V. S. Rzhevskaya, A. V. Omelchenko, D. A. Panov

INFLUENCE OF SELENIUM NANOPARTICLES ON BASIC CULTIVATION PARAMETERS AND PHYTOSTIMULATING PROPERTIES OF LACTOCOCCUS LACTIS

V. I. Vernadsky Crimean Federal University, Simferopol, Russia Received 13 January 2025 Accepted 23 March 2025 doi: 10.5922/vestniknat-2025-2-9

To cite this article: Rzhevskaya V.S., Omelchenko A.V., Panov D.A., 2025, Influence of selenium nanoparticles on basic cultivation parameters and phytostimulating properties of *Lactococcus lactis, Vestnik of Immanuel Kant Baltic Federal University. Series: Natural Sciences.* № 2. P. 142 – 154. doi: 10.5922/vestniknat-2025-2-9.

The aim of this study was to investigate the effects of selenium nanoparticles on the key cultivation parameters and phytostimulatory properties of Lactococcus lactis IMB B-7352. Cultivation of L. lactis IMB B-7352 was carried out in MRS medium supplemented with nanoselenium at concentrations of 0.25, 0.5, 0.75, and 1.0 mg/L (based on selenium content). The antagonistic activity of L. lactis IMB B-7352 against cultures of phytopathogenic bacteria was assessed using the agar block method. It was found that nanoselenium at a concentration of 0.75 mg/L exhibited optimal stimulatory activity in terms of biomass accumulation by L. lactis IMB B-7352. Examination of the antagonistic activity of L. lactis IMB B-7352 revealed that the inhibition zones against Xanthomonas campestris B-4102 and Agrobacterium tumefaciens B-8833 measured 55.0 mm and 20.0 mm, respectively. The addition of nanoselenium to the MRS medium did not result in statistically significant differences in the inhibition zones of phytopathogenic bacteria. A stimulatory effect of L. lactis IMB B-7352, cultivated on MRS medium supplemented with nanoselenium, was demonstrated on the growth parameters of wheat and pea plants. It was established that nanoselenium concentrations of 0.25 -0.5 mg/L in the MRS medium are optimal for promoting plant growth. For the first time, the effectiveness of nanoselenium supplementation in the cultivation medium for Lactococcus species used for plant growth stimulation has been demonstrated. The experiments conducted are promising for the development of microbial preparations for crop production.

Keywords: nanoselenium, strain, *Lactococcus lactis*, phytopathogenic bacteria, *Xanthomonas campestris*, *Agrobacterium tumefaciens*, antagonistic activity, plant growth stimulation

Introduction

Growth regulators and plant protection products play a decisive role in the development of agriculture. Today these groups of drugs are mainly represented by chemical compounds that can be toxic to humans and fauna, as well as pollute reservoirs, groundwater and soil [1]. Biological remedies account for only a few percent of the total volume of such drugs. Their disadvantage is their narrow specificity, limited conditions of use (temperature,

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speed of action, number of treatments) [2]. A promising solution to this problem is the development of complex biological products with both protective and stimulating effects based on microorganisms and nanoparticles of biogenic elements [3; 4].

Selenium occupies a significant place as nanocomposite materials used to stimulate plant growth [5; 6]. Unlike ionic forms, elementary selenium in nanoparticle form is less toxic and bioavailable. The biological activity of selenium nanoparticles, like other biogenic elements, depends not only on the size, shape, and stability of nanoparticles, but also on the properties of the stabilizing matrix [7; 8].

In recent studies, it has been shown that the presence of selenium in the medium has a positive effect on the accumulation of lactic acid bacteria biomass [9; 10]. Bacteria of the genus Lactococcus are capable of producing substances with antibiotic activity, which allows them to exhibit pronounced antagonism against various microorganisms, including phytopathogenic ones. The antagonistic activity of lactobacilli is due to such metabolites as organic acids (mainly lactic acid), as well as antimicrobial and antibiotic-like compounds — lysozyme, hydrogen peroxide, bacteriocins (lactacins), diacetyl, histamine and other amines [11—13].

