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THE INFLUENCE OF BIOTHIN ON GROWTH AND DEVELOPMENT OF BACTERIA LACTOBACILLUS PLANTARUM

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Abstract

Objective*: the aim of the study was to study the effect of biotin on the vital activity of lactic acid bacteria.* ***Methods:*** *a study of the effect of vitamin H on lactic acid bacteria Lactobacillus plantarum probiotic "Flactonia" was carried out in a periodic culture on selective nutrient media: MPC and cabbage. Biotin was added at a concentration of 5 mg/L to the media to stimulate the growth of Lactobacillus plantarum. It was used nutrient media (MRS and cabbage media) without growth promoter as a control. The study included the investigation of the dynamics of accumulation of bacteria Lactobacillus plantarum. To achieve this goal, sampling was carried out within 60 hours. The determination of biomass, active acidity and kinetic parameters of crop growth was carried out every 6 hours. Such kinetic parameters of bacteria growth as the rate of exponential growth (μ, h^{-1}), constant of fission rate (V, h^{-1}), generation time (g, hour). Isolation of lactic acid microorganisms was carried out by the method of multiseries dilutions. Incubation was carried out for 2-3 days at a temperature of 37 ° C. The number of colonies was counted by the last three dilutions, in which the growth of lactic acid bacteria was observed.The pH was determined potentiometrically using a membrane pH meter. Bacterial biomass was determined by nephelometric method. The optical density of the suspension was measured at 590 nm. The amount of biomass was expressed in mg of dry cells per mL of medium. A calibration curve for the dependence of the optical density of the culture fluid on biomass was used for the calculation.* ***Results and discussion:*** *the effect of the biotin stimulator (vitamin H) on vital activity, biomass accumulation and cultivation parameters of lactic acid bacteria Lactobacillus plantarum is investigated in the article. The results of the research indicate that when the biotin was introduced into the cabbage medium and the MRS medium, the cell division constant increases and the generation time decrease. In these media with a growth stimulator, the amount of biomass increased by 76% in cabbage medium and 50% in MRS compared to control. The number of colony-forming units (CFU) in the cabbage medium was 42%, and in the MRS medium was 28% higher than in the control (without biotin).*

Keywords: active acidity; biomass; biotin; CFU; constant of fission rate; generation time; lactic acid bacteria Lactobacillus plantarum; vitamin H.

1. Introduction

According to modern concepts, probiotics are living microorganisms and substances of microbial origin that determine positive effects on the physiological, biochemical and immune responses of the hosts organism (human or animal) under the natural mode of introducing due to the optimization and stabilization of its microbiota [1,4].

Bifidobacteria and lactobacilli are used most often in bacteriotherapy, as well as in the production of functional foods . These probiotics are called classic, because their active principle is the strains isolated from the human gut that dominate in the gut since the first days of life. In addition, the lactobacilli, and bradbery inherent ability to colonize epithelial tissues. In addition, the ability to colonize the epithelial tissue is characterized by lactobacilli and bifidobacteria [2,3].

Currently, special attention in the development of microbial biotechnology is given to selection of

strains of lactic acid bacteria and bifidobacteria to obtain a probiotic and enzyme dairy products [5].

The standard formulation of nutrient media recommended for use for cultivation of industrial strains of lactobacilli, usually require additional adjustments to give them the desired growth properties [6]. Therefore, an important task in the process of optimizing nutrient media is not only the choice of available and inexpensive raw materials and semi-finished products, but also obtaining of high-quality and competitive probiotics on their basis.[6,7].

Lactobacilli are chemo-organoheterotrophs, which are very demanding on power supplies. They predominantly ferment hexoses (glucose, fructose, mannose, galactose) among carbohydrates and disaccharides (lactose, maltose, sucrose). Only heterofermentative species ferment pentoses (ribose, xylose, arabinose). Lactose is a disaccharide, so it must be broken down by the enzyme galactosidase to glucose and galactose before taking the path of catabolism. Then galactose is phosphorylated to glucose-6-phosphate [8,9].

Lactobacilli require for their development in addition to carbohydrates , also various growth factors: amino acids, vitamins, nucleotides.

Probiotic "Lactona" contain lactic acid bacteria *Lactobacillus plantarum*, which participate in the formation of the optimal composition of the intestinal microbiota. *Lactobacillus plantarum* require the full culture media for their growth and development, so vitamins as additional sources of energy are added to nutrient media. According to the literature, biotin is used in the concentration of 1-10 mg / l to stimulate growth and development and increased production of metabolites for *Lactobacillus plantarum* [10].

