CROP PRODUCTION

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PCR IDENTIFICATION AND CHARACTERIZATION OF ENZYMATIC ACTIVITY AND ANTAGONISTIC PROPERTIES OF BACTERIA OF THE GENUS PSEUDOMONAS ISOLATED FROM NATURAL SOURCES

Research article

Abstract

The molecular genetic identification of bacterial strains of genus *Pseudomonas* isolated from soil and vegetative material of potato plants (Solanum tuberosum) was carried out. PCR analysis using the genus-specific OpriF / OpriR primer showed that 9 samples of a pure bacterial culture were characterized by the presence of one amplicon characteristic of *Pseudomonades*. Affiliation of selected bacteria to genus *Pseudomonas* was confirmed by a comparative analysis of the deciphered nucleotide sequence of the 16S rRNA gene with the reference nucleotide sequences of typical strains from the RDP database. A wide range of enzymatic activity and pronounced antagonistic properties in relation to phytopathogenic micromycetes of genera *Alternaria* and *Fusarium* were established in selected strains of bacteria.

Keywords: *Pseudomonas*, *Alternaria, Fusarium,* molecular-genetic identification, molecular typing, enzymatic activity, antifungal activity.

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ПЦР ИДЕНТИФИКАЦИЯ И ХАРАКТЕРИСТИКА ФЕРМЕНТАТИВНОЙ АКТИВНОСТИ И АНТАГОНИСТИЧЕСКИХ СВОЙСТВ БАКТЕРИЙ РОДА *PSEUDOMONAS* ВЫДЕЛЕННЫХ ИЗ ПРИРОДНЫХ ИСТОЧНИКОВ

Научная статья

Аннотация

Проведена молекулярно-генетическая идентификация штаммов бактерий рода *Pseudomonas*, выделенных из почвы и травянистой части растений картофеля (*Solanum tuberosum*). ПЦР-анализ с использованием родоспецифичного праймера OpriF/OpriR показал, что 9 образцов чистой культуры бактерий характеризовались наличием одного ампликона, характерного для бактерий рода *Pseudomonas*. Принадлежность отобранных бактерий к роду *Pseudomonas* подтверждена сравнительным анализом секвенированной нуклеотидной последовательности гена 16S рРНК с референтными нуклеотидными последовательностями типовых штаммов из базы данных RDP. Установлены широкий спектр ферментативной активности и выраженные антагонистические свойства в отношении фитопатогенных микромицетов родов *Alternaria* и *Fusarium* у отобранных штаммов бактерий.

Ключевые слова: *Pseudomonas*, *Alternaria, Fusarium,* молекулярно-генетическая идентификация, молекулярное типирование, ферментативная активность, антифунгальная активность.

1. Introduction

Bacteria of genus *Pseudomonas* are one of the most diverse and environmentally significant microbial groups belonging to plant-growth promoting rhizobacteria (PGPR). Certain types of pseudomonads colonizing plant rhizosphere are able to stimulate the growth and development of crops [1], effectively complete the processes of nitrogen fixation and denitrification [2, 3] owing to the synthesis of extracellular metabolites. Pseudomonads can degrade a wide spectrum of toxic compounds, like herbicides, insecticides, pesticides containing chlorine, fluorine, mercury, bromine atoms. Some representatives of the genus possess the capacity to break down surfactants.

Antagonistic activity of bacteria affects their survival in natural associations - multicomponent systems consisting of dominant and associative organisms involved in generation and maintenance of stable and productive symbiosis. Meanwhile, antagonistic activity of the dominant microbiota is controlled by regulatory strains and is mediated by the synthesis of antibiotics, bacteriocins and lytic enzymes [4]. Despite the accumulated data on microbial antagonism, properties of pseudomonads in the course of competition with pathogenic species are not sufficiently characterized. The biotic regulation of bacterial antagonism governed by enzymes lipases, proteases and amylases may also be defined as scarcely studied phenomenon. Due to high metabolic and physiological activity, bacteria of genus *Pseudomonas* arouse keen interest as potential significant producers of biologically active substances [5, 6]. Nowadays, researchers and manufacturers are focused on the development and fabrication biological products based on various microbial consortia, diming to achieve beneficial effect in cultivation of staple crops for national agricultures [3]. In this regard, the selection of high-quality experimental material for isolation of microbial cultures directly on site in the tilled field is of vital importance. The development of genetic tools and methodological approaches to the identification of microorganisms, allows today to set promptly the taxonomic diagnosis of microorganisms isolated from natural sources. Methods based on nucleic acid amplification using genus-specific primers are widely used for molecular genetic identification of microorganisms due to high sensitivity and rapid results.

