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Control of magnetic susceptibility of probiotic strain *Lactobacillus rhamnosus* GG

Svitlana Gorobets[1], *Oksana Gorobets*[2], *Liubov Kuzminykh*[1]✉

[1]*Department of Bioenergy, Bioinformatics and Environmental Biotechnology, Faculty of Biotechnology and Biotech Engineering, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", pr. Peremohy 37, Kyiv 03056, Ukraine*
[2]*Faculty of Physics and Mathematics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", pr. Peremohy 37, Kyiv 03056, Ukraine*

✉*Corresponding author: l.kuzminykh-2022@kpi.ua*

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Abstract

The paper investigates the increase in the natural magnetically controlled properties of probiotic microorganisms *Lactobacillus rhamnosus* GG (LGG) and their ability to form magnetically sensitive inclusions (MsI). The magnetic susceptibility of LGG was increased by modifying the nutrient medium composition and by cultivating the probiotic culture in a constant magnetic field. Therefore, this study can be useful for their further use as magnetically controlled vectors since it is being extensively researched for their use in targeted drug delivery in cancer treatment, for the prevention of chemotherapy complications, etc. The results of this study indicate that growing microorganisms with natural magnetically-guided properties in a modified medium with iron chelate and in an external magnetic field leads to an increase in the magnetic susceptibility of LGG by 1.8 and 2.6 times, respectively, compared with the control. The best magnetic susceptibility was recorded for LGG suspensions, which were grown in a constant magnetic field and cultured in modified medium with iron chelate. The LGG suspension, grown both in a constant magnetic field and on a modified medium with iron chelate, had the highest magnetic susceptibility, and it was 4.8 times larger than the magnetic susceptibility in the control.

Introduction

Probiotics are used for treating and preventing diseases of the gastrointestinal tract (Parian *et al.* 2018), strengthening the immune system (Mendi *et al.* 2016), overcoming the effects of antibiotic therapy (Saarela *et al.* 2000), for the prevention and treatment of tumor diseases (Bedada *et al.* 2020; Lamichhane *et al.* 2020), as vectors for targeted drug delivery (Duong *et al.* 2019). In particular, studies show a number of advantages while using probiotic microorganisms as vectors comparing to other microorganisms that can have toxic effects on the body (Zhou *et al.* 2018; Laliani *et al.* 2020). The strain of lactic acid bacteria *Lactobacillus rhamnosus* GG or *L. rhamnosus* ATCC 53103 (LGG) is the most studied strains of probiotics due to its valuable properties. The microorganism colonises the gastrointestinal tract and thus protects against pathogens and increases the body's resistance to infections (Capurso 2019). It is known that LGG is used as a vector in targeted drug

delivery in the treatment of pathologies and tumors (Tangney 2010; Bedada *et al.* 2020). The use of probiotics as vectors for targeted drug delivery has a number of advantages, in particular, the non-invasiveness of probiotic cultures and the affinity for tumor tissue of some probiotic microorganisms (Bedada *et al.* 2020). Probiotic microorganisms can specifically target a tumor, causing intra-tissue regression (Tangney 2010).

