UDC [579.864:579.873.1]:546.33'234

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.

ТЕХНОЛОГІЯ ВИРОБНИЦТВА БІОЛОГІЧНО АКТИВНОЇ ДОБАВКИ НА ОСНОВІ СЕЛЕНВМІСНОЇ КУЛЬТУРИ БІФІДОБАКТЕРІЙ

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Анотація. У статті наведено дані стосовно позитивного впливу есенціального мікроелементу селену на здоров'я людини. Охарактеризовано здатність пробіотичних мікроорганізмів накопичувати неорганічні форми селену (селеніти, селенати), перетворюючи їх в органічні. Наведено дані стосовно впливу концентрацій селеніту натрія на приріст біомаси біфідобактерій. Визначено оптимальні умови максимального накопичення селеновмісних культур біфідобактерій. На основі отриманих даних розроблено технологію селеновмісної біологічно активної добавки.

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

DOI: http://dx.doi.org/10.15673/fst.v11i1.296

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 his 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 selenmetionin and selenotsysteyin) in which selenium replaces sulfur [2]. Selenium is a part of enzymes (glutationperoksidase, yodtironindeyodinase, tioreduktase), 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 selenotsysteyin-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 selenotsysteyine through enzyme selenotsysteyinsyntaza. 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 selenoproteyine. 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-I.* Cultivation of bifidobacteria was carried out on corn-lactose environment. As a source of selenium, used sodium selenite Na_2SeO_3 (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 cultuvation 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 yeald of biomass of microorganisms 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 КУО/sm^3; x – C^{Se}; y – t.

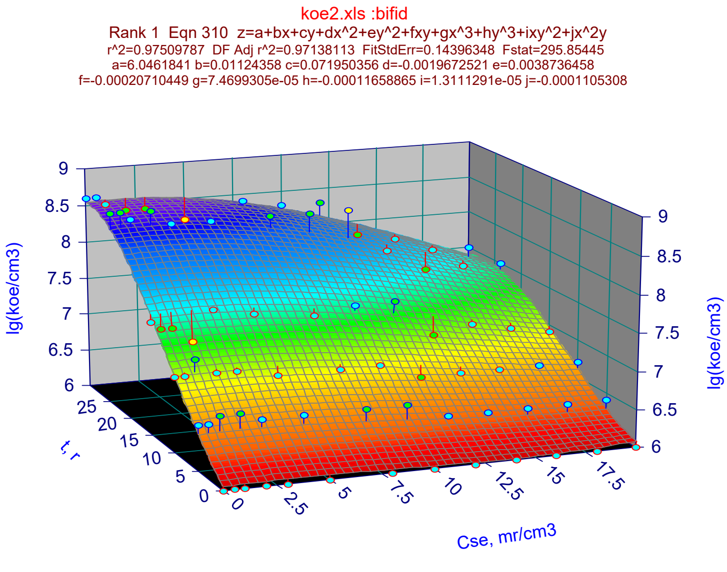

Fig. 1. Cultivation of bifidobacteria in corn-lactose environment

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:

$$\begin{cases} \frac{dz}{dx} = 0 \\ \frac{dz}{dy} = 0 \end{cases} \quad (1)$$

$$\frac{d}{dx}Z = \frac{d}{dx}\left(a + b\cdot x + c\cdot y + d\cdot x^2 + e\cdot y^2 + f\cdot x\cdot y + g\cdot x^3 + h\cdot y^3 + i\cdot x\cdot y^2 + j\cdot x^2\cdot y\right) \quad (2)$$

$$\frac{d}{dy}Z = \frac{d}{dy}\left(a + b\cdot x + c\cdot y + d\cdot x^2 + e\cdot y^2 + f\cdot x\cdot y + g\cdot x^3 + h\cdot y^3 + i\cdot x\cdot y^2 + j\cdot x^2\cdot y\right) \quad (3)$$

$$3\cdot g\cdot x^2 + 2\cdot j\cdot x\cdot y + 2\cdot d\cdot x + i\cdot y^2 + f\cdot y + b = 0 \quad (4)$$

$$j\cdot x^2 + 2\cdot i\cdot x\cdot y + f\cdot x + 3\cdot h\cdot y^2 + 2\cdot e\cdot y + c = 0 \quad (5)$$