Lactic acid bacteria are capable of converting insoluble phosphorus salts into compounds available to plants [14], accelerating mineralization processes in soil [15], and stimulating plant growth processes [16; 17]. In recent decades, lactic acid bacteria, due to the synthesis of phytohormones and / or their precursors and metabolites that suppress phytopathogens, have been considered as plant-growth-promoting rhizobacteria (capable of stimulating plant growth (PGPR)) [18].

Materials and methods

The aim of this work is to investigate the effect of selenium nanoparticles on the main parameters of cultivation and phytostimulating properties of *Lactococcus lactis* IMB B-7352.

The research tasks included: 1) selection of the optimal concentration of nanoselen for cultivation of *L. lactis* IMB B-7352 strain according to biomass accumulation; 2) study of the antagonistic activity of the *L. lactis* IMB B-7352 strain in relation to phytopathogenic bacteria *Xanthomonas campestris* and *Agrobacterium tumefaciens*; 3) determination of the influence of *L. lactis* IMB B-7352 strain on the growth performance of cultivated plants at early stages of development.

The material for the study was a strain of lactic acid streptococcus: *Lactococcus lactis* (Lister 1873) [19] IMB B-7352, isolated from the epiphytic sphere of plants. The *Lactococcus lactis* strain IMB B-7352 is a facultatively anaerobic mesophilic homofermentative streptococcus. The antagonistic activity of streptococcus was evaluated on the culture of phytopathogenic bacteria and *Xanthomonas campestris* B-4102 and *Agrobacterium tumefaciens* B-8833, obtained from the All-Russian collection of industrial microorganisms.



A water-soluble composition of selenium nanoparticles was obtained according to an original method developed at the Crimean Federal University named after V.I. Vernadsky [20].

To select the optimal amount of nanoselenium, the cultivation of lacto-coccus was carried out on a Man-Rogosa-Sharpe (MRS) nutrient medium [18]. Nanoselenium was added to a sterile medium cooled to 40 °C at concentrations of 0.25; 0.5; 0.75; and 1.0 mg/l (for selenium). MRS medium was used as a control, where an equivalent amount of distilled water was added instead of selenium solutions. For inoculation of the nutrient medium, a 24-hour culture of lactococcus with a titer of 1.6×10^7 was used. Microorganisms were cultured in a 96-well plate in a Multiskan FC photometer (Thermo Fisher Scientific, United States) at a temperature of 30 °C with constant shaking. The determination of the optical density of the medium was carried out at a wavelength of λ = 620 nm with a frequency of 1 hour for 70 hours.

The antagonistic activity of *L. lactis* IMB B-7352 against *X. campestris* B-4102 and *A. tumefaciens* B-1111 was studied by the method of agar blocks [21].

The effect of lactococcus on plants was studied in laboratory vegetation experiments in water culture. Wheat seeds of the Nador variety and the Madonna variety of peas were used as a test object. The seeds were sterilized in 1% KMnO₄ solution, and then 30 seeds were laid out in Petri dishes on filter paper soaked in distilled water (control 1), 0.1% solution of L. lactis IMB B-7352 culture liquid (control 2) and 0.1% solutions of L. lactis IMB B-7352 culture liquid grown on MRS nutrient medium with the addition of nanoselenium at concentrations of 0.25 to 1.0 mg/l. Petri dishes were placed in a thermostat at a temperature of $25\pm1\%$ C. Three replicates were used in each variant. The measurements of the growth parameters of plants (the length of the roots and the aerial part) were carried out on the 7th day using a metal ruler with a step of 0.05 cm. The growth parameters of plants were expressed as % of the control [22]. The experiments were carried out in 3-fold biological replication.

Statistical analysis of experimental data was carried out using Excel programs (Microsoft, USA) and Statistica 6.0 package. The results of the study were considered statistically significant at p < 0.05 [23].

