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INFLUENCE OF DIFFERENT CONCENTRATIONS OF SUGARS ON THE STOPHYLOCOCCUS AUREUS BIOFILM FORMATION

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Abstract

The article analyzes the influence of such sugars as glucose, sucrose, lactose and galactose on the formation of biofilms by *S. aureus* strains isolated from the reproductive tract of women with dysbiosis. The investigation reveals a stimulatory effect characterized as the increase of the number of cells more than 7.76 times and the biofilm formation index – 1.4 times, when glucose was added into the synthetic medium in the amount of 1.5%,. The research fixes the negative influence on the formation of biofilm when the investigated sugars were added into the medium in the concentrations of 2.5% or more.

Keywords: *S. aureus*, biofilm, formation, sugars.

Introduction

The problem of staphylococcal biofilms today is positioned as one of the most important in medical practice. In the first place, this is usually due to the fact that in the form of film microorganisms acquire both an enhancement of their inherent qualities and new features, especially when it includes to polymicrobial communities [5, 7, 10]. In particular, there is an increase of resistance to environmental factors such as antibiotics, antiseptics and disinfectants, pH, irradiation and other [2, 3, 8, 14]. Particular interest have the study of staphylococcal biofilms, which are among the most common while cases of lesions, especially in view of the ability of staphylococci to colonize all biotopes of the human body and for a long time, and sometimes for life, persist

in the organisms, causing relapse of lesions [6, 9, 12, 13]. Formation of biofilm is not an obligatory for the living of microorganisms, however, this form significantly increases the survival of microorganisms in the environment [18], that is related to the structure of the biofilm, in particular, with the presence of the matrix layer around the cell cluster.

The study of the effect of sugars on the formation of biofilms have a particular interest, since the effect of the formation of the exopolymeric matrix depends on the sugars, which is known in staphylococci [20], preferably containing polysaccharides. Monomers for the synthesis of these polysaccharides are external sugars, which can be diluted after absorption by staphylococci as a source of energy and as a plastic material [13].

The aim of the research was to investigate the effect of different concentrations of sugars on the activity of biofilm formation by strains of *Staphylococcus aureus*.

Materials and methods of research

For the research the 96-well plastic plates (Sarstedt, Germany) were used. The number of viable cells and the biofilm-formation index of *S. aureus* strains (n = 7) isolated from the reproductive tract of women with dysbiosis were determine. In each well of the vertical rows of the plate the 200 μl of a universal synthetic medium was applied (g/l: citric acid – 10, asparagine – 3, K_2HPO_4 – 6, $ZnSO_4$ – 0,5, $MgSO_4$ – 0,7, $FeSO_4$ – 0,1; NaCl – 6; NA_2HPO_4 – 1,5; glycine – 1,0; amber acid – 3,0 and glycerin – 40-50 ml. Distilled water was added to 1 liter. The pH of the medium for growing staphylococci was set as 7.5-7.6 [19]) with different concentrations of sugars: glucose, sucrose, lactose and galactose.

Preparation of medium with different concentrations of sugars:

1) to 99.5 ml of a sterile base of the medium the 0.5 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 0.5% sugar;

2) to 99.0 ml of a sterile base of the medium the 1.0 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 1.0% sugar;

3) to 98.5 ml of a sterile base of the medium the 1.5 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 1.5% sugar;

4) to 98.0 ml of a sterile base of the medium the 2.0 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 2.0% sugar;

5) to 97.5 ml of a sterile base of the medium the 2.5 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 2.5% sugar;

6) to 97.0 ml of a sterile base of the medium the 3.0 g of a certain sugar were added: glucose, or sucrose, or lactose, or galactose, and sterilized, receiving an medium containing 3.0% sugar.

In a nutrient medium the 100 μl of a bacterial suspension containing 1.0×10^4 CFU / ml. As a control the well with 200 μl of sterile base medium and 100 μl of bacterial suspension containing 1.0×10^4 CFU / ml was used: it was a control of biofilm-formation. Another control was well with 200 μl of sterile base of the medium and 100 μl of isotonic solution (0.5% NaCl): it was a control of the nutrient medium.

Preparation of suspensions of microorganisms with a determined concentration of microbial cells was made in a sterile isotonic solution (0.5% NaCl) and with the next inoculation of dilutions prepared from the obtained bacterial suspension to determine the amount of CFU / ml.

The analysis of film growth was made 72 h after inoculation of the cell suspension into the wells of the plate.

To determine the number of viable cells in the formed biofilm the residues of the nutrient medium were removed by micropipette from the wells of the plate, and then the biofilm was washed twice with isotonic solution (0.5% NaCl). The biofilm was homogenized. From the obtained bacterial suspension the 10-fold dilution were made and inoculated on Petri dishes with meat-peptonic broth. After 24 h of incubation, the number of CFU / ml was calculated.

