ANTIMICROBIC ACTIVITY OF FRUIT AND VEGETABLES' NATURAL MICROFLORA AS A SOURCE OF RECEIVING BIOPRESERVATIVES

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Abstract: During the researches the microorganisms showing antagonistic activity towards the pathogenic microflora were allocated on the surface of vegetables. After the allocated strains' identification for further researches four strains of the microorganisms which are not pathogenic have been selected: *Bacillus stratosphericus*, *Bacillus endophyticus*, *Bacillus pumilus*, *Bacillus subtilis*. Antagonistic activity of the chosen strains towards the testing cultures on the solid medium was studied by the diffusive method. Isolates show high inhibitory potential against the pathogens causing various human diseases. It should be noted that all isolates except *Bacillus stratosphericus* show antagonistic activity towards Salmonella enterica from 25 to 38%, while *Bacillus stratosphericus* does not inhibit the growth of *Bacillus stratosphericus*. Strains *Bacillus stratosphericus*, *Bacillus endopheticus*, *Bacillus subtilis inhibit* the development of *Alcaligenes faecalis* (52–54%). Strains *Bacillus endophyticus* (18%) and *Bacillus pumilus* (30%) are inhibitors of *P. aeruginosa*.

Keywords: microorganism, fresh fruit and vegetables, antagonist, pathogenic microorganism, lactobacilli, antimicrobial activity

INTRODUCTION

Recently significant increase in amount of fruit and vegetables which cause diseases of food origin is observed. Fresh fruit and vegetables, leaves, roots and tubers are one of the most perishable products on the markets. These products are rich with carbohydrates and are low in proteins, they have pH in the range from 7.0 to slightly acidic environment and are adequate habitat for some bacteria, yeasts and mould.

Nowadays a lot of ways of shelf life extension of fruit and vegetables are known (ultrasound, protective covering, conservation). But in connection with constantly growing demand for fresh and healthy products consumers also became more critical to use of synthetic additives for food storage. Thereby the most popular method of shelf life extension of products is conservation [1].

Conservation is set of measures like isolation of product, destruction of bacteria and spores which are in it, change of its structure and storage conditions for prevention of microorganisms development, product protection against destruction under high temperature and sun rays stress. There are a lot of ways of conservation, but no one of them is performed when using lactic bacteria.

Lactobacillales living on plants' surface began to draw attention both of Russian and foreign researchers long since. And though the data of some authors were quite often contradictory, the analysis of literary data in general allows to gain rather complete idea about lactobacillales content in vegetable microflora and about their specific structure. Some researchers [1, 2] found on plants (vegetable, grain, leguminous) to 10^4–10^6 lactic bacteria cells per 1 g of plant material.

Lactobacillales are the bacteria included in the group of gram-positive, non-sporing, represent cocci or rods which make lactic acid as the main final product of fermentation of carbohydrates. Lactobacillales are choosy to food, the need carbohydrates, amino acids, acids, peptides, nucleinic acids and vitamins. This definition includes more wide range of bacteria, than strains of lactobacillales recognized by science (historically acknowledged). This list was narrowed subsequently, specifying a positive role which is played by lactobacillales in the processes of fermentation proceeding in foodstuff production. Despite frequent changes of taxonomical classification of lactic acid bacteria the most studied are: *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus. Tetragenococcus, Vagococcus, Weissella* [3].

Lactic acid bacteria are industrially important, they possess enzymatic properties, are also good to people's health and have nutritional value. The types used for food production: *Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus, Carnobacterium*. These microorganisms have been allocated from grain, green plants, dairy and meat products, vegetables and mucous membranes of animals.

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These microorganisms are used for delay of spoil and preserving products by natural fermentation, they have found application as ferments in dairy products, pastries, in production of meat, vegetable products and in alcoholic beverage industry.

Analyses of lactic acid bacteria's capacity for antimicrobic activity have been carried out on meat, fermented milk products, fermented vegetables, dairy products and fish. Some strains of lactic bacteria are capable to produce protein compounds with antimicrobic effect which are known as bacteriocins. Some of them are very specific, others have a wide range of antimicrobic effect. Preserving effect of lactic bacteria on food and drinks is explained by combined action of a range of the antimicrobic metabolites such as organic acids, diacetyl tartaric acid, hydrogen peroxide, bacteriocins produced during fermentation. These components not only influence on taste, smell, color and texture of food, but also prevent undesirable microflora development. Thus, lactic acid bacteria and their products flavour fermented products with distinctive savor, texture, prevent spoilage, prolong shelf life and inhibit pathogenic organisms [4, 5].

The purpose of this work is to develop production technology of biopreservative on the basis of lactobacilli bacteriocins in order to increase the expiry dates of fruit and vegetables.

Relevance of the work is determined by the need of progressive technologies implementation and increase of healthy food production on the basis of natural components.

Scientific novelty of the conducted researches consists in using microorganisms antagonists allocated directly from foodstuff which produce antimicrobic metabolites – bacteriocins as the biopreserving environment for minimally processed vegetables and fruit.

Research objectives:

– selection of lactic microorganisms strains of the different taxonomical groups allocated from vegetable raw materials, showing antimicrobic activity;

– physiological and biochemical properties of the selected strains of microorganisms.

MATERIALS AND METHODS

Objects of researches are fresh vegetables: onion, garlic, eggplant, tomato, bell pepper. And also microorganisms allocated from the surface of fresh vegetables: *Bacillus pumilus*, *Bacillus endophyticus*, *Bacillus stratosphericus*, *Bacillus subtilis*.

All vegetables were carefully rinsed with distilled water, chopped in sterile conditions and brought in a small amount (about 5 g) in test tubes with 5 ml of liquid nutrient mediums, or ground small piece on a dish surface with agar medium. Dishes and test tubes were permanently incubated in three temperature conditions (30, 37 и 45^{o}C) within 1–5 days.

For primary allocation milk medium (MM) – sterile skim milk; MRS broth, brain heart infusion broth (BHIB); milk agar (MA) – milk and 3% agar in the ratio of 1 : 1; fish-and-peptone agar (FPA); MRS agar were used.

