TECHNOLOGY OF PRODUCTION BIOLOGICAL ACTIVE ADDITIVE BASED ON SELENIUM CONTAINING CULTURE OF BIFIDOBACTERIUM

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Annotation. The article presents data on the positive impact of essential microelement selenium on the human body. It was characterized the ability to accumulate inorganic forms of selenium (such as selenites and selenates) into the organic forms by probiotic microorganisms. The article presents data concerning sodium selenite concentration impact on biomass growth of bifidobacterium culture. It was fined optimum conditions for accumulation maximum selenium containing biomass of microorganisms. Based on experiments it was created selenium containing biologically active additive.

Keywords: bifidobacterium, sodium selenite, inoculums, biological active additive.

INTRODUCTION

Nowadays in Ukraine and many countries in the world there is a serious problem of inadequate supplying of microelement selenium to the human body. Selenium content in foods is low that’s why supply of selenium trace from food does not meet the daily needs of it. Selenium is a trace mineral essential microelement. It plays an important role in the flow of many physiological processes in human organism. It is performs a catalyst redox reactions which protect the body from harmful free radicals. Selenium belongs to the trace elements which adequate spacing and maximum permissible level of consumption relatively narrow and largely depends on the form in which selenium is ingested.

In foods of animal and vegetable origin selenium presents almost in organic form, and its form have a less toxic than inorganic forms [1]. Therefore important area of research is to develop new methods of preventing and overcoming selenium deficit in population.

In China artificially making inorganic forms of selenium in the soil, leading to its accumulation by plants. Other ways of overcoming the trace element selenium is enrichment of directly foodstuffs. An example serves selenium enriched production of tea, salt, water, eggs and meat, mushrooms, sprouts cereals. Perspective area of researching is to create dietary supplements based on probiotic selenium enriched microorganisms. This products has a double positive effect on the human body. On the one hand due to the content of organic forms of selenium is better absorbed by the body, and on the other – due to probiotic effect on microorganisms.

Formulation of the problem

Biological synthesis of organic forms of selenium compared to other methods that require little energy and is environmentally friendly, eliminating the possibility of harmful by-products. Today important area of research is the development of new food products enriched with organic forms of selenium. Therefore selection of the optimal conditions for cultivation selenium containing microorganisms is an important task aimed in creating selenium containing products [2].
Literature review

Selenium is a 34-th element in the periodic table of elements Mendeleev, it is located in the 4-th period, 6-th main group. Selenium was investigated by J. Berzelius in 1817. It is able to form organic and inorganic structures. To inorganic forms include selenites and selenates. Organic forms presents by so-called organic selenoaminoacides (such us selenometionin and selenotsysteyin) in which selenium replaces sulfur [2]. Selenium is a part of enzymes (glutationperoksidase, yodtironіndeyodinase, tіoreduktase), proteins, and it can deposit into all organs in a human body [3].

Published data indicates that bacteria can accumulates and biotransformates ions of metals after entry its into the cultivation medium [4]. Adding selenium to the culture medium of microorganisms improves their redox potential through the formation of its organic forms [5].

Synthesis of selenocysteine carried out in specialized tRNA, which also include it in the growing peptide chain. Primary and secondary structure selenotyrsine-specific tRNA tRNKSec differ from those of standard tRNAs in several respects. Thus, the acceptor region contains 8 pairs in bacteria and 10 – in eukaryotes and longer T-loop; In addition, the replacement of several typical tRNKSec fairly conservative base pairs. First tRNKSec binds to serine by the enzyme seryl-tRNA ligase, but created complex Ser-tRNKSec not enter into broadcasting because broadcasting is not recognized by normal factors (EF-Tu in bacteria and in eukaryotes eEF1A). Serine residue associated with tRNAs, turns on the balance with selenotyrsine through enzyme selenotyrsinysyntaza. This complex is formed Sec-tRNKSec that binds specifically to an alternative translational factor (SelB or mSelB (or eEFSec), which specifically delivers it to the ribosome translates mRNAs for selenoprotein. The specificity of the delivery due to the presence of additional protein domain (bacteria, SelB) or more subunits (SBP2 for eukaryotic mSelB/eEFSec), which binds to the item mRNA secondary structure formed part SECIS [6-8].