The index of biofilm formation was determined by measuring of the amount of crystalline violet associated dye on a photo-electro-colorimeter of KFK-2MP. After the cultivation, the residue of the nutrient medium was removed by a micropipette. The surface of the biofilm was washed with 0.01 M phosphate buffer (pH = 7.2) and stained with 0.1% crystalline violet for 10 min. The surface of the biofilm was washed with isotonic solution (0.5% NaCl) and dried at room temperature, then the dye was bound to the biofilm was extracted with ethanol [17]. Then, the measurements of the optical density of ethanol extracts on a photo-electro-colorimeter at a wavelength of 590 nm in a cuvette with an optical path length of 1 cm was done.

The growth of the biofilm of the studied strains under the influence of various concentrations of sugars was evaluated by the presence of a difference in optical density between the experimental and control samples, followed by the calculation of the biofilm formation index (BI) by the formula [15]:

$$BI=OD_{592}(\text{crystalline violet})\times OD_{592}(\text{planctonic cells})/OD_{592}(\text{inoculating dose}).$$

Results were processed statistically using the program MS Exell.

Results and discussion

Figure 1 shows the changes of the number of cells in *S. aureus*

biofilms that were formed in media with different concentrations of sugars after 72 h from inoculation of bacteria.

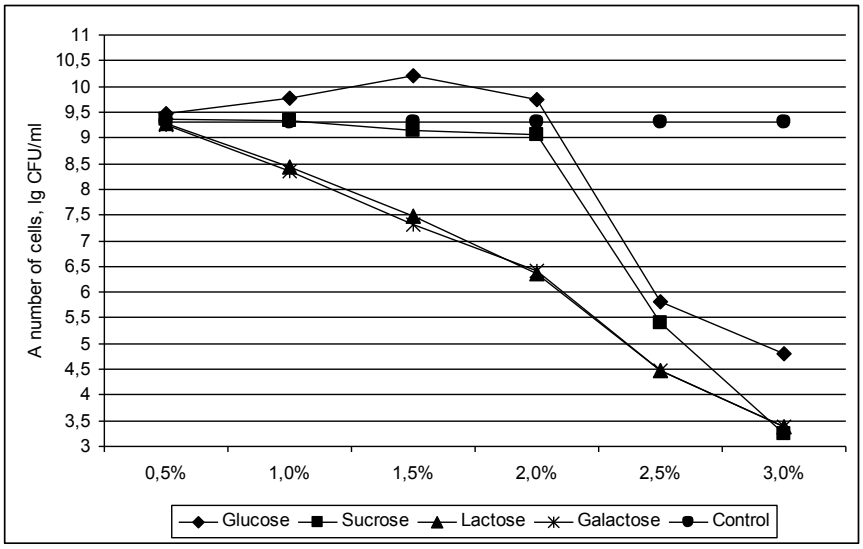

Fig. 1 A number of cells in *S. aureus* biofilms during the cultivation in synthetic media with various concentrations of sugars (72 h)

From the presented data, it may conclude that glucose is the only sugar that has a certain stimulative effect on the biofilm growth: the addition of it in the culture medium at concentrations from 0.5 to 2% resulted in an increase of the number of viable cells for 72 h of cultivation compare to control. Consequently, the largest number of cells in the film accumulated during cultivation in a medium with 1.5% glucose, was 7.76 times higher than the control value. The amount of CFU in the control biofilm was 9.31 lg CFU / ml on average. Addition of glucose to 1% and 2% also contributed to an increase in cells in the biofilm by more than 2.75 times compared with the control.

When the content of any of the listed sugars in the nutrient medium at 0.5% concentration did not show significant stimulatory or inhibitory effects: the number of cells was within the range close to the control value, and in the presence of glucose and sucrose, an increase in the number of cells was observed in excess of 1.15 times compared with control, and when using lactose or galactose – a 1.05-fold decrease in cell counts.

During glucose and sucrose use, the marked inhibitory effect was observed at concentrations of these sugars of 2.5 and 3%, when the number of cells decreased more than 3,000 times compared to the control. The presence in the medium of lactose or galactose coincided with the decrease in the number of

cells in the film, starting with the concentration of sugars 1%: while there was a decrease of more than 7.76 times. The minimal number of cells in the film was observed when 3% of each of these sugars were added into the culture medium, which was accompanied by a decrease in the number of cells at more than 8.31 $\times 10^4$ times compared with the control.

The effect of all sugars on the biofilm-formation index (BI) of *S. aureus* strains is shown in Fig. 2.