From test tubes with visible growth of microorganisms (turbidity or milk coagulum existence) and from total lawns on dishes exhaustive inoculations were carried out. The allocated microorganisms were cultivated on the agar mediums (FPA, MRS and MA), cloned to pure cultures and carried out their genetic identification and the analysis of antimicrobic properties.

DNA allocation of microorganisms was carried out using a set of "PROBA-NK" reagents for DNA allocation from biological material ("DNA technology", Moscow).

Electrophoresis in agarose gel was carried out using cameras for SE-2 horizontal electrophoresis with the Elf-4 power supply ("Helicon", Russia). For preparation of 2% agarose for 1 g of agarose 50 ml of TBE single buffer were added and mixed thoroughly. The received solution was placed in the microwave oven (for 2–5 min. depending on furnace power, watched intensity of suspension boiling) or boiled on water bath within 15 min until the complete decomposition of agarose. The melted agarose was cooled to 56°C and 5 mcl of ethydium bromide (10 mg/ml concentration) were added, and then mixed carefully. The melted agarose with ethydium bromide was poured in the prepared form. Gel thickness was about 0.5–0.7 cm. In 30–40 min. the comb was removed. The prepared gel was used directly or stored in the one-fold buffer in the refrigerator at 4°C. For electrophoresis 2 mcl of buffer for plotting and 10 mcl of reaction mixture were mixed in a separate test tube. Then put mixture into gel holes (the ratio of the buffer/ reactionary compound is 2/8). Marker of molecular weight (100 bp) was entered to one of the holes. The filled gel was placed into the gel box filled with buffer. Buffer layer thickness over a surface of gel is about 2–3 mm. In the mode of constant voltage of 100 W the electrophoresis lasted about 70–90 min. For reliability check of the received results in one of the holes 100 bps of DNA marker ("Sibenzim", Novosibirsk) were placed. Structure of the buffer for storage: 10 mM Tris HCl (pH 8.0); 1 mM EDTA; 50 mM NaCl.

Polymerase chain reaction was carried out with use of primers which were specially picked up and synthesized by Sintol firm (Moscow). Temperature condition was selected taking into account the length of amplificated fragment, length and structure of the used primers.

Allocation and cleaning of PCR-products were carried out with the use of GTG agarose (Lonza Rockland, USA). Test tubes with amplification products were consistently put in tripod, 1/5 of crystal violet (MERCK, Germany) was added and mixed. 20 mcl tests were selected under the oil layer and brought in gel holes, and then camera cover was closed. The camera was hooked up to direct-current power supply, observing polarity, and the source was switched on. Source parameters were set: voltage is 250 V, amperage is 100 mA, and duration is 7 min. Optimum electric field intensity of herewith is 10 V/cm.

Upon electrophoresis completion gel was placed on a transilluminator, having arranged strips horizontally, and holes on top. The strip of agarose gel containing

the PRC reaction product of the necessary size was carefully cut out by the surgical scalpel which is previously processed by 70% solution of ethyl alcohol, trying to take as little gel as possible and to place it in a test tube with the corresponding number. Before cutting each following fragment the surgical scalpel was processed by 70% ethyl alcohol in order to avoid samples contamination. The cut-out strips of agarose gel containing PRC reaction products were placed inside the cut-off filtering tips in amount of 200 mcl. Test tubes were centrifuged within 5 min at maximum speed.

Cleaning quality and quantity of the allocated sample were estimated. For this purpose horizontal electrophoresis was repeated in agarose gel. Sequencing of 16S RNA gene fragment of the allocated microorganisms was made by Sanger method with use of reagents set of "ABI Prism Big Dye Terminator Cycle Sequincing Ready Reaction Kits", ("Applied biosistems", USA), using the GS Junior 454 sequenator ("Roche", Switzerland). Sanger reaction products' cleaning was performed with use of Sephadex G50 Superfine sorbent (Bio-sciences, Sweden).

The choice of test strains for antimicrobic activity research is caused by the fact that the tested strains are the representatives of pathogenic and opportunistic microflora of vegetables and fruit causing damage of fruit or human diseases: *Pseudomonas fluorescens* – pathogenic bacterium causing soft rot on vegetable raw materials; *Pseudomonas aeruginosa* – opportunistic bacterium causing nosocomial human infections; *Candida albicans* – microfungus, the originator of opportunistic human infections; *Alcaligenes faecalis* – opportunistic bacterium causing intra-abdominal infections, septical fever and meningitis; *Leuconostoc mesenteroides* – opportunistic bacterium causing infectious diseases; *Escherichia coli* – opportunistic bacterium causing a gastroenteritis; *Enterobacter ludwigii* – opportunistic bacterium causing infectious diseases of kidneys and urinary tract, genital organs, respiratory system; *Erwinia aphidicola* – pathogenic bacterium causing soft rot on vegetable raw materials; *Micrococcus luteus* – pathogenic bacterium causing fruit and vegetables diseases; *Salmonella enteric* – pathogenic bacterium causing gastroenteritis; *Listeria monocytogenes* – pathogenic bacterium causing gastroenteritis and sepsis; *Yersinia spp.* – the pathogenic bacterium causing yersiniosis; *Staphylococcus aureus* – pathogenic bacterium causing pneumonia, meningitis, osteomyelitis, endocarditis, infectious-toxic shock and sepsis.

RESULTS AND DISCUSSION

At the first stage of pilot studies from fresh vegetables the groups of microorganisms presented in table 1 were allocated.

As a result of sequencing on 16S rRNA the following nucleotide sequences have been received.

