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Section 1. Biology

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Efficiency of probiotic autosymbions association (A. viridans and B. subtilis 3 strains) research

Introduction. Range of indications, covered using of probiotics is well — spread: in order to stimulate cellular and humoral factors of immunity, activates of metabolism and normalize digestion, treatment and prevention of dysbiosis, intestinal infectious diseases and nutritional etiology, normalization of digestive tract microflora after treatment with antibiotics and other antibacterial remedies. This range of probiotics application in the clinical practice would be significantly extended an antimicrobial therapy of the exterior purulent-inflammatory processes[1].

Probiotics could contain both types of bacteria as well as monobiotics and associated strains several types of microorganisms (from 2 to 30) — associated probiotics. Some types of probiotics have a vital importance in the humans, animals, birds, fish vital activities, i. e. wouldn't depend on an organism of their primary residence, where these probiotics strains were selected, a so — called heterobiotics. Mostly, common useage have the so — called homobiotics, carried out from biological material of the given type of animal or human organism. Recently in medical practice autobiotics were provided, carried out from the normal strains of microflora, which should be isolated from an individual organism, in order to correct its microecology[2].

The autoprobiotic remedies composition advantages would be focused on several strains of probiotics, which properties complement each other, souldn't carried out mutual antagonism[3].

Primary importance have been shown autoprobiotic remedies composition, which covered physiological, biochemical, antagonistic and other properties. Finaly, interaction research between these probiotic associations should affect into the remedies properties.

Aerococcus — carried out from normal microflora of humans and animals, having a well — spread antagonistic action[4]. Antagonistic properties of aerococcus sould be connected for a long time with hydrogen peroxide — basic issue of an industrial aerococcus strain production, as well as a final product "A — bacterin" — which have been carried out in Microbiology, Virology, Immunology and Epidemiology Department at the SE "Dnipropetrovsk Medical Academy MHU"[5].

A series of experiments *in vitro* carried out in order to research spectrum of antagonistic action *A. viridans 167* and clinical research of "A — bacterin", have been shown a high inhibitory activity this type of bacteria towards nosocomial infections sources and microorganisms, involved in the pathological microflora formation in a human intestine[6].

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Research of the liquid forms of probiotic association was carried out on the basis of autostrain *Aerococcus viridans* (microaerophilous metabolism) and *Bacillus subtilis 3* (rVMV 105) — aerobic metabolism), as well as normal human microflora strains, in our point of view, should have a vital importance.

Materials and methods. In accordance with method[1] have been carried out allocation and identification of autostrain *A. viridans* in the experimental animals.

In an experimental research liquid association carried out from probiotic complex of autostrains *A. viridans (a) № 1* and industrial strain *B. subtilis 3* in order to microbiological background an exterior purulent-inflammatory processes antimicrobial therapy model: staphylococcal infection, is carried out on white mice and burns and wounds treatment, caused by *Pseudomonas aeruginosa,* should be appointed on the rats.

Modeling of staphylococcal infection in white mice was carried out by injecting 0,1 ml 1 billion suspension of *S. aureus* daily culture, isolated from a patient with purulent discharge, subcutaneously. This model should obtain distinct symptoms of inflammation, developed in mice in 2–3 days after infection and continued 10–13 days. Staphylococcus, appointed percutaneously, whould intensively growth caused local manifestations of these disease.

In order to research ability of probiotic complex autostrain *A. viridans (a) № 1* and industrial strain *B. subtilis 3* ("PK"), carried out before exposure of Staphylococcus subcutaneously dose (1 ml 1 billion suspension) should appointed to 35 white mice in different terms as well as before and after Staphylococcus 0,1 ml "PK" (2 billion cells per one suspension). Control group covered 10 mice. Observed time focused on the local symptoms of the disease and an intensity of their development.

Experimental model of burns, infected with *Pseudomonas aeruginosa* animals, provided under general anesthesia with thiopental sodium, after that should be injured demaged side of animals as well as metal ring (diameter 2 cm) with a cotton swab dipped in alcohol and set on fire. Wound was dried with a sterile swab and an infected (1 ml 10^9 cells) dose of *P. aeruginosa* carried out into the center of a burn, waiting the full absorption.

Action of aerococcus and bacillus probiotic association were conducted on 44 rats, i. e. 20 animals was included in an experimental group and 24 — to control group. In the experimental group infected wounds of animals were treated daily, applying "PK" (2 billion in 1 ml) under recovery. "PK" was applied as well as the instillation on wound surface (exposure time 2 minutes). Wounds' surface in the control group of animals was treated with isotonic sodium chloride same way, every day, carried out same exposure time. Observed period should be provided 3 weeks. Indicators of "PK" action are the following: intensity of the inflammation manifestations, terms of death or healing the wound surface.

