THE SORBTION OF LPS TOXIC SHOCK BY NANOPARTICLES ON BASE OF CARBONIZATED VEGETABLE RAW MATERIALS

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Abstract

Immobilization of lactobacillus on high temperature carbonizated vegetable raw material (rice husk, grape stones) increases their physiological activity and the quantity of the antibacterial metabolits, that consequently lead to increase of the antagonistic activity of lactobacillus. It is implies that the use of the nanosorbents for the attachment of the probiotical microorganisms are highly perspective for decision the important problems, such as the probiotical preparations delivery to the right address and their attachment to intestines mucosa with the following detoxication of gastro-intestinal tract and the normalization of it's microecology.

Besides that, thus, the received carbonizated nanoparticles have peculiar properties – ability to sorbtion of LPS toxical shock and, hence, to the detoxication of LPS.

1. Introduction

Last years the number of patients suffering from serious diseases caused by endotoxins of gram negative bacteria tends to increase. These endotoxins are nonsecreted termostable lipopolysaccharides (LPS), the main component of external membrane of these bacteria. LPS consist of variable polysaccharide parts and conservative lipid part called Lipid A [1].

The release of endotoxins takes place during the death of the pathogenic microorganisms and leads to generalized infection endotoxical shock as the highest reflection.

Also the constant source of LPS in host organism is gram negative intestine microflora. However, in healthy organism the entrance of small portion of LPS into the blood consider to be necessary for maintaining physiological immunity.

The changes of gastro-intestinal tract microecology can lead to increased reproduction of gram negative bacteria. This follows by the increasing LPS production and even bacteria translocation into the other organs and tissues, which in turn leads to increased releasing of endotoxins into blood circulation.

The toxic effect of LPS can be neutralized by using the various approaches. One of them is the endotoxin removal by sorption on nanoparticles [2].

In Combustion Problems Institute the new materials were obtained by high-temperature carbonization of vegetative raw material (rice husk, grape stones, apricot stones and other). These materials possessed the properties of active sorbents. It is important to note that the formation of nanosize particles was observed, when the temperature of carbonization was about 500-700^{o}C. By electronic microscopy it was established that the nanoparticles surface had numerous pores and folds which could play role of binding sites.

Materials with nanostructure appear unexpected properties thanks to which they can be used in various fields of science and technology [3].

Especially the utilization of carbonized nanoparticles is important for obtaining new medicines, for example, for normalization of intestine microecology or for the treatment of the intestine disorders known as disbacteriosis. Various infections, antibiotic treatment and other factors can cause these diseases. Bacterial therapy by microorganisms with probiotical action is used for the recovery of normal intestine's function. The majority of probiotics contain lactic acid bacteria and bifidobacteria [4]. It is intended to improve microecological situation in thick intestine. The calculating activity of preparations decreases considerably when probiotical drug passes across stomach and thin intestine. Therefore, principal new methods are required to create and deliver it to target organ.

The decision of the drugs addressed delivery problem to different organs and tissues in necessary doses may be connected with new biotechnologies with the using of carbonizated nanoparticles. The great perspectives in this direction were connected with the creation of the immobilizated probiotical preparations, in which bacterial cells were distributed on nanoparticles carrier surface [5]. Carbonizated nanoparticle, such as activated coal, demonstrated detoxical properties and is of special interest for using as carriers for microorganisms-probiotics and for detoxication of the different toxins. It may be expected that intestine microecology can be recovered quickly and effectively by the increasing of the probiotical activity, as a consequence of bacteria delivery in time.

In this article the results of the investigation of probiotical activity of free and attached microbial cells and processes of linkage of the LPS-toxic shock by carbonizated nanoparticles.

2. Materials and methods

Lactic acid bacteria *Lactobacillus fermentum AK-2* isolated from intestine of healthy children was used in our investigations. It showed probiotical properties with high antagonistic and adhesival activities [6].

Carbonizated in the Institute of Burning Problem of the al-Farabi Kazakh National University rice husk (RH) and grapes stones (GS) on 650 ºC were used as nanostructural sorbents [7].

Immobilization of microbial cells on nanocarriers was made by method [8].

Scan microanalysated apparate JCXA-7334 was used to evaluate the surface of modified sorbents and the interactions between sorbents and cells [9]. Experimental disbacteriosis at breedless rats was inducted by antibiotic – ciprofloxacin [6]. Sorbtional activity of the nanosorbents in relation LPS was studied in model experiments.