Selenium enriched microorganisms are able to provide antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory effect on the host organism and inhibit the growth of pathogens [2].

Main part

The aim of researches was the selection of optimal cultivation conditions, which would have made it possible to get the maximum yield of biomass selenium enriched bifidobacteria and create on their basis of dietary supplements.

The experiments used the museum culture Bifidobacterium bifidum-1. Cultivation of bifidobacteria was carried out on corn-lactose environment. As a source of selenium, used sodium selenite Na2SeO3 (Hemel). Sodium selenite dissolved in sterile distilled water and added to the culture medium at concentrations from 0.5 mg/cm 3 to 20 g/cm 3. As a control served medium without adding sodium selenite. Inoculum of daily culture brings in flasks with the cultivation medium in quantities of 5 %.

Initially, the study determined the optimal conditions for the accumulation of biomass of selenium containing bifidobacteria. The criteria were chosen optimality has some key indicators: indicators of colony forming units; quantitative content of selenium in the culture medium; cultivation. Indicator of kolonony forming units makes possible to determine the number of viable microorganisms and their enzymatic activity, and describes the yield of biomass on completion of cultivation process.

According to the data found mapping function as criteria dependent on the parameters. Surfaces described polynomial degree 3, Fig. 1. Where, z – lg KVO/sm3; x – CSe; y – t.

Fig. 1. Cultivation of bifidobacteria in corn-lactose environment
With Table Curve 3D we received coefficients. The relationship between the criteria of differentiated and continuous optimization in all areas of the field definition parameters. So it makes sense to use the classic methods of optimization. Extremum functions located at the points where the partial derivatives are equal to 0:

\[
\begin{align*}
\frac{dZ}{dx} &= 0 \\
\frac{dZ}{dy} &= 0
\end{align*}
\]  
(1)

\[
\begin{align*}
\frac{dZ}{dx} &= \frac{d}{dx} \left(a + b \cdot x + c \cdot y + d \cdot x^2 + e \cdot y^2 + f \cdot x \cdot y + g \cdot x^3 + h \cdot y^3 + i \cdot x \cdot y^2 + j \cdot x^2 \cdot y\right)
\end{align*}
\]  
(2)

\[
\begin{align*}
\frac{dZ}{dy} &= \frac{d}{dy} \left(a + b \cdot x + c \cdot y + d \cdot x^2 + e \cdot y^2 + f \cdot x \cdot y + g \cdot x^3 + h \cdot y^3 + i \cdot x \cdot y^2 + j \cdot x^2 \cdot y\right)
\end{align*}
\]  
(3)

\[
\begin{align*}
3 \cdot g \cdot x^2 + 2 \cdot j \cdot x \cdot y + 2 \cdot d \cdot x + i \cdot y^2 + f \cdot y + b &= 0
\end{align*}
\]  
(4)

\[
\begin{align*}
j \cdot x^2 + 2 \cdot i \cdot x \cdot y + f \cdot x + 3 \cdot h \cdot y^2 + 2 \cdot e \cdot y + c &= 0
\end{align*}
\]  
(5)

Decision:

\[
\begin{align*}
y &= \frac{f - 2 \cdot e + \sqrt{f^2 - 4 \cdot e \cdot f + 4 \cdot e^2 + 12 \cdot b \cdot h - 4 \cdot b \cdot i - 12 \cdot c \cdot h + 4 \cdot c \cdot i}}{6 \cdot h - 2 \cdot i}
\end{align*}
\]  
(6)

\[
\begin{align*}
x &= \frac{d + \sqrt{d^2 + 2 \cdot d \cdot j \cdot y + j^2 \cdot y^2 - 3 \cdot g \cdot i \cdot y^2 - 3 \cdot f \cdot g \cdot y - 3 \cdot b \cdot g + j \cdot y}}{3 \cdot g}
\end{align*}
\]  
(7)

It was defined extremes where – cultivation and x – sodium selenite concentration. Thus, in the cultivation of bifidobacteria in corn-lactose environment we identified that optimum time of cultivation is 24.5 hours and quantitative of sodium selenite – 1.6 g/cm^3.