Bacillus pumilus:

CTAATACATGCAGTCGAGCGGACAGAAGG
GAGCTTGCTCCCGGATGTTAGCGGCGGACGGG
TGAGTAACACGTGGGTAACCTGCCTGTAAGAC
TGGGATAACTCCGGGAAACCGGAGCTAATACC
GGATAGTTCCTTGAACCGCATGGTTCAAGGAT
GAAAGACGGTTTCGGCTGTCACTTACAGATGG
ACCCGCGGCGCATTAGCTAGTTGGTGGGGTAA
TGGCTCACCAAGGCGACGATGCGTAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCC

TACGGGAGGCAGCAGTAGGGAATCTTCCGC
AATGGACGAAAGTCTGACGGAGCAACGCCGC
GTGAGTGATGAAGGTTTTCGGATCGTAAAGCT
CTGTTGTTAGGGAAGAACAAGTGCGAGAGTAA
CTGCTCGCACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTGTCCGGAATTA
TTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTA
AGTCTGATGTGAAAGCCCCCGGCTCAACCGGG
GAGGGTCATTGGAAACTGGGAAACTTGAGTGC
AGAAGAGGAGAGTGGAATTCCACGTGTAGCG
GTGAAATGCGTAGAGATGTGGAGGAACACCA
GTGGCGAAGGCGACTCTCTGGTCTGTACTGAC
GCTGAGGAGCGAAAGCGTGGGGAGCGAACAG

Table 1. Results of microorganisms allocation from vegetables

№	Name of strain	Allocation source
1	*Bacillus stratosphericus*	onion
2	*Bacillus endophyticus*	tomato
3	*Bacillus pumilus*	onion
4	*Bacillus subtilis*	pepper
5	*Enterococcus casseliflavus*	pepper
6	*Leuconostoc mesenteroides*	pepper
7	*Serratia plymuthica*	onion
8	*Pseudomonas azotoformans*	onion
9	*Staphylococcus warneri*	onion
10	*Staphylococcus epidermidis*	tomato
11	*Bacillus cereus*	eggplant
12	*Microbacterium foliorum*	onion
13	*Staphylococcus aureus*	eggplant
14	*Staphylococcus caprae*	tomato
15	*Erwinia persicina*	pepper
16	*Erwinia aphidicola*	pepper

Bacillus stratosphericus:

CTATACATGCAAGTCGAGCGGACAGAAGG
GAGCTTGCTCCCGGATGTTAGCGGCGGACGGG
TGAGTAACACGTGGGTAACCTGCCTGTAAGAC
TGGGATAACTCCGGGAAACCGGAGCTAATACC
GGATAGTTCCTTGAACCGCATGGTTCAAGGAT
GAAAGACGGTTTCGGCTGTCACTTACAGATGG
ACCCGCGGCGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCGACGATGCGTAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGACGAAAGTCTGACG
GAGCAACGCCGCGTGAGTGATGAAGGTTTTCG
GATCGTAAAGCTCTGTTGTTAGGGAAGAACAA
GTGCAAGAGTAACTGCTTGCACCTTGACGGTA
CCTAACCAGAAAGCCACGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TGTCCGGAATTATTGGGCGTAAAGGGCTCGCA
GGCGGTTTCTTAAGTCTGATGTGAAAGCCCCC
GGCTCAACCGGGGAGGGTCATTGGAAACTGGG
AAACTTGAGTGCAGAAGAGGAGAGTGGATTTC
CACGTGTAGCGGTGAAATGCGTAGAGATGTGG
AGGAACACCAGTGCGAAGCGACTCTCTGGTCT
GTACTGACGCTGAG

Bacillus endophyticus

CTAATACATGCAAGTCGAGCGGAGTTTTGG
AAAAGCTTGCTTTTCAAAACTTAGCGGCGGAC
GGGTGAGTAACACGTGGGCAACCTGCCCTTGA
GACGGGGATAACTCCGGGAAACCGGAGCTAAT
ACCGGATAACACATATCTTCGCATGAGGATAT
GTTAGAAGGTGGCTTTTAGCTACCACTCAAGG
ATGGGCCCGCGGCGCATTAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCGACGATGCGTAGCC
GACCTGAGAGGGTGATCGGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTAGGGAATCTTCCGCAATGGACGAAAGTCT
GACGGAGCAACGCCGCGTGAGTGATGAAGGTT
TTCGGATCGTAAAGCTCTGTTGTTAGGGAAGA
ACAAGTACCTGTTGAATAAGCAGGTACCTTGA
CGGTACCTAACCAGAAAGCCACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGAATTATTGGGCGTAAAGCGC
GCGCAGGCGGTTCCTTAAGTCTGATGTGAAAG
CCCACGGCTCAACCGTGGAGGGTCATTGGAAA
CTGGGGAACTTGAGTGCAGAAGAGGAGAGCG
GAATTCCACGTGTAGCGGTGAATTGCGTAGAG
ATGTGGAGGAACACCAGTGGCGAAGGCGGCTC
TCTGGTCTGTAACTGACGCTGAGGCGCGA

Bacillus subtilis

CTATACATGCAAGTCGAGCGGACAGATGGG
AGCTTGCTCCCTGATGTTAGCGGCGGACGGGT
GAGTAACACGTGGGTAACCTGCCTGTAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCG
GATGGTTGTTTGAACCGCATGGTTCAAACATA
AAAGGTGGCTTCGGCTACCACTTACAGATGGA
CCCGCGGCGCATTAGCTAGTTGGTGAGGTAAC
GGCTCACCAAGGCAACGATGCGTAGCCGACCT
GAGAGGGTGATCGGGCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGACGAAAGTCTGACG
GAGCAACGCCGCGTGAGTGATGAAGGTTTTCG
GATCGTAAAGCTCTGTTGTTAGGGAAGAA

Enterococcus casseliflavus:

CTATACATGCAGTCGAACGCTTTTTCTTTCA
CCGGAGCTTGCTCCATCGAAAGAAAAAGAGTG
GCGAACGGGTGAGTAACACGTGGGTAACCTGC
CCATCAGAAGGGGATAACACTTGGAAACAGGT
GCTAATACCGTATAACACTATTTTCCGCATGGA
AGAAAGTTGAAAGGCGCTTTTGCGTCACTGAT
GGATGGACCCGCGGTGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCAACGATGCATAG
CCGACCTGAGAGGGTGATCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCGGCAATGGACGAAAGT
CTGACCGAGCAACGCCGCGTGAGTGAAGAAG
GTTTTCGGATCGTAAAACTCTGTTGTTAGAGAA
GAACAAGGATGAGAGTAAAATGTTCATCCCTT
GACGGTATCTAACCAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGC
GAGCGCAGGCGGTTTCTTAAGTCTGATGTGAA
AGCCCCCGGCTCAACCGGGGAGGGTCATTGGA
AACTGGGAGACTTGAGTGCAGAAGAGGAGAG
TGGAATTCCATGTGTAGCGGTGAATGCGTAGA
TATAT

