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Effects of bacterial isolates separated from the rhizosphere of halophytic plants on plant growth

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Abstract. The plant development is affected by various factors such changes in soil and plant microflora, and the use of more chemical pesticides. The adaptation of halophytes to the harsh environment may be facilitated by the pertinent characteristics of microorganisms that reside in the intercellular space of the tissues of salt-tolerant desert plants. In addition to solving the primary issue of comprehending their function in adaptation, research on the rhizosphere bacteria of salt-tolerant plants is practically significant for the production of biofertilizers that support the growth of agricultural crops in saline soils. In this paper, plant growth-promoting rhizobacteria (PGPR) are in close association with roots to enhance host plant adaptation to their environment and the relative abundance of *Arthrobacter, Bacillus, Massilia*, and *Devosia* in the rhizosphere and *Bacillus, Oceanobacillus*, *Lactococcus*, and *Massilia* in roots during tillering and ripening stages and these genera are described as important rhizobacteria. 70 bacterial isolates were isolated from the rhizosphere of different plants growing in saline soils. 22 isolates were identified as salt-tolerant based on their growth characteristics in feeds with 15-20% NaCl and were selected as a result of one-step screening. Antifungal activity of 22 selected bacterial isolates against pathogenic fungi was identified. It was found that isolates 5, 13, 3.1, 34.1, 19, 10, 4 and 30 are more active than others and have a better ability to inhibit pathogenic fungi.

1 Introduction

Soil salinity is a serious environmental issue covering over 1 billion hectares of land worldwide [1]. It makes up over 25% of arable lands, and approximately 50% of irrigated land suffers from human-induced salinization to varying degrees [2]. Soil salinization is an escalating issue across various climatic zones, significantly impacting agricultural productivity and environmental health [3]. However, its impact on irrigated agriculture is devastating in arid and semi-arid areas [4, 5, 6]. Soil salinity negatively impacts on 120.8 million hectares in the Commonwealth of Independent States (CIS) [7]. Uzbekistan, as a

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member of the CIS, has more than 50% salt-affected areas. According to statistics, weakly saline soils 30%, moderately saline soil 16% and highly saline soils 4% [8].

Microorganisms living in the intercellular space of the tissues of salt-tolerant desert plants also have relevant properties, which may be involved in the adaptation of halophytes to the stressful conditions of their habitat. At the same time, the study of rhizosphere microorganisms of salt-tolerant plants is of practical importance for obtaining biofertilizers that promote the growth of agricultural crops in saline lands, but also solves the main problem of understanding their role in adaptation.

A number of scientific research aimed at accelerating the growth and development of medicinal plants by using rhizobacteria, protecting them from various external influences, diseases, and improving the quality of the grown crop was conducted by a number of foreign scientists J.K. Vessey [9], V.N. Conducted by Hemavathi [10], Ch. Hongda [11], A. Fatma [12], M. Yadegari [13] and others. Plant microbiota can originate mainly from the soil and to a lesser extent from the air sphere [14]. Beneficial bacteria can also protect plants against pathogens directly, such as production of antimicrobial compounds, competition for nutrients and colonization sites, or indirect systemic resistance mechanisms. [15, 16]. First, microbial communities do not form randomly [17, 18].

Research is currently being conducted on rhizobacteria found in the roots of Ferula tadshikorum Pimonov plants [19, 20, 21]. In this regard, phyllosphere communities exhibit strong and reproducible consistent dynamics and are mainly colonized by bacterial members of the phyla Proteobacteria, Actinobacteria, and Bacteroidetes. [22, 23]. Rhizosphere bacteria of the genus Pseudomonas have been most studied for their ability to synthesize indolyl-3-acetic acid, which is known to stimulate plant root development. Studies of IAA synthesis in Pseudomonas PGPR indicate the possibility of obtaining genetically modified strains of Pseudomonas PGPR capable of increasing IAA synthesis [24]. Different composition and components of root secretion can serve as attractants for plant pathogens. The growth of root rhizosphere bacteria (including mycorrhizal fungi) necessarily reduces the amount and diversity of organic matter released by the roots, resulting in reduced susceptibility to plant pathogens [25]. Many antibiotics are produced by rhizobacteria, including phenazine, carboxylic acid, diacetyl phloroglucinol, oomycin A, pyocyanin, pyrroles, pyoluteorin, pyrrolnitrine, iturin A, surfactin, and others [26, 27]. Phytopathogens also produce their own siderophores, but unlike pseudomonad siderophores, they bind to iron ions much more slowly. Therefore, Pseudomonads dominated the competition with phytopathogens for the essential iron element. In summary, binding of iron to pseudomonad siderophores resulted in limiting phytopathogen proliferation and improved plant growth [28, 29]. Bacillus sp ST-PGPR strain improved maize root growth and development through accumulation of proline and soluble sugars under drought and salinity [30]. Many studies have shown that siderophores are found in plant-associated bacterial strains. Notably, bacteria belonging to the genus Enterobacter and Burkholderia found in roots are siderophore-producing bacteria.