The optimum cultivation conditions were the basis for a dietary supplement based on selenium containing bifidobacteria. Fig. 2,3 reflect the changing parameters of active and titrated acidity in the accumulation of selenium containing biomass of bifidobacteria. Determined that in the 24 hour of accumulation selenium containing biomass indicators of active acidity were at pH 5.1, and the titration acidity – 60.5 °T.

The quantitative content of selenium containing biomass of bifidobacteria on 24 hour cultivation amounted to 4,4×10^9 CFU/cm^3. Selenium containing biomass of microorganisms separated from the culture medium by centrifugation at 10,000 rev^1 for 15 min. The received biomass separated from the non accumulated sodium selenite, which was contained in the culture medium by washing in sterile water, followed by centrifugation at 10,000 rev^1 for 15 min. The next step was the introduction of a protective environment which incorporates contained milk, sucrose and zhelatoza followed by freeze-
drying. Microbiological parameters obtained product are shown in Table 1.

The data in Table 1 indicate that quantitative bifidobacteria in the finished product meets the probiotic content without of Coliform bacteria and pathogenic microorganisms. Organoleptic characteristics derived selenium containing product presents in the Table 2.

Table 1 – Microbiological characterization of product

| Indicator                          | Characteristics          |
|------------------------------------|--------------------------|
| The quantitative content of        | 4.4 ×10⁸ CFU /sm³        |
| bifidobacteria                     |                          |
| Mold fungi CFU /sm³                | missing                  |
| Pathogenic microorganisms,         | missing                  |
| including salmonella               |                          |
| Coliform bacteria 0.1 g            | missing                  |

The resulting dietary supplement characterized beige powdery structure, specific taste and smell. Quantitative selenium content in the finished product was 200±1 mg/g. Technological scheme of production selenium containing product shown in Fig. 4.

Table 2 – Organoleptic characteristics of the product

| Indicator                          | Characteristics          |
|------------------------------------|--------------------------|
| Appearance                         | Powdered or crystalline porous mass |
| Taste and smell                    | Specific                  |
| Color                              | Beige                    |
| Quantitative selenium content, mg/g| 200±1                    |

Conclusions

As follows was developed technology for dietary supplements based on selenium containing bifidobacteria. During the studies found that the best time for cultivation culture of Bifidobacterium in corn-lactose medium is 24.5 hours, and the optimal content of sodium selenite – 1.6 g/cm³. It was created biologically active additive based on selenium containing Bifidobacterium culture with quantitative content of microorganisms at 4.4×10⁸ CFU/cm³.

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ТЕХНОЛОГІЯ ПРОИЗВОДСТВА БІОЛОГІЧНО АКТИВНОЇ ДОБАВКИ ИЗ СЕЛЕНСОДЕРЖАЩЕЙ КУЛЬТУРЫ БИФІДОБАКТЕРІЙ

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Аннотація. В статті приведені дані про позитивне впливання ессенціального мікроелементу селена на здоров'я людини. Охарактеризована можливість пробіотичних організмів накопичувати неорганічні форми селена (селенати, селенити), преврашати їх в органічні. Приведені дані про вплив концентрацій селеніту на прирост біомаси біфідобактерій. Опреділені оптимальні умови максималного накопичення селенсодержащей культури бифідобактерій. На основі отриманих даних розроблена технологія селенсодержащей біологічно активної добавки.

Ключові слова: біфідобактерії, селеніт натрію, інокулят, біологічно активна добавка.

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