In the last few years, the field of biotechnology and medicine has been intensively developing to create minimally invasive systems for the targeted delivery of drugs and genes using biohybrid microrobots – conjugates of bacteria, magnetic nanoparticles, and the target cargo (liposomes, etc.). Such microrobots should possess high-throughput fabrication, efficient motility, tissue penetration, multifunctional operation, and external stimuli-responsive control (including control and trigger-triggered cargo release). Magnetic nanoparticles in the composition of microrobots are used to provide the possibility of remote control by means of magnetic field application and, accordingly, targeted delivery to the target organ. Many bacterial biohybrid microrobots suffer from low to moderate conjugation yields, with only a small fraction of cells carrying artificial cargo. In particular, since bacteria have a short doubling time (e.g., 20 min for wild-type *E. coli* under optimal conditions), some of the payload will be lost during the growth phase, which results in a loss of payload efficiency and weakens magnetic controllability over long periods of time. Thus, for effective magnetic control in medical applications, a high-performance conjugation process and stability of the properties of microrobots for a long time are required. In addition to throughput, the conjugation approach should not adversely affect either the bacteria or the functional properties of the artificial media. In addition, as a result of conjugation, the large size of the microrobot can seriously hinder its mobility and thus affect the performance of the bacterial microrobot. The problem of increasing the motility of the bacterial microrobot is related to the difficulty of penetration of the bacterial vector through an environment with high density and hierarchical structure in real tumors (Akolpoglu *et al.* 2022). A significant part of the biohybrid designs of microrobots reported in the literature have insufficient characteristics of mobility, stability, and controllability. In this regard, further investigation of the mechanical motility of bacteria in confined spaces is needed, together with the possibilities of artificial taxis, such as magnetophoresis, which may create more opportunities for effective penetration and transport of anticancer agents into solid tumors. Microorganisms that synthesize biogenic magnetic nanoparticles (BMNs) or magnetosomes can be used as magnetically-guided vectors to transfer a large number of therapeutic substances (Kuzajewska *et al.* 2020). These microorganisms lack the disadvantages of bacterial biohybrids as their large size, insufficient stability, and loss of magnetic controllability over time. Magnetotactic bacteria are the best-known producers of biogenic magnetic nanoparticles. The genetic apparatus of BMNs biosynthesis is unique in all kingdoms of living organisms, as shown by genetic analysis (Gorobets *et al.* 2014a). However, magnetotactic bacteria (MTB) are used with caution as bacterial vectors for drug delivery because their effect on living organisms is not fully studied and because of the lack of clinical trials and regulatory documentation (Mathuriya 2015). Also, MTB is difficult to cultivate and maintain viability since their habitat is significantly different from the internal environment of a human or animal organism (Müller *et al.* 2020). BMNs of non-magnetotactic microorganisms have been studied in comparison with BMNs of MTB (Vainshtein *et al.* 2002 and 2014; Gorobets 2012). BMNs of non-magnetotactic microorganisms are perspective as bacterial magnetically guided vectors because they manifest magnetophoresis in gradient magnetic fields (Vainshtein *et al.* 2002 and 2014; Gorobets and Koralewski 2017). A number of probiotic and non-magnetotactic microorganisms can produce BMNs, as summarized in the review of the experimental data (Gorobets *et al.* 2014b). As a result, the use of probiotics that produce BMNs and magnetically sensitive inclusions (MsI) is a promising solution for this class of problems (Vainshtein *et al.* 2002 and 2014). Bacteria from the genus *Lactobacillus* synthesize MsI when grown on special media with the addition of ferric ions (Ariskina 2003). Also, magnetically-guided vectors based on LGG can have additional

advantages, as they can control their velocity to the tumor site and are well concentrated in the desired area (Mokriani *et al.* 2021). It is known that tumors can produce an increased amount of BMNs compared to healthy tissues (Brem *et al.* 2006; Chekchun *et al.* 2011; Gorobets 2017). Magnetically-guided vectors are more efficiently attached to tumor cells and accumulate in the target area due to both aerotaxis to hypoxic regions of the tumor and magnetic dipole-dipole interactions of bacterial BMN and BMN of the tumor (Mikeshyna *et al.* 2018; Gorobets *et al.* 2022). Magnetically-guided vectors in the tumor area can serve as a magnetic material for therapeutic magnetic hyperthermia, which together with the therapeutic effect of the delivered drugs, can increase the effectiveness of treatment several times (Gorobets *et al.* 2013; Felfoul *et al.* 2016).

Based on the above issues, the purpose of this work is as follows: the approach is developed increasing the magnetic susceptibility of bacterial vectors not due to loading (conjugation) with artificial magnetic nanoparticles but due to, firstly, the selection of bioinformatics methods of bacterial strains that have genetic mechanism of biomineralization of biogenic magnetic nanoparticles (BMN) and, secondly, due to the modification of the environment for growing bacteria (adding iron salts to the medium during cultivation) and growing conditions (applying an external magnetic field during cultivation) to activate the biomineralization mechanism of BMN.

Thus, the study of ways to control the magnetic susceptibility of LGG is relevant for the creation of magnetically-guided vectors based on the natural magnetically-guided properties of LGG.

Experimental

Physical methods and software

The physical methods of the research were optic microscopy, magnetophoresis, and methods of magnetostatics. The SciPy package of Python programming language was used for the numeric simulation of magnetophoresis in bacterial agglomerates.

Cultivation of microorganisms

The probiotic lyophilized drug Acidolac® (1 sachet of the drug contains 4×10^9 CFU *Lactobacillus rhamnosus* GG) was used for the study. Pure cultures of microorganisms were isolated and cultured on Lactobacilli-MRS Agar medium. The microorganisms were cultured on the standard agar medium and modified medium with the addition of iron chelate for 48 h at a temperature of 36 ± 1 ^{o}C. LGG was grown on MRS medium prepared according to a standard recipe. The modified medium was prepared to enhance the natural magnetically-guided properties with the addition of iron chelate at a concentration of 64 mg.L^{-1}. The media were prepared according to the recipe and sterilized by pressure in a steam sterilizer at 121 ^{o}C for 15 –20 min. The control group of microorganisms (LGG contr.) was grown on a standard medium under standard conditions. LGG was grown to enhance the natural magnetically-guided properties with modification of the cultivation conditions: grown on a standard medium in a constant magnetic field (LGG + M), grown on a modified medium with the addition of iron chelate (LGG + Fe), grown on a modified medium with the addition of iron chelate in a constant magnetic field (LGG + Fe + M). The purity of the LGG culture was checked by microscopic examination.