2 Materials and methods

Average soil sample was prepared by mixing individual samples. Areas up to 100 m^2 are sampled from three points, areas larger than 100 m^2 are sampled from 5 points (envelope method), and areas larger than 1 hectare are sampled from 15 points. The top 2 cm layer of plowed land is removed, and the genetic horizons of non-plowed land are sampled using a sterilized shovel or knife. Soil samples are placed in previously prepared sterile and resealable glass jars or sterile paper bags, and these containers are labeled with the place of sampling, horizon, date of collection, etc. The analysis of samples is carried out during the first day. If necessary, they can be stored in the refrigerator for two days. The analyzed soil is thoroughly mixed following the rules of asepsis and antiseptics, plant roots and other objects are removed from it.

To separate the above-ground part (phyllosphere) and underground part (rhizosphere) of the plant, as well as soil bacteria, the plant tissues were crushed and placed in a 1.5 ml test tube. 1 ml of phosphate salt buffer (NaCl - 0.137 M, KCl - 0.0027 M, Na2HPO4 - 0.01 M; KH2PO4 - 0.0018 M; pH=7.4) was added to test tubes and mixed in a vortex apparatus. At the next stage, the test tubes were diluted in nutrient agar (0.5% peptone, 0.3% beef extract, 1.5% agar, pH=6.8) at levels 101, 102, 103, 104, 105, 106 and planted with a sterile glass spatula. Inoculated Petri dishes were placed in a thermostat at 250 C for 24 hours. Each single bacterial colony was separated according to its morphological state and pure cultures were isolated. Pure culture colonies were selected based on colony color in the randomization condition. In this case, one Petri dish was divided into 24 parts, each pure bacterial culture was planted in it, incubated in a thermostat and placed in a refrigerator for long-term storage.

3 Results and discussion

In Uzbekistan, halophyte plants have a variety, they are well studied, a source of nutrients, bioremediants, objects of landscape design, and a source of useful substances (oil, medicinal compounds, etc.). In recent years, studies of the rhizosphere and microorganisms of salt-tolerant plants and their role in salinity adaptation of halophytes have begun to emerge.

Currently, by studying the microflora of the soil of the island region, the interaction of microorganisms in the microflora is considered important in the formation of the ecosystem there. Studying antagonistic properties of antagonistic bacteria against plant pathogenic fungi is one of the urgent issues for agriculture. Plant fungal pathogens are one of the important negative factors that cause serious damage to agricultural crops and reduce productivity. Biological control of fungal pathogens is a suitable alternative to chemical control and has no negative impact on the environment. The study of microbiological activity in saline arid soils showed that an increase in soil salinity is accompanied by a decrease in the biomass of microorganisms and an increase in their metabolic rate at the same time as their respiration. Soil salinity also results in a decrease in the C/N ratio of microbial biomass, indicating the predominance of bacteria in saline soils. Therefore, we started our research by looking for samples, for this, soil samples were taken from the rhizosphere part of local halophyte plants Aeluropus littoralis, Climacoptera crassa, Halocnemum strobilaceum, Suaeda heterophylla species growing in saline lands from the vegetation area of the island in order to study halotolerant rhizosphere microorganisms.

