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VIRULICIDAL EFFECT OF THE PROBIOTIC DRUG "SVITECO-MULTI" ON POLIOVIRUS TYPE 1 AND INFLUENZA VIRUS

The experimental work shows the antiviral activity of the probiotic drug "Sviteco-Multi" which contains bacteria of the genus Bacillusin model system in cell culturesMDCKandHEp-2, against influenza A (H1N1)pdm2009 virus and vaccine poliovirus type 1, which allows to recommend it for use, in particular, as an alternative to traditional antiviral disinfectants.

Keywords: probiotic drugs, antiviral activity, Bacillus subtilis.

Introduction. Probiotics are considered to be living microorganisms that, when introduced into the body in adequate quantities, have a beneficial effect on the host's body. The category of probiotics includes various microorganisms, in particular representatives of *Lactobacillus* and *Bifidobacterium*, as well as bacteria of the genus *Bacillus*. The latter genus includes a group with a wide variety of species that has been known to scientists for over 100 years, including *B. subtilis, B. pumilus, B. licheniformis, B. amyloliquefaciens* and *B. megatherium*.

It turns out that probiotics of the genus *Bacillus* have an antifungal and antiphlogistic effect due to creation of biofilm, which prevents the reproduction of fungi and pathogenic bacteria. Currently probiotics are used in medical practice, though the history of their use in European countries began in the fields of animal husbandry and poultry breeding for prevention of spread of diseases, which are caused by bacteria and fungus to decrease the usage of antibiotics. Proven, that probiotics due to the ability to produce interferon and enzymes (protease and lipase etc.) increase local immunity and accelerate wound healing and burns. But the systematic study of biofilms' effect on biosafety of human and animals began only in the last two decades.

Relevance. Relatively recently, new trends have emerged in the world – the so-called "probiotic antiseptics" and "probiotic disinfection". These new approaches are especially important against the background of the frequently developed antibiotic-resistant forms of microorganisms, namely – multidrug-resistant. The presence of multidrug-resistant forms of microorganisms makes it impossible to use antibiotics due to their ineffectiveness. And it is probiotics that can play a key role in the treatment of burns and wound healing. Probiotic disinfection is the use of probiotics for disinfection, in particular to eliminate foci of nosocomial infections [1].

A number of microbiological preparations are produced on the basis of strains of *Bacillus subtilis* designed to protect garden, indoor and greenhouse plants from a complex of fungal and bacterial diseases.

There are also works which dedicated to influence of *Bacillus subtilis* on host longevity including extended lifespan, when they are administered or present in adequate quantities. However, the mechanisms by which probiotics stimulate host longevity remain unclear and very poorly understood [2].

A separate important issue is the use of probiotics where there is a risk of formation of biofilms of pathogenic microorganisms, the formation of which leads to ineffective use of conventional disinfectants.

If the antibacterial action of probiotics has been studied for a long time, then there are only some reports concerning their antiviral action [3-5]. There were the fully successful results of study on high effectiveness of antiviral activity of crude extracts from *B. licheniformis* against porcine epidemic diarrhea virus (PEDV). This study was performed both *in vivo* and *in virto* [6]. Anotherstudy was devoted the investigation the activity of probiotic strain *B. subtilis* against the influenza virus. The antiviral effect of the extracted peptide from this strain (P18) has been demonstrated *in vitro* and *in vivo*as highly effective against influenza virus [7].

In another research demonstrated that peptide P34, which obtained from *B. subtillis,* reduced viral titer of equine arteritis virus from $10^{4.5}$ $TCID_{50/ml}$ to $10^{2.75}$ $TCID_{50/ml}$, showing a percent of inhibition of 98.6 % [8].

In more resent investigation was shown the mediated influence the *B. subtilis* to SARS-CoV-2 virus, especially in terms of symptomatic management where linked to acute respiratory infections [9].

Bacillus bacteriahave been shown to release peptide glycan, a so-called surfactin [10-12], which is a natural antibiotic.The mechanism of surfactin action is destroys the virion*in vivo* condition.

Our study was aimed to evaluate potential virucidal activity of the probiotic drug "Sviteco-Multi" against poliovirus type 1 and influenza A/California/07/2009 A (H1N1) pdm2009 in experiment in vitro.

It should be noted that the drug "Sviteco-Multi" includes a complex of bacteria of the genus *Bacillus*, in particular – *Bacillus subtilis and Bacillus megatherium*.

Materials and methods. The investigation was performed according the EN 14476+A2 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area [13].

The research was performed with influenza A(H1N1)pdm2009 virus (hasRNAgenome, hasenvelope), which was isolated in epidemiological season 2019/2020, with highin factiousactivity. $TCID_{50/ml}$ of this virus was 10^{-7}. There was also used poliovirus type 1 (has RNA genome and has no envelope) with infectious activity $TCID_{50/ml}$, that is equal to a 10^{-6}. Vaccine poliovirus type 1 is a standard virus, which is used to control the action of disinfectants according to international standards.