2. Analysis of the latest research and publications

To date, much attention is paid to the study and improvement of probiotics on the basis of lactic acid bacteria. Many works are devoted to the role of probiotics for human health. In the works of foreign and domestic scientists (S. Salminem, E. Isolauri, T. Onnela, T. Vakhitov, L. Petrov, V. Bondarenko, J. Magnusson, K. Str?m, St. Roos, J. Sj?gren, J. Schn?hrer) the main questions of the theory regarding preparations that normalize the composition and function of the gastrointestinal tract

3. The influence of biothin on biomass growth, active acidity and growth of culture *Lactobacillus plantarum.*

A study of the dynamics of bacterial growth of *Lactobacillus plantarum* showed that active growth of biomass on a cabbage medium with vitamin H was observed for 24 hours of cultivation (fig.1). So the value of the optical density with the growth of microorganisms on a medium with a vitamin is three times higher than the result of growth on a control medium.

The value of the optical density was 50% higher on media with biotin starting from 22 hours than in the control (fig. 2). This indicates that in the medium of MRS with the growth stimulator were accumulated more cells of the culture.

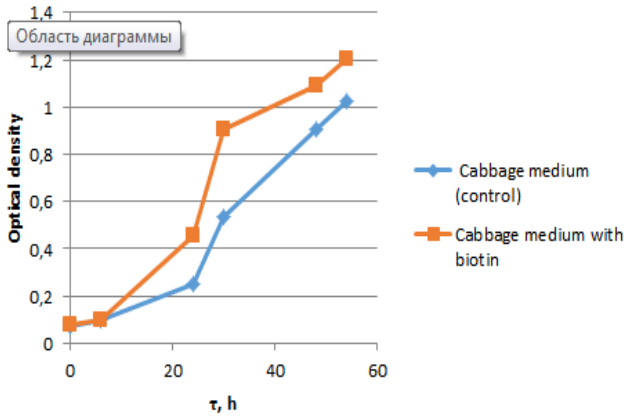

Fig.1. Dynamics of the optical density change from the time of cultivation of *L. plantarum* on a cabbage medium

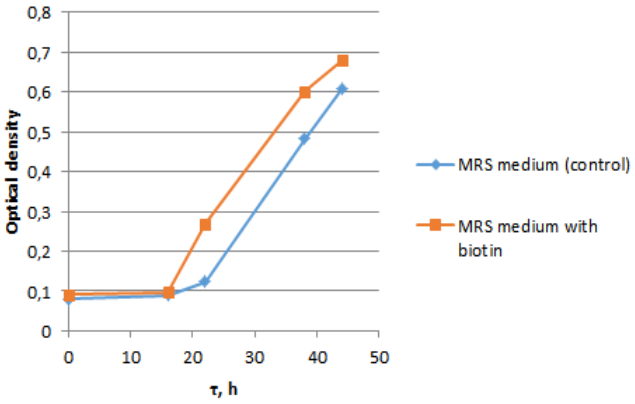

Fig.2. Dynamics of the optical density change from the time of cultivation of *L. plantarum* on the MRS

The number of viable cells of *Lactobacillus plantarum* on the medium of MRS (Fig. 3) and the cabbage medium (Fig. 4) with vitamin H at the end

of the cultivation process is 28% and 42% higher than in media without stimulant.

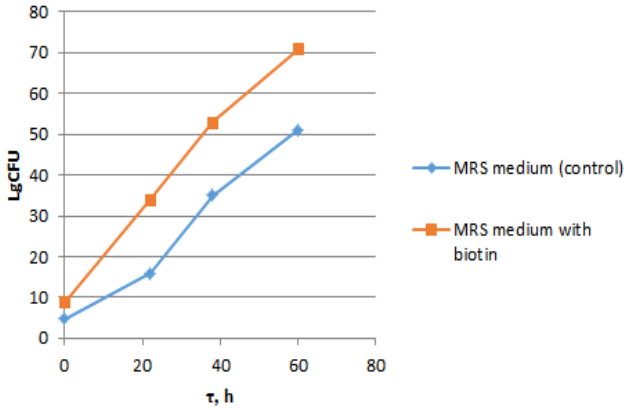

Fig.3. The number of viable cells of *L. plantarum* on the MRS medium

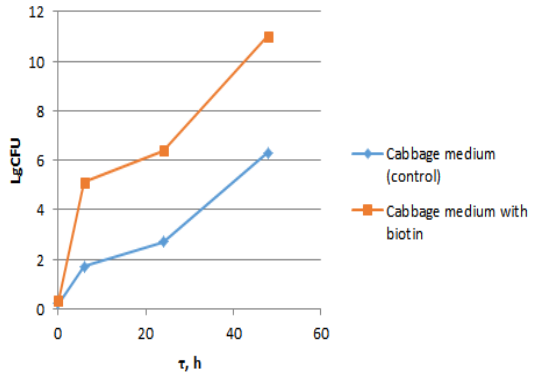

Fig.4. The number of viable cells of *L. plantarum* on cabbage medium

In addition, we studied the changes of active acidity under the influence of biotin on the growth and development of lactic acid bacteria. It was determined that the value of active acidity was significantly lower on the medium of MRS with the growth stimulator starting from 22 h. This indicates that acid was accumulated in the medium with biotin in 12% more than in the medium without it (Fig. 5).