Research of microorganisms with a two-magnet system

The magnetic susceptibility of the LGG suspension was investigated using a system of two magnets according to the method already described (Wosik *et al.* 2018). A drop of suspension with a culture of microorganisms is applied to the glass located on the contact surface of the system of two permanent magnets. The system of two permanent magnets is created so that the highest gradient of the magnetic field is created in the area of their joint. The magnetic particles in the suspension move to the contact line of the magnets, forming a strip. Magnetic susceptibility was studied in two ways: concentrating the bacterial suspension on the contact line to determine the width of the formed band and determining the velocity of cell agglomerates to the contact line of the system of two permanent magnets.

Bandwidth measurements of an LGG cell suspension formed at the contact surface of a system of 2 permanent magnets

An aqueous suspension of probiotic strain LGG was applied to a 0.13 mm thick cover slide, placed on a two-magnet system until the liquid was completely dry to measure the width of the strip formed. After drying the product, the bandwidth of the suspension was evaluated using a digital microscope and a microline. Series of digital microscope images were taken, and the average width of the formed strip was determined using the IrfanView software.

Determination of the velocity and the average magnetic susceptibility of particles of the LGG suspension

A drop of LGG suspension was applied to a glass placed on the contact surface of the system of two permanent magnets. The movement of conglomerates of LGG suspension was recorded on video using a digital microscope. The video was filmed in several repetitions to ensure the reliability of the results. Microorganisms were taken from different parts of the Petri dish, a suspension was prepared, and individual videos were made moving to the line of contact of the system of two permanent magnets. The experiment was repeated several times with separate generations of LGG cultures to obtain reliable results. An average of 15 videos were made for each type of microorganism cultivation. Particles with similar sizes were selected, the motion of which was clearly visible in the video, for the experiments. The number of particles examined was: LGG contr. - 57 particles, LGG + M - 78 particles, LGG + Fe - 83 particles, LGG + Fe + M - 139 particles. Particles were selected that were clearly visible, 40-110 μm in size, the average particle size was approx. 70 μm. The magnetic susceptibility of the LGG suspension was determined using Python and the formulas in the section Results and Discussion.

The program IrfanView has been used to determine the particle movement (μm) to the contact line of the magnets and the particle diameter (μm). The time (s) of movement of the particle was determined by dividing the video into frames by the Free Video to JPG Converter software. The average velocity ($\mu m.s^{-1}$) of the particles was determined by dividing the path (μm) by the time (s) of movement of the particles to the contact line of the magnets. Table 1 presents the physical parameters taken to determine the magnetic susceptibility of the LGG suspensions.

Table 1. Physical parameters of the system that were used to calculate the average magnetic susceptibility of suspensions in the study with a system of two magnets.

Parameter	Dimension	Value, units
Saturation magnetization of magnets in a system of two permanent magnets	M_s	1600 G [a]
Dynamic viscosity of water	η	0.01 g/(cm·s)
Magnet length	a	0.65, cm
Glass thickness	h	0.13, mm

Note: a - the unit is part of the Gaussian system of units or CGS-EMU, 1 G = 10^{-4} tesla (SI system).

Results and Discussion

Determination of bandwidth and velocity of suspension particles LGG

The microscopy of the studied of probiotic strain LGG is shown in Fig. 1.

The video files are linked to Fig. 1:

1) The video file “L.rhamnosus GG contr”: the control group of microorganisms (LGG contr.) was grown on a standard medium under the standard conditions;
2) the video file “L.rhamnosus GG + Fe”: LGG was grown to enhance the natural magnetically-guided properties with modification of the cultivation conditions grown on a modified medium with the addition of iron chelate (LGG + Fe);
3) The video file “L.rhamnosus GG + M”: LGG was grown to enhance the natural magnetically-guided properties with modification of the cultivation conditions: grown on a standard

medium in a constant magnetic field (LGG + M);

4) The video file "L.rhamnosus +Fe +M": LGG was grown to enhance the natural magnetically-guided properties with modification of the cultivation conditions: grown on a modified medium with the addition of iron chelate in a constant magnetic field (LGG + Fe + M).