3. Results

It is known that new linkage sites appear in high temperature carbonization of RH and GS which may be specified for lactobacteria cells.

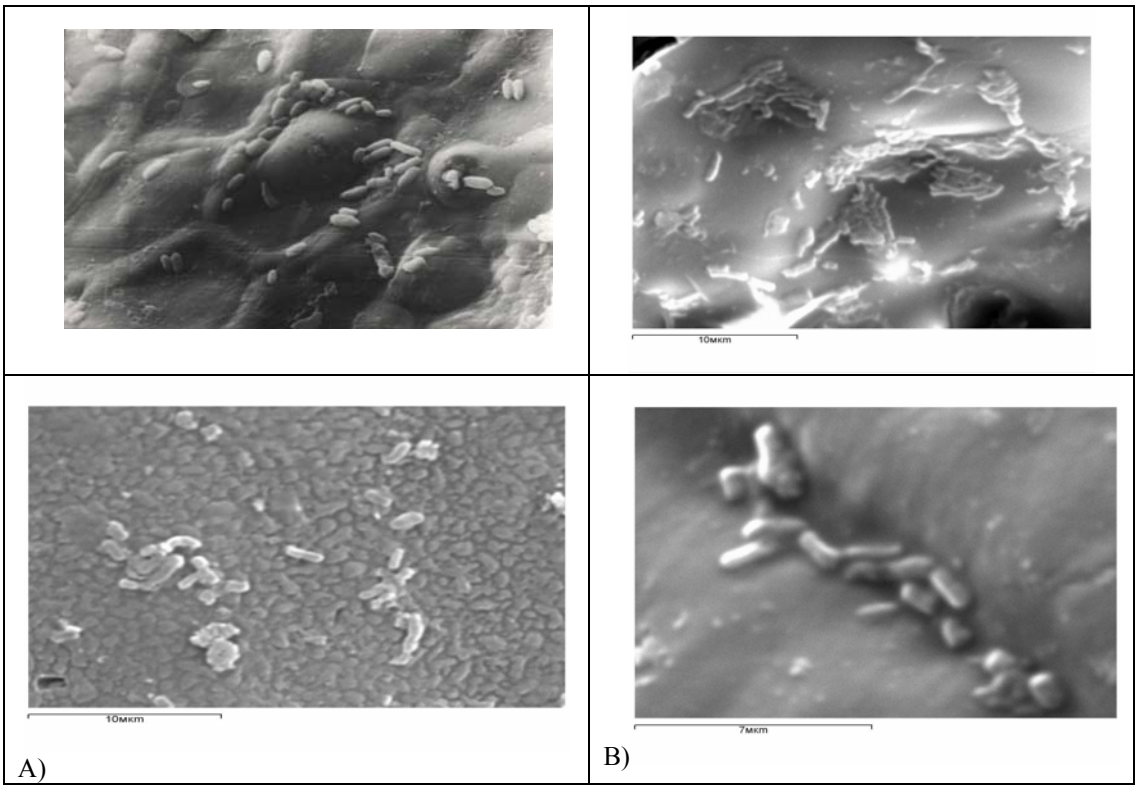

Figure 1. Electron-microscopes pictures with microcolonies on nanocarriers: A) GS; B) RH.

It was pointed that 35-43 % of lactobacteria cells were attached on GS and 55-62 % - on RH. It means that RH is more appropriate for immobilization of lactobacillus cells. This fact can support the assumption that immobilizated lactobacteria cells successfully pass stomach and form sorbent colonies, which were adhesed on the intestines mucosa. This phenomenon leads to the accelerated therapy effect of probiotics for the disbacteriosis elimination. Particular attention attracts disbacteriosis when the number of gram negative bacteria, for example *Esherichia coli,* increases [4].

It was interest to examine the effectiveness of lactobacillus immobilizated cells in these conditions. I.Savitskaya proposed the model to receive experimental disbacteriosis in laboratory rats by antibiotic – ciprofloxacin, which induced disbacteriosis with deficit of bifido - and lactobacteria and excessive amount of *Esherichia coli* [6]. Proibiotics therapy were carried out by the immobilized cells of probiotical microorganisms. Intestinal microflora was investigated during antibiotic entering, in 1 and 15 days (Table 1).