The goal of this study was to screen bacteria isolated from soil under potato culture (*Solanum tuberosum*), as well as from the potato herb for affiliation to genus *Pseudomonas* using PCR analysis with a genus-specific pair of primers amplifying a fragment of the lipoprotein I gene of Pseudomonads. Investigation of enzymatic activity and antagonistic properties of the selected bacterial cultures belonging to genus *Pseudomonas*.

2. Materials and methods

Bacterial strains isolated from soil samples and potato herb material served as the objects of study. Strains of mycelial fungi *Fusarium graminearum* BIM F-601, *F. proliferatum* BIM F-602, *Fusarium sp.* BIM F-603, *F. oxysporum* BIM F-618, *Alternaria brassicae* BIM F-621 provided from the Belarusian collection of non-pathogenic microorganisms (BIM acronym of the collection) were chosen as phytopathogenic agents.

To isolate the bacteria, the sample material was thoroughly mixed with sterile distilled water, and a series of tenfold dilutions was conducted. Bacterial suspension (100 µl) from dilutions 10^{-3} - 10^{-7} was inoculated on meat-peptone agar and incubated at 28 ° C for 48 hours. Isolation of bacterial genome DNA was performed using commercial Jena Bioscience kits (Germany) according to the attached instructions. Primers and reagents manufactured by ThermoScientific and PrimeTech were used in the experiments. The reaction mixture comprised 1X AM buffer for Taq polymerase, 200 µM of each dNTP, 1.25 units of Taq polymerase, 10 pmol of each primer, 10–20 ng of chromosomal DNA. Amplification was performed on SureCycler 8800 gradient thermal cyclers (Agilent Technologies and Eppendorf Mastercycler ep GradientS, Germany). The list of primers and amplification details are presented in table 1.

Table 1 - List of primers

<table>
<tr><th>Primers</th><th>Nucleotide sequence</th><th colspan="3">Time-temperature amplification regime</th></tr>
<tr><td rowspan="3">OpriF[6]</td><td rowspan="3">5’-ATGAACAACGTTCTGAAATTCTCTGCT-3’</td><td>1 cycle</td><td>95 oC</td><td>5 min</td></tr>
<tr><td rowspan="3">30 cycles</td><td>95 oC</td><td>30 sec</td></tr>
<tr><td>60 oC</td><td>30 sec</td></tr>
<tr><td rowspan="2">OpriR[6]</td><td rowspan="2">5’-CTTGCGGCTGGCTTTTTCCAG-3’</td><td>72 oC</td><td>20 sec</td></tr>
<tr><td>1 cycle</td><td>72 oC</td><td>5 min</td></tr>
</table>

To facilitate screening of 29 bacterial isolates for affiliation to the Pseudomonas genus, a couple of primers OpriF/OpriR was engaged, yielding an amplicon of 249 bp size and amplifying a fragment of lipoprotein I gene of Pseudomonads [7]. Purification of the amplified DNA fragments was performed using the commercial kit “Jena Bioscience” (Germany) according to the attached instructions. The DNA concentration was measured using a commercial kit QuantiFluor dsDNA System (“Promega” USA), at Promega Quantus portable fluorimeter (“Promega” USA). The sequencing reaction was carried out according to the Sanger method with the aid of Jena Cycle Sequencing Kit (JenaBiosciences) in compliance with the manufacturer's instructions. Separation and analysis of sequencing products were performed at Li-COR 4300 DNA Analyzer. Computer processing of results, their editing and presentation in the FASTA format was performed using e-Seq ™ Software. A comparative analysis of the homology between the deciphered nucleotide sequence of the 16S rRNA gene and the reference sequences of GenBank database and the Ribosomal Database Project (RDP) was conducted. Taxonomic affiliation of the examined microorganism to the definite species was confirmed at the level of homology of its nucleotide sequence with the

reference nucleotide sequences of representatives of the same species over 97%. Enzymatic activity of the isolated bacteria was studied using the commercial api®ZYM and api®20NE kits (BioMerieux, France) according to the attached instructions. The antifungal activity was tested by the well diffusion method [7].