Experiment with influenza viruses conducted on MDCK cell culture (epithelial caninekidney cells) received from Centers for Disease Control and Prevention (CDC – Atlanta,

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USA) which is highly sensitive to influenza viruses. For propagate of cell culture growth medium on the basis of DMEM cultural medium with 5 % fetal bovine serum used. Maintaining medium, which is used for infected and probably infected cell culture, is serum-free and contains serum albumin (7.5 %) and purified trypsin Sigma (T-1426).

Experiment with poliovirus type 1 is conducted on HEp-2 cell culture, which is sensitive to poliovirus reproduction. The same growth medium is used for both cell cultures HEp-2 and MDCK, but maintaining medium for HEp-2 contains 2 % fetalbovineserum instead of serum albumin.

Prior to the main experiment, Petri dishes with meat-peptone agar were treated with "Sviteco-Multi" by spraying from an aerosol can. After treatment, the dishes were kept at a temperature of 37^0C for 24 hours, which allowed toforms a microbial biofilm on the surface of the agar, formed by bacteria of the genus *Bacillus*.

After that on one part of Petri dishes on the surface of the agar in certain places (loci) virus-containing liquid was applied containing 2.5$TCID_{50/ml}$ (10^{-2}) and 5$TCID_{50/ml}$ (10^{-1}) of influenza virus A/California/07/2009 A(H1N1)pdm2009, 100 µl per each locus. At intervals of 30 seconds, 2 and 5 minutes, washes were taken from the treated loci and added to MDCK cell culture.

Virus-containing fluid containing 1$TCID_{50/ml}$ (10^{-6}) of vaccine poliovirus type 1, 100 µl per locus, was applied to other "Sviteco-Multi"-treated Petri dishes at selected loci. At intervals of 5, 10, 15 and 30 minutes, washes were taken from the treated loci and introduced into HEp-2 cell culture.

The results were calculated by assessing the cytopathic effect of the viruses during 2-4 days after inoculation of viruses. Cytopathic effect (viability of viruses)was expressed in %.

The viral percent of inhibition (PI) were calculated by PI = [1- (Titer of treated/ Titer of controls)] x 100 [14].

Results and discussion. The experiment showed that aerosol application of undiluted probiotic drug "Sviteco-Multi" on meat-peptone agar, followed by application of a dose of vaccine poliovirus type 1 1$TCID_{50/ml}$ (10^{-6}) leads to its inactivation 30 minutes after application.

The graphic image is given in Fig. 1.

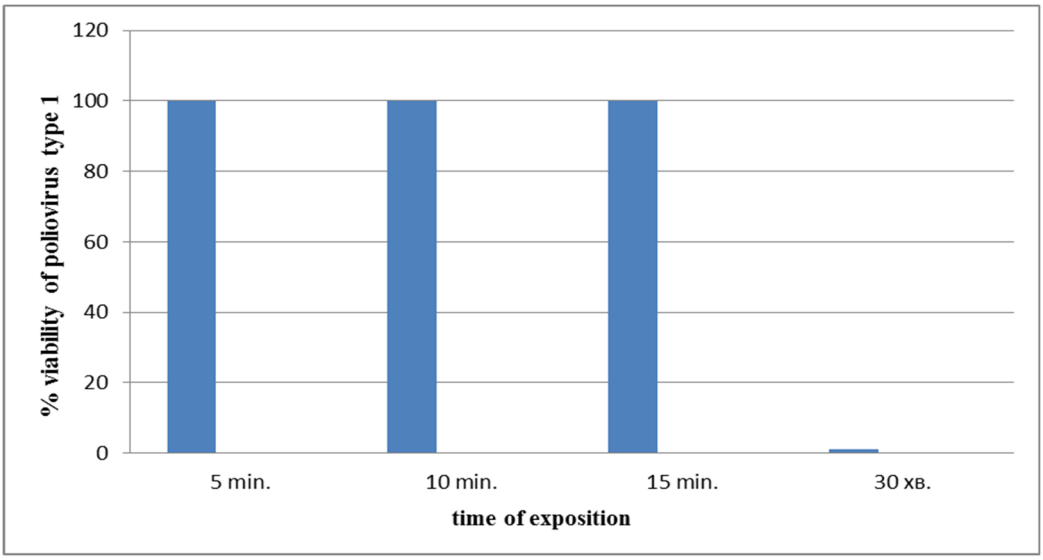

Fig 1. The viability of poliovirus type 1 on the biofilm which generated by bacterium *Bacillus*

As can be seen from the Figure 1 in case of exposition 5, 10 and 15 minutes the vaccine poliovirus type 1 does not have time to be inactivated and remains viable. But after 30 minutes it can be observed the inactivation of poliovirus on the surface of biofilm created by bacteria genus *Bacillus*.

Theexperimentwith influenza virus showed that treatment by aerosol application of undiluted probiotic drug "Sviteco-Multi" on meat-peptone agar, followed by application of a dose of influenza virus A/California/07/2009 A(H1N1)pdm2009 2,5$TCID_{50/ml}$ (10^{-2}) leads to its inactivation after 30 seconds.