Results and discussion

The introduction of MRS nanoselenium into the nutrient medium at concentrations of 0.25-1.0~mg/l influenced both the accumulation of biomass of the culture of the *L. lactis* IMB B-7352 strain and the timing of lactococcus passage through the main stages of development. In the lag phase (0-3~hours) between the control and experimental variants of the *L. lactis* IMB B-7352 strain, differences began to appear: the optical density of the variants of nanoselenium at a concentration of 0.5 and 0.75 mg/l nanoselenium exceeded the control by 10.8 % and 3.4 %, respectively (Fig. 1). In the growth acceleration phase (4-5~hours), the optical density of the culture in the variant -0.5~mg/l was 25.1 % higher than the control, in the variants of nanoselenium



at a concentration of 0.25 and 0.75 mg/l - by 17.9% and 19.1%, respectively. The least stimulating activity on the *L. lactis* IMB B-7352 strain was shown by nanoselenium at a concentration of 1.0 mg/l: the excess of the control indicator in the lag phase was 6.1%.

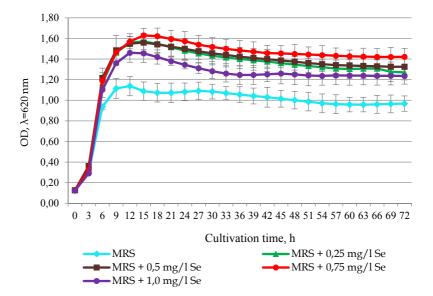


Fig. 1. Accumulation of *L. lactis* strain IMB B-7352 biomass on MRS nutrient medium nanoselenium

In the exponential growth phase, the optical density of variants with 0.25-0.75 mg/l nanoselenium exceeded the control by 27.8-32.0%, while those with 1.0 mg/l nanoselenium exceeded the control by 20.1%. In the phase of growth retardation in the experimental variants (0.25-0.75 mg/l nanoselenium), the optical density increased by 32.2-33.3%, in the variant with 1.0 mg/l nanoselenium — by 23.6%. In the stationary phase, the maximum value of optical density in the variants with 0.25-0.75 mg/l nanoselenium exceeded the control value by 37.3%, 36.4%, and 43.1%, respectively, in the variant with 1.0 mg/l nanoselenium — by 28.3%. A similar tendency for an increase in optical density persisted for 60 hours of cultivation: for variants with 0.25-0.75 mg/l nanoselenium, it was 36.2%, 39.6%, and 49.0%, respectively. A nanoselenium concentration of 1.0 mg/l was inhibitory compared to 0.75 mg/l. There was no significant difference between the concentrations of 0.25 and 0.5 mg/l. All investigated concentrations have a significant difference with the control variant.

As a result of the studies, it was shown that the introduction of nanoselenium into the nutrient medium MRS did not affect the antagonistic activity of *L. lactis* IMB B-7352 in relation to *X. campestris* B-4102 and *A. tume-faciens* B-8833 (Table, Fig. 2). There were no significant differences in the zones of suppression of phytopathogenic microorganisms between different variants of the content of nanoselenium in the nutrient medium during cultivation of *L. lactis* IMB B-7352.



Antagonistic activity of *L. lactis* IMB B-7352 strains to *X. campestris* B-4102 and *A. tumefaciens* B-8833

Experience options	Zones of suppression of growth inhibition by <i>L. lactis</i> strain IMB B-7352	
	X. campestris B-41026	A. tumefaciens B-8833
MRS (control)	55.0±0.2	20.0±0.3
MRS + Se 0.25 mg/1 Se ⁰	55.0 ± 0.6	19.5±0.1
MRS + Se 0.50 mg/1 Se ⁰	55.0 ± 0.8	21.0±0.7
MRS + Se 0.75 mg/1 Se ⁰	55.0±0.5	20.0±0.5
MRS + Se 1.00 mg/1 Se ⁰	55.0±0.4	20.0±0.9

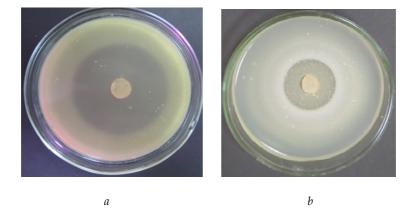


Fig. 2. Antagonistic activity of *L. lactis* IMB B-7352 to *X. campestris* B-4102 (*a*) and *A. tumefaciens* B-8833 (*b*)