It was established that after 5 days ciprofloxacin entering the noticeable increase of the amount of *Esherichia coli* was observed in thin and thick intestines which remained even after 15 days after the abolition of antibiotic. Another situation was observed when rats received immobilizated probiotics, in particularly, when RH was used as carrier for microorganisms cells.

Table 1. The content of the *Enterobacteriacea* microflora at breedless rats with ciprofloxacin - induced disbacteriosis after probiotical therapy by *L. fermentum AK-2* immobilized on nanosorbents

Group of animals	Number of bacteria in 1g (M±m)			
	Large intestine		Small intestine	
	wall	contents	wall	contents
Before the enter of antibiotic				
The control	$(3,0\pm0,3)x10^4$	$(6,9\pm0,7)x\ 10^6$	$(1,1\pm0,2)x10^2$	$(1,5\pm0,4)x10^3$
1 day after abolition of antibiotic				
Without probiotics	$(2,9\pm0,5)x10^5$	$(7,9\pm0,6)x10^7$	$(2,9\pm0,3)x10^4$	$(1,2\pm0,4)x10^5$
Probiotics on GS	$(9,7\pm0,6)x10^4$	$(1,2\pm0,3)x10^7$	$(7,8\pm0,8)x10^2$	$(8,9\pm0,2)x10^3$
Probiotics on HR	$(6,9\pm0,4)x10^4$	$(9,5\pm0,2)x10^6$	$(0,9\pm0,3)x10^2$	$(5,3\pm0,8)x10^3$
15 day after abolition of antibiotic				
Without probiotics	$(2,4\pm0,4)x10^5$	$(2,1\pm0,3)x10^8$	$(8,4\pm0,2)x10^4$	$(9,5\pm0,8)x10^5$
Probiotics on GS	$(1,2\pm0,7)x10^5$	$(9,6\pm0,6)x10^7$	$(2,9\pm0,4)x10^3$	$(2,8\pm0,4)10^4$
Probiotics on HR	$(3,4\pm0,4)x10^4$	$(8,5\pm0,7)x10^6$	$(1,2\pm0,3)x10^3$	$(1,2\pm0,7)x10^3$

This may be connected with the stimulation of the lactobacills antibiotical activity by RH (fig.2) and perhaps with the intensification processes of LPS adsorbtion on RH. It is shown that immobilization on RH increases antimicrobial activity of lactobacills by 25-60% in comparison with antimicrobial activity of lactobacills immobilizated on GS.

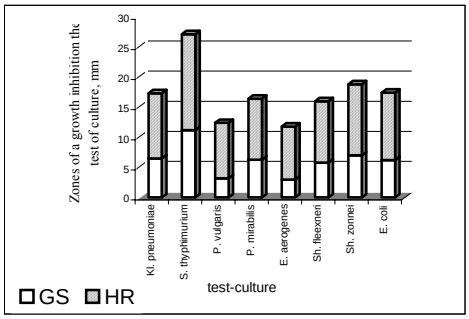

Figure 2. Antimicrobic activity of the cells of *L. fermentum AK-2* immobilizated on carbonized nanosorbents

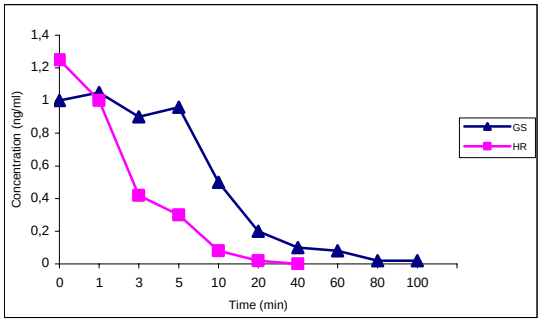

Figure 3. LPS adsorption on GS and HR

Nanoparticle on the basis of carbonizated vegetative raw material (rice husk, apricot stones and the others) were used for sorbtion of LPS. In accordance with receiving results 100% sorbtion of LPS on carbonized HS nanoparticle is observed in 20 minutes, on GS – in 80 minute (fig 3).

4. Conclusion

Immobilization of lactobacills on high temperature carbonizated vegetable raw material (rice husk, grape stones) increases their physiological activity and the quantity of the antibacterial metabolits, that consequently lead to increase of the antagonistic activity of lactobacills. It is implies that the use of the nanosorbents for the attachment of the probiotical microorganisms are highly perspective for decision the important problems, such as the probiotical

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