In order to research effect different doses of associated remedies, covered bacilli and aerococcus, through peroral way of transfer into digestive tract of mice mucous membrane should be appointed non –physiological large doses (10 billion and 5 billion once a day for 10 days "per os" to the mice, CBA type), as well as repeated each 2 months application 1 x 10^9 microbial cells.

For statistical analysis used software package Statistica v6.1*. Quantitative indicators should be presented as meaning with its standard error (M±m). Comparison an average meaning of these values was provided through criteria Student (t), relative values — Chi — square Pearson (χ^2). Statistically significant differences whould be carried out while value $p \leq 0,05$.

Results and discussion. Analysis of the results as well as impact PK in the model of Staphylococcus infection have been shown such terms of remedies intake: 5 hours earlier carrieed out for Staphylococcus, in 5 minutes and 5 hours after Staphylococcus application, which contributes antagonistic effect and prevents development of infiltration. Application of "PK" in a dose 0.1 ml 2 billion daily probiotic culture association in 24 and 48 hours, should reduce manifestations of the disease. In these cases, since 3–4 days, local process at experimental mice was significantly diminished in contrast to control group of animals, where well-defined infiltration carried out 8 days of observation period (table 1, fig. 1, 2).

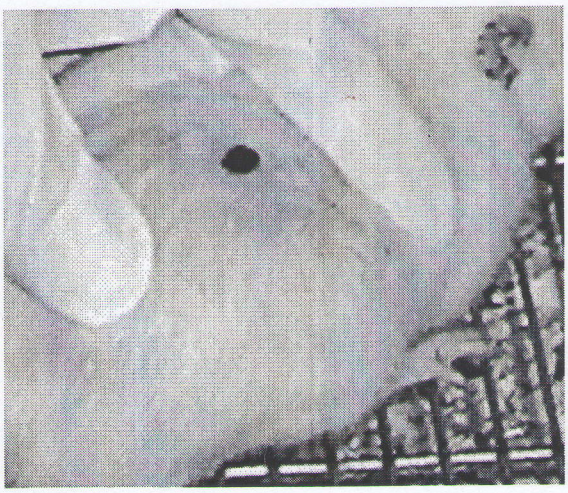

Fig. 1. Action of "PK" in a dose $2,0x10^9$ cells in the experimental group of animals, 4 day of observation

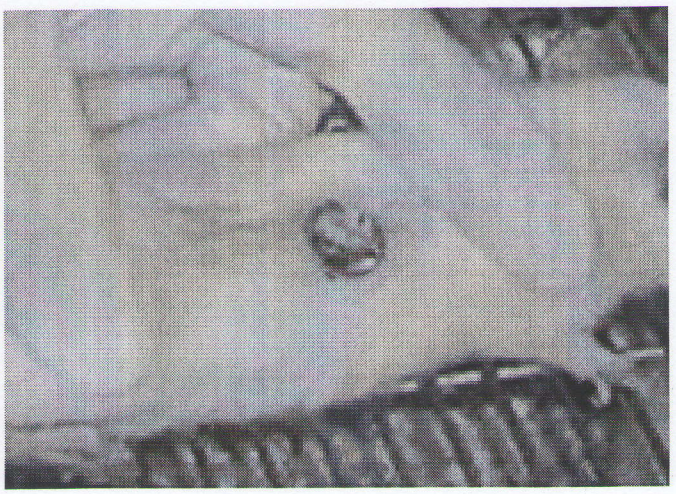

Fig. 2. Wound surface in the control group of animals, 4 day of observation

Result of bacteriological examination purulent inflammation have been showed in the groups of mice, which were injected with associated complex, along with disappearance local symptoms of inflammation should decrease Staphylococcus amount, taken from the abscess. Staphylococcus disappeared on the 7th day. In the control group staphylococci whould visualise on 11 days of observation.

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Table 1. – Development of local inflammatory process in white mice towards exposition period and combinations Staphylococcus and "PK" subcutaneous injection

"PK" injected subcutaneously	Animals amount	Appearance and intensity of inflammation (day)										
		1	2	3	4	5	6	7	8	9	10	11
a) in 5 hours befor Staphylococcus injection	5	–	–	–	–	–	–	–	–	–	–	–
b) in 5 hours and 3 hours befor Staphylococcus injection	5	–	–	–	–	–	–	–	–	–	–	–
a) in 5 minutes after Staphylococcus injection	5	–	–	–	–	–	–	–	–	–	–	–
b) in 5 minutes and 3 hours after Staphylococcus injection	5	–	–	–	–	–	–	–	–	–	–	–
c) in an hour and 5 hours after Staphylococcus injection	5	–	–	–	–	–	–	–	–	–	–	–
d) in 24 hours after Staphylococcus injection	5	++++	+++	++	++	++	++	+	–	–	–	–
e) in 48 hours after Staphylococcus injection	5	++++	++++	+++	+++	++	++	+	–	–	–	–
control animals	10	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	+

Notes: "-" inflammation symptoms are absent; "+" — subtle hyperemia; "++" — mild hyperemia and edema; "+++" — during autopsy carried out pyon; swelling and redness; "++++" — redness and swelling spread over a large area of skin, during autopsy — mostly pyon.