The study of the influence of lactococcus on the growth performance of wheat plants of the Nador variety and the pea variety Madonna showed that the L. lactis IMB B-7352 strain, depending on the concentration of nanoselenium in the nutrient medium, has a stimulating effect on the length of roots and shoots. Thus, the L. lactis strain IMB B-7352 (control 2) stimulates the growth of roots and shoots by 11.51 and 7.1 % compared to control 1 (Fig. 2). The concentration of nanoselenium influenced the change in the morphometric parameters of wheat seedlings: the content of 0.25-0.50 mg/l Se⁰ in the medium increased the root length by 39.25 % and 47.43 % compared with control 1, and shoots — by 21, 6 % and 8.3 %. At the same time, the content of nanoselenium at a concentration of 0.75 mg/l of nanoselenium in the cultivation medium of L. lactis IMB B-7352 stimulated root growth by 14.01 % compared with control 1, while the shoot length decreased by 23.78 % compared to control 1 (Fig. 3, 4). L. lactis strain IMB B-7352 cultivated on a medium with 1.0 mg/l nanoselenium inhibited the growth of both roots and shoots of wheat.

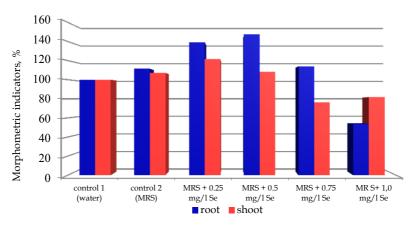


Fig. 3. Influence of *L. lactis* strain IMB B-7352, cultivated on MRS medium with different concentrations of nanoselenium, on the morphometric parameters of wheat plants of variety Nador

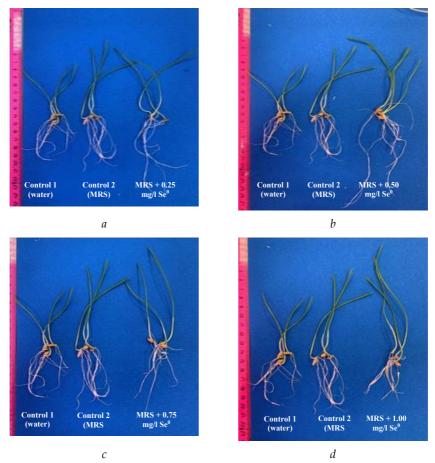


Fig. 4. Wheat plants of the variety Nador grown in a 0.1 % solution of *L. lactis* IMB B-7352, cultivated in MRS medium with various concentrations of nanoselenium: $a-0.25~{\rm mg/1\,Se^0}, b-0.50~{\rm mg/1\,Se^0}, c-0.75~{\rm mg/1\,Se^0}, d-1.00~{\rm mg/1\,Se^0}$



Thus, during the cultivation of L. lactis IMB B-7352 on a nutrient medium with nanoselenium at a concentration of 0.25-0.50 mg/l, stimulation of the growth of shoots and roots of wheat plants was observed, and concentrations of 0.75 and 1.0 mg/l of nanoselenium in the nutrient medium had an inhibitory effect.

It was shown that all the studied concentrations of nanoselenium, introduced into the nutrient medium MRS with *L. lactis* IMB B-7352, had a stimulating effect on the growth processes of the Madonna variety (Fig. 5).

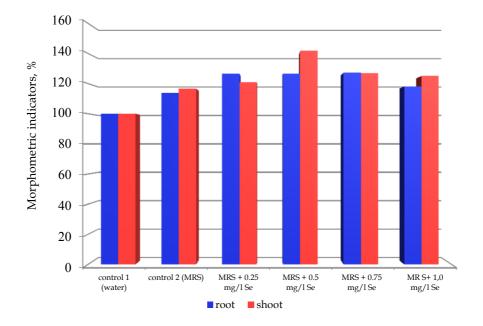
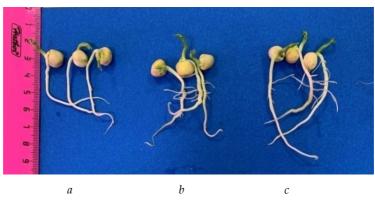


Fig. 5. Influence of *L. lactis* strain IMB B-7352, cultivated on MRS medium with different concentrations of nanoselenium on the morphometric parameters of pea plants of the Madonna variety

The concentration of nanoselenium in the nutrient medium 0.25-0.5 mg/l stimulated the growth of roots by 26%, and shoots — by 20.83% and 41.67% compared to control 1. The content of nanoselenium at a concentration of 0.75 mg/l during cultivation of L. lactis IMB B-7352 strain stimulated root growth by 27.18%, and shoot — by 27.08% compared to control 1 (Fig. 6). Concentration of 1.0 mg/l nanoselenium in comparison with control 1 stimulated the growth of roots and shoots of peas by 18.06% and 25.0%, respectively.

