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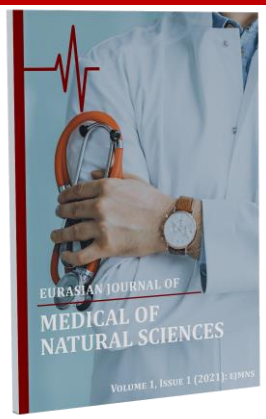

CICHORIUM INTYBUS LISOLATION OF ENDOPHYTIC MICROORGANISMS FROM PLANTS AND IDENTIFICATION OF BIOTECHNOLOGICAL POTENTIAL

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ABSTRACT

In this study, in order to isolate endophytic microorganisms isolated from Cichorium intybus L, samples were collected from 3 districts of Samarkand region: Pastdargom, Urgut, Nurabad. The results of identification microorganisms grown in nutrient media for the isolation of endophytic microorganisms from Cichorium intybus L was also evaluated. Biological potential was assessed.

Topic relevance: The symbiotic, antagonistic relations of plants and microorganisms in the world have been studied by scientists around the world, the connection between plants and microorganisms, their has Bern described various medicinal properties . It was found that endophytic microorganisms spend a certain cycle of life in plant tissues, improving the plant immune system, their broad-spectrum effect against various pathogenic microorganisms and pests. To date, the study of the chemical and microbiological composition of medicinal plants and the assessment of their biotechnological potential, the acquisition of biopreparations on their basis determines the relevance of the topic.

The purpose of the study.*Asteraceae*Isolation of endophytic microorganisms with antimicrobial and antagonistic properties from the plant Cichorium intybys belonging to the family
Identification of isolated microorganisms based on Bergy classification and MALDI TOFF.
Study of morphological, cultural and biochemical properties of microorganisms with antagonistic properties.

Determination of biotechnological potential in pharmaceuticals and agriculture from isolated strains Bacsillus cereus, Bacsillus pumilus and Bacsillus thuringiensis.

A number of tasks are identified based on the purpose of the study.

Study of morphological, cultural and biochemical properties of microorganisms with antagonistic properties.

Determination of biotechnological potential in pharmaceuticals and agriculture from isolated strains Bacsillus cereus, Bacsillus pumilus and Bacsillus thuringiensis.

To study the effect of isolated active endophytes on the growth and development of medicinal plants.

Scientific significance

The results of the study are intended to isolate endophytic bacteria from plants and assess their biotechnological potential. Various scientific approaches to the interaction of endophytic microorganisms with plants have not been elucidated fully yet .These studies are aimed at fully disclosing the interaction between "plants and bacteria".

Subject and object of research.The object of study was selected from the vegetative organs of the plant Cichorium intybus, belonging to the family Asteraceae. Bacterial isolates isolated from the surface of the vegetative organs of the plant Cichorium intybus were used as the object. A number of tools were used in the development of this technology.The subject of research is the process of isolation of endophytic microorganisms from medicinal plants, morphological and cultural characteristics of endophytic microorganisms, based on which the technology of the process of obtaining biopreparations.

ANALYSIS OF THE LITERATURE ON THE SUBJECT

Cichorium intybus L. is a perennial concrete herb of the genus Cichorium. The chemical composition of the saccharin plant. In roots and leaves: 11% inulin (polysaccharide), 4% protein, glycoside intubin - this substance gives a bitter taste, organic acids, vitamins - thiamine, ribovlafin, vitamin C. Biological healing properties: Improves have the digestive system and antimicrobial properties, improves the activity of the central nervous system, improves heart function.

Streptomyces sp was obtained from endophytic microorganisms in a growing plant in Australia, and its properties were studied, their bioactivity against gram-positive bacteria, their activity against malaria parasites [25].

Endophytic fungi from Cichorium intybus L. growing in Eastern China Xylaria sp. YX-2 is isolated. A biologically active compound identified as 7-amino-4-methylcumarin was isolated from this endophyte, and it showed significant antimicrobial activity against several food and food-degrading microorganisms. S. aureus, E. coli, S. typhia, S. typhimurium. , S. enteritidis, A. hydrophila, Yersinia sp., V. anguillarum, Shigella sp., V. parahaemolyticus, C. albicans, P. expansum, A. hydrophila, and A. niger, and it is suggested that this compound may be used [26].