Leuconostoc mesenteroides:

CTAATACATGCAAGTCGAACGCACAGCGAA
AGGTGCTTGCACCTTTCAAGTGAGTGGCGAAG
GGTGTGTAACACGTGGACAACCTGCCTCAAGG
CTGGGGANAACATTTGGAAACAGATGCTAATA
CCGAATAAAACTTAGTGTCGCATGACACAAAG
TTAAAAGGCGCTTCGGCGTCACCTAGAGATGG
ATCCGCGGTGCATTAGTTAGTTGGTGGGGTAA
AGGCCTACCAAGACAATGATGCATAGCCGAGT
TGAGAGACTGATCGGCCACATTGGGACTGAGA
CACGGCCCAAACTCCTACGGGAGGCTGCAGTA
GGGAATCTTCCACAATGGGCGAAAGCCTATGG
AGCAACGCCGCGTGTGTGATGAAGGCTTTCGG
GTCGTAAAGCACTGTTGTATGGGAAGAACAGC
TAGAATAGGAAATGATTTTAGTTTGACGGTAC
CATACCAGAAAGGGACGGCTAAATACGTGCCA
GCAGCCGCGGTAATACGTATGTCCCGAGCGTT
ATCCGGATTTATTGGGCGTAGAGCGAGCGCAG
ACGGTTTTATTAAGTCTGATGTGAAAGCCCCG
GAGCTCAAC

Serratia plymuthica:

CTACACATGCAGTCGAGCGGTAGCACGGGA
GAGCTTGCTCTCTGGGTGACGAGCGGCGGACG
GGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATAC
CGCATGATGTCGCAAGACCAAAGTGGGGGACC
TTCGGGCCTCACGCCATCGGATGTGCCCAGAT
GGGATTAGCTAGTAGGTGGGGTAATGGCTCAC
CTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGCACAATGGGCGCAAGCCTGATGCAGCCATG
CCGCGTGTGTGAAGAAGGCCTTAGGGTTGTAA
AGCACTTTCAGCGAGGAGGAAGGCGTTGTAGT
TAATAGCTGCAACGATTGACGTTACTCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCG
CGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
GTTAAGTCAGATGTGAAATCCCCGAGCTTAAC

TTGGGAACTGCATTTGAAACTGGCAAGCTAGA
GTCTTGTAGAGGGGGGTAGAATTCCAGGTGTA
GCGGTGAAATGCGTAGAGATCTGGAGGAATAC
CGGTGGCGAAGGC

Pseudomonas azotoformans:

CTACACATGCAGTCGAGCGGTAGAGAGAA
GCTTGCTTCTCTTGAGAGCGGCGGACGGGTGA
GTAATGCCTAGGAATCTGCCTGGTAGTGGGGG
ATAACGTTCGGAAACGGACGCTAATACCGCAT
ACGTCCTACGGGAGAAAGCAGGGGACCTTCGG
GCCTTGCGCTATCAGATGAGCCTAGGTCGGAT
TAGCTAGTTGGTGAGGTAATGGCTCACCAAGG
CGACGATCCGTAACTGGTCTGAGAGGATGATC
AGTCACACTGGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGAAAGCCTGATCCAGCCATGCCGCG
TGTGTGAAGAAGGTCTTCGGATTGTAAAGCAC
TTTAAGTTGGGAGGAAGGGTTGTAGATTAATA
CTCTGCAATTTTGACGTTACCGACAGAATAAG
CACCGGCTAACTCTGTGCCAGCAGCCGCGGTA
ATACAGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAA
GTTGGATGTGAAATCCCCGGGCTCAACCTGGG
AACTGCATTCGAAACTGACTGACTAGAGTATG
GTAGAGGGTGGTGGAATTTCCTGTGTAGAGGT
GAAATGCGTAGATATACGAAGGAACACCAGTG
GCGAAGGCGACCACCTGGACTANTACTGAC

Staphylococcus warneri:

CTATACATGCAAGTCGAGCGACAGATAAGG
AGCTTGCTCCTTTGACGTTAGCGGCGGACGGG
TGAGTAACACGTGGATAACCTACCTATAAGAC
TGGGATAACTTCGGGAAACCGGAGCTAATACC
GGATAACATATTGAACCGCATGGTTCAATAGT
GAAAGGCGGCTTTGCTGTCACTTATAGATGGA
TCCGCGCCGTATTAGCTAGTTGGTAAGGTAAC
GGCTTACCAAGGCAACGATACGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGGCGAAAGCCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGTCTTCGG
ATCGTAAAACTCTGTTAT
CAGGGAAGAACAAATGTGTAAGTAACTGT
GCACATCTTGACGGTACCTGATCAGAAAGCCA
CGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGT
CTGATGTGAAAGCCCACGGCTCAACCGTGGA
GGGTCATTGGAAACTGGAAAACTTGAGTGCA
GAAGAGGAAAGTGGAATTCCATGTGTAGCGG
TGAATGCGCAGAGATATGGAGGACACCAGTG
GCGAAT

Staphylococcus epidermidis:

GCTATACATGCAGTCGAGCGACAGACGAGG
AGCTTGCTCCTCTGACGTTAGCGGCGGACGGG
TGAGTAACACGTGGATAACCTACCTATAAGAC
TGGGATAACTTCGGGAAACCGGAGCTAATACC
GGATAATATATTGAACCGCATGGTTCAATAGT
GAAAGACGGTTTTGCTGTCACTTATAGATGGA
TCCGCGCCGCATTAGCTAGTTGGTAAGGTAAC
GGCTTACCAAGGCAACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGGCGAAAGCCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGTCTTCGG
ATCGTAAAACTCTGTTATTAGGGAAGAACAAA
TGTGTAAGTAACTATGCACGTCTTGACGGTAC
CTAATCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
ATCCGGAATTATTGGGCGTAAAGCGCGCGTAG
GCGGTTTTTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGAA
AACTTGAGTGCAGAAGAGGAAAGTGGAATTCC
ATGTGTAGCGGTGAAATGCGCAGAGATATGGA
GGAACACCAGTGGCGAAGGCGACTTTCTGGTC
TGTAACTGACGCTGATGTGCGAAAGCGTGGGG
ATCAAACA

Bacillus cereus:

CTATACATGCAAGTCGAGCGGACAGAAGG
GAGCTTGCTCCCGGATGTTAGCGGCGGACGGG
TGAGTAACACGTGGGTAACCTGCCTGTAAGAC
TGGGATAACTCCGGGAAACCGGAGCTAATACC
GGATAGTTCCTTGAACCGCATGGTTCAAGGAT
GAAAGACGGTTTCGGCTGTCACTTACAGATGG
ACCCGCGGCGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCGACGATGCGTAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGACGAAAGTCTGACG
GAGCAACGCCGCGTGAGTGATGAAGGTTTTCG
GATCGTAAAGCTCTGTTGTTAGGGAAGAACAA
GTGCAAGAGTAACTGCTTGCACCTTGACGGTA
CCTAACCAGAAAGCCACGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TGTCCGGAATTATTGGGCGTAAAGGGCTCGCA
GGCGGTTTCTTAAGTCTGATGTGAAAGCCCCC
GGCTCAACCGGGGAGGGTCATTGGAAACTGGG
AAACTTGAGTGCAGAAGAGGAGAGTGGATTTC
CACGTGTAGCGGTGAAATGCGTAGAGATGTGG
AGGAACACCAGTGCGAAGCGACTCTCTGGTCT
GTACTGACGCTGAG

Microbacterium foliorum:

CTACACATGCAGTCGAACGGTGAACACGG
AGCTTGCTCTGTGGGATCAGTGGCGAACGGGT
GAGTAACACGTGAGCAACCTGCCCCTGACTCT
GGGATAAGCGCTGGAAACGGCGTCTAATACT
GGATACGAGTAGCGACCGCATGGTCAGTTACT
GGAAAGATTTATTGGTTGGGGATGGGCTCGCG
GCCTATCAGCTTGTTGGTGAGGTAATGGCTCA
CCAAGGCGTCGACGGGTAGCCGGCCTGAGAG
GGTGACCGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGCACAATGGGCGCAAGCCTGATGCAG
CAACGCCGCGTGAGGGATGACGGCCTTCGGG
TTGTAAACCTCTTTTAGCAGGGAAGAAGCGAA
AGTGACGGTACCTGCAGAAAAAGCGCCGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTA
GGGCGCAAGCGTTATCCGGAATTATTGGGCGT
AAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCT
GTGAAATCCGGAGGCTCAACCTCCGGCCTGCA
GTGGGTACGGGCAGACTAGAGTGCGGTAGGG
GAGATTGNCN

Staphylococcus aureus:

GCTATACATGCAGTCGAGCGAACGGACGAG
AAGCTTGCTTCTCTGATGTTAGCGGCGGACGG
GTGAGTAACACGTGGATAACCTACCTATAAGA
CTGGGATAACTTCGGGAAACCGGAGCTAATAC
CGGATAATATTTTGAACCGCATGGTTCAAAAG
TGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAA
CGGCTTACCAAGGCAACGATGCATAGCCGACC
TGAGAGGGTGATCGGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGGCGAAAGCCTGACG
GAGCAACGCCGCGTGAGTGATGAAGGTCTTCG
GATCGTAAAACTCTGTTATTAGGGAAGAACAT
ATGTGTAAGTAACTGTGCACATCTTGACGGTA
CCTAATCAGAAAGCCACGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TATCCGGAATTATTGGGCGTAAAGCGCGCGTA
GGCGGTTTTTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGA
AAACTTGAGTGCAGAAGAGGAAAGTGGAATTC
CATGTGTAGCGGTGAAATGCGCAGAGATATGG
AGGAACACCAGTGGCGAAAGGCGACTTTCTGG
TCTGTAACTGACGCTGATGTGCGAAAGCGTGG
GGATC

Staphylococcus caprae:

CTATACATGCAGTCGAGCGAACAGACGAGG
AGCTTGCTCCTCTGACGTTAGCGGCGGACGGG
TGAGTAACACGTGGATAACCTACCTATAAGAC
TGGGATAACTTCGGGAAACCGGAGCTAATACC
GGATAATATATTGAACCGCATGGTTCAATAGT
GAAAGACGGTTTTGCTGTCACTTATAGATGGA
TCCGCGCCGCATTAGCTAGTTGGTAAGGTAAC
GGCTTACCAAGGCAACGATGCGTAGCCGACCT
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ACGGTCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGGCGAAAGCCTGACGG
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GCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
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GCGGTTTTTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGAA
AACTTGAGTGCAGAAGAGGAAAGTGGAAATTC
CATGTGTAGCGGTGAAATGCGCAGAGATATGG
AGGAACACCAGTGGCGAAGG

Erwinia persicina strain:

GCTACACATGCAGTCGAACGGTAGCACAGA
GAGCTTGCTCTTGGGTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCCGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACC
GCATAACGTCTTCGGACCAAAGTGGGGGACCT
TCGGGCCTCACACCATCGGATGTGCCCAGATG
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TAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAA
GTACTTTCAGTGGGGAGGAAGGCGAAGAGGTT
AATAACCTTTTCGATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGC
GGTAATACGGAGGGTGCAAGCGTTAATCGGAA
TTACTGGGCGTAAAGCGCACGCAGGCGGTCTG
TCAAGTCGGATGTGAAATCCCCGGGCTCAACC
TGGGAACTGCATTCGAAACTGGCAGGCTAGAG
TCTTGTAGAGGGGGGTAGAATTCCAGGTGTAG
CGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGG

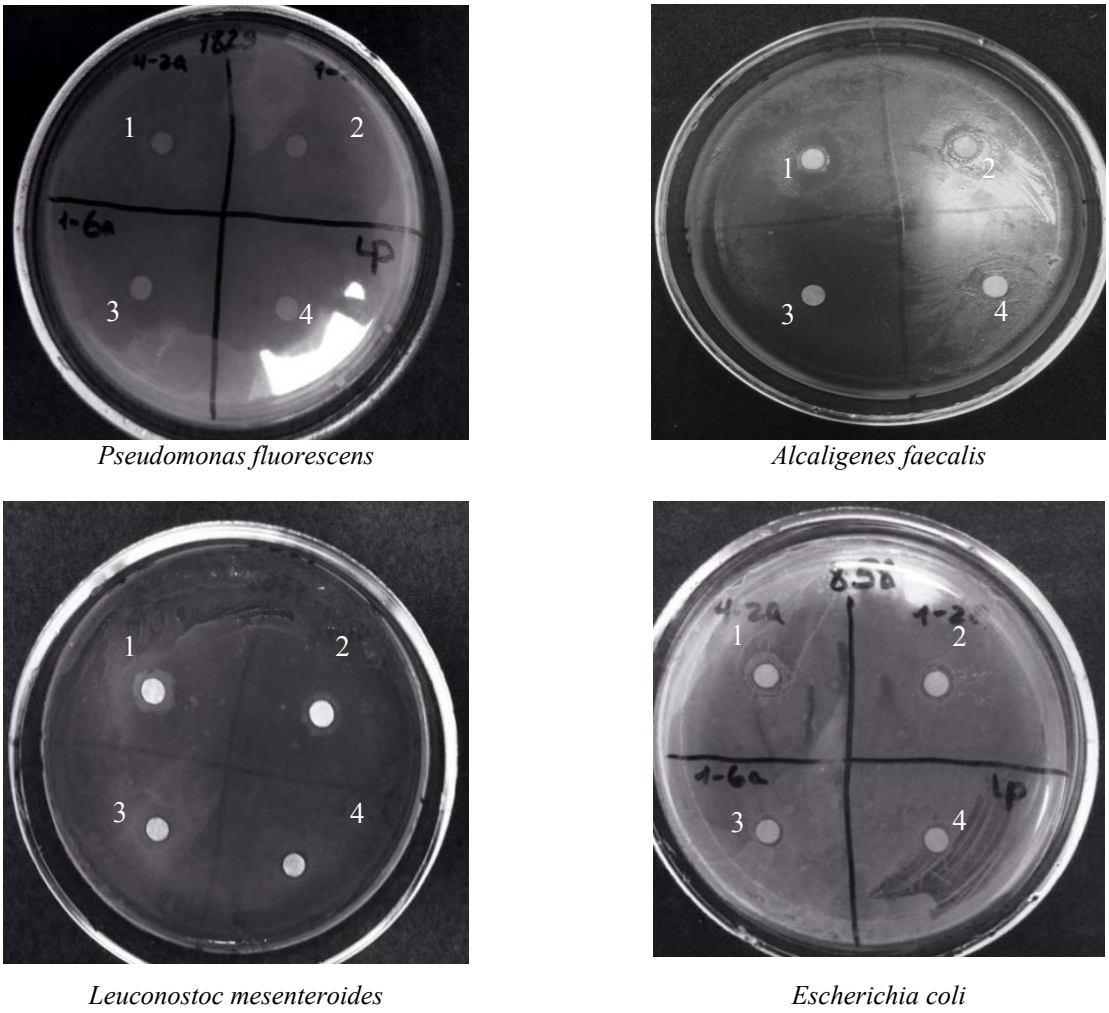
Erwinia aphidicola:

GCTACACATGCAGTCGAACGGTAGCACAGA
GAGCTTGCTCTTGGGTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCCGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACC
GCATAACGTCTTCGGACCAAAGTGGGGGACCT
TCGGGCCTCACACCATCGGATGTGCCCAGATG
GGATTAGCTAGTAGGTGGGGTAACGGCTCACC
TAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAA
GTACTTTCAGTGGGGAGGAAGGCGATGAAGTT
AATAGCTTCGTCGATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGC
GGTAATACGGAGGGTGCGAGCGTTAATCGGAA
TTACTGGGCGTAAAGCGCACGCAGGCGGTCTG
TCCAGTCGGATGTGAAATCCCCGGGCTCAGCC
TGGGAACTGCGTTCGAAACTGGCAGGCTAGAG
TCTTGTAGAGGGGG

For further researches four strains of microorganisms which are not pathogenic are selected: *Bacillus stratosphericus*, *Bacillus endophyticus*, *Bacillus pumilus*, *Bacillus subtilis*.

One of the main properties of the chosen strains of microorganisms are antimicrobic properties which are caused by formation of secondary metabolites: organic acids, ethanol, diacetyl, H_2O_2 and protein compounds which are known as bacteriocins. Antagonistic activity of the chosen strains was studied in relation to test cultures on the solid medium by diffusive method. The test strain was taken on agar nutrient medium (ANM) as lawn and at the same time paper disks impregnated with bacteria metabolites allocated from vegetables surface (10 mcl/disk) were put on the lawn. The disks were put so that the distance between their centers was not less than 24 mm. After putting the disks on agar they were pressed by a sterile needle or forceps until the full contact with medium surface. As a control the disk with MRS medium was used, as a comparison drug the disk with ciprofloxacin antibiotic (from a standard set) was used. Dishes were incubated at 37°C within 24 hours. Results were considered on existence and size (in mm) of a transparent zone of lack of microorganisms' growth around a disk.

Results of bacteria's antagonistic activity determination on solid nutrient medium are presented in Fig. 1 and in Table 2.