Thus, cultivation on a nutrient medium L. lactis IMB B-7352 with nanoselenium at a concentration of 0.25-0.75 mg/l stimulates the growth of pea plants of the seed variety Madonna.





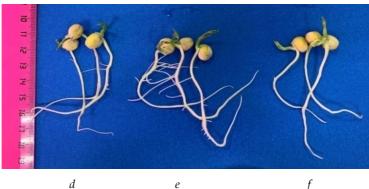


Fig. 6. Plants of the Madonna variety, grown in a 0.1 % solution of *L. lactis* IMB B-7352, cultivated in MRS medium with various concentrations of nanoselenium: $a-\text{control 1}, b-\text{control 2}, c-0.25 \, \text{mg/l Se}^0, d-0.50 \, \text{mg/l Se}^0, \\ e-0.75 \, \text{mg/l Se}^0, f-1.00 \, \text{mg/l Se}^0$

Discussion

Microorganisms can assimilate elemental selenium Se⁰ and form organoselenium compounds [24]. Selenium is a component of glutathione peroxidase, thyroid reductase, and selenocysteine of the 21st amino acid, which is involved in the biosynthesis of cysteine (Cys) and redox reactions [25]. However, there is a narrow range between the concentration of Se⁰ at which it is beneficial and that at which it is toxic. In our studies, it was shown that cultivation of *L. lactis* IMB B-7352 on a MRS nutrient medium with 0.25-0.50 mg/l of nanoselenium led to the stimulation of the growth parameters of both the bacterial culture itself and test cultures of plants grown on it.

Studies carried out on *B. subtilis* IMV B-7392 showed that nanoselenium at concentrations of 0.2 mg / 1 did not cause changes in the growth of the bacterial culture, but significantly increased the yield of biologically active substances. Incubation of *B. subtilis* IMV B-7392 with nanoselenium resulted in its rapid uptake by the culture of *B. subtilis* IMV B-7392. At the same time, the permeability of the outer membrane of the cell temporarily increased



without rupture, nanoparticles penetrated into the cell and attached to their cell structures. After a 30-minute incubation, bubble formation was observed on the cell surface [26].

The introduction of nanoselenium into nutrient media for bacteria of the genus Lactobacillus significantly (up to 37.5%) increased the antimicrobial activity of lactobacilli in relation to phytopathogens [26], fungi of the genus Candida. Selenium can induce the production of such exometabolites as lactic and acetic acids, hydrogen peroxide, carbon monoxide (IV), bacteriocins and / or induce the synthesis of new antimicrobial compounds [28].

Today some types of lactobacilli can biotransformation inorganic selenium into its organic derivatives. Lactic acid bacteria can naturally synthesize selenium nanoparticles and assimilate exogenous nanoparticles [29–32]. Due to the fact that lactic acid bacteria can ensure the conversion of inorganic selenium into its organic forms, they can be used as selenium-containing probiotics [33–35]. At the same time, lactic acid bacteria stimulate the growth and development of plants, since they have a positive effect on the efficiency of absorption of nutrients by the plant, suppression of soil pathogens, and a decrease in the impact of stress factors [18; 36]. The inoculation of agricultural crops with microorganisms or microbial preparations enriched with nanoselenium, against the background of a reduction in the use of mineral fertilizers, herbicides and insecticides, is an urgent and promising direction, which is becoming increasingly important in modern agriculture.

Conclusion

It has been shown that the introduction of MRS nanoselenium into the nutrient medium at a concentration of 0.25-0.75 mg/l is not toxic to the culture of *L. lactis* IMB B-7352 and does not exhibit bactericidal or bacteriostatic action. The experiments have shown the possibility of using nanoselenium as a component of a nutrient medium for cultivation of *L. lactis* IMB B-7352 in order to use the strain for the production of a microbial preparation aimed at stimulating plant growth processes.