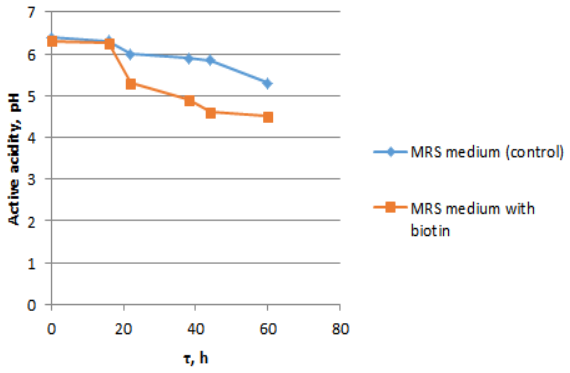

Fig.5. The dynamics of changes in acidity microorganisms *L. plantarum* on MRS medium

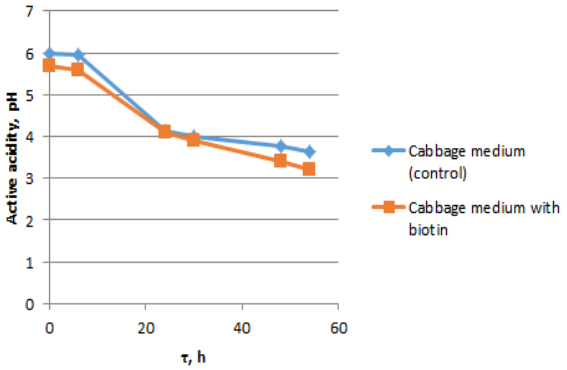

Fig.6. Dynamics of change of active acidity by *L. plantarum* microorganisms on cabbage medium

The pH values practically did not differ from each other under the conditions of culture growth in the cabbage medium with vitamin and without (Fig. 6). According to the presented data, it can be seen that the addition of biotin to the nutrient medium leads to a significant increase in the productivity of *Lactobacillus plantarum* with an increase in lactic acid production.

4. The kinetic parameters of growth of bacteria *L. plantarum*

The kinetic parameters of growth were determined for a more detailed characterization of growth and development of bacteria on the medium of MRS and cabbage medium with biotin (Table 1).

Table 1

The kinetic parameters of the exponential growth of lactic acid bacteria *L. plantarum*

Medium of *L. plantarum* cultivation	V, h^{-1}	g, h	μ, h^{-1}
Cabbage medium (control)	0,55	1,81	0,3
Cabbage medium with biotin.	0,76	1,31	0,32
MRS medium (control)	0,63	1,58	0,31
Medium MRS with biotin.	0,84	1,19	0,34

It can be seen from the obtained data that *Lactobacillus plantarum* on a medium with a growth stimulator are characterized by the highest constant of fission rate (v) - 0.84 h^{-1}, at the shortest generation time (g) - 1.19 h. The culture of *Lactobacillus plantarum* developed at the slowest rate on a cabbage medium without vitamin H, as the

value of constant of fission rate (v) was 0.55 h^{-1}. The culture had the longest generation period (g) - 1.81 h on this variant of the nutrient medium. The specific rate of exponential growth fluctuated in the same range for all variants of the experiment and amounted 0.30-0.34.

5. Conclusions

The study showed a positive effect of biotin on the growth and development of lactic bacteria *Lactobacillus plantarum*

It was determined that the introduction of biotin into the cabbage medium and MRS the amount of biomass increased by 76% and 50%, respectively, compared with the control variants of these media.

It has been shown that the addition of biotin to cabbage medium and MRS medium leads to a decrease in the active acidity. These data demonstrate that the number of cells in the culture is increased, since the amount of organic acids becomes larger when the nutrient medium is acidified.