Decision:

$$y = \begin{pmatrix} \dfrac{f - 2\cdot e + \sqrt{f^2 - 4\cdot e\cdot f + 4\cdot e^2 + 12\cdot b\cdot h - 4\cdot b\cdot i - 12\cdot c\cdot h + 4\cdot c\cdot i}}{6\cdot h - 2\cdot i} \\ -\dfrac{2\cdot e - f + \sqrt{f^2 - 4\cdot e\cdot f + 4\cdot e^2 + 12\cdot b\cdot h - 4\cdot b\cdot i - 12\cdot c\cdot h + 4\cdot c\cdot i}}{6\cdot h - 2\cdot i} \end{pmatrix} \quad (6)$$

$$x = \begin{pmatrix} -\dfrac{d + \sqrt{d^2 + 2\cdot d\cdot j\cdot y + j^2\cdot y^2 - 3\cdot g\cdot i\cdot y^2 - 3\cdot f\cdot g\cdot y - 3\cdot b\cdot g} + j\cdot y}{3\cdot g} \\ -\dfrac{d - \sqrt{d^2 + 2\cdot d\cdot j\cdot y + j^2\cdot y^2 - 3\cdot g\cdot i\cdot y^2 - 3\cdot f\cdot g\cdot y - 3\cdot b\cdot g} + j\cdot y}{3\cdot g} \end{pmatrix} \quad (7)$$

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.

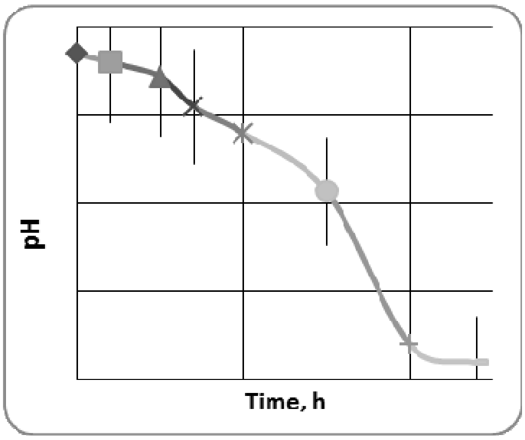

Fig. 2. Changing the parameters active acidity

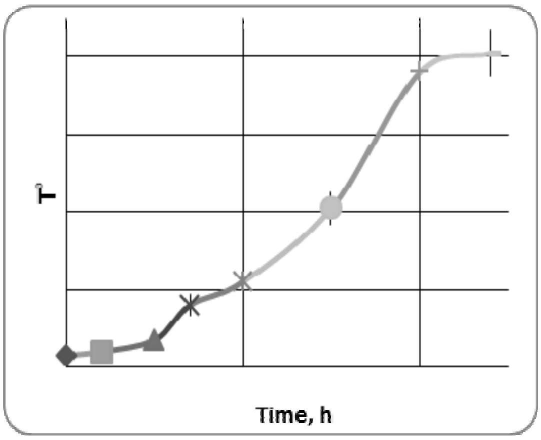

Fig.3. Changing the parameters titration acidity

The quantitative content of selenium containing biomass of bifidobacteria on 24 hour cultivation amounted to 4,4×10^8 CFU/cm^3. Selenium containing biomass of microorganisms separated from the culture medium by centrifugation at 10,000 rev^{-1}for 15 min. The recived 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} aor 15 min. The next step was the introduction of a protective environment which incorporates contained milk, sucrose and zhelatoza followed by freezedrying. 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 bifidobacteria	$4{,}4 \times 10^8$ CFU /sm^3
Mold fungi CFU /sm^3	missing
Pathogenic microorganisms, including salmonella	missing
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