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The authors

Viktoriya S. Rzhevskaya, graduate student, V.I. Vernadsky Crimean Federal University, Russia.

E-mail: viktoriyar45@mail.ru ORCID: 0000-0003-4788-0407 SPIN-код: 1399-5542

Dr. Aleksandr V. Omelchenko, Associate Professor, V.I. Vernadsky Crimean Federal University, Russia.

E-mail: omelchenko_tnu@mail.ru ORCID: 0000-0002-9490-622X

SPIN-код: 7161-8836

Dr. Denis A. Panov, Associate Professor, V.I. Vernadsky Crimean Federal University, Russia.

E-mail: despanov@gmail.com ORCID: 0000-0001-7186-7531 SPIN-код: 3874-4860

В. С. Ржевская, А. В. Омельченко, Д. А. Панов

ВЛИЯНИЕ НАНОЧАСТИЦ СЕЛЕНА НА ОСНОВНЫЕ ПАРАМЕТРЫ КУЛЬТИВИРОВАНИЯ И ФИТОСТИМУЛИРУЮЩИЕ СВОЙСТВА LACTOCOCCUS LACTIS

Крымский федеральный университет им. В.И. Вернадского, Симферополь, Россия Поступила в редакцию 13.01.2025 г. Принята в печать 23.03.2025 г. doi: 10.5922/vestniknat-2025-2-9

Для цитирования: *Ржевская В. С., Омельченко А. В., Панов Д. А.* Влияние наночастиц селена на основные параметры культивирования и фитостимулирующие свойства *Lactococcus lactis* // Вестник Балтийского федерального университета им. И. Канта. Сер.: Естественные науки. 2025. № 2. С. 142—154. doi: 10.5922/vestniknat-2025-2-9.

Цель исследования — изучение влияния наночастиц селена на основные параметры культивирования и фитостимулирующие свойства Lactococcus lactis IMB B-7352. Культивирование L. lactis IMB B-7352 проводили на питательной среде MRS с добавлением наноселена в концентрации 0,25; 0,5; 0,75 и 1,0 мг/л (по селену). Антагонистическую активность штамма L. lactis IMB B-7352 оценивали по отношению к культурам фитопатогенных бактерий методом агаровых блоков. Установлено, что оптимальной стимулирующей активностью по накоплению биомассы культуры L. lactis IMB B-7352 обладает наноселен в концентрации 0,75 мг/л. Изучение антагонистической активности L. lactis IMB B-7352 показало, что зона угнетения роста X. Campestris B-4102 составила 55,0 мм, а А. tumefaciens B-8833 — 20,0 мм. При добавлении МРС-наноселена в питательную среду достоверных различий в зонах подавления фитопатогенных бактерий не выявлено. Показано стимулирующее влияние штамма L. lactis IMB B-7352, культивируемого на среде MRS с наноселеном, на параметры роста рас-

тений пшеницы и гороха. Установлено, что концентрации наноселена в среде MRS 0,25 – 0,5 мг/л являются оптимальными для стимуляции роста растений. Впервые показана эффективность внесения наноселена в питательную среду для выращивания лактококков, используемого для стимуляции роста растений. Проведенные эксперименты перспективны для разработки микробных препаратов для растениеводства.

Ключевые слова: наноселен, штамм, Lactococcus lactis, фитопатогенные бактерии, Xanthomonas campestris, Agrobacterium tumefaciens, антагонистическая активность, стимуляция роста растений

Об авторах

Виктория Степановна Ржевская — аспирант, Крымский федеральный университет им. В.И. Вернадского, Россия.

E-mail: viktoriyar45@mail.ru ORCID: 0000-0003-4788-0407

SPIN-код: 1399-5542

Александр Владимирович Омельченко — канд. биол. наук, доц., Крымский федеральный университет им. В. И. Вернадского, Россия.

E-mail: omelchenko_tnu@mail.ru ORCID: 0000-0002-9490-622X

SPIN-код: 7161-8836

Денис Александрович Панов — канд. хим. наук, доц., Крымский федеральный университет им. В.И. Вернадского, Россия.

E-mail: despanov@gmail.com ORCID: 0000-0001-7186-7531

SPIN-код: 3874-4860