of NaCl, the increase in the concentration of tyrosine in the culture fluid, due to the hydrolytic activity of bacterial enzyme systems, occurred much later compared to the control. So at a temperature of 10 °C -- 6 hours after incubation for a substrate with a mass fraction of NaCl 2 %, and in 12 hours for 6 and 8% salt concentration, at 30 °C, an increase in tyrosine concentration was noted after 12 hours of exposure.

The lengthening of the period of hydrolytic processes' predominance occurrence is due to the direct effect NaCl makes on the hydrate shells of hydrolytic proteins-enzymes.

The concentration of tyrosine during incubation at 10 °C in the substrate with the addition of NaCl varied from 271.2 to 2351.6 µmol/L and was 160 %. At the initial stage of cultivation, there was a significant decrease in tyrosine in the medium (from 40 to 55 %). The maximum was observed after 12 hours of cultivation and exceeded the initial level by 75--80 %.

Changes in the content of tyrosine in the substrate with the addition of different salt concentrations at an incubation temperature of 30 °C ranged from 169 % of the initial level (from 103.1 to 2298.4 mmol/l). The maximum values were observed after 12 hours of exposure, and increased by 75% in the medium with the addition of 2% salt and 45% in the medium with 8% of NaCl.

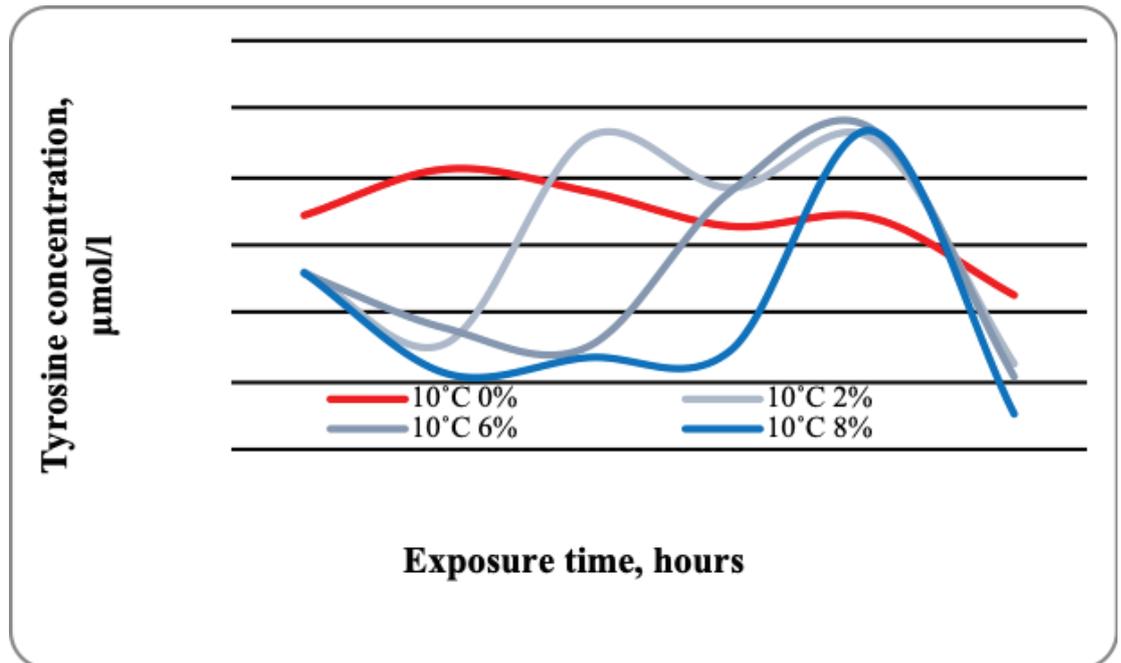


Figure 4: Changes in tyrosine level in samples during incubation of *Propionibacterium* culture with concentration of 10^9 cells/ ml at 10 °C depending on the mass fraction of NaCl in the substrate.

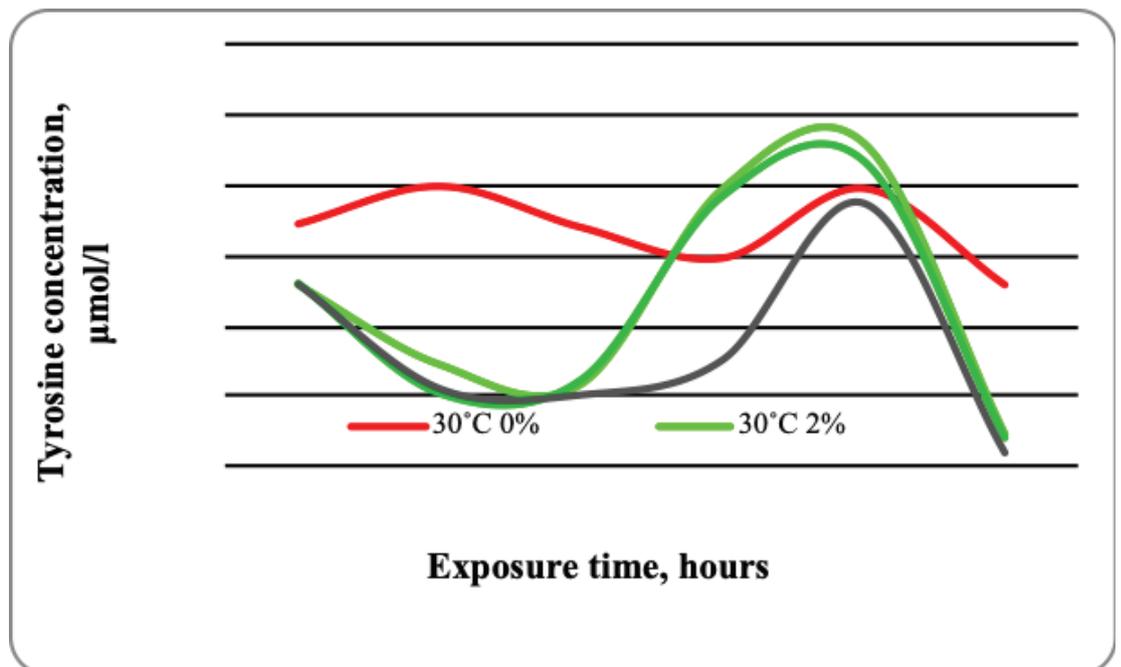


Figure 5: Change of tyrosine level in samples during incubation of *Propionibacterium* culture with concentration of 10^9 kl / ml at temperature of 30 °C depending on the mass fraction of NaCl in the substrate.

4. Conclusion

A comparative analysis of the possibility of using individual protein fractions from fish muscle tissue as a substrate to assess the enzymatic activity of *Propionibacterium*

freudenreichii subsp. *shermanii* bacteria made by the spectrophotometry method found that the sarcoplasmic fraction has advantages in terms of the results' correctness.

As a characteristic of the total enzymatic activity of microorganisms, it is advisable to use the relative change in the tyrosine concentration during incubation.

The correlation between the intensity of proteolytic processes during incubation of bacterial cells in media with a pH different from the normal value is shown.

It was noted that at the initial stage of cultivation, the substrate containing sarcoplasmic fraction and NaCl demonstrated a significant predominance of tyrosine withdrawal from the medium and a slowdown in proteolytic activity.

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