In particular, the study of the composition simple endophytes growing in South Africa revealed that most of them are a group of microorganisms belonging to the genus Bacillus. A total of five endophyte compositions were identified, endophytic microorganisms morphologically were described, and various studies were conducted

to determine their species tracking. To do this, they determined that the endophytes obtained by sequencing the 16S RNA gene belonged to the Bacillus genus, two of which were Bacillus thuringiensis strain PWI-44, Bacillus pumilus strain 2XWM-ARB08, Bacillus cereus strain TR11, Bacillus cereus strain BC2]. In this case, the antimicrobial properties of saccharin showed antimicrobial properties against cultures Bacillus cereus, Staphylococcus aureus, Staphylococcus prepared from their leaves. Extracts from the root showed significant activity against Bacillus cereus and minimal activity against Staphylococcus epidermidis. It has been found also that this plant has various phytochemical compounds that confirm its antimicrobial activity and its use as a medicinal plant [22].

Cichorium intybus growing in Iran has been isolated from microorganisms such as Staphylococcus aureus, Enterococcus faecalis, A. Pseerococcus, baumannii [12]. Proof of this is the similarity of metabolites in plants [12]. Therefore, it is important to study the medicinal properties of saccharin plants and the composition of endophytes in order to facilitate the use of these medicinal properties on an industrial scale. On this basis, Pelargonium hortorum and Portulaca oleracea were isolated from a plant growing in Iran and their antagonistic relationship against Staphylococcus aureus, Acinetobacter baumanni was studied [13]. A total of seven groups of endophytes were found to dominate the Cichorium intybus and were located in various organs throughout the plant. In particular, six of them were found in the leaves of the plant and one in the branches of the plant. They can be described on the basis of micromorphological, gram staining and enzyme tests. In this case, the composition of the endophytes of the upper part of the sac was examined [14]. In addition, scientists have shown that based on the study of the antagonistic properties of the plant, it is possible to predict the types of endophytic microorganisms in it. In particular, studies to determine the antimicrobial properties of Cichorium intubus revealed that it was used against six bacteria: Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermis, Methicillin-resistant Staphylococcus aureu, Klebsiella pneumoniaeuiae Bacillus subtilis have been studied. These studies have shown that saccharin has good antibacterial properties. [15] Analysis of the endophytes of saccharin around the world shows that there are differences between the dominant endophytes of Cichorium untybus growing in different climates and affect their characteristics.

METHODOLOGY

The effectiveness of surface sterilization can be verified using a trace of a sterilized tissue sample. [44] Different plant tissues differ in nature and thickness, so the stages and concentrations of sterilizers can vary from plant to plant and tissue. The selected isolation medium differs depending on the type of isolated endophytes (fungi, bacteria and actinomycetes). [45]

№	Sterilization means, dilution and duration	Source
1.	Ethanol 96%, 30 seconds to 1 minute; NaOCl 2.5–10% Cl, 1–10 min; ethanol 70–96%, 10–30 s; washing in sterile water.	Petrini and Müller (1979), Schulz et al. (1993), Espinosa-Garcia and Langenheim (1990), Mishra et al. (2012), Verma et al. (2014)

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2.	Formaldehyde 37–40%, 1–5 min; NaOCl, 10% available Cl, 5 min.	Schulz et al. (1993)
3.	Ethyl alcohol 75–96%, 30 s, sterile	Fisher et al. (1994)
4.	Ethanol 70%, 1 min; H2O2 15–35%, 15 min .; ethanol 70–99%, 1 min; wash with sterile water.	Bissegger and Sieber (1994), Hata and Futai (1995)
5.	Ethanol 96%, 1 min; peracetic acid 0.35%, 3-5 min; ethanol 96%, 30 s	Fisher and Petrini (1990)
6.	Ethanol 75–96%, 30 s, rinse with sterile water	Fisher et al. (1986), Petrini and Fisher (1988)
7.	HgCl2 0.001–0.1%, 1–5 min; ethanol 70%, 1 min; sterile water, 1 min	C. Booth (1971), Cabral (1985)
8.	NaOCl 3–8% available Cl, 20–40 min; rinse three times with sterile distilled water	Gond et al. (2015b), Verma et al. (2017)

General microbiological, biotechnological and agronomic research methods are used in this research work. The isolated microorganisms were identified based on the Bergy classification and MALDI TOFF. Study of morphological, cultural and biochemical properties of microorganisms with antagonistic properties. Isolated strains of Bacsillus cereus, Bacsillus pumilus and Bacsillus thuringiensis were used to determine the biotechnological potential of pharmaceuticals and agriculture.

CONCLUSION DISCUSSION

The following nutrient media were used to isolate endophytic microorganisms from the common sagebrush (Cichorium intybus L).