3. Results and discussion

29 bacterial cultures were isolated from soil samples under potato cultivars and vegetative material of *Solanum tuberosum*. To confirm the genetic identity of bacterial cultures to the genus *Pseudomonas*, PCR was performed using the genus-specific OpriF/OpriR primers, is used as an express method for detection of pseudomonads among microbial isolates. Using the NCBI Primer Blast server and the GenBank database, specificity of these primers for Pseudomonas genus was confirmed and experimentally proved for the cultures from the Belarusian collection of non-pathogenic microorganisms identified by analyzing the homology of the 16S rRNA nucleotide sequences and the reference sequences of the GenBank database and the Ribosomal Database Project (RDP).

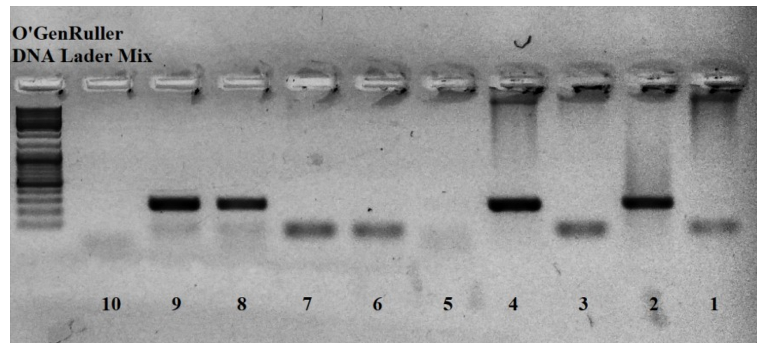

Fig. 1 - Electrophoregram of PCR products of bacterial DNA amplification (primers OpriF / OpriR). 1. *Arthrobacter arilatensis; 2. Pseudomonas koreensis;* 3. *Chryseobacterium nakagawai;* 4. *Pseudomonas argensinensis;* 5. *Bacillus licheniformis;* 6. *Bacillus cereus;* 7. *Bacillus subtilis;* 8. *Pseudomonas extremorientalis;* 9. *Pseudomonas asplenii;* 10. *reagent purity control.*