The addition of biotin in both nutrient media leads to an increase in the rate constant of cell division and a decrease in generation time in comparison with the control. This indicates that biotin stimulates the vital activity of lactic acid bacteria *Lactobacillus plantarum.*

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Вплив біотину на ріст та розвиток бактерій *Lactobacillus plantarum*

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Мета: вивчити вплив біотину на життєдіяльність молочнокислих бактерій. **Методи:** об'єктом дослідження були молочнокислі бактерії *Lactobacillus plantarum*, які виділили з пробіотика «Флактонія». Для культивування бактерій використовували селективні поживні середовища: МРС та капустяне. Для стимуляції росту бактерій Lactobacillus plantarum в середовища додавали 5 мг/л біотину. Контролем були вище названі середовища без стимулятору росту. Дослідження включали вивчення динаміки накопичення бактерій *Lactobacillus plantarum*. Для цього протягом 60 годин проводили відбір проб: кожні 6 годин визначали біомасу, активну кислотність та кінетичні параметри

росту культур: швидкість експоненціонального росту (μ, год$^{-1}$), константа швидкості ділення (V, год$^{-1}$), час генерації (g, год). Виділення молочнокислих мікроорганізмів проводили методом багатосерійних розведень. Посіви інкубували протягом 2-3 діб при температурі 37 ^{0}С. Кількість колоній підраховували по трьом останнім розведенням, в яких спостерігали ріст молочнокислих бактерій. pH визначали потенціометрично за допомогою мембранного pH-метра. Бактеріальну біомасу визначали нефелометричним методом. Оптичну густину суспензії вимірювали при 590 нм. Біомасу виражали в мг сухих клітин на мл середовища. Для розрахунку використовували калібрувальну криву залежності оптичної густини культуральної рідини від біомаси. **Результати:** у статті досліджено вплив стимулятора росту біотину (вітамін H) на життєдіяльність, накопичення біомаси та параметри культивування молочнокислих бактерій *Lactobacillus plantarum*. Результати досліджень свідчать про те, що при внесенні біотину в капустяне середовище та середовище МРС збільшується константа швидкості ділення клітини та зменшується час генерації. В даних середовищах зі стимулятором росту кількість біомаси збільшилась в капустяному на 76% і в МРС на 50% порівняно з контролем. Кількість колонієутворюючих організмів (КУО) на капустяному середовищі, була на 42%, а на середовищі МРС на 28% вища ніж в контролі (без біотину).

Ключові слова: активна кислотність; біомаса; біотин; КУО; вітамін H; молочнокислі бактерії *Lactobacillus plantarum*; швидкість експоненціального росту; константа швидкості ділення; час генерації.

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Влияние биотину на рост и развитие бактерий *Lactobacillus plantarum*

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Цель: изучить влияние биотина на жизнедеятельность молочнокислых бактерий. **Методы:** объектом исследования были молочнокислые бактерии *Lactobacillus plantarum*, которые выделили из пробиотика «Флактония». Для культивирования бактерий использовали селективные питательные среды: МРС и капустное. Для стимуляции роста бактерий *Lactobacillus plantarum* в среду добавляли 5 мг/л биотина. Контролем были вышеназванные среды без стимулятора роста. Исследования включали изучение динамики накопления бактерий *Lactobacillus plantarum*. Для этого в течение 60 часов проводили отбор проб: каждые 6 часов определяли биомассу, активную кислотность и кинетические параметры роста культур: скорость експоненционального роста (μ, ч$^{-1}$), константу скорости деления (V, ч$^{-1}$), время генерации (g,ч). Выделение молочнокислых микроорганизмов проводили методом многосерийных разведенний. Посевы инкубировали в течение 2-3 суток при температуре 37 ^{0}С. Количество колоний подсчитывали по трем последним разведением, в которых наблюдали рост молочнокислых бактерий. pH определяли потенциометрически с помощью мембранного pH-метра. Бактериальную биомассу определяли нефелометрическим методом. Оптическую плотность суспензии измеряли при 590 нм. Биомассу выражали в мг сухих клеток на мл среды. Для расчета использовали калибровочную кривую зависимости оптической плотности культуральной жидкости от биомассы. **Результаты**: в статье исследовано влияние стимулятора роста биотина (витамин H) на жизнедеятельность, накопления биомассы и параметры культивирования молочнокислых бактерий *Lactobacillus plantarum*. Результаты исследований свидетельствуют о том, что при внесении биотина в капустную среду и среду МРС увеличивается константа скорости деления клетки и уменьшается время генерации. В данных средах со стимулятором роста количество биомассы увеличилась в капустном на 76% и в МРС на 50% по сравнению с контролем. Количество колониеобразующих организмов (КОЕ) на капустной среде, была на 42%, а на среде МРС на 28% выше, чем в контроле (без биотина).

Ключевые слова: витамин H; биотин; КОЕ; биомасса; молочнокислые бактерии *Lactobacillus plantarum*; активная кислотность; скорость экспоненциального роста; константа скорости деления; время генерации.

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