The cover glass with the bacterial culture was manually shifted several times during the video capture with the purpose to show magnetophoretic movement of bacterial agglomerates to the contact line of two magnets at several typical microscope fields. The method of video capture was the same as for video capture of the magnetophoretic movement of bacterial agglomerates in the reference (Gorobets *et al.* 2023).

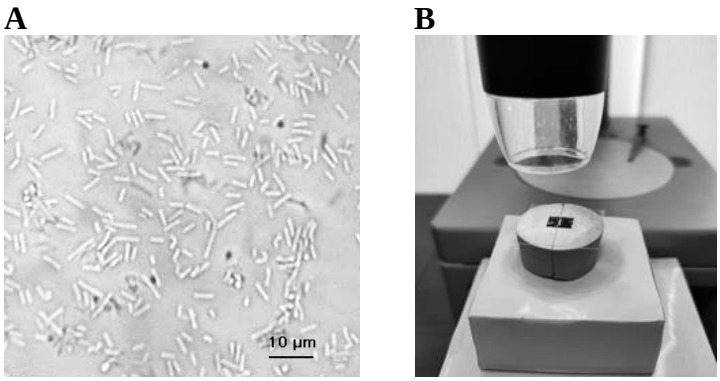

Fig. 1. Probiotic bacteria *L. rhamnosus* GG, cultured from Acidolac® on MRS medium (**A**). Digital microscope examination of the magnetophoretic mobility of *L. rhamnosus* GG: a system of two permanent magnets is presented, where a cover glass with a culture concentrating on the contact line is located on top (**B**).

Fig. 2 shows a preparation from a suspension of LGG, placed over a system with two magnets. The images show the conglomerates of bacterial cells located along the contact line of the magnetic system.

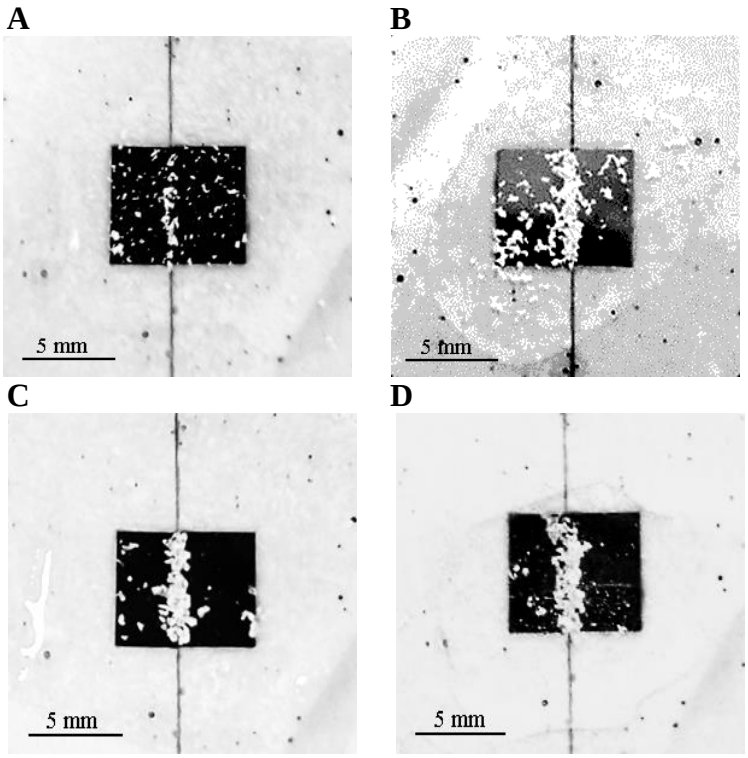

Fig. 2. Stripes from a suspension of probiotic strain *L. rhamnosus* GG, which formed above the contact line of the system of two magnets: LGG contr. (**A**), LGG +M (**B**), LGG + Fe (**C**), LGG + Fe + M (**D**).

Wider and less branched strips form above the contact line when the natural magnetically-guided properties of the LGG culture are enhanced by iron ions and a constant magnetic field. The strip that is formed after cultivation under standard conditions (LGG contr.) is thin, indistinct, with gaps and branches, many particles are not on the line of contact and do not move towards the line. The strip, formed by cultivation in a constant magnetic field (LGG + M) is distinct along its entire length but rather branched; a certain number of suspension particles do not move towards the contact line. The strip that is formed after cultivation on a modified medium with iron chelate (LGG + Fe) is somewhat narrower compared to the strip formed by LGG + M. The strip formed for LGG + Fe on the contact line of two magnets is distinct, rather uniform, the particles are concentrated on the line, therefore, they form a denser strip with few branches; there are few particles that remain outside the strip and do not move towards the contact line. The strip for LGG + Fe is narrower than the strip for LGG + M due to the fact that the particles are more densely concentrated on the contact line. The strip formed during cultivation on a modified medium with the addition of iron chelate and in a constant magnetic field (LGG + Fe + M) is the widest strip compared to the strips formed for other cultivation conditions. A distinct, wide strip is formed for LGG + Fe + M rather homogeneous and has few branches; few particles remain outside the strip and do not move towards the contact line.