The formation of biofilm by strains of *S. aureus* occurs when all investigated sugars are added to media. However, the gradual decrease of the index with increasing of concentrations indicates a reduction in the intensity of film-forming processes. At the same time, it can be noted that the fluctuations of the index within the values close to the control index may indicate a slight correlation between the processes of biofilm formation and the presence of sugars in the medium, at least about the unexpressed stimulatory effect of sugar. The only exception is glucose: the addition of it at concentrations from 1% to 2% was accompanied by an increase in BI, as well as shown in the previous study, and an increase in the number of cells.

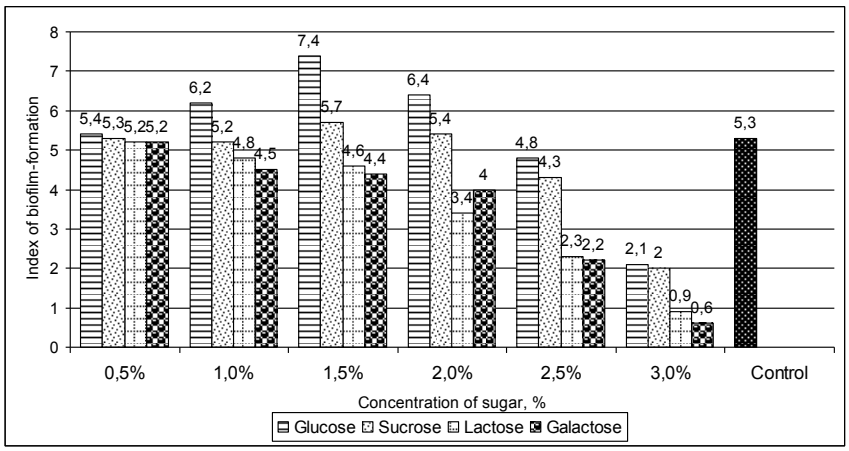

Fig. 2 Influence of different concentrations of sugars on biofilm-formation index of *S. aureus* strains

The maximal BI at 72 h of cultivation was determined for a glucose concentration of 1.5%: this index exceeded the control by 1.4 times. Instead, the inhibitory effect, set for concentrations of more than 2% of sugars, was shown on the BI. This index was significantly lower compared to control, especially for concentrations of 3%. The lowest dependence of BI on the concentration of sugars was noted for glucose and sucrose: a decrease of 2.52 times and 2.65

times respectively, while the maximum dependence was detected for galactose – a decrease of 8.83 times.

The dynamics of the decrease of the BI coincided with the earlier established dynamics of the decrease of the number of cells in film, indicating the reproducibility of the results of the experiment.

Consequently, the study of the influence of carbon sources on the formation of biofilms showed that the type of sugar and its concentration may show a positive, neutral or negative effect on the growth characteristics of staphylococcal biofilms. Thus, the neutral effect was observed when using the smallest of the studied concentrations of 0.5% of any of the four studied sugars – glucose, sucrose, lactose or galactose, which is confirmed in the some experiments [11]. An addition of any of the studied sugars in the medium at a concentration of 2.5% and more, done an inhibitory effect on the formation of biofilm. There is evidence that the only sugar necessary for biofilm formation is glucose, which is even used in film growth modeling [4, 16]. We recorded its stimulatory effect during the addition of 1.5% glucose into the nutrient medium, which was accompanied by the largest relative to control by the growth of the number of cells and the index of biofilm formation: the number of cells increased 7.76 times compared with the control of the film grown on a medium without sugar. Also, a slight stimulatory effect of concentrations of 1% and 2% of glucose and sucrose should be noted. Lactose and galactose, from a concentration of 1%, had a negative effect. In particular, the results obtained by us confirm the experiments of Korobov and co-auth. [11]. At the same time, it should be noted that the presence of sugars in the culture medium can influence on the formation of the biofilm matrix depending on the microorganisms. Thus, in studies of staphylococcal strains, it was determined that the role of sucrose is not significant, while for *Listeria monocytogenes*, its presence in the medium with the Congo red is necessary for development of strains, able to exopolysaccharide biosynthesis [1].

Conclusions

1. The stimulatory effect of addition of 1.5% glucose into the nutrient medium has been established, which resulted in a 7.76-fold increase in the number of cells and a 1.4-fold increase in the biofilm-formation index compared with the control of the film grown on a sugar-free medium.

2. A slight stimulatory effect of glucose and sucrose concentrations of 1% and 2% was noted.

3. The negative influence on the formation of biofilm of glucose, sucrose, lactose and galactose in concentrations of 2.5% and more was shown.

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