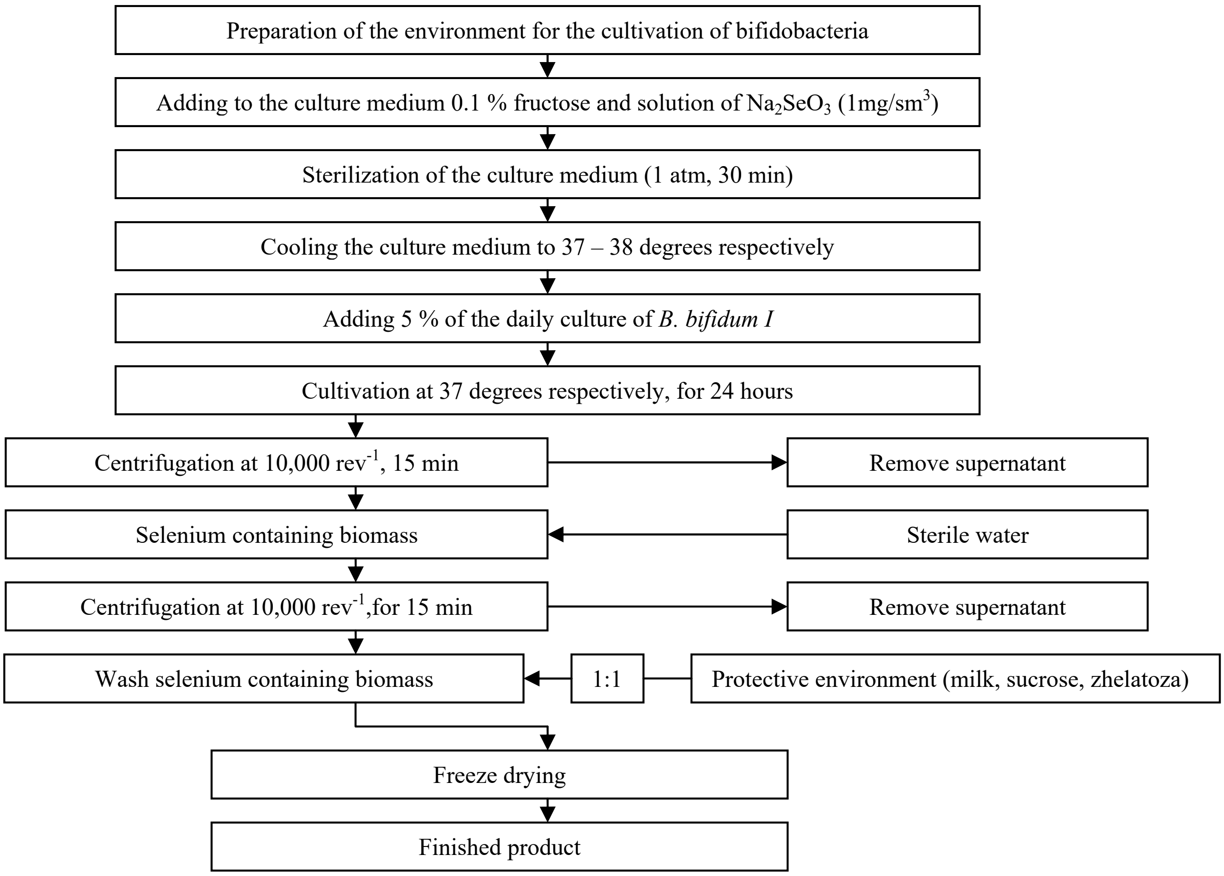

Fig. 4. Technological scheme for selenium containing product

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/cm^3. It was created biologically active additive based on selenium containing Bifidobacterium culture with quantitative content of microorganisms at 4,4×108 CFU/cm^3.

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ТЕХНОЛОГИЯ ПРОИЗВОДСТВА БИОЛОГИЧЕСКИ АКТИВНОЙ ДОБАВКИ ИЗ СЕЛЕНСОДЕРЖАЩЕЙ КУЛЬТУРЫ БИФИДОБАКТЕРИЙ

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Аннотация. В статье приведены данные о положительном воздействии эссенциального микроэлемента селена на здоровье человека. Охарактеризована способность пробиотических микроорганизмов накапливать неорганические формы селена (селениты, селенаты), превращая их в органические. Приведены данные о влиянии концентраций селенита натрия на прирост биомассы бифидобактерий. Определены оптимальные условия максимального накопления селенсодержащей культуры бифидобактерий. На основе полученных данных разработана технология селенсодержащей биологически активной добавки.

Ключевые слова: бифидобактерии, селенит натрия, инокулят, биологически активная добавка.

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Отримано в редакцію 14.12.2016 Received 14.12.2016
Прийнято до друку 25.01. 2017 Approved 25.01. 2017