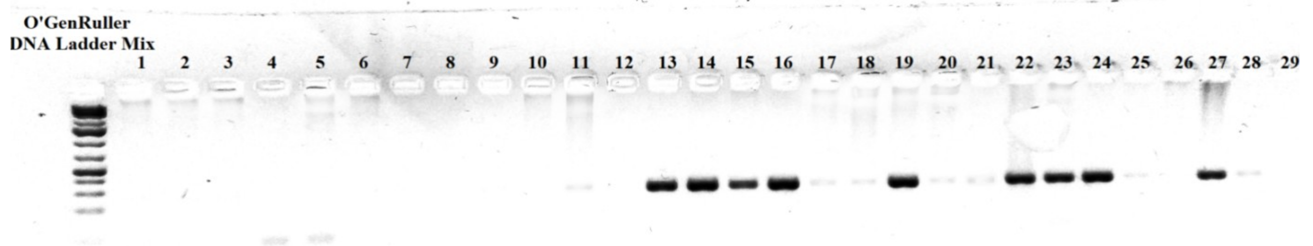

Fig. 2 - Electrophoregram of PCR products of DNA samples amplification primers OpriF / OpriR. DNA samples of bacterial isolates are denoted by corresponding lane number: 1. KaTL1-1б; 2. KaTL1-1a; 3. KaTL2-4; 4. KaTL2-3; 5. KaTL2-2; 6. KaTL2-1; 7. KaTL1-7; 8. KaTL1-6; 9. KaTL1-5; 10. KaTL1-4; 11. KaTL1-3; 12. KaTL1-2; 13. KaTp2-14; 14. KaTp2-13; 15. KaTp2-11; 16. KaTp2-6; 17. KaTp2-4; 18. KaTp2-3; 19. KaTp2-1; 20. KaTp1-13; 21. KaTp1-12; 22. KaTp1-11; 23. KaTp1-10; 24. KaTp1-9; 25. KaTp1-6; 26. KaTp1-5; 27. KaTp1-4; 28. KaTp1-3; 29. KaTp1-1.

The collection bacterial cultures of the species: *Arthrobacter arilatensis*, *Chryseobacterium nakagawai*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus subtilis* were chosen as a negative control, whereas collection cultures *Pseudomonas koreensis*, *Pseudomonas argensinensis*, *Pseudomonas extremorientalis, Pseudomonas asplenii* were taken as a positive control. Electrophoregram of PCR products is presented in Figure 1. Amplification resulted in amplicons sized approximately 269 bp for 4 DNA samples belonging to bacteria of genus *Pseudomonas* and matching the size of the lipoprotein I gene fragment. Using PCR with genus-specific primer, 29 bacterial cultures isolated from soil samples: KaTL1-2, KaTL1-3, KaTL1-4, KaTL1-5, KaTL1-6, KaTL1-7, KaTL2-1, KaTL2-2, KaTL2-3, KaTL2-4, KaTL1-1a, KaTL1-1b and vegetative material of *Solanum tuberosum*: Tp1-1, KaTp1-3, KaTp1-4, KaTp1-5, KaTp1-6, KaTp1-9, KaTp1-10, KaTp1-11, KaTp1-12, KaTp1-13, KaTp2-1, KaTp2-3, KaTp2-4, KaTp2-6, KaTp2-11, KaTp2-13, KaTp2-14 were screened for affiliation to Pseudomonas genus. The amplification results are shown in Figure 2.

Following amplification by primers OpriF and OpriR of twenty-nine cultures of bacteria isolated from soil samples and from the vegetative part of *Solanum tuberosum*, amplicons sized about 269 bp were derived for 9 DNA samples, corresponding to isolates from vegetative part: KaTp2-14, KaTp2-13, KaTp2-11, KaTp2-6, KaTp2-1, KaTp1-12, KaTp1-11, KaTp1-10, KaTp1-9, KaTp1-4. To confirm the taxonomic diagnosis of nine bacterial cultures, sequencing of the 16S rRNA gene was performed. Bacterial cultures were identified by a comparative analysis of the homology of the nucleotide sequence of 16SrRNA gene and the reference sequences of the GenBank database and the Ribosomal Database Project (RDP). The identification results are presented in table 2.

Table 2 - Results of molecular-genetic identification of bacterial cultures isolated from the herbaceous part of *Solanum tuberosum*

Isolate designation	Similarity to reference sequences from the GenBank database	Species affiliation
KaTp1-4	*Pseudomonas koreensis* (97%)	*Pseudomonas koreensis*
KaTp1-9	*Pseudomonas ficuserectae* (99%)	*Pseudomonas ficuserectae*
KaTp1-10	*Pseudomonas argentinensis* (99%)	*Pseudomonas argentinensis*
KaTp1-11	*Pseudomonas koreensis* (99%)	*Pseudomonas koreensis*
KaTp2-1	*Pseudomonas argentinensis* (99%)	*Pseudomonas argentinensis*
KaTp2-6	*Pseudomonas fulva* (99%) *Pseudomonas parafulva* (99%)	*Pseudomonas sp.*
KaTp2-11	*Pseudomonas costantinii* (99%) *Pseudomonas extremorientalis* (99%) *Pseudomonas simiae* (99%)	*Pseudomonas sp.*
KaTp2-13	*Pseudomonas fulva* (99%)	*Pseudomonas fulva*
KaTp2-14	*Pseudomonas umsongensis* (99%) *Pseudomonas migulae* (99%) *Pseudomonas baetica* (99%)	*Pseudomonas sp.*

According to the results of molecular-genetic analysis, nine bacterial isolates: KaTp1-4, KaTp1-9, KaTp1-10, KaTp1-11, KaTp2-1, KaTp2-6, KaTp2-11, KaTp2-13, KaTp2-14 are classified as *Pseudomonas*, which supports the results of PCR amplification with specific OpriF / OpriR primers.