Fig. 3 shows the results of the average velocity of particles at the line of contact of the system of two magnets. The average velocity (μm.s^{-1}) of the particles was obtained by dividing the path of the particle by time.

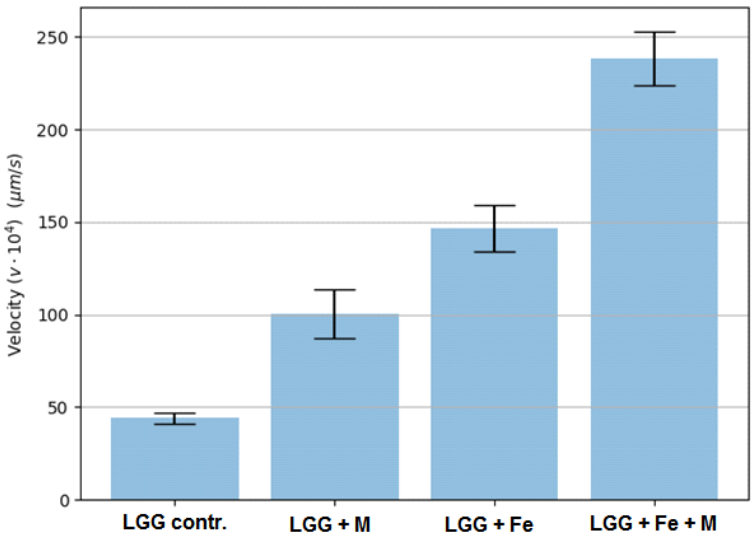

Fig. 3. Dependence of the average velocity of the particles in the suspension of *L. rhamnosus* GG depending on the cultivation conditions. The statistical significance of the results is 0.95.

The results in this paper show that the speed of the suspension particles in the two-magnet system increased as the growing conditions changed, and the culture medium was modified.

Calculation of the magnetic susceptibility of the suspension LGG

The main idea of modifying the magnetic properties of bacteria with natural magnetically-guided properties is to grow these bacteria under special conditions, namely with the addition of iron chelates to the environment and under the application of an external constant magnetic field during cultivation. This idea is based on experimental data on the influence of cultivation conditions on the amount of BMNs in magnetotactic bacteria. The BMNs of magnetotactic bacteria increase up to several times due to an increase in the concentration of iron ions in the cultivation medium and the application of an external magnetic field with an induction of about 0.1 T during cultivation. The magnetic susceptibility of the biomass of magnetotactic

bacteria also increases (Wang *et al.* 2009). According to Gorobets *et al.* (2014b) and Mokriani *et al.* (2021), it can be assumed that the same influencing factors that regulate the amount of BMNs in magnetotactic bacteria will similarly affect the number of BMNs and the magnetic susceptibility of non-magnetotactic bacteria with natural magnetically-guided properties. In addition, the novelty of this work is the choice of non-magnetotactic bacteria with natural magnetically-guided properties that are used as vectors for drug delivery (Zhou *et al.* 2018; Bedada *et al.* 2020).

In recent years, methods for creating strong magnetic fields have been intensively developed using systems of highly anisotropic permanent magnets connected in such a way that the directions of the magnetization vectors of these magnets are different at the boundary between them. In this case, strong magnetostatic fields are created at the "sharp angles" of each of the magnets, having a logarithmic divergence at certain points in space.

This divergence is not distorted due to the bending of the magnetization vector, if the material from which the magnet is made has a large magnetic anisotropy. Akhiezer *et al.* (1968) and Samofalov *et al.* (2004; 2013) calculated the divergence of the tangential component of the magnetic field outside a uniformly magnetized rectangular parallelepiped. The magnetostatic field increases logarithmically when approaching the edges of the parallelepiped and can exceed the value $4\pi M_s$ that is the limiting value for permanent magnets of traditional designs.

Fig. 4 presents an option of implementation of this idea. The combination of several highly anisotropic ferromagnets of various shapes with different directions of magnetization in one magnet to create strong magnetic fields near their common "sharp angle".

Fig. 4 presents a magnet where two ferromagnets are connected in such a way that one of them is magnetized along the axis OZ and the other one in the opposite direction.