According to the literature, halophyte plants are divided into 4 groups according to the level of salt resistance: hyperhalophytes, euhalophytes, hemigalophytes, haloglycophytes. In this context, Climacoptera crassa and Halocnemum strobilaceum belong to hyperhalophytes because they can grow in high salinity soils. High salt concentration in the soil is the most serious abiotic factor that negatively affects the growth and development of crops. Salt-induced disturbances are observed at all stages of plant growth, and many physiological and biochemical parameters are vulnerable to NaCl, such as water status, photosynthetic capacity, mineral nutrition, stomatal closure, protein synthesis, lipid metabolism, and membrane integrity. Several biotechnological methods have been used to increase salt tolerance of plants, but these methods are both time-consuming and expensive. In this regard, various technologies are increasingly used to increase the number of microorganisms in the rhizosphere of plants.

The rhizosphere is a relatively nutrient-rich environment, including amino acids, sugars, fatty acids, and other organic compounds. In plants, roots act as a link between them and the soil, when salt stress occurs, the osmotic pressure of the soil solution is higher than that of the plant cells, and therefore the plant cannot get enough water. Root system, i.e., root length, root diameter, number of roots, number of root hairs, xylem vessel diameter, root bark width and suberin deposition are negatively affected under salt stress. Research was conducted on the isolation of salt-resistant rhizosphere bacteria from selected local halophyte plants. Different solutions of NaCl salt were used for the isolation of rhizosphere bacteria, experiments were carried out in the amount of 5% and 20%. 70 rhizosphere bacteria were initially isolated by multiple re-inoculations on meat peptone agar, peptone agar and potato dextrose agar media. Studying the ability of isolated bacteria to grow under salt stress of 5% to 20% NaCl showed that most of the isolates had unchanged growth characteristics in 5% and 10% salt media, therefore, high salt concentrations inhibited the growth of bacteria to one degree or another. inhibited, including 25 bacteria stopped growing, and 45 isolates (about 80%) were found to have the ability to grow stably in saline media. In general, 3 times more isolates were isolated from 5% NaCl medium than from 10% NaCl medium. It can be seen that a large number of isolates were isolated from the rhizosphere of Climacoptera crassa and *Halocnemum strobilaceum* (23 and 18, respectively), and 14 and 15 isolates were isolated from Aeluropus littoralis and Suaeda heterophylla, respectively. The results show that out of 50 isolated isolates, 5 and 10% salt can grow, and 30 isolates can grow in 15% salt. 20 isolates showed signs of growth in 20% NaCl feed, only 20 bacteria showed stable growth: these bacteria were studied. (shown in Table 4) Characterization of bacteria and their level of halotolerance were analyzed after 4 days of inoculation of bacterial strains. The apical region of roots grown under saline conditions is characterized by extensive vacuolization and absence of typical apical tissue organization. Epidermis and bark parenchyma cells shrink under the influence of salt. Salt stress inhibits root growth, prevents cell division and elongation. Mineral nutrition of plants affects the ability to adapt to acute stress. Imbalance of nutrition hinders the growth and development of plants. The morpho-cultural characteristics of the isolated bacteria were studied, and 70 strains were identified based on colony growth, color, and observation under a microscope. In order to isolate bacteria showing antifungal properties from plant phyllosphere and rhizosphere and soil bacteria, half nutrient agar and potato dextrose agar (potato starch 4 gm L-1, dextrose 20 gm L-1, and agar 15 gm L-1, pH 5.6) was mixed. For this purpose, 15 economically important pathogenic fungi stored in the collection of the Institute of Genetics and Experimental Plant Biology of the RFA were propagated on potato dextrose agar, and fungal mycelium with a diameter of 5 mm was cut from them and placed in the middle of the above mixed agar petri dish and placed in the nutrient medium at a distance of 2.5 cm from its four sides. planted It was cultured with bacteria and placed in a thermostat. Antagonistic properties of bacteria were determined by inhibiting the growth of mycelium of the fungus and measuring the distance between them. Investigation of the stimulation of isolated bacteria to the germination and root and stem growth of wheat and cotton seeds in Petri dishes with filter paper at different NaCl salinities. Soil samples were taken in sterilized test tubes from the vegetation cover soil in saline lands throughout the island. Microflora in suspension were grown in peptone nutrient medium with different concentrations of NaCl at 30 °C for 1-2 days and pure cultures were obtained. Pathogenic fungi were grown in KDA medium at 25-26 °C for 7 days. Representatives of the pathogenic fusarium family: *F. oxysporum* f.sp. Vasinfectum, F. graminearum, was obtained from the collection of Phytopathogenic and other microorganisms unique scientific object of the Institute of Genetics and Plant Experimental Biology.The antagonistic effect of the isolates was evaluated by dual-culture nutrient medium assay [31].

