Part 3. Biosafety

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STUDY OF THE EFFECT OF THE PREPARATION BASED ON THE BACTERIOCIN NISIN ON PATHOGENIC BACTERIA

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Summary. The bactericidal effect of the nisin-based preparation on the bacterial cultures (*Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715) was studied *in vitro*. Two postbiotic concentrations were tested. Based on *in vitro* studies, the expressed antimicrobial effect of the test drug on *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 was established. In addition, postbiotic variant 1 was active in relation to *Listeria ivanovii* ATCC 19119, variant 2 — in relation to *Yersinia enterocolitica* ATCC 23715. The obtained results point to the prospect of further study of the postbiotics effect *in vivo* for the purpose of their application in the treatment of infections caused by pathogenic bacteria with acquired resistance to antibiotics.

Keywords: postbiotics, drug, strains, *in vitro*, sensitivity

Introduction. Postbiotics are bacterial metabolites and breakdown products of probiotic bacteria (mostly gram-positive). Postbiotics include organic acids and biologically active substances — bacteriocins, which effectively counteract pathogenic microorganisms, even in acidic media. The range of antimicrobial postbiotics is ambiguous. It can be both as very narrow as extremely wide. Most of them are produced by gram-positive bacteria (Sorg et al., 2016; Abee, 1995; Collier-Hyams and Neish, 2005; Morowitz et al., 2010; Mack and Lebel, 2004).

Bacteriocin nisin has a broader antimicrobial spectrum than most other bacteriocins. It is non-toxic, and used in the food industry as a natural preservative (E234). Researchers believe that postbiotics affect the inner wall of the colon, but the mechanisms of health influence are not sufficiently clear yet (Neish et al., 2000; Abee, Krockel and Hill, 1995; Aguilar-Toalá et al., 2018).

However, postbiotics are like acid oxidants, because the effective digestion of nutrients requires adequate acidity in the stomach. It has been proved that acidic media (with low pH values) cannot withstand most pathogenic bacteria, but it is favorable for growth and reproduction of lactic acid bacteria. Pepsinogen is converted into a proteolytic enzyme pepsin only in a very acidic medium (pH 2–4). Consequently, when the high-nutritious diet ingested in the stomach, the splitting of proteins is disturbed, which contributes to protein fermentation with the formation of toxic biogenic amines (Amalaradjou and Bhunia, 2013; Amaretti et al., 2013; Anvari, Khayati and Rostami, 2014; Kucheruk and Zasiekin, 2013).

The aim of the study was to determine the bactericidal effect of the nisin-based preparation on the bacterial cultures (*Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715) *in vitro*.

Materials and methods. Tests of the postbiotic drugs were carried out by the of the drug diffusion in agar solutions in accordance with the methodological recommendations (ISO, 2014; Holovko et al., 2013; MHU, 2017).

Two postbiotic concentrations were tested:

Variant 1 — postbiotic formulation (0.05 g of nisin, 10 ml of 40% lactic acid, 89.95 ml of distilled water);

Variant 2 — postbiotic formulation (0.10 g of nisin, 10 ml 40% lactic acid, 89.90 ml distilled water).

Reference strains of microorganisms were used as test cultures: *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715.

Results. The effectiveness of the two drug variants (with different concentrations of the active substance) was tested in relation to the microorganisms of different groups: gram-negative sticks, representatives of the family Enterobacteriaceae — *Escherichia coli* ATCC 25922 (F 50) (in concentrations 4.3×10^9 CFU/cm^3, 4.3×10^8 CFU/cm^3, 4.3×10^7 CFU/cm^3), *Yersinia enterocolitica* ATCC 23715 (in concentration 9.0×10^8 CFU/cm^3), gram-positive bacillus asporogenous — *Listeria ivanovii* ATCC 19119 (in concentration 5.0×10^8 CFU/cm^3), gram-positive cocci — *Staphylococcus aureus* ATCC 25923 (in concentrations 5.5×10^9 CFU/cm^3, 5.5×10^8 CFU/cm^3, 5.5×10^7 CFU/cm^3), gram-positive spore-forming bacillus — *Bacillus subtilis*

ATCC 6633 (in concentrations 3.5×10^9 CFU/cm^3, 3.5×10^8 CFU/cm^3, 3.5×10^7 CFU/cm^3).

The efficacy of the drug test variants was determined in the native state and in successive ten-time dilutions from 1:100 to 1:1,000,000 (Tables 1 and 2).

The results showed the effectiveness of the drug variant 1 native solution in relation to the test cultures; the dilution of the inhibitory agent in relation to the test cultures was not observed (Table 1).

There was a slight dependence of the *Escherichia coli* ATCC 25922 (F 50) test culture concentration on the efficacy of the test drug: for the ten times test culture concentration gradual reduction, the growth inhibition diameters of the crops increased around the application of the drug. Diameters of growth inhibition of *Escherichia coli* ATCC 25922 (F 50) test culture in concentration 4.3×10^9 CFU/cm^3 around the drug variant 1 application area constituted 8 mm; for 4.3×10^8 CFU/cm^3 — 9–15 mm; for 4.3×10^7 CFU/cm^3 — 15 mm. In the test cultures *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 the correlation of the test culture concentration on the drug effect was not observed. The diameters of the inhibition of the *Bacillus subtilis* ATCC 6633 test culture growth for concentrations 3.5×10^9 CFU/cm^3, 3.5×10^8 CFU/cm^3, 3.5×10^7 CFU/cm^3 around the zones of the application of the drug variant 1 was 15 mm. The diameters of the inhibition of *Staphylococcus aureus* ATCC 25923 test culture growth for concentrations 5.5×10^9 CFU/cm^3, 5.5×10^8 CFU/cm^3, 5.5×10^7 CFU/cm^3 around the application area of the drug variant 1 was 18 mm. The *Listeria ivanovii* ATCC 19119 culture was tested in concentration 5.0×10^8 CFU/cm^3, zones of drug oppression were 36 mm; the *Yersinia enterocolitica* ATCC 23715 culture tested in the concentration 9.0×10^8 CFU/cm^3, the zones of inhibition were 13 mm.