The expression for the tangential component of the magnetostatic field for the Kittel's domain structure of two magnets has the form (Eq. 1) (Akhiezer *et al.* 1968; Samofalov *et al.* 2004 and 2013):

$$H_x^{(m)}(x,y,z) = M_s\left[\ln\left(x^2+2ax+a^2+z^2\right)+\ln\left(x^2-2ax+a^2+z^2\right)-2\ln\left(x^2+z^2\right)\right] \quad (1)$$

where contains a logarithmic divergence at $z = 0$ and $x \to 0$.

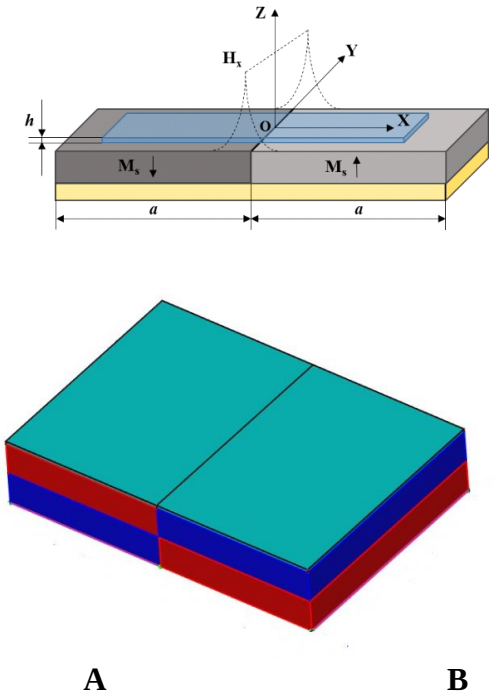

Fig. 4. Scheme of Kittel's open domain structure of two magnets (**A**). Schematic representation of magnetic poles of the Kittel's open domain structure of two magnets (**B**): red – south pole, blue – north pole.

Therefore, a magnet of this shape, made of highly anisotropic material, can be used to create strong magnetic fields in localized volumes of space.

The normal component of the magnetostatic field for the Kittel's domain structure of two magnets has the form (Eq. 2) (Samofalov *et al.* 2004 and 2013):

$$H_z^{(m)}(x,y,z) = 2M\left(\operatorname{arctg}\left(\frac{a+x}{z}\right)-\operatorname{arctg}\left(\frac{a-x}{z}\right)-\operatorname{arctg}\left(\frac{x}{z}\right)\right) \quad (2)$$

Analysis of the existing theories of particle deposition in a gradient magnetic field has revealed that the theory of magnetophoresis of para- and diamagnetic particles in high-gradient fields has been quite fundamentally developed. The choice of a model to describe the efficiency of the magnetic separator depends on the size of the particles that must be isolated from the working medium. There are two approaches which depend on particle size (more or less some critical size), the particle approach and the continuous medium approach. The continuous medium approach is used to model

the separation of particles, usually less than one micron in size. The statistical method is used when it is impossible to estimate the spontaneous Brownian motion of each particle and the suspension is considered as a continuous medium. The particle approach gives good results for particles of sufficient size to be neglected in Brownian motion. The model using the particle approach was first developed in (Friedlander *et al.* 1981; Plyavin' and Blum 1983; Vincent-Viry *et al.* 2000; Gorobets and Mikhailenko 2014). This model takes into account only the gradient magnetic force $\vec{F}_m$ and the force of hydrodynamic resistance $\vec{F}_{st}$. The force of inertia is neglected, and the balance of forces is written in the following form (Eq. 3 and 4):

$$\vec{F}_m = \vec{F}_{st} \tag{3}$$

$$\vec{F}_m = \frac{1}{2}\chi \operatorname{grad} \vec{H}^2 V_p \tag{4}$$

$\vec{H}$ – the strength of the magnetic in the working volume of the magnetic separator, χ – effective magnetic susceptibility, which is equal to the difference between the susceptibilities of the liquid and the particle $\chi = \chi_p - \chi_f$, V_p - the volume of the particle.

The gradient of the magnetic force is directly proportional to the gradient of the magnetic energy density. Thus, if the field is locally homogeneous, it means that no force acts on the particles. A non-zero gradient magnetic force exists in an inhomogeneous magnetic field. If the motion can be characterized by small Reynolds numbers, the Stokes force acting on a spherical particle has the form (Eq. 5):

$$\vec{F}_{st} = 6\pi\eta b \vec{v} \tag{5}$$

Assuming that the shape of a particle trapped in an inhomogeneous magnetic field is a sphere of b radius which is moving in a liquid with dynamic viscosity η, $\vec{v}$ the velocity difference between liquid and particle in a stationary medium. b is the radius of bacterial cell agglomerates in magnetophoretic experiments, in the case of b about 3 microns, the estimated value of the ratio b/a is about 0.001. However, the numeric simulation of mag/netophoresis of the agglomerates of bacterial cells was carried out not for the estimated value of b/a but for an exact one based on the measurement of the radius of every bacterial cell agglomerate using video recording of optic microscopy of magnetophoretic movement of bacterial agglomerates.