Pseudomonas fluorescens *Alcaligenes faecalis*

Leuconostoc mesenteroides *Escherichia coli*

Fig. 1. Results of allocated microorganisms' antimicrobic activity studying on the solid medium: 1 – *Bacillus subtilis*, 2 – *Bacillus stratosphericus*, 3 – *Bacillus endophyticus*, 4 – *Bacillus pumilus.*

Table 2. Results of microorganisms' antimicrobial activity determination allocated from fresh vegetables surface

Testing culture	Strains			
	Bacillus endophyticus	*Bacillus stratosphericus strain*	*Bacillus pumilus*	*Bacillus subtilis*
	Diameter of growth inhibition zones, mm			
Pseudomonas fluorescens -	0	8	0	0
Pseudomonas aeruginosa ATCC 9027 -	Growth inhibition	0	Growth inhibition	0
Candida albicans	0	0	0	0
Leuconostoc mesenteroides +	0	0	8	0
Arthrobacter cumminsii +	0	0	0	10
Alcaligenes faecalis-	10	0	0	8
Escherichia coli ATCC 25922 -	0	8	0	8
Enterobacter ludwigii -	0	10	8	0
Erwinia aphidicola-	0	0	0	8
Micrococcus luteus+	0	8	0	0
Salmonella enterica-	0	12	8	8
Listeria monocytogenes+	0	0	0	0
Yersinia spp.-	8	10	10	8
Staphylococcus aureus+ ATCC 25923	8	8	8	8

In the analysis of the data presented in Table 2 it is possible to see that strains *Bacillus subtilis* and *Bacillus stratosphericus strain* possess wider range of antimicrobic activity in comparison with the others as they suppress growth of the majority of pathogenic microorganisms. Strains' data inhibit the development of gram-positive and gram-negative bacteria. *Bacillus subtilis* strain shows antagonistic activity against: *Arthrobacter cumminsii, Staphylococcus aureus, Alcaligenes faecalis*, *Escherichia coli* ATCC 25922, *Erwinia aphidicola*, *Salmonella enterica*, *Yersinia spp. Bacillus stratosphericus strain* of microorganisms possesses antimicrobic activity against the following bacteria: *Pseudomonas fluorescens, Escherichia coli* ATCC 2592, *Enterobacter ludwigii, Salmonella enterica*, *Yersinia spp., Staphylococcus aureus* ATCC 25923 and micrococcaceae: *Micrococcus luteus.*

The allocated strain of *Bacillus endophyticus* microorganisms is the antagonist in relation to the following strains: *Alcaligenes faecalis*, *Yersinia spp., Staphylococcus aureus* ATCC 25923. *Bacillus pumilus* strain suppresses the development of *Leuconostoc mesenteroides*, *Enterobacter ludwigii, Salmonella enterica*, *Yersinia spp., Staphylococcus aureus* ATCC 25923.

Bacteriocins are the bacterial proteins or peptides synthesized on ribosomes. Unlike the known antibiotics bacteriocins have rather narrow range of action since they are active against bacteria of the same or phylogenetic sibling species. It is especially typical for the substances emitted from gram-negative bacteria. More broad activity spectrum is typical for bacteriocins of gram-positive bacteria. From this point of view the research of antagonistic activity of the produced bacteriocins is of great importance. It can be achieved by the neutralization of other metabolites.

Research results of antimicrobic activity of microorganisms' allocated strains in liquid nutrient medium are presented in Fig. 2–5.

The data given in Fig. 2–5 demonstrate that all isolates inhibit *Pseudomonas fluorescens* growth (from 53 to 88%). Strong antagonistic activity is shown by *Bacillus pumilus* and *Bacillus endophyticus* in relation to *Arthrobacter cumminsii* and *Staphylococcus aureus* (from 55 to 85%). *Bacillus endophyticus* shows the strong inhibiting activity in relation to *Micrococcus luteus* and *Listeria monocytogenes* (from 55 to 91%). *Bacillus endophyticus* shows considerable inhibition of *Yersinia spp.*, *Escherichia coli* ATCC 25922 (from 54 to 76%). All isolates show the degree of inhibition from 28 to 49% in relation to *Enterobacter ludwigii* and *Erwinia aphidicola*, respectively.

Isolates show the high inhibiting potential against the pathogens causing various human diseases. It should be noted that all isolates except for *Bacillus stratosphericus* show antagonistic activity in relation to *Salmonella enterica* from 25 to 38%, while *Bacillus stratosphericus* does not inhibit the growth of *Salmonella enterica*.

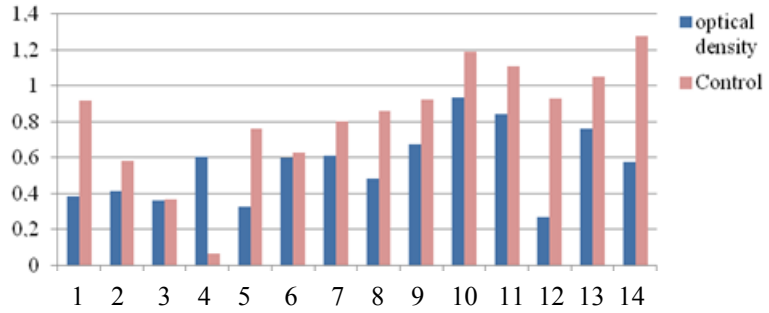


Fig. 2. Antagonistic activity of Bacillus pumilus strain : 1 – *Pseudomonas fluorescens*; 2 – *Pseudomonas aeruginosa*; 3 – *Candida albicans*; 4 – *Leuconostoc mesenteroides*; 5 – *Arthrobacter cumminsii*; 6 – *Alcaligenes faecalis*; 7 – *Escherichia coli*; 8 – *Enterobacter ludwigii*; 9 – *Erwinia aphidicola*; 10 – *Micrococcus luteus*; 11 – *Salmonella enterica*; 12 – *Listeria monocytogenes*; 13 – *Yersinia spp.*; 14 – *Staphylococcus aureus*.

