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Antifungal Activity of Effective Straws of Endophitic Bacteria of Kochia Prostrata Plant

Shohzod Axanbayev[1]* and Z. F. Ismailov[2]

[1]*Basic doctoral student of Samarkand State University named after Sharof Rashidov, Uzbekistan*
[2]*Samarkand State University named after Sharof Rashidov, Doctor of Biological Sciences, Uzbekistan*

**Corresponding author*

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A B S T R A C T

The article presents the results of experiments on the isolation, identification and antifungal activity of endophytic bacteria of *Kochia prostrata* against plant pathogens (*Fuzarium oxysporum*, *Rhizoctonia solani*). The findings suggest that endophytic bacteria associated with the *Kochia prostrate* may be used in biological control of phytopathogens in agricultural crops in the future. These data are preliminary data on the antifungal activity of promising strains of endophytic bacteria of the *Kochia prostrata*.

Introduction

Vitamins It is known that the use of various chemical compounds - pesticides, herbicides and fungicides in the fight against phytopathogens in world agricultural practice is not only effective in protecting plants from disease, but also poses a serious threat to human health due to the release of various toxic substances into the environment. At present, the use of biological protection products is important in reducing the damage caused by plant pathogens. In this regard, endophytic bacteria living in plant tissues play an important role in increasing plant resistance to diseases by releasing extracellular hydrolytic enzymes, siderophores, lipopeptides, antibiotics, and volatile metabolites (Shurugin *et al.*, 2022; Alikulov *et al.*, 2022). The present study is aimed at studying the antifungal activity of 5 endophytic bacterial strains associated with *Kochia prostrata*, which grows in the arid regions of Uzbekistan.

Today, the protection of agricultural crops from various diseases is one of the most pressing issues. It is estimated that about 16% of the world's agricultural output is affected by various diseases (Luzmaria *et al.*, 2020). Studies by foreign and local scientists have shown that endophytic bacteria have the ability to eliminate many diseases caused by various plant pathogens (Li *et al.*, 2018; Slama *et al.*, 2019; Egamberdieva *et al.*, 2020). Studies by Ryan and others have shown that endophytic

bacteria such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Burkholderia*, which clone in plant tissues, have antagonistic activity against phytopathogens such as *Fusarium oxysporum* and *Rhizoctonia solani*.

Endophytes belonging to the genus *Pseudomonas* have been shown to produce many antibiotics in the prevention of plant diseases (Park *et al.*, 2017). P. flourescens from this generation produces 2,4-diacetylfluoroglycinol to prevent tobacco root rot (Luzmaria *et al.*, 2020). Endophytes of the genus *Bacillus* and *Burkholderia* are the most effective isolates for the control of bacterial and fungal pathogens in vitro. Some studies have suggested that endophytic bacteria play an important role in protecting plants from soil pathogens (Daniele *et al.*, 2021).

Materials and Methods

Bacteria were isolated and cultured using Priti *et al.*, 2020 and Dashti *et al.*, 2009. The extracted DNA 16S rRNA was analyzed for gene analysis by polymerase chain reaction (PCR) using the following primers: 27F 5'-GAGTTTGATCCTG GCTCTAG-3 '(Sigma-Aldrich, St. Louis, MO) and 1492 R 5'-GAATCAAGGAAG (GAAGCAAGGA AG) -Aldrich, St. Louis, MO). PCR products were tested by electrophoresis using GelRed.

ABI PRISM BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) was used for sequencing. The obtained sequences were compared with a sequence of the closest species of the genus from the National Biotechnology Information Center (NCBI) (http: //www.ncbi. Nlm.nih.gov/).

In our study, in vitro testing of 5 endophytic bacterial strains of phytopathogenic *Fusarium oxysporum* and *Rhizoctonia solane* fungi, previously selected and identified by 16S rRNA analysis, was performed. To do this, potato dextrose agar PDA (chopped potatoes, 200 g / l; dextrose, 20 g / l; agar, 18 g / l) was cut into discs and transferred to the center of the culture medium to store 5 mm of fungal phytopathogens in the nutrient medium. LB was then evaluated by measuring the inhibition distance of strains grown in agar medium by incubation in 3 corners of the nutrient medium in petri dish and grown at 28^{o}C for 7 days.

Results and Discussion

The selected strains were identified by 16S rRNA gene sequential analysis (Table 1) and the resulting sequences were registered with the National Biotechnology Information Center (NCBI) at ON567219-ON567223 (http://www.ncbi.nlm.nih.g).

Studies have shown that antifungal activity of phytopathogens varies between promising strains of endophytic bacteria of *Kochia prostrate* (Figure 1-2).

In experiments, the inhibition distance of *Bacillus amyloliquefaciens* KoPr101 strain was 26 mm relative to *Rhizoctonia solani*, 24 mm to *Fusarium oxysporum*, the *Bacillus pumilus* KoPr113strain was found to be 22 and 16 mm, the *Priestia aryabhattai* KoPr118 strain was found to be 18 and 25 mm, the *Pseudomonas putida* CoPr129 strain was found to be 25 and 27 mm, and the *Priestia endophytica* CoPr 131 strain was found to be 28 and 26 mm.

