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## STUDY OF AUTOSYMBIONT AEROCOCCUS VIRIDANS ADHESIVE PROPERTIES ON EPITHELIAL CELLS MODEL

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**Annotation.** *Homo- and heteroadhesive activity of Aerococcus viridans autosymbiotic strains on the model of intestinal epithelial cells of laboratory animals was evaluated in this study. It was shown that adhesive activity of autosymbiotic aerococci strains varies and depends on the microorganism origin and species of epithelial cells host. Aerococcus viridans autosymbionts have higher adhesive activity in terms of their contact with epithelial cells of the host and lower activity in terms of heteroadhesion.*

**Keywords:** *Aerococcus viridans, autosymbionts, adhesion.*

Adhesive activity is the ability of bacteria to attach to the intestinal epithelium and multiply before the cells of the mucous layer are renewed.

To provide this function, bacteria synthesize a number of structures (pili / fimbriae) through which they attach to the epithelial cell. These structures are called colonization or adhesion factors, in some microorganisms they are related to pathogenicity factors. Bacteria are able to express different types of pili, which are encoded by various chromosomal and plasmid genes. This genetic diversity allows the cells to adapt to a changing environment and use this opportunity towards the different surface structures of the host.

The mechanism of specific adhesion includes two phases: reversible and irreversible. A reversible phase can be provided by hydraulic interaction, electrostatic attraction, Brownian motion, as well as atomic and molecular vibrations. An irreversible specific phase is provided by a number of lock-key bonds between complementary molecules on each of the cellular surfaces. As soon as these bonds have formed, the attachment of bacteria becomes irreversible.

Adhesion and colonization of microorganisms to host tissues is the first and obligatory step of infection. This adhesion is considered not only as a microorganism attachment to the substrate, but also as a process where both components of the system «epithelial cell - organism» actively interact with each other [4].

Considering the high adhesiveness as a pathogenicity factor for pathogenic and conditionally pathogenic microorganisms [9], it is necessary to remember, that strains of probiotic microorganisms with excessively high adhesiveness, can not be recommended for the production of probiotics [6].

In addition, the structure, stability and protective properties of intestine biofilms,

which create a protective barrier for pathogens, largely depend on the adhesive properties of microorganisms. [8]

The use of personalized probiotic bacteria auto-strain therapy is a perspective direction [1, 7], first of all, in our opinion, in terms of optimum adhesive properties of used microorganisms, which should have higher affinity to epithelium of macro organism.

Antagonistic and adhesive properties of the Museum *Aerococcus viridans* 167 strain are studied well [3]. In addition, there is an evidence of homo- and heteroadhesive properties of autosymbiont *Aerococcus* kind, studied in red blood cells of humans and animals [2]. In view of the autoprobiotic therapy problem development and prophylaxis by symbiont *Aerococcus viridans* strains, the parameters of aerococci adhesiveness to the epithelial cells of the organism from which they were isolated, are need.

The purpose this study was to evaluate the homo- and heteroadhesive activity of autosymbiont *Aerococcus viridans* strains on the model of laboratory animals intestinal epithelial cells.

**Materials and methods.** Autosymbiont *Aerococci* strains, isolated from mice, rats and humans feces were used for evaluation of mono and hetero adhesion [5].

Adhesiveness of autosymbiont to epithelial cells of the colon of outbred rats and mice was studied. Rats and mice were anesthetized by ether, pieces of 5 x 5 mm, isolated from the intestine, were carefully washed from mucus and content in phosphate buffered saline (PBS). Epithelial cells were obtained from pieces of intestinal mucosa on vibrator IKA VORTEX Genius 3.

The cells were washed with cooled PBS (pH 7,2) by centrifugation at 800 rpm for 10 min twice. After determining the cell suspension concentration with the Goryaeva camera under a light microscope it was adjusted to  $2 \times 10^6$  cells / ml. *Aerococci* culture suspension of  $2 \times 10^9$  CFU / ml concentration was prepared.

0.5 ml of bacterial culture of isolated auto symbionts and *Aerococcus viridans* 167 museum strain suspension was applied on 0.5 ml of epithelial cell suspension. Cell-bacterial mixture was incubated in the thermostat at  $(37 \pm 1)^\circ \text{C}$  for 30 min., periodically shaking. Then the mixture was washed with PBS from of microbes that did not adhered at 600 rpm for 10 min three times. All manipulations were carried out in the cold. 1 - 2 drops of PBS were added to the sediment and smears on the glass were prepared. Preparations were fixed  $96^\circ$  alcohol and stained by Romanovsky-Gimze.