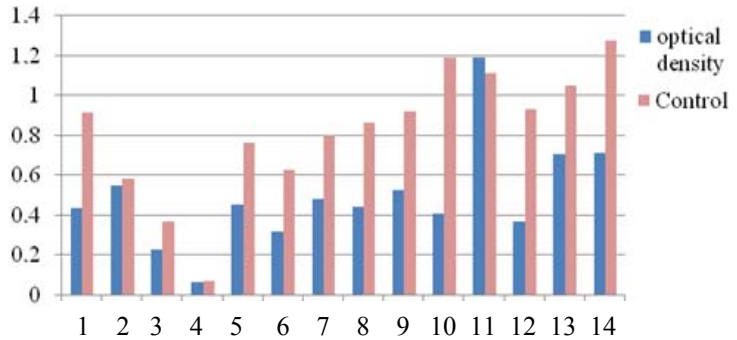


Fig. 3. Antagonistic activity of Bacillus stratosphericus strain: 1 – *Pseudomonas fluorescens*; 2 – *Pseudomonas aeruginosa*; 3 – *Candida albicans*; 4 – *Leuconostoc mesenteroides*; 5 – *Arthrobacter cumminsii*; 6 – *Alcaligenes faecalis*; 7 – *Escherichia coli*; 8 – *Enterobacter ludwigii*; 9 – *Erwinia aphidicola*; 10 – *Micrococcus luteus*; 11 – *Salmonella enterica*; 12 – *Listeria monocytogenes*; 13 – *Yersinia spp.*; 14 – *Staphylococcus aureus*.

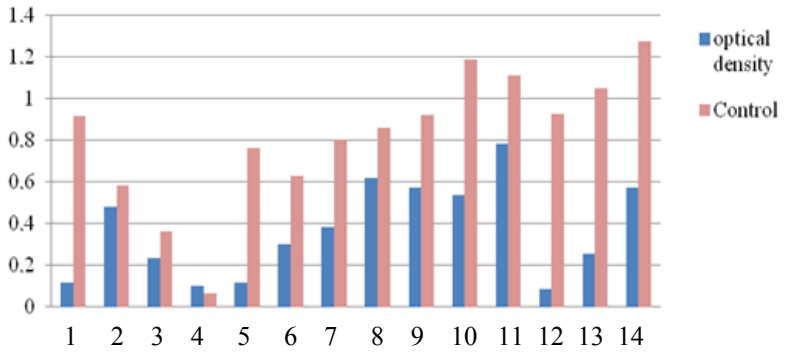


Fig. 4. Antagonistic activity of Bacillus endophyticus strain: 1 – *Pseudomonas fluorescens*; 2 – *Pseudomonas aeruginosa*; 3 – *Candida albicans*; 4 – *Leuconostoc mesenteroides*; 5 – *Arthrobacter cumminsii*; 6 – *Alcaligenes faecalis*; 7 – *Escherichia coli*; 8 – *Enterobacter ludwigii*; 9 – *Erwinia aphidicola*; 10 – *Micrococcus luteus*; 11 – *Salmonella enterica*; 12 – *Listeria monocytogenes*; 13 – *Yersinia spp.*; 14 – *Staphylococcus aureus*.

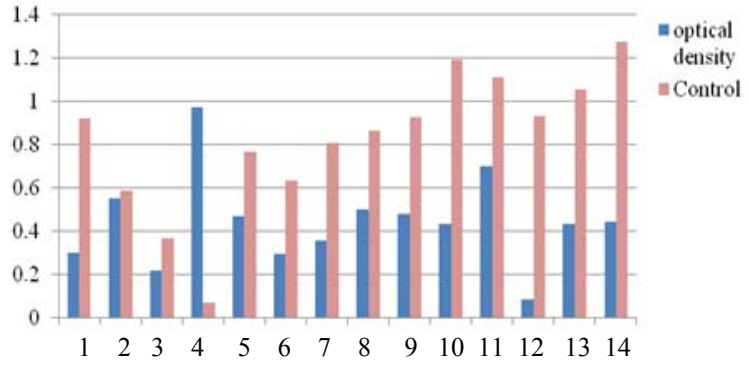


Fig. 5. Antagonistic activity of Bacillus subtilis strain: 1 – *Pseudomonas fluorescens*; 2 – *Pseudomonas aeruginosa*; 3 – *Candida albicans*; 4 – *Leuconostoc mesenteroides*; 5 – *Arthrobacter cumminsii*; 6 – *Alcaligenes faecalis*; 7 – *Escherichia coli*; 8 – *Enterobacter ludwigii*; 9 – *Erwinia aphidicola*; 10 – *Micrococcus luteus*; 11 – *Salmonella enterica*; 12 – *Listeria monocytogenes*; 13 – *Yersinia spp.*; 14 – *Staphylococcus aureus*.

Bacillus stratosphericus, Bacillus endopheticus and Bacillus subtilis strains suppress the development of Alcaligenes faecalis (52–54%). Inhibitors of P. aeruginosa are Bacillus endophyticus (18%) and Bacillus pumilus (30%) strains.

CONCLUSION

As a result of research of allocated microorganisms' antimicrobic activity on the liquid nutrient medium it is found out that all isolates inhibit the growth of *Pseudomonas fluorescens* (from 53 to 88%). Strong antagonistic activity is shown by *Bacillus pumilus* and *Bacillus endopheticus* in relation to *Arthrobacter cumminsii* and *Staphylococcus aureus* (from 55 to 85%). *Bacillus methylotrophicusus*, *Bacillus endopheticus* and *Bacillus safensis* show strong inhibiting activity in relation to *Micrococcus luteus* and *Listeria monocytogenes* (from 55 to 91%). *Bacillus endopheticus* and *Bacillus safensis* show considerable inhibition of *Yersinia spp.*, *Escherichia coli* ATCC 25922 (from 54 to 76%). All isolates show the degree of inhibition from 28 to 49% in relation to *Enterobacter ludwigii* and *Erwinia aphidicola*, respectively.

The obtained data suggest the possibility of using the allocated microorganisms in order to study the inter-stain interactions and on the basis of these data to develop the biological product possessing the preserving properties.

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