1.CHAPAKA - The composition of the chapaka nutrient medium used to isolate endophytic microorganisms from the common cichorium (Cichorium intybus L) MgSO4 7H2O 0.5 gr, KCL 0.5 gr, NaNO3 0.2 gr, K2HPO4 1.0 gr, FeSO4 0.01 gr , Sucrose 30.0 g, Agar 15.0 g, 1 liter of distilled water pH- 7.3

2-PDA-Potato glucose agar (PDA) nutrient medium used to isolate endophytic microorganisms from Cichorium intybus L

Preparation: Dilute 39.00 g of powder in 100 ml of distilled water, heat and sterilize in an autoclave at 1.1 atm at 121 C for 15 minutes. Then 10% tartaric acid is added to pH 3.5 (1 ml of acid is added to 100 ml of nutrient medium), it is impossible to heat after the addition of tartaric acid.

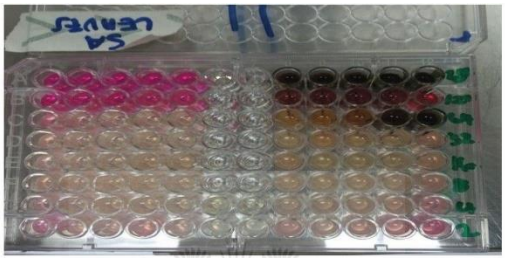

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Figure 1. Planting in nutrient media to isolate endophytic microorganisms from the common cichorium (Cichorium intybus L)

Research progress

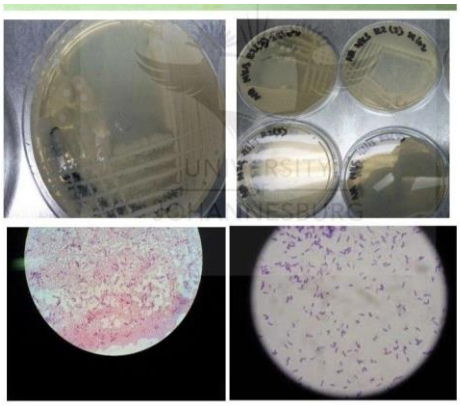
In the practical part of this study, the roots and leaf stalks of Cichorium intybus L, which grew in different climatic environments, were collected from 3 districts of Samarkand, Pastdargom, Urgut, and Nurabad. In sterilization to isolate endophytic microorganisms from

Ethanol 96% 30 C 1 min

NaOCL 2.5 to 10% was used

In the next stage, the samples were purified by repeated transplanting in 2 different media PDA, CHAPAKA, as a result of which the presence of microorganisms Bacillus cereus, Bacillus pumilis, Bacillus thuringiensis

(Root Bacsillus. Cereus, leaf extract Bacsillus cereus)



2. Identification of microorganisms grown in nutrient media to isolate endophytic microorganisms from the common cichorium (Cichorium intybus L)

The study also examined the anatomical properties of Bacillus thuringiensis

A comparative assessment revealed that the dominant strain was Bacillus thuringiensis

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>gb|KJ937088.1| Bacillus thuringiensis strain PWI-A4 16S ribosomal RNA gene, partial sequence
Length=1414
Score = 1770.7 bits (893), Expect = 0E00
Identities = 897/899 (100), Gaps = 0/899 (0)
Strand = Plus/Plus
Query 1 GTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACA 60
Sbjct 1 GTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACA 60
Query 61 CGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATA 120
Sbjct 61 CGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATA 120
Query 121 ANATTTTGAACNGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGA 180
Sbjct 121 ACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGA 180
Query 181 CCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCG 240
Sbjct 181 CCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCG 240
Query 241 ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC 300
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Query 301 AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAT 360
Sbjct 301 AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAT 360
Query 361 GAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGC 420
6
Sbjct 361 GAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGC 420
Query 421 TGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT 480
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Sbjct 481 AATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTT 540
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Sbjct 541 CTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGAC 600
Query 601 TTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGG 660
Sbjct 601 TTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGG 660
Query 661 AGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCG 720
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Sbjct 721 TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGT 780
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Sbjct 841 TACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 899

Figure 3. Results of identification of microorganisms grown in nutrient media for the isolation of endophytic microorganisms from the common cichorium (Cichorium intybus L) Bacillus thuringiensis strain

CONCLUSION

Our goal was to isolate and identify endophytic microorganisms from the common sap. Based on the results obtained, several endophytes were isolated from the roots of this plant. Their genotypic composition indicates that they all belong to the same generation of bacteria. The literature shows that these endifits have important properties for plant life.

It was also found that the importance of their secondary metabolites as biotechnological potential of endophytes is significant.

The study of their endophytic microorganisms, the phytochemical properties of endophytes secondary metabolites determine the medicinal properties of saplings and the biotechnological ability of endophytes.

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