Dual-culture medium was prepared by mixing KDA and nutrient agar (OA) in a 1:1 ratio. A 5 mm diameter disk cut from the pathogenic fungal culture was placed in the center of a petri dish with dual-culture medium, and the bacterial isolates were seeded in 4 places at a distance of 2 cm from the center.

3.1 Morphological characteristics of selected bacterial isolates and their effect on plant growth.

22 isolates selected as a result of one-step screening isolated from the rhizosphere of plant cover throughout the island were tested for their antagonistic properties against these two fungi. The morpho-cultural characteristics of 22 selected isolates were studied in more detail. When 22 salt-resistant strains were selected and their morpho-cultural characteristics were studied under a light microscope, it can be seen that they are mainly composed of single or chain rod-shaped cells, and it was observed that most of them belong to the *Bacillaceae* and *Pseudomonadaceae* families. Bacteria create physiologically active substances for plants, break down residual substances and, in turn, affect higher plants. Rhizosphere bacteria use substances from plant roots. Epiphytic microflora bacteria are found in the leaves and branches of higher plants. Among them, the most common representative is Vast herbicola.
Currently, the use of microorganisms and the products they produce are increasingly used in agriculture, industry, medicine, and pharmaceuticals. In addition, the use of chemicals against pests in the cultivation of agricultural products causes serious damage to the composition of fruits and vegetables, which directly affects human health. In the first stage, the effect of selected isolates on cotton and wheat was studied. Microorganisms are the most diverse organisms found in nature, and the strains with the best properties are selected and used as biological control for plant protection against pests, diseases, and cultivation. Rhizobacteria help improve stress tolerance by enhancing plant growth; stimulating the production of phytohormones, siderophores and soluble phosphates; reducing the level of ethylene; and regulation of dehydration response and antioxidant gene expression. In this experiment, secondary metabolites can be used in plant cultivation as an additional mechanism used by microorganisms against abiotic stress. When we tested our 22 isolates on the growth and development of wheat plants, the experiments were carried out in pots, and 6 wheat seeds per pot were treated with bacteria before planting. Isolate No. 4 was more active than the others and had a significant effect on wheat germination. All the wheat seeds germinated and when germination was observed for 5 days, 1st day 4.76cm, 2nd day 7.71cm, 3rd day 10.6cm, 4th day 12.2cm and 5th day 14, It was 4 cm. Isolate number 3.1 followed with 5.81 cm on day 1, 7.33 cm on day 2, 9.78 cm on day 3, 11.9 cm on day 4 and 12.6 cm on day 5. did In addition, isolates 5, 10, 13, 19, 30, 34.1 gave twice higher results compared to the control (Table 1).

Table 1. Effect of rhizosphere halophyte microorganisms on wheat plant growth

Wheat plant growth was calculated in cm							
	Number of isolates	Day 1	Day 2	Day 3	Day 4	Day 5	Germinatio n of seeds
1.	1	1,93	2,8	3,35	3,95	4,28	4
2.	2	1,80	2,78	3,13	3,85	5,16	4
3.	3,1	5,81	7,33	9,78	11,9	12,6	6
4.	3,2	2,96	5,53	6,1	8,10	11,2	6
5.	4	4,76	7,71	10,6	12,2	14,4	6
6.	5	2,06	5,11	6,51	8,18	11,0	6
7	5,1	2,11	3,56	6,40	7,56	9,88	6
8.	10	2,63	6,48	6,9	9,78	16,0	5
9.	11	1,56	3,53	4,33	5,30	6,96	3
10.	13	1,86	4,5	5,15	5,45	8,95	6
11.	15	2,33	5,58	6,73	8,48	10,8	6