The enzymatic activity of 9 strains of bacteria belonging to the genus *Pseudomonas* was studied. Test results are presented in Table 3. It was found that all examined bacterial cultures show a positive reaction to esterase, lipase (C8), naphthol-AS-BI-phosphohydrolase; a capable to assimilate sugars: glucose, arabinose, mannose, as well as malate and potassium gluconate. Assimilation of sodium citrate, mannitol, enzymatic activity toward capric acid, one of three main saturated fatty acids, as well as the presence of acid phosphatase and enzymes hydrolyzing proteins and peptides: leucine and valine arylamidase recorded for eight bacterial cultures. Seven bacterial strains are characterized by the activity of one of the major hydrolytic enzymes: alkaline phosphatase. In bacteria, alkaline phosphatase is active in the periplasmic space, outside the cytoplasmic membrane.

Alkaline phosphatase of bacteria is relatively resistant to inactivation, denaturation and degradation. One of phosphatase functions of is the removal of phosphate from organic molecules, since many phosphorylated compounds cannot penetrate through plasma membrane. Alkaline phosphatase is used in molecular biological studies for the cleavage of phosphate groups at the 5'-end and to prevent their ligation. Removal of terminal phosphates also makes it possible to introduce a radioactive label. Alkaline phosphatase is most widely used in enzyme immunoassay [9].

Table 3 - Enzymatic activity of strains belonging to the genus *Pseudomonas*

Reaction/enzyme	KaTp								
	1-4	1-9	1-10	1-11	2-1	2-6	2-11	2-13	2-14
Alkaline phosphatase	+	+/-	+/-	+	+	-	+	-	-
Esterase (C4)	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Esterase Lipase (C8)	+	+	+/-	+	+/-	+/-	+/-	+/-	+
Lipase (C14)	+/-	-	-	-	-	-	-	-	-
Leucine arylamidase	+	+	-	+	+	+	+/-	+	+
Valine arylamidase	+/-	+/-	-	+/-	+/-	+/-	+/-	+/-	+/-
Cystine arylamidase	-	-	-	-	-	-	-	-	-
Trypsin	-	-	-	-	-	-	-	-	-
α-chymotrypsin	-	-	-	-	-	-	-	-	-
Acid phosphatase	+	+/-	-	+	+/-	+/-	+	+/-	+/-
Naphthol-AS-BI-phosphohydrolase	+	+/-	+/-	+	+/-	+	+/-	+	+/-

Continiation of Table 3 Enzymatic activity of strains belonging to the genus Pseudomonas

α-galactosidase	-	-	-	-	-	-	-	-	-
β-galactosidase	-	-	-	-	-	-	-	-	-
β-glucuronidase	-	-	-	-	-	-	-	-	-
α-glucosidase	-	-	-	-	-	-	-	-	-
N-acetyl-β-glucosaminidase	-	-	-	-	-	-	-	-	-
α-mannosidase	-	-	-	-	-	-	-	-	-
β-fucosidase	-	-	-	-	-	-	-	-	-
Nitrate reduction to nitrites	-	-	-	-	-	+	+	-	-
Indole production	-	-	-	-	-	-	-	-	-
Acidification	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	+/-	+/-	+/-	-	+	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-
Hydrolysis (β-glucosidase)	-	-	-	-	-	+	+	+	-
Hydrolysis (protease)	+	+	+	-	-	+	-	+	+
Assimilation (glucose)	+	+	+	+	+	+	+	+	+
Assimilation (arabinose)	+	+	+	+	+	+	+	+	+
Assimilation (mannose)	+	+	+	+	+	+	+	+	+
Assimilation (mannitol)	+	+	+	-	+	+	+	+	+
Assimilation (N-acetyl-glucosamine)	+	+	+	-	-	+/-	-	+/-	+/-
Assimilation (maltose)	-	-	-	-	-	+/-	-	-	-
Assimilation (gluconate)	+/-	+	+	+	+	+	+	+	+
Assimilation (caprate)	-	+	+	+	+	-	+	+	+/-
Assimilation (adipate)	-	-	-	-	-	-	-	-	-
Assimilation (malate)	+	+	+	+	+	+/-	+	+	+
Assimilation (citrate)	+	+/-	+	+	+	-	+	+	+
Assimilation (phenyl-acetate)	-	-	-	-	+	-	-	-	-

