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Proceeding Paper

Evaluation of the safety of immobilized microorganisms *Lysobacter sp.* on inorganic media †

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Abstract: It is known that the immobilization of microorganisms on carriers of various nature increases their safety. Inorganic matrices: sodium carboxymethyl cellulose technical brand "KMC 85/500"; colloidal silicon dioxide in the form of a commercial preparation "Polysorb"; sodium form of montmorillonite from the Podgorenskoye deposit, Voronezh region. Bacterial cells were immobilized by adding *Lysobacter sp.* solid sterile carrier with constant mechanical stirring in the ratio "carrier:biomass", equal to 1:(2-4). During the experiment, it was found that the mineral montmorillonite is a promising material for the immobilization of bacterial cells in order to obtain biocompositions based on them, since a positive trend in the preservation of bacterial cells was revealed.

Keywords: inorganic matrices; immobilization; microorganisms; cell safety

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1. Introduction

Currently, a promising direction in biomedicine is the creation of biofilms and compositions based on microorganisms-antagonists of pathogenic microflora on carriers of various nature [1-3].

The aim of the study was to identify a rational inorganic carrier for the immobiliza-tion of *Lysobacter sp.*

2. Materials and Methods

As a model bacterial culture for immobilization, a *Lysobacter* culture isolated from the soil of the city of Belgorod using classical biotechnology methods with confirmation of generic affiliation by 16S rRNA sequencing (1484 nucleotides) was used, as a result of which a unique nucleotide sequence of the strain among those presented in GenBank was revealed: the maximum percentage of similarity observed with strain *L-43* (MT229166.1) and *Lysobacter enzymogenes* M497-1 (AP014940.1) - 99.7% each.

As a growth substrate, a liquid nutrient medium containing 0.2 wt% casein and 0.1 wt% yeast extract was used; T = $30^{o}C$; log phase - 24 hours. Metabolic products: chitosan, beta-1,4-glucanase, protease.

To study the effect of immobilization of microorganisms and their enzymes, solid carriers were used:

1. Sodium carboxymethyl cellulose (NaCMC) technical brand "KMC 85/500" produced by LLC "Davos-Trading". TU 2231-001-53535770-2010 (with change No. 1.2): degree of substitution for carboxymethyl groups 80-90; degree of polymerization 500-550; pH value (pH) of an aqueous solution with a mass fraction of CMC 1% in the range of 8-12; dynamic viscosity of a 2% CMC solution at a temperature of 25 °C mPa*s according to the Brookfield method, not less than 100.

2. Colloidal silicon dioxide in the form of a commercial preparation "Polysorb" produced by JSC "Polysorb". Polysorb MP (medical oral) is an inorganic, non-selective, multifunctional enterosorbent based on highly dispersed silica with particle sizes up to 0.09 mm, with the chemical formula SiO_2. The sorption capacity of the drug for internal use is 300 m^2/g.

3. Sodium form of montmorillonite (NaMMT) from the Podgorenskoye deposit, Voronezh region [4, 5], obtained by introducing soda ash (4 wt%) into a native rock suspension (5 wt%), followed by sedimentation enrichment and drying (t=95±3°C). The quantitative content of montmorillonite, determined according to GOST 28177-89 - 79.35±0.14 wt.%. As a result of the modification, the crystal lattice parameters changed: for the native form of Ca-montmorillonite a = 5.16 Å, b = 8.94 Å, c = 15.02 Å; for modified montmorillonite a = 5.22 Å, b = 9.04 Å, c = 13.82 Å. Specific surface 60 m^2/g, specific pore volume 0.083 cm^3/g; average pore size 55.5Å.

The immobilization of bacterial cells was carried out by adding *Lysobacter sp*. in the logarithmic phase of growth, a solid sterile carrier with constant mechanical stirring in the ratio "carrier : biomass", equal to 1:(2-4), at a temperature of 30°C; the mixture was thoroughly mixed for at least 40 minutes, frozen at minus 40°C and then freeze-dried at minus 40-45°C for 24 hours to a level of 3-7% moisture content of the composition. The dry compositions obtained were then stored in sterile flacons at room temperature.

Survival after immobilization of microorganisms *Lysobacter sp.* on solid carriers was determined by the Pour Plate method, in which the samples were suspended in a Petri dish using molten agar cooled to about 40-45°C (just above the solidification point to minimize heat-induced cell death). After the nutrient agar solidified, the plates were incubated for 24 hours and the number of colony forming units (CFU) was determined by the serial dilution method.

The degree of preservation (α, %) was determined by the formula:

$$\alpha = (1 - \left(\frac{CFU_{ref} - CFU_n}{CFU_{ref}}\right) \times 100\ \%, \quad (1)$$

where: CFU_{ref} - the number of colony-forming units in the biocomposition immediately after immobilization;

CFU_n - the number of colony-forming units in the biocomposition after storage on the n-th day.

3. Results

The results of assessing the viability of immobilized *Lysobacter sp.* are presented in Table 1, and Figure 1 shows the dynamics of the preservation of bacterial culture.