12.	16	0,80	1,67	1,96	2,48	3,33	3
13.	17,1	0,45	0,66	0,70	0,87	1,41	3
14.	19	4,03	5,28	8,88	9,96	12,2	6
15.	25	4,88	5,05	7,46	9,38	10,9	6
16.	26	0,31	0,66	1,25	1,46	3,41	4
17.	30	2,63	3,91	4,40	6,0	9,06	5
18.	31,1	0,51	1,80	2,15	2,36	4,03	3
19.	36	0,18	1,9	2,96	3,15	5,7	4
20.	37	3,03	6,51	7,16	7,66	9,66	4
21.	39	2,56	3,75	3,93	4,05	6,46	5
22.	50	2,21	5,83	6,76	8,40	10,8	6
23	Control	1,80	1,80	3,03	3,95	5,31	5

Acceleration of plant growth under the influence of hormones produced by bacteria helps to form a larger area of leaves, which leads to increased evaporation of water from their surface and can reduce water content. Therefore, it was important to study the effect of rhizosphere bacteria on plant growth and water exchange. The ability of rhizosphere bacteria to synthesize plant hormones is an important mechanism for stimulating plant growth under their influence. In our experiments, an increase in the level of hormones in plants under the influence of bacteria can stimulate their growth. Given the role of these pigments in photosynthesis, increased chlorophyll levels should significantly contribute to plant biomass accumulation and may explain the superior growth-promoting ability of *B. subtilis* IB 22 (Fig. 1).

Fig. 1. Effect of rhizosphere halophyte microorganisms on wheat plant growth

Changes in the composition of root exometabolites during the growing season of a plant have been shown to lead to the predominance of microorganisms associated with different ecological strategies at different stages of its development. At the beginning of the growing season (spring and first half of summer), r-strategists (non-spore-forming rhizobacteria) dominate in the rhizosphere, and at the end of the season (second half of summer and autumn).

During the growth of new roots, exudates secreted by the elongation zone near the end of the root support the growth of colonizers that mainly use easily digestible sugars and organic acids. In older root zones, rhizodeposits are mainly released as exfoliating cells and consist of more difficult-to-digest materials, including lignocellulose and hemicellulose, so these zones are home to fungi and bacteria adapted to oligographic conditions. Affects the lateral root germination zone, which is relatively rich in food sources.

As a result of studies carried out under conditions simulating the wheat rhizosphere, it was found that bacilli consume less wheat root exudates as a sole source of carbon than pseudomonads. This indicates that Pseudomonas is more dependent on the studied plants. The growth of Pseudomonas bacteria in the rhizosphere of diploid wheat lines is more intense than in the rhizosphere of hexaploids, which is related to the specific characteristics of the quantitative and qualitative composition of root exometabolites of these plants. As a result of many biocontrol experiments with antibiotic-producing rhizobacteria, it has been shown that their effect on various phytopathogens is specific and depends on the type of plant affected by this pathogen. Our task was to determine which substances are responsible for the antifungal activity of the studied strains of Pseudomonas and Bacillus and to determine the role of root secretions in the production of these metabolites. We studied the effect of the selected isolates not only on wheat, but also on cotton, as a result, four seeds were planted in each pot. (Fig. 2).

Fig. 3. Effect of rhizosphere halophyte microorganisms on cotton plant growth

The application of nitrogen fertilizers is one of the most important agricultural practices that has contributed to the growth of global crop production over the past half century. Previous studies have shown that excess N fertilization above a certain threshold value does not increase crop productivity, but can cause large N losses and cause a number of environmental problems.

Because of the important function of bacteria and fungi in nitrogen metabolism in the root zone, the responses of root-associated microbiomes to N fertilization need to be comprehensively studied, especially N uptake in relation to root exudation, which is very important for the plant.