The average number of bacteria that stick to the 25 colon cells were calculated under a light microscope.

The study was repeated at least 3 times when counting the adhesion of each microorganism strain. The average adhesion (AA), the number of microbes in one cell - microbe / cell in 10 fields of vision, according to the results of all studies, was evaluated.

The level of bacteria adhesion was conventionally differentiated into four stages:

- Not adhesive (AA = 0);
- Slightly adhesive (AA = 1 - 5);
- Medium adhesive (AA = 5 - 10);

- Highly adhesive (AA higher than 10).

Statistical processing of the results was carried out with the use of the package of licensed applications STATISTICA (6.1, AGAR909E415822FA serial number). Type of parameters distribution was analyzed by Shapiro-Wilk test. Significant differences between the indices, taking into account the type of distribution were determined by Student's t-test. The threshold level of statistical significance of the results was  $p < 0,05$ . Results are presented as the arithmetic mean (M) and the standard error of the mean ( $\pm$  SD).

The work was conducted in compliance with the regulations of the medical ethics commission, designed on the base of provisions of the European Convention Council "Protection of human dignity in biomedical aspect" (1997) and the Helsinki Declaration of the World Medical Association (2008).

### Results and Discussion.

8 aerococci isolates were selected from outbred rats (1-8k) feces, 7 aerococci isolates from outbred mice (1-7m) feces. Morphological, cultural, biochemical, antagonistic properties of isolates were studied. Two most active biochemically and antagonistically isolates were selected for the experiment: 5m2015 isolated from mice and 3k2015 isolated from rats.

Tables 1 and table 2 present the results of autosymbiont *Aerococcus viridans* adhesion to epithelial cells of rats and mice.

Table 1

Types of autosymbionts (isolate)	Number of epithelial cells	AA
Rats (3k2015)	25	4,11 $\pm$ 0,16*
Mice (5m2015)	25	9,02 $\pm$ 0,18
<i>Aerococcus viridans</i> 167	25	5,21 $\pm$ 0,19*

Note. \* -  $P < 0,005$  compared with isolates of mice.

As it is shown in Table 1, the higher adhesive properties in relation to the epithelial cells of mice had autosymbionty isolated from the body of mice (isolate 5m2015). Adhesive activity of *A. viridans* 3k2015 to epithelial cells of mice can be regarded as weakly adhesive while the adhesive activity of *A. viridans* 5m2015 to epithelial cells of mice can be considered medium adhesive, closer to the highly adhesive. Adhesive activity of *A. viridans* 167 to epithelial cells of mice can be considered medium with minimal adhesive performance.

Table 2

**Indicators of autotymbiont *Aerococcus viridans* strains adhesion to epithelial cells of rats (M ± SD)**

Types of autotymbionts (isolate)	Number of epithelial cells	AA
Rats (3k2015)	25	9,42±0,12
Mice (5m2015)	25	4,89±0,18*
<i>Aerococcus viridans</i> 167	25	5,42±0,13*

Note. \* -  $p < 0,005$  compared to rats isolates.

As is evident from Table 2, autotymbiont isolated from the body of rats (isolate 3k2015) had a significant adhesive properties with respect to the red blood cells of rats. *A. viridans* 3k2015 adhesive activity to the epithelial cells of rats can be considered as medium, closer to highly adhesive, while the adhesive activity of *A. viridans* 5m2015 can be considered a weakly adhesive, closer to medium adhesive. Adhesive activity of *A. viridans* 167 to the epithelial cells of rats can be considered as medium with minimal adhesive indexes.

**Conclusions.** *Aerococcus viridans* autotymbionts is a component of the normal intestinal flora of animals.

Autotymbiont aerococci strains adhesive activity varies depending on the origin of microorganism and epithelial cells host type. *Aerococcus viridans* autotymbionts have higher adhesive activity in studying under conditions of contact with epithelial cells of the host and less activity in terms of heteroadhesion.

Aerococci homoadhesive activity against the epithelial cells is medium adhesive closer to the highly adhesive and is higher than heteroadhesive. Thus, autotymbionts didn't have excessively high rates of adhesion, which could contradict the requirements to probiotics. Therefore, these autotymbionts properties can become a precondition for closer contact of microorganism with the macroorganism and more rapid and qualitative normalization of microbiota.

Due to more optimal adhesive ability the use of *Aerococcus viridans* autotymbionts can be theoretically reasonable to correct dysbiotic conditions of humans and animals and requires further study.

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