Table 1. Viability of immobilized *Lysobacter* cells.

Biomass : carrier ratio	**CFU * (g/l) after storage**			
	Day 2	Day 15	Day 31	Day 92
Freeze culture	2,4±0,04 · 10^5	2,3±0,06 · 10^5	2,0±0,04 · 10^5	0,9±0,05 · 10^5
NaCMC 1:2	3,4±0,06 · 10^5	3,7±0,02 · 10^5	3,9±0,05 · 10^5	2,1±0,02 · 10^5
NaCMC 1:3	3,5±0,04 · 10^5	3,8±0,03 · 10^5	4,1±0,07 · 10^5	2,7±0,07 · 10^5
NaCMC 1:4	3,3±0,07 · 10^5	3,5±0,03 · 10^5	3,6±0,05 · 10^5	1,9±0,03 · 10^5

Polysorb 1:2	3,5±0,02 · 10^5	3,2±0,02 · 10^5	2,7±0,01 · 10^5	1,8±0,02 · 10^5
Polysorb 1:3	3,2±0,03 · 10^5	3,1±0,01 · 10^5	2,5±0,05 · 10^5	1,7±0,01 · 10^5
Polysorb 1:4	3,3±0,03 · 10^5	3,1±0,05 · 10^5	2,4±0,07 · 10^5	1,5±0,04 · 10^5
NaMMT 1:2	3,6±0,03 · 10^5	3,8±0,02 · 10^5	4,0±0,04 · 10^5	4,2±0,06 · 10^5
NaMMT 1:3	3,5±0,05 · 10^5	3,7±0,06 · 10^5	3,9±0,07 · 10^5	4,1±0,03 · 10^5
NaMMT 1:4	3,4±0,01 · 10^5	3,8±0,04 · 10^5	4,0±0,06 · 10^5	4,1±0,03 · 10^5

*Std. Deviation

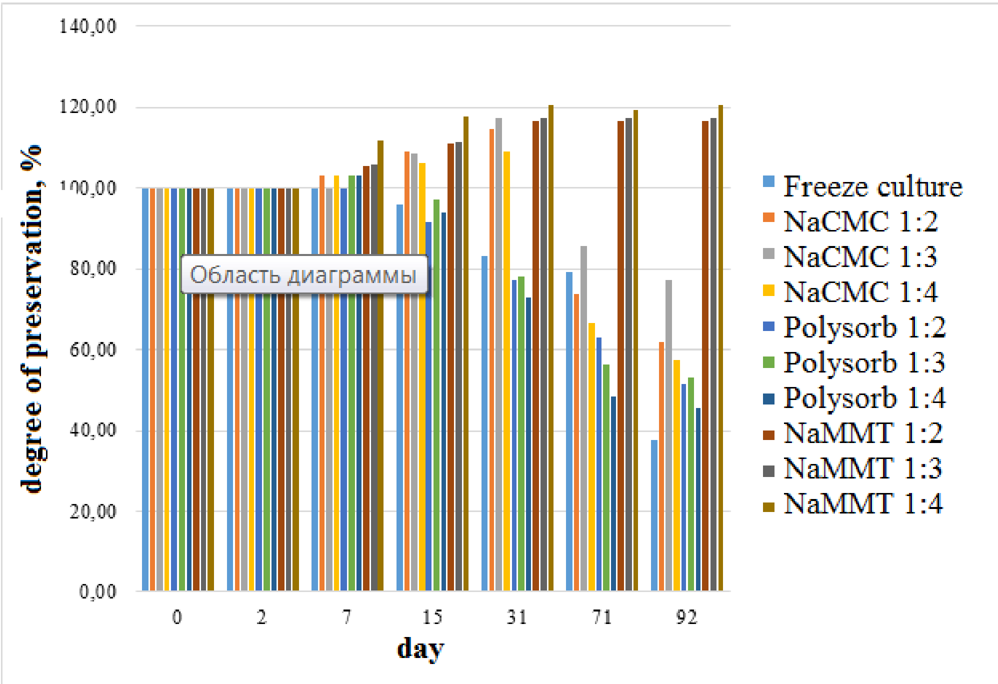

Figure 1. Dynamics of preservation of the bacterial culture *Lysobacter sp.*

After three months (92 days) of storage of lyophilizates, the following results were obtained:

1. Lyophilization of the bacterial culture of Lysobacter sp. without immobilization on the matrix leads to a decrease in safety to 37.5%.

2. Cell immobilization on sodium carboxymethyl cellulose allows to increase safety up to 65%; on colloidal silicon dioxide (Polysorb) - up to 50%.

3. When immobilized on the mineral montmorillonite, not only the preservation of microorganisms is manifested, but also an increase in the number of cells by 18%.

4. Conclusions

Thus, a positive trend in the preservation of bacterial cells during immobilization on solid carriers was revealed.

It has been established that the most effective matrix for immobilization of *Lysobacter sp.* is the sodium form of montmorillonite.

The obtained research results can be used to create biocompositions based on bacterial cultures for various purposes.

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