INDUCTION OF BIOCHEMICAL ACTIVITY OF *LACTOCOCC US LACTIS* SSP. *LACTIS* UNDER THE INFLUENCE OF ULTRAVIOLET

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Introduction. Among the various types of radiation the most common are UV-rays, which capable to induce of biochemical processes in bacteria. UV-radiation (UVR) is acted on the cells mainly proteins and nucleic acids. In proteins the primary photochemical reaction is the ionization of aromatic amino acids, the dissociation of the -S-S- bonds, which are present in the nisin structure producing by *L. lactis* ssp.*lactis*. In this case, free radicals can form, a cascade of reactions occurs, leading to a change in its structure, as well as for other protein components. In the initial stage of the development of culture, the enzymes of the intracellular fund are activated, and are produced "SOS" proteins, which protect the cell from unfavorable factors.

The aim of the research is to induce of biochemical processes of lactococci under the influence of UVR.

Materials & Methods. A natural strain of *L.lactis* ssp.*lactis* 229 with a bacteriocin-synthesizing activity (as Nisin) of 2500 IU/mL was isolated from the cow milk (GenBank accession no. DQ 255954). Prior to irradiation, the cells were separated from the culture broth by centrifugation, washed twice with ammonium citrate buffer (pH 6.4), irradiated for 1, 5, 10 min, which corresponded to 7.6-7600 erg/mm². The grown colonies were chopped by a replicator into agar medium MRS supplemented with 1.5% agar and into agar medium with 200 µg/ml nisin in order to accelerate the selection process according to the specified characteristic. The nisin product was provided by diffusion method on the agar plate with *Bacillus coagulans* 429 as an indicator culture. The spontaneous and UV-induced variability of the strains was determined on the basis of antimicrobial activities. Colonies with the greatest inhibitory zones were selected. Quantitative determination of the antimicrobial activity was performed by measuring the zones of *B.coagulans* growth suppression with subsequent calculation involving a calibration plot for standard nisin solutions (3000 IU/ml, Nisaplin, Aplin& Barrett,Ltd.,UK).

Results. Only 11,1% "+" variants were isolated after treatment of the bacterial suspension for 10 minutes with an activity level higher than the initial level (Tabl.1)

The population of strain 229 is heterogeneous in the synthesis of nisin. clones had antimicrobial activity in the range from 500 to 2900 IU/ml (Tabl.2). When adding nisin to the medium, low-active variants were separated. Effective dose of strain 229 should be considered 10 min, at which 12% of the clones had had antimicrobial activity in the range from 3100 to 3350 IU/mL.

As a result, it was obtained variant 6 with antibiotic activity of 3350 U/mL, which is 27.7% higher.

Table 1. Effect of UV-rays on nisin-synthesizing activity of *L. lactis* subsp. *lactis* strain 229

Time, min	Distribution of options for nisin synthesizing activity, %			
	Lost activity	Below - control level	Control	"+" variants
0	0	24,0±1,1	66,0±2,2	0
1	11,8±0,7	34,5±1,2	54,7±1,8	0
5	29,3±0,2	36,0±1,5	34,2±1,3	0
10	53,1±2,1	12,0±0,5	23,8±0,7	11,1± 0,3

The "+" variant No 12 with activity of 3850 IU/ml was isolated after double UV-treatment for 10 min. It was showed that they somewhat changed the rate of their growth, the accumulation of nisin. With regard to fermentation of carbohydrates showed similarity to the initial strain for the consumption of pentoses, but became more resistant to antibiotics inhibiting protein synthesis.

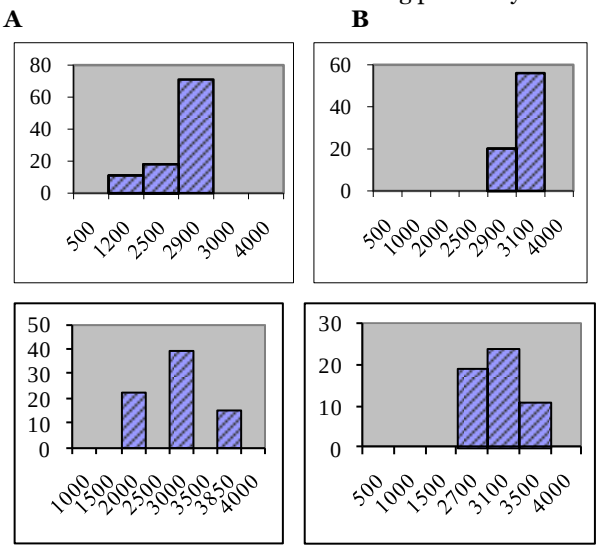

Fig. 1. The spontaneous and UV-induced variability (%) of the strain 229 on the basis of nisin producing activities (IU/mL).

A-spontaneous variability of the activity of the strain 229 on agar MRS medium; B- variability of the activity on agar MRS medium with nisin; C –variability after UV-rays treatment for 10 min; D - by stepwise, double UV-treatment for 10 min.

The No 6 lost its acquired properties after lyophilization and storage, reversed to wild type, but No 12 retained the biochemical stability.

Table 2.Antimicrobial action of wild strain 229 and studied UVR "+" variants in comparison with nisin

Test-microorganisms	Strains			
	229	12	6	Nisaplin
	Diameter of inhibition zone, mm			
Bacillus coagulans	17.0	23.0	20.0	17.0
Staphylococcus aureus	20.0	21.5	19.0	25.0
Listeria monocytogenes	15.0	17.0	14.0	15.0
Salmonella Typhimurium	0	0	0	0
Escherichia coli	0	10,0	0	0
Proteus vulgaris	0	9.0	0	0
Klebsiella pneumoniae	0	0	0	0
Enterobacter aerogenes	0	0	0	0
Alcaligenes faecalis	0	0	0	0
Aspergillus niger	0	10	0	0
Rhodotorula aurantiaca	0	10.0	0	0
Candida guellermondii	0	9.0	0	0

Conclusion: the UVR induce the biochemical processes in natural strain *L.lactis* ssp.*lactis* 229. It was selected effective strain with twice activity from the initial; the acquired biochemical properties are stable during long-term storage.

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