The capture process is determined as a result of the competition between the magnetic force $\vec{F}_m$ and the Stokes force $\vec{F}_{st}$(Friedlander *et al.* 1981). The magnetic field strength is described by expressions (Eq. 1), Eq. 2 in the case when an inhomogeneous magnetic field is created by a system of two magnets shown in Fig. 4. We assume that a thin layer of liquid on the surface of the system of magnets is stationary, then the velocity of spherical particles in the liquid on the surface of the system of magnets along the axis, and the characteristic radius of the particle. Eq. 3 can be converted to the next dimensionless form (Eq. 6):

$$\frac{dX}{d\tau} = f(X,Z) \tag{6}$$

where dimensionless coordinates

$$X = \frac{x}{a},\quad Y = \frac{y}{a},\quad Z = \frac{z}{a} \text{ and } \tau = \frac{\chi M_s^2 b^2}{9a^2\eta}\cdot t$$

and notation are introduced (Eq. 7):

$$f(X,Z) = \frac{d}{dX}\left\{4\left(\operatorname{arctg}\left(\frac{1+X}{Z}\right) - \operatorname{arctg}\left(\frac{1-X}{Z}\right) - \operatorname{arctg}\left(\frac{X}{Z}\right)\right)^2 + ln\frac{\left(1+2X+X^2+Z^2\right)\left(1-2X+X^2+Z^2\right)^2}{\left(X^2+Z^2\right)^2}\right\} \tag{7}$$

As a result of integrating Eq. 7, we find the dimensionless time τ_X required for the particle to move in the magnetic field of the magnet system from a point with dimensionless coordinate $-X$ to the line of contact of two magnets with $\frac{b}{a}$ dimensionless coordinate, as a function of dimensionless particle coordinates Z:

$$\tau_x\left(X,Z,\frac{b}{a}\right) = \int_{-X}^{\frac{b}{a}} \frac{dX}{f(X,Z)} \tag{8}$$

The suspension of particles sedimented in the gravitational field before being introduced into the magnetic field, therefore $Z = \frac{b+h}{a}$ the dimensionless

coordinate of the particle was chosen for the calculation, where *h* is the thickness of the glass on the surface of the system of two magnets. This means that the center of the spherical particle is at a height above the surface of the magnet system equal to the radius of the particle with the addition of the thickness of the glass. It can be assumed with great accuracy that $Z=0$ if $b+h=a$.

$$\tau_x\left(X,\frac{b}{a}\right)=\int_{-X}^{\frac{b}{a}}\frac{\mathrm{d}X}{f\left(X,0\right)} \tag{9}$$

Calculation $\left\langle\frac{\mathrm{d}X}{\mathrm{d}\tau}\right\rangle$ of the average dimensionless velocity of the particle along $0x$ the axis in $\left[-X;\frac{b}{a}\right]$ the region based on expression (Eq. 10):

$$\left\langle\frac{\mathrm{d}X\left(X,Z,\frac{b}{a}\right)}{\mathrm{d}\tau}\right\rangle=\frac{X}{\tau_x\left(X,Z,\frac{b}{a}\right)} \tag{10}$$

The dependence of $V=\left\langle\frac{\mathrm{d}X\left(X,Z,\frac{b}{a}\right)}{\mathrm{d}\tau}\right\rangle$ average dimensionless velocity on *X* when *Z*=0 is presented in Fig. 5.

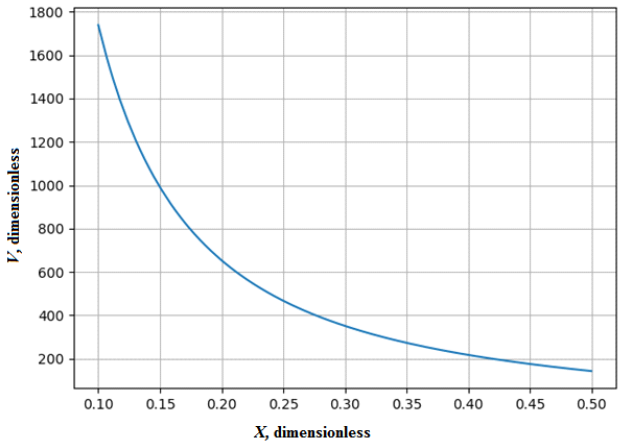

Fig. 5. Dependence of the average dimensionless velocity of the particle on *X* at *Z*=0.001 and at $\frac{b}{a}=0.001$ calculated by Eq. 10.