Application of 10 times higher dose of influenza virus – 5$TCID_{50/мл}$ (10^{-1}) leads to its inactivation after 2 minutes on the surface of the biofilm formed by cultures of the genus *Bacillus*.

It can be fully explained because the influenza virus has much lower resistance to environmental factors and disinfectants than poliovirus type 1.

The graphic imageisgivenin Fig. 2.

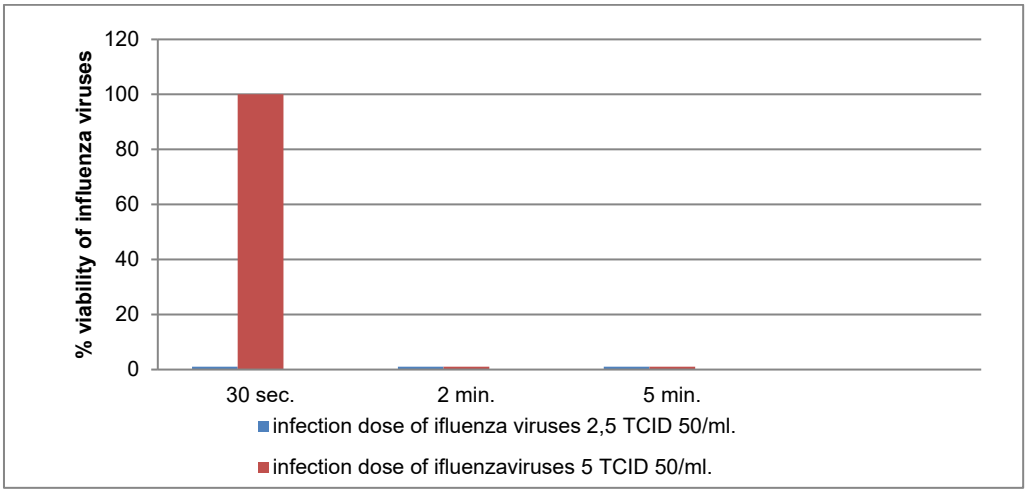

Fig 2. The viability of influenza virus A(H1N1)pdm2009 on the biofilm which generated by bacterium *Bacillus*

For today the mechanism of probiotics' action has not been clarifies. The majority of researchers come to the conclusion that in an organism the mechanism of their action is combined and is realized by different ways. It has been revealed that the metabolites released by probiotics display important protective activities to inhibit viral infections. These metabolites form a micro-environment that is not conducive to viral reproduction [15-18].Besides probiotic bacteria may binddirectly to the virus and inhibit virus attachment to the host cell receptor. Another mechanism – is adhesion of probiotics on the epithelial surfacemay block viral attachment in organism. Another way-induction of low-grade nitric oxide (NO) production and dehydrogenase production may also have antiviral activities. Probiotics is able to act by modulation of immune response through epithelial cells or activation of immune responses through macrophages and dendritic cells. It can realise upon activation, CD8+ T lymphocytes differentiate into cytotoxic T lymphocytes (CTLs), which destroy virus-infected cells. CD4+ T lymphocytes differentiate into Th1 and Th2 cells. 10 T-helper cells type 1 (Th1) activates phagocytes, promoting virus killing [19].

Another mechanism of antiviral activity of *B. subtillis* is containing in its metabolites. Particularly – it has been shown that surfactin, a cyclic lipopeptide producedby *B. subtilis*, is a molecule able to counteract both SARS-CoV-1, MERS-CoV and HCoV-229E coronaviruses. One of the last studies showed the potential antiviral activity of surfactin against SARS-CoV-2.The antiviral activity of surfctinwas tested in vitro in a cellular model of infection on Vero E6 cells [20]. One else investigation confirmed that surfactin from *B. subtillis* can also suppress the proliferation ofporcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) inepithelial cells at a relatively low concentration range [3].

Based on experimental studies, it can be assumed that biofilms formed by bacteria of the genus *Bacillus* may also inhibit the reproduction of other viruses. However, confirmation of this requires further research.

Conclusion. Therefore, the probiotic preparation "Sviteco-Multi", which contains bacteria of the genus *Bacillus*, has antiviral activity against influenza A(H1N1)pdm2009 virus and poliovirus type 1, which makes it possible to recommend its use, in particular as an alternative to traditional disinfectants.

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ВІРУЛІЦИДНА ДІЯ ПРОБІОТИЧНОГО ПРЕПАРАТУ "SVITECO-MULTI" НА ПОЛІОВІРУС ПЕРШОГО ТИПУ ТА ВІРУС ГРИПУ

В експериментальній роботі в модельній системі на культурах клітин MDCK та HEp-2 показана противірусна активність пробіотичного препарату "Sviteco-Multi", який містить бактерії роду Bacillus, щодо вірусу грипу A(H1N1) pdm 2009 та вакцинного поліовірусу першого типу, що дає змогу рекомендувати його для використання, зокрема як альтернативи традиційним дезінфікуючим противірусним засобам.

Ключові слова: пробіотчні препарати, противірусна активність, Bacillus subtilis.