Action of aerococcus and bacillus probiotic association was evaluated in another model as well as Pseudomonas aeruginosa infection, carried out on the rats with severe inflammation local manifestations of the pathological process. Results have been shown protective effect towards complex *A. viridans (a) № 1* and *B. sutilis 3* at the daily application, carried out once per day under period of recover.

Perifocal inflammation should be less significant by its intensity in the experimental group of animals. On the other hand, wounds were quickly released from pyon and covered with a dry scab (Fig. 3). Wounds at 60,0% of rats in the experimental group were completely healed with rejection of scab before 11th day of observation. In the control group of animals similar pattern was observed only in 25,0% of cases (χ^2=5,5; p<0,05) (Fig. 4).

Fig. 3. Wound surface in the experimental group of animals, treated with "PK" in a dose 2,0.10^9 cells, 7 day of observation

Fig. 4. Wound surface in the control group of animals, 7 day of observation

Amount of *P. aeruginosa* contained in a discharge from wounds of treated rats, since remedy injection was on the lowest level than in the control group (table 2). Among those animals, who were treated with probiotic complex, *P. aeruginosa* contamination of wounds was below a critical level in a 4 day of treatment, i. e. in 14 day the pathogen from wounds shouldn't sown. In the control group tendency to clean wounds was less significant. Reducing of seeding below a critical level should be carried out in a 7 day. On a 14 day microbial cleaning of animals wounds shouldn't observed.

Table 2. – Pseudomonas aeruginosa cells per 1 ml, could been sown from the rats wounds (M±m, n=10)

Day of observation	Experimental group	Control group
1	*2*	*3*
1	4,6±0,1×10^6	4,5±0,6×10^6
2	4,0±0,6×10^6	3,6±0,4×10^6

1	2	3
5	$3,5\pm0,1\times10^3$	$4,3\pm0,4\times10^5$ *
6	$1,9\pm0,5\times10^3$	$4,8\pm0,3\times10^4$ *
7	$7,8\pm0,3\times10^2$	$6,5\pm0,1\times10^3$ *
8	$4,5\pm0,1\times10^2$	$6,7\pm0,6\times10^3$ *
9	$2,3\pm0,1\times10^2$	$5,5\pm0,2\times10^3$ *
10	$1,5\pm0,4\times10^2$	$5,7\pm0,5\times10^3$ *
11	94±14	$8,5\pm0,4\times10^2$
12	37±15	$5,3\pm0,3\times10^2$
13	8±4	$1,5\pm0,1\times10^2$ *
14	-	$0,2\pm0,1\times10^2$ *

Note. * — p<0,001 in comparison with control group

Results of an experiment have been shown, that in 100% cases, carried out after treatment with probiotic complex, aerococcus and bacillus from wounds should appear in 7 days after research remedy application.

In the histological preparations was shown dynamics of changes mucous membrane in the digestive tract after 1, 5, 10 and 30 days of the suspension selected strain final application.

Research of sections, taken from an intestine of mice, carried out with electron microscopy, couldn't found any differences towards mucosa in the experimental and control group of mice. Finaly, biological association *A. viridans* (k) and (z) shouldn't have antagonistic activity, focused on the museum strain *A. viridans 167* and could be provided in order to prepare associated probiotic complexes *B. sutilis 3*.

Conclusions. Research protective action towards liquid probiotic mixture, have been selected from autostrain *A. viridans (a) № 1* and *B. subtilis 3* (autoprobiotic complex), carried out in the experimental models as well as external inflammatory processes, caused by Staphylococcus and *Pseudomonas aeruginosa*, have been shown its effectiveness, probably, correspond to the probiotic properties due to synergistic effect.

Probiotic complex should be appointed in a dose 0.1 ml 2 billion of the daily probiotic culture association in 24 and 48 hours, reduced manifestations of the disease. In these case, since 3–4 days localization process in experimental group of mice was significantly diminished in contrast with control group of animals, where well-defined infiltration should persiste untill 8 days of observation.

Artificial way *per os* probiotic complex injection in a dose 5 billion cells per 10 days couldn't show any effects towards an intestinal microflora in the experimental group of animals.

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