Note: "+" a positive reaction, "+/-" a weak positive reaction, "-" a negative reaction

The KaTp2-1 strain possesses arginine dihydrolase activity. Two cultures of bacteria KaTp2-6 and KaTp2-11 are able to reduce nitrates to nitrites. Strain KaTp2-1 assimilates phenylacetic acid. It should be noted that phenylacetic acid may be released into the environment during production of b-lactam antibiotics as a metabolic intermediate of aromatic amino acids, lignin, styrene [10]. Microorganisms are known to use phenylacetic acid as a carbon source for growth and development. *P. putida* bacteria utilize phenylacetic acid in metabolism via hydroxylation of the aromatic ring [11, 12].

Bacterial strains: KaTp1-4, KaTp1-9, KaTp1-10, KaTp2-1, KaTp2-6, KaTp2-11, KaTp2-13 possess a wide spectrum of enzymatic activity. For these bacterial cultures, activity was recorded in 17 of 38 biochemical tests.

The antifungal activity of isolated pseudomonads against phytopathogenic fungi of the genera *Alternaria* and *Fusarium* was evaluated. Strains of filamentous fungi from the Belarusian collection of non-pathogenic microorganisms: *Fusarium graminearum* BIM F-601, *F. proliferatum* BIM F-602, *Fusarium sp.* BIM F-603, *F. oxysporum* BIM F-618, *Alternaria brassicae* BIM F-621 were used in the experiments. Bacterial cultures KaTp1-11 and KaTp2-1 displaying pronounced antagonistic properties against phytopathogenic micromycetes strain and the bacterial culture KaTp1-4 inhibiting growth of mycelium and sporulation in most studied micromycetes were revealed. Bacteria *Pseudomonas sp.* KaTp1-11 inhibited growth of mycelium in phytopathogen *Fusarium graminearum* BIM F-601 for 5 days and formed inhibition zone ~ 13-17 mm in diameter. Strain

Pseudomonas argentinensis KaTp2-1 demonstrated inhibition zone of *F. proliferatum* BIM F-602 sporulation ~ 13-17 mm 7 days. Antagonistic properties of the strain *Pseudomonas koreensis* KaTp1-4 in relation to the studied cultures of micromycetes are presented in table 4.

Table 4 - Characterization of antagonistic properties of strain *Pseudomonas koreensis* KaTp1-4 in relation to phytopathogenic micromycetes

Antagonistic properties	Strain of micromycetes				
	Fusarium graminearum БИМ F-601	*Fusarium proliferatum* БИМ F-602	*Fusarium sp.* БИМ F-603	*Fusarium oxysporum* БИМ F-618	*Alternaria brassicae* БИМ F-621
Size of growth and sporulation inhibition zone	*	**	***	–	**
The pattern of inhibition	I^S	I^S	I^R	–	I^R
Duration of antagonistic action	+	+	+++	–	++

Note: * - diameter of inhibition zone is in the range of 7-12 mm; ** - 13-17 mm; *** - 18-25 mm; IR - inhibits growth of mycelium; IS - inhibits sporulation of phytopathogens; zones of growth inhibition and sporulation remain for: + - 5 days; ++ - 7 days; +++ - 8 or more days.