Table 6. Effect of rhizosphere halophyte microorganisms on cotton plant growth

Cotton plant growth was calculated in cm								
	Isolates	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.	1	1,70	1,33	1.55	-	-	-	-
2.	2	2,86	3,65	4.88	4,71	4,91	5,25	5,80
3.	2,1	-	-	-	-	-	-	-
4.	3,1	3,33	4,45	4.81	5,88	6,16	6,83	7,41
5.	4	4,43	5,26	5.8	6,09	6,36	6,73	7,08
6.	5	4,40	4,85	5.4	6,38	6,50	6,60	6,93
7.	5,1	4,11	4,78	5.66	5,86	6,11	6,06	6,41
8.	10	5,4	6,01	6.48	6,55	6,75	6,98	7,56
9.	13	4,45	5,48	5.9	6,41	6,73	6,93	7,63
10.	15,1	2,95	4,46	5.28	5,58	4,9	6,36	6,76
11.	15,2	4,30	4,66	5.1	4,76	4,96	5,06	6,26
12.	16	2,80	3,31	4.76	6,16	6,41	7,08	7,53
13.	17,1	2,75	2,23	3.55	3,96	4,35	4,73	5,11
14.	17,3	-	-	-	-	-	-	-
15.	19	3,63	4,02	4.66	1,90	4,78	4,85	5,18
16.	19,1	-	-	-	-	-	-	-
17.	25	5,36	6,70	7.33	7,9	8,35	8,65	9,10
18.	30	0,25	0,56	0,75	1,33	1,58	1,90	2,58
19.	31,1	0,58	1,06	1.18	3,30	2,78	2,91	3,28
20.	34,1	2,45	4,38	5.18	5,36	5,30	5,83	6,01
21.	36	1,33	2,86	3.55	3,96	3,96	4,26	4,90
22.	50	3,83	4,40	5.06	5,25	5,48	5,83	6,31
23.	Control	1,26	2,15	4.06	3,15	3,33	3,41	3,81

Rhizosphere Microorganisms These root-associated microbiomes play an important role in plant nutrition and productivity. It is known that the increase of synthetic fertilizers in our country's agricultural land in the last year has not only increased the yield, but also led to environmental problems, but we do not have a complete understanding of how the crops with high content of nutrients shape the root-associated microbial communities. When we conducted research in cotton, it was very important to determine the amount and composition of root metabolites and exudates.

Our results show that plant growth status and N input alter the microbial community composition in the root zone of cotton and wheat. Plant growth stage had a stronger effect on bacteria than on fungal community composition. A number of bacterial genera characterized as plant growth-promoting rhizobacteria responded positively to normal fertilization and their abundance was significantly correlated with organic acid levels, suggesting that organic acid secretion may be an evolved strategy by plants. To deal with high N levels, beneficial microbes need to be recruited in the root zone. These results provide new insight into associations between increased N availability, changes in carbon availability, and shifts in plant roots and rhizosphere microbial communities of intensive agricultural ecosystems.
Plant growth-promoting rhizobacteria (PGPR) are in close association with roots and can enhance host plant adaptation in their environment. In this study, the relative abundance of Arthrobacter, Bacillus, Massilia , and Devosia in the rhizosphere and Bacillus, Oceanobacillus , Lactococcus, and Massilia in the roots were higher at the jointing and ripening stages than at the treatment stage, and these genera were described as important rhizobacteria.

4 Conclusion

70 bacterial isolates were isolated from the rhizosphere of different plants growing in saline soils. 22 isolates were identified as salt-tolerant based on their growth characteristics in feeds with 15-20% NaCl and were selected as a result of one-step screening. Antifungal activity of 22 selected bacterial isolates against pathogenic fungi was determined. It was found that isolates 5, 13, 3.1, 34.1, 19, 10, 4 and 30 are more active than others and have a better ability to inhibit pathogenic fungi. Including 13 isolates of *F. graminearum* and *F. oxysporum* f.sp. Vasinfectum fungi were inhibited by 33.0 and 33.5 mm respectively, followed by 30 isolates at 27.63 and 27.5 mm, and thirdly by 34.1 isolates at 24.5 and 26.2 mm. The effect of selected bacterial isolates on the growth of wheat and cotton plants under saline conditions was studied. When our 8 isolates were applied to wheat and cotton roots, isolates number 4, 5, 10, 30, 34.1 had the best effect on plant root development. The morphological and cultural characteristics of the selected most active bacteria were studied and identified by the MALDI-TOF method. As a result, *Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Pseudomanas puti 1, Pseudomanas puti, Cryptococcus neoformans, Bacillus cereus* strains were found to be the most active.

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