The average velocity of a particle measured in seconds is determined through the dimensionless average velocity by the formula (Eq. 11):

$$\left\langle\frac{\mathrm{d}x}{\mathrm{d}t}\right\rangle=\frac{\chi M_s^2b^2}{9a\eta}\left\langle\frac{\mathrm{d}X\left(X,Z,\frac{b}{a}\right)}{\mathrm{d}\tau}\right\rangle \tag{11}$$

The effective magnetic susceptibility of a particle moving in a liquid on glass on the surface of a system of two magnets can be determined from the last expression (Eq. 12):

$$\chi=\frac{9a\eta}{M_s^2b^2}\cdot\frac{\left\langle\frac{\mathrm{d}x}{\mathrm{d}t}\right\rangle}{\left\langle\frac{\mathrm{d}X\left(X,Z,\frac{b}{a}\right)}{\mathrm{d}\tau}\right\rangle} \tag{12}$$

The dimensionless coordinate of the particle is chosen for calculation, as already mentioned: $Z=\frac{b+h}{a}$. The results of the calculation of the average effective magnetic susceptibility of particles depending on the cultivation conditions by formula (Eq. 12) are presented in Fig. 6.

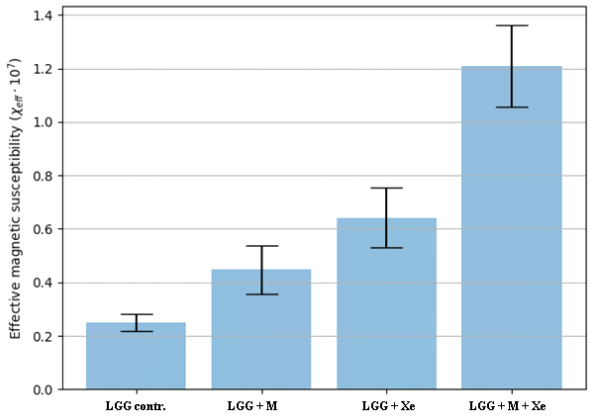

Fig. 6. The average effective magnetic susceptibility of the particles of the *L. rhamnosus* GG suspension depending on the cultivation conditions according to formula Eq. 12. The error was determined using the SDM. The statistical significance of the results is 0.95.

The approach of increasing of magnetic susceptibility of bacteria for drug delivery applications proposed in this work has the following advantages compared to methods based on conjugation:

- In this approach, the rapid division of bacteria does not lead to the loss of increased magnetic susceptibility by daughter cells, so a homogeneous and stable process of distribution

of magnetic susceptibility in the bacterial culture occurs.

- The size of the bacteria in this approach does not change, so the mobility of the bacterial vector is not reduced, which will facilitate the penetration of the bacterial vector through the environment with high density and hierarchical structure in real tumors.
- The proposed approach is also promising as the first stage of creating a bacterial vector with increased magnetic susceptibility, with the aim of further using the method of conjugation with magnetic nanoparticles or magnetoliposomes. In this case, the magnetodipole interaction between the BMN of the bacterial vector and artificial magnetic nanoparticles will create additional magnetic forces that bind this nanocomposite.

Conclusion

The results show that the speed of the suspension particles in the two-magnet system increased as the growing conditions changed and the culture medium was modified. The effective magnetic susceptibility for the LGG control sample is $(0.25 \pm 0.03) \times 10^{-7}$. The magnetic susceptibility of the LGG bacterial culture growing in a constant magnetic field was 1.8 times greater in comparison with the magnetic susceptibility for the control sample. The magnetic susceptibility of the LGG bacterial culture growing at the medium modified with iron chelate was 2.6 times greater in comparison with the magnetic susceptibility for the control sample. The magnetic susceptibility of the LGG bacterial culture growing at the medium modified with iron chelate in a constant magnetic field was 4.8 times greater in comparison with the magnetic susceptibility for the control sample. Thus, modification of the growth medium with iron chelate and/or cultivation in a constant magnetic field improves the ferrimagnetic properties of the bacterial culture LGG, which can be useful in creating vectors of magnetically-guided delivery. A significant increase in the magnetic susceptibility of LGG bacterial culture was observed when both methods are used together i.e., modification of the medium with iron chelate and cultivation in a constant magnetic field.

Conflict of Interest

The authors declare that they have no conflict of interest.

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