Table 1 — Test of the variant 1 (0.05 g of nisin, 10 ml of 40% lactic acid, 89.95 ml of distilled water) postbiotic drug effectiveness

Test cultures	Concentration, CFU/cm^3	Diameters of the culture growth inhibition (mm) according to dilutions					
		native	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Escherichia coli ATCC 25922 (F 50)	4.3×10^9	8	—	—	—	—	—
	4.3×10^8	9–15	—	—	—	—	—
	4.3×10^7	15	—	—	—	—	—
Bacillus subtilis ATCC 6633	3.5×10^9	15	—	—	—	—	—
	3.5×10^8	15	—	—	—	—	—
	3.5×10^7	15	—	—	—	—	—
Staphylococcus aureus ATCC 25923	5.5×10^9	18	—	—	—	—	—
	5.5×10^8	18	—	—	—	—	—
	5.5×10^7	18	—	—	—	—	—
Listeria ivanovii ATCC 19119	5.0×10^8	36	—	—	—	—	—
Yersinia enterocolitica ATCC 23715	9.0×10^8	13	—	—	—	—	—

For the variant 2 application of the drug in relation to the test cultures, similar results were recorded: the native sample of the drug was effective, its ten-times dilution of inhibitory activity in relation to the test cultures was not shown (Table 2). In addition, there was a certain correlation between the concentration of test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and the efficacy of the test sample preparation: for a gradual reduction of the ten-times concentration of the cultures, the growth inhibition diameters of the cultures around the application of the drug increased. Diameters of the growth inhibition of test culture *Escherichia coli* ATCC 25922 (F 50) for the concentration 4.3×10^9 CFU/cm^3 in the area of application of the drug variant 2 were < 5 mm; for 4.3×10^8 CFU/cm^3 — 15 mm; for 4.3×10^7 CFU/cm^3 — > 15 mm. The diameters of inhibition of growth of test culture *Bacillus subtilis* ATCC 6633 for the concentration 3.5×10^9 CFU/cm^3 in the area of application of the drug variant 2 were 13 mm; for 3.5×10^8 CFU/cm^3 — 14–20 mm; for 3.5×10^7 CFU/cm^3 — 20 mm. The diameters of the growth inhibition of test culture *Staphylococcus aureus* ATCC 25923 for the concentration 5.5×10^9 CFU/cm^3 in the area of application of the drug variant 2 were 12 mm; for 5.5×10^8 CFU/cm^3 — 15 mm; for 5.5×10^7 CFU/cm^3 — 17 mm. The *Listeria ivanovii* ATCC 19119 culture was tested in concentration 5.0×10^8 CFU/cm^3, zones of drug oppression were 13 mm; the *Yersinia enterocolitica* ATCC 23715 culture tested in the concentration 9.0×10^8 CFU/cm^3, the zones of inhibition were 22 mm.

Table 2 — Test of the variant 2 (0.10 g of nisin, 10 ml of 40% lactic acid, 89.90 ml of distilled water) postbiotic drug effectiveness

Test cultures	Concentration, CFU/cm^3	Diameters of the culture growth inhibition (mm) according to dilutions					
		native	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Escherichia coli ATCC 25922 (F 50)	4.3×10^9	< 5	—	—	—	—	—
	4.3×10^8	15	—	—	—	—	—
	4.3×10^7	> 15	—	—	—	—	—
Bacillus subtilis ATCC 6633	3.5×10^9	13	—	—	—	—	—
	3.5×10^8	14–20	—	—	—	—	—
	3.5×10^7	20	—	—	—	—	—
Staphylococcus aureus ATCC 25923	5.5×10^9	12	—	—	—	—	—
	5.5×10^8	15	—	—	—	—	—
	5.5×10^7	17	—	—	—	—	—
Listeria ivanovii ATCC 19119	5.0×10^8	13	—	—	—	—	—
Yersinia enterocolitica ATCC 23715	9.0×10^8	22	—	—	—	—	—

Comparing the effect of variants 1 and 2 of the test drug, it should be noted that in relation to the test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 the effectiveness of the options manifested itself at approximately the same level.

In relation to the test culture *Listeria ivanovii* ATCC 19119 variant 1 of the drug was much more effective; in relation to the test culture *Yersinia enterocolitica* ATCC 23715 — variant 2.

Conclusions. 1. It was established that the tested variants 1 and 2 of postbiotics containing 0.05 g and 0.10 g of nisin respectively exhibited pronounced inhibitory effect *in vitro* on the growth of test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923.

2. Postbiotic variant 1 was active in relation to *Listeria ivanovii* ATCC 19119, variant 2 — in relation to *Yersinia enterocolitica* ATCC 23715.

3. The obtained results point to the prospect of further study of the postbiotics effect *in vivo* for the purpose of their application in the treatment of infections caused by pathogenic bacteria with acquired resistance to antibiotics.

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