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DETERMINATION OF COMPOSITION OF BACILLUS STRAINS PRODUCING BACTERIOCINS FOR PROSPECTIVE OF ANTIBIOTICS CREATION

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Abstract

The problem of microbial resistance towards many types of antibiotics is very widespread presently. The discovering of new types of antimicrobial substances may help to resolve this problem. Bacteriocins are widely studied by many scientists in the world as one of the prospective alternative to antibiotics. *Bacillus* strains are one of the main producers of bacteriocins and in this research the activity of several *Bacillus* strains was studied in the different combinations. The antagonistic action of these strains was measured against *Escherichia coli* by the diffusion method. Results of the research allowed to determine the combination of starins, which shows the maximum activity.

Keywords: bacteriocins, combination, antimicrobial activity, zone of inhibition

Introduction

Multiple resistance of microorganisms to antibiotics is a global public health problem all over the world [1]. Strains of microorganisms causing infectious are increasingly becoming multi resistant and, therefore the treatment of diseases caused by known antibiotics is ineffective [2].

The wide spread of antibiotic-resistant strains of microorganisms becomes threatening pace and demonstrates the growing "crisis of antibiotic therapy" [3]. As a consequence, there is a need to search for, and a detailed study of the introduction into medical practice of new, alternative antibiotic antimicrobial agents

Currently some antimicrobial agents that represent alternative antibiotics were studied, including bacteriophages [4], probiotic bacteria [5], antimicrobial peptides [6] and bacteriocins [7]. The bacteriocins are among the more promising components for the development of antibiotics.

The bacteriocins are natural substances, which are not harmful for human and possess broad spectrum of activity. This makes them promising for application in the food industry and in medicine.

Bacteriocins represent a large class of heterogeneous bacterial antagonists which vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action.

Bacillus strains possess the ability to synthesize a wide variety of bacteriocins. Main producers of bacteriocins are strains *Bacillus licheniformis, Bacillus pumilus, Bacillus circulans, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Bacillus amyloliquefaciens* [8, 9, 10]. Thus, strains of the genus *Bacillus* are producers of peptide and lipopeptide antibiotics [11], and have the ability to synthesize a wide range of bacteriocins such as batsilin and fungumitsin [12] plipastatin and sufraktin [13] koagulin [14] tochiin [15] amilolichin [16].

Broad spectrum antimicrobial activity of bacteriocins produced by *Bacillus* strains, allows them not only inhibit the growth of Gram-positive bacterias, but also to inhibit Gram-negative bacteria, yeasts and fungi which are pathogenic to man and animals.

Thus, the ability of the genus Bacillus strains synthesize large amounts of antimicrobial agents with a broad spectrum of antimicrobial activity, confirms the relevance of research of these strains for discovering new ways of antibiotics creation. There is a huge possibility that these strains have the higher level of activity during cultivation in combination. Thereby the antagonistic activity of different combinations of *Bacillus* strains was studying.

Materials and methods

The strains were isolated from the vegetables. For isolation of strains the cultivation was conducted in the liquid medium at the temperatures 30, 37, 40 °C with the subsequent cultivation of isolated colonies on the solid nutrient medium. The strains were identified by the studying their morphological, biocemical properties and by sequencing of 16S rRNA gene. Determination of antagonistic activity was performed by measuring the optical density. The test-culture of *E.coli* was grown during 24 hours at 37 ± 2 °C. Cells from the agar were resuspended in NaCL solution in the concentration 10^9. The isolated strains were grown during 24 hours at 37 ± 2 °C. The culture liquid was centrifuged at 7000 rpm during 10 min and the supernatant was separated. For separation the cell supernatant was filtered through Millex-GV filter (0.22 um, Millipore, USA). Were conducted neutralizing the supernatant by adding sodium hydroxide. The mix of supernatants of *Bacillus* strains was mixed with the solution of test culture.

The mix was incubated at 37 ± 2 °C for 24 hours. As a control the sterile medium mixed with the solution of the pathogenic strain was used. Absorbance of the mixture was measured during 24 hour of cultivation. All experiments were performed in triplicate.

Results and discussion

For determination of the combination of isolated strains bacteriocins which have the maximum activity against *E.coli* the optivcal density was mwasured during 24 hours of incubation. The results obtained after measuring the optical density, during incubation are presented in the table 1.

Table 1 - The changing of optical density, during incubation in the different combinations

Combination of strains	Optical density			
	Time of cultivation, hours			
	0	2	4	24
Bacillus subtilis, Bacillus endophyticus	0,403	0,462	0,530	0,847
Bacillus subtilis, Bacillus stratosphericus	0,295	0,376	0,479	0,871
Bacillus subtilis, Bacillus pumilus	0,098	0,396	0,490	0,596
Bacillus endophyticus, Bacillus stratosphericus	0,113	0,353	0,449	0,551
Bacillus endophyticus Bacillus pumilus	0,323	0,411	0,497	0,796
Bacillus stratosphericus Bacillus pumilus	0,302	0,411	0,477	0,812
Bacillus subtilis, Bacillus stratosphericus, Bacillus pumilus	0,116	0,344	0,422	0,559
Bacillus endophyticus, Bacillus stratosphericus, Bacillus pumilus	0,097	0,391	0,482	0,604
Bacillus subtilis, Bacillus stratosphericus, Bacillus endophyticus	0,112	0,255	0,302	0,461
Bacillus subtilis, Bacillus pumilus, Bacillus endophyticus	0,104	0,212	0,310	0,365
Bacillus subtilis, Bacillus pumilus, Bacillus endophyticus, Bacillus stratosphericus	0,108	0,291	0,381	0,434
Control	0,090	0,346	0,470	0,876

The antimicrobial activity was evaluated comparing the optical density of combinations to the optical density of control. Also the level of inhibition was counted in percentage for proper evaluation. We can conclude that the optical density was almost the same as control in the following combinations *Bacillus subtilis, Bacillus endophyticus*; *Bacillus subtilis*, *Bacillus stratosphericus*; *Bacillus stratosphericus, Bacillus pumilus*. It means that these combinations didn't show antimicrobial activity. The lowest optical density compare to control was observed in the combinations: *Bacillus subtilis, Bacillus endophyticus*, *Bacillus stratosphericus; Bacillus subtilis, Bacillus endophyticus, Bacillus pumilus; Bacillus subtilis, Bacillus endophyticus*, *Bacillus stratosphericus, Bacillus pumilus* (the level of inhibition from 48 to 69%).It means that growth of *E.coli* was suppressed more in these combinations. The other combinations of strains inhibited the *Escherichia coli* growth from 10 to 36%.

Conclusion

Results of conducted research allowed us to determine the combination of *Bacillus* strains which had the highest antimicrobial activity. Counting the level of inhibition in percentage, we observed that the highest percentage of inhibition of *Escherichia coli* was in the combination *Bacillus subtilis, Bacillus endophyticus, Bacillus pumilus*. This combination of strains suppressed the growth of Escherichia coli on the 69%. Therefore it is prospective to use the microbial strains in this combination for creation of new antimicrobial drugs.

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