Bacteria *P. koreensis* KaTp1-4 exhibit a high level of antifungal activity against phytopathogenic micromycetes of the genera *Fusarium* and *Alternaria*. Growth inhibition zones of mycelial fungi of genus *Fusarium* lay in the range ~ 7–25 mm, while this bacterial strain, inhibited both growth of fungal mycelium and sporulation processes and the effect lasted for 8 days or more. The antifungal effect of bacteria on micromycetes of genus *Alternaria* was expressed as suppression of mycelium growth for 7 days with diameter of growth inhibition zone ~ 13-17 mm.

4. Conclusion

Screening of twenty-nine bacterial cultures isolated from soil samples and plant material of *Solanum tuberosum*, for affiliation to genus *Pseudomonas* using PCR analysis with OpriF / OpriR genus-specific primer amplifying a fragment of the lipoprotein I gene resulted in selection of 9 isolates generation PCR product matching the size of the amplified fragment of the lipoprotein I gene. Molecular-genetic identification based on the analysis of the nucleotide sequences of the 16S rRNA gene confirmed that nine bacterial isolates belong to genus *Pseudomonas*. The selected strains were screened for the spectrum of enzymatic activity and antagonistic properties against phytopathogenic micromycetes of the genera *Fusarium* and *Alternaria*. *P. koreensis* KaTp1-4 strain possessing elevated level of antifungal activity was selected. It may potentially lay the basis for the development of biological agents to control fungal diseases in cultivars.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

References

1. Microbial Mediated Plant Functional Traits. [Electronic resource] / Maren L. Friesen, Stephanie S. Porter, Scott C. Stark and others // Friesen Annual Review of Ecology, Evolution, and Systematics. – 2011. - Vol. 42. - P. 23-46. – Mode of access: https://doi.org/10.1146/annurev-ecolsys-102710-145039 . – Date of access: 20.02.2019.
2. Dessaux, Y. Engineering the Rhizosphere. / Y. Dessaux, C. Grandclément, D. Faure // Trends in Plant Science. – 2016. Vol. 21, № 3. – P. 266-278.
3. The contribution of genome mining strategies to the understanding of active principles of PGPR strains. [Electronic resource] / J. Paterson, Ghazaleh Jahanshah, Yan Li and others // FEMS Microbiol Ecol. - 2017. – Article number: 93. – P. 1–31. – Mode of access: https://doi.org/10.1093/femsec/fiw249. - Date of access: 20.02.2019.
4. Вахитов Т.Я. Регуляторные функции бактериальных экзометаболитов на внутрипопуляционном и межвидовых уровнях: 03.00.23 защита диссертации: 20.04.07 / Вахитов Тимур Яшевич.- 2007. - 561 с.
5. Silby, M.W. Pseudomonas genomes: diverse and adaptable / Silby M. W., Winstanley C., Godfrey S. A. and others // FEMS Microbiol Rev. – 2011. – Vol. 35. – P.652–680.
6. Sitaraman, R. Pseudomonas spp. as models for plant-microbe interactions // Front. Plant Sci. – 2015. – Vol. 6, article 787. - Mode of access: https://doi.org/10.3389/fpls.2015.00787. - Date of access: 20.02.2019.

7. Gholami, A. PCR-based assay for the rapid and precise distinction of Pseudomonas aeruginosa from other Pseudomonas species recovered from burns patients / A. Gholami, A. Majidpour, M. Talebi-Taher and others // J PREV MED HYG. – 2016. Vol.57. – P.81-85.

8. Методы почвенной микробиологии / Сэги Й. – М.: Колос, 1983.– 253 с.

9. Tamás, L. Effect of carboxymethyl chitin-glucanon the activity of some hydrolytic enzymes in maize plants. / L. Tamás,

10. J. Huttová, I. Mistrík and others // Chem. Pap. – 2002. Vol. 56, № 5. - P. 326—329.

11. Shrivastava, R. Metabolism and Preferential Utilization of Phenylacetic acid and 4-Hydroxyphenylacetic Acid in Pseudomonas putida CSV86. / R. Shrivastava, H. Purohit, PS. Phale // J. Bioremed Biodegrad. - 2011. - Vol. 2:- P. 2-7.