As can be seen from the images, the inhibition distance of the promising strains of endophytic bacteria of the *Kochia prostrata* against the fungus *Rhizoctonia* ranged from 18 mm to 28 mm. *Priestia endophytica* KoPr 131 strain showed high levels of antifungal activity against this fungus, and *Priestia aryabhattai* KoPr118 strain showed minimal levels.

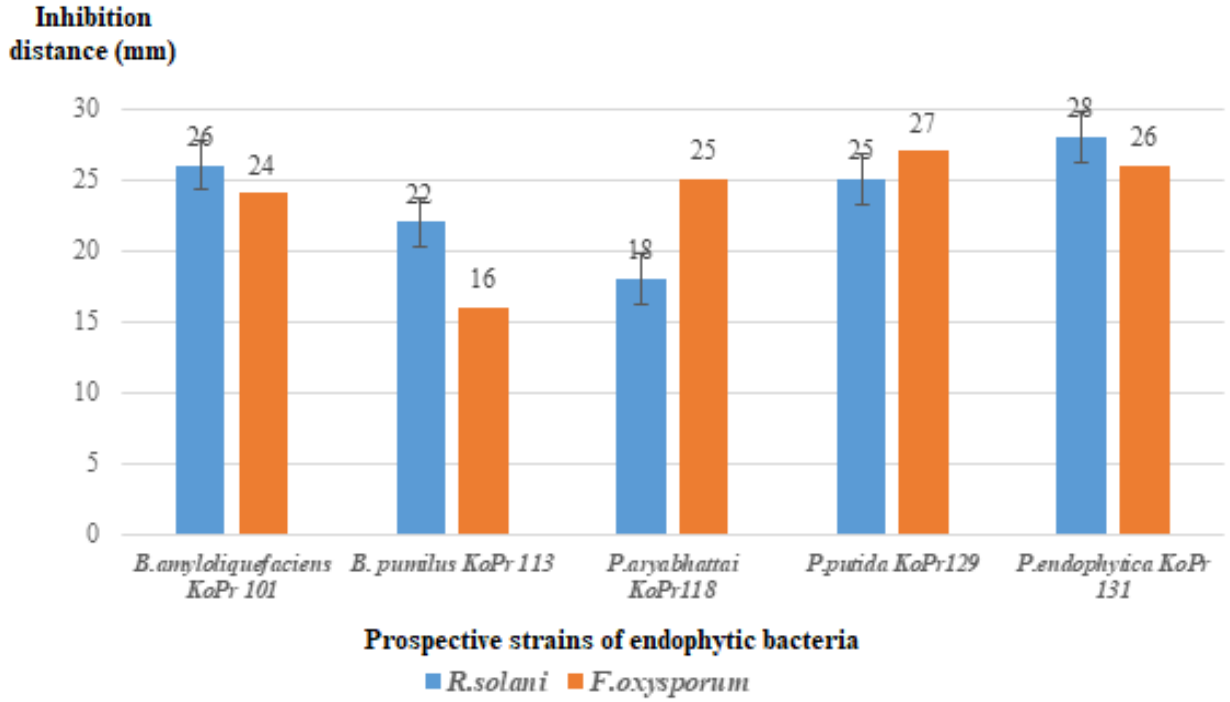
Prospective strains of endophytic bacteria of the *Kochia prostrata* ranged from 16 mm to 27 mm against *Fusarium oxysporum*. The high level of antifungal activity against this fungus was shown by the strain *Pseudomonas putida* KoPr129 and the minimum level was shown by the strain *Bacillus pumilus* KoPr113.

Table.1 The effective plant growth promoting endophytes isolated from *Kochia prostrata* and their closest relatives from GenBank

Isolated strains deposited to GenBank			Closest match (16S rRNA genes) (GenBank)		
Strain	**Length (bp)**	**Accession number**	**Reference strains**	**Accession number**	**Percent identity**
KoPr101	1448	ON567219	*Bacillus amyloliquefaciens*	LN864483.1	99.72
KoPr113	1479	ON567220	*Bacillus pumilus*	AB212862.2	99.66
KoPr118	1467	ON567221	*Priestia aryabhattai*	MG757377.1	99.73
KoPr129	1484	ON567222	*Pseudomonas putida*	MW522571.1	99.73
KoPr131	1479	ON567223	*Priestia endophytica*	OM960594.1	99.59

Fig.1 In vitro screening for antifungal activity of endophytic bacterial strains of Kochia prostrata against phytopathogens

Fig.2 Antifungal activity endophytic bacterial strains of *Kochia prostrata* against some phytopathogens



Among the promising strains of endophytic bacteria of the *Kochia prostrata* plant, the antifungal activity of *Priestia endophytica* KoPr 131 and *Pseudomonas putida*KoPr129 in was stronger than other strains.

Prospective strains of endophytic bacteria of the *Kochia prostrata* range from 18 mm to 28 mm relative to the *Rhizoctonia solani* and from 16 mm to 27 mm relative to the *Fusarium oxysporum*. These data suggest that the endophytic bacteria of the *Kochia prostrata* have antifungal activity against phytopathogenic fungi.

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