12. Mohamed, M. Aerobic metabolism of phenylacetic acids in Azoarcus evansii. / M. Mohamed, W. Ismail, J. Heider and others // Arch Microbiol. - 2002. - Vol. 178. P. 180-192.

13. Luengo, J. The Catabolism of Phenylacetic Acid and Other Related Molecules in Pseudomonas putida. / J. I. Jiménez, Nogales, J. L. García // Handbook of Hydrocarbon and Lipid Microbiology. - 2007. - P. 1297-1325.

References in English

1. Maren L. Friesen Microbial Mediated Plant Functional Traits. [Electronic resource] / Maren L. Friesen, Stephanie S. Porter, Scott C. Stark and others // Friesen Annual Review of Ecology, Evolution, and Systematics. – 2011. - Vol. 42. - P. 23-46. – Mode of access: https://doi.org/10.1146/annurev-ecolsys-102710-145039 . – Date of access: 20.02.2019.

2. Dessaux Y. Engineering the Rhizosphere. / Y. Dessaux, C. Grandclément, D. Faure // Trends in Plant Science. – 2016. Vol. 21, № 3. – P. 266-278.

3. Paterson J. The contribution of genome mining strategies to the understanding of active principles of PGPR strains. [Electronic resource] / J. Paterson, Ghazaleh Jahanshah, Yan Li and others // FEMS Microbiol Ecol. - 2017. – Article number: 93. – P. 1–31. – Mode of access: https://doi.org/10.1093/femsec/fiw249. - Date of access: 20.02.2019.

4. Vakhitov T. Y. Regulyatornyye funktsii bakterial'nykh ekzometabolitov na vnutripopulyatsionnom i mezhvidovykh urovnyakh: [Regulatory functions bacterial ehkzometabolitov at intrapopulation and interspecific levels]: dis. of PhD in Biology sciences: 03.00.23 defense of the thesis: 20.04.07 / VakhitovTimur Yashevich.- 2007. - 561 p. [in Russian]

5. Silby M.W. Pseudomonas genomes: diverse and adaptable / Silby M. W., Winstanley C., Godfrey S. A. and others // FEMS Microbiol Rev. – 2011. – Vol. 35. – P.652–680.

6. Sitaraman R. Pseudomonas spp. as models for plant-microbe interactions // Front. Plant Sci. – 2015. – Vol. 6, article 787. - Mode of access: https://doi.org/10.3389/fpls.2015.00787. - Date of access: 20.02.2019.

7. Gholami, A. PCR-based assay for the rapid and precise distinction of Pseudomonas aeruginosa from other Pseudomonas species recovered from burns patients / A. Gholami, A. Majidpour, M. Talebi-Taher and others // J PREV MED HYG. – 2016. Vol.57. – P.81-85.

8. Metody pochvennoy mikrobiologii [Methods of soil microbiology] / Segi Y. – M.: Kolos, 1983.– 253 c. [in Russian]

9. Tamás L. Effect of carboxymethyl chitin-glucanon the activity of some hydrolytic enzymes in maize plants. / L. Tamás,

10. J. Huttová, I. Mistrík and others // Chem. Pap. – 2002. Vol. 56, № 5. - P. 326—329.

11. Shrivastava R. Metabolism and Preferential Utilization of Phenylacetic acid and 4-Hydroxyphenylacetic Acid in Pseudomonas putida CSV86. / R. Shrivastava, H. Purohit, PS. Phale // J. Bioremed Biodegrad. - 2011. - Vol. 2:- P. 2-7.

12. Mohamed M. Aerobic metabolism of phenylacetic acids in Azoarcus evansii. / M. Mohamed, W. Ismail, J. Heider and others // Arch Microbiol. - 2002. - Vol. 178. P. 180-192.

13. Luengo, J. The Catabolism of Phenylacetic Acid and Other Related Molecules in Pseudomonas putida. / J. I. Jiménez, Nogales, J. L. García // Handbook of Hydrocarbon and Lipid Microbiology. - 2007. - P. 1297-1325.