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Isolation and study of plant growth promoting rhizobacteria from Triticosecale Wittmack growing in Almaty region

Abstract. This article presents data on isolation and characterization of native plant growth-promoting rhizobacteria (PGPR) from the rhizosphere and root-endosphere of X Triticosecale Wittmack growing in Almaty region. A total of 22 distinct (eighteen gram-negative and four gram-positive) bacterial isolates were identified, classified according to their spore-forming activity: eighteen non-spore-forming and four spore-forming bacteria. Bacterial isolates were screened *in vitro* for PGPR characteristics and evaluated for their beneficial effects on the early growth of triticale variety X Triticosecale Wittmack. The findings of our research indicated isolation of four growth-promoting bacteria (AS4, AS6, AR1, TS6), three antifungal bacteria (AR2 and AS7), and one strain of combined action, i.e. high growth-promoting and fungistatic activity (AR6). Research has demonstrated that PGPR can be utilized as biofertilizers for crop production. Study marks the beginning of a larger investigation into the bacterial diversity of the triticale variety X Triticosecale Wittmack in the Almaty region.

Key words: root-endosphere, rhizosphere, rhizobacteria, PGPR, growth stimulation.

Introduction

Chemical fertilizers are widely used all over the world for delivery of indispensable nutrients to the soil-plant system. However, it is known that the price, availability and environmental impact of chemical fertilizing, especially nitrogen and phosphate fertilizing, especially nitrogen and phosphorus fertilizers, cause significant problems in modern agriculture [1]. Thus, there is an urgent need to identify alternative strategies that guarantee the yield of competitive crops and ensure ecological security, long-term environmental balance and sustainability within the framework of the agro-ecosystem.

Plant growth-promoting rhizobacteria (PGPR) are free-living soil microbes that aggressively colonize the rhizosphere and the roots of plants and, when applied to seeds or plants, enhance growth and yield of the plant. They have the ability to synthesize plant growth regulators. i.e., phytohormones, including indoleacetic acid (IAA), cytokinins, and gibberellins [2]; realize the possibility of strengthening the nitrogen (N_2) fixation process [3]; are able to decompose inorganic phosphate and mineralize organic phosphorus and other nutrients [4]; can synthesize siderophores necessary for plant metabolism; are able to synthesize antibiotics, enzymes and fungicidal compounds and develop antagonistic effect against phytopathogenic microorganisms due to competition with harmful microorganisms [5].

Positive effects of rhizobacteria can be observed in a multitude of ways in various crops [6-8]. For example, one of the most crucial plant growth-promoting effects of *Pseudomonas* was observed in a notable enhancement of the root biomass (20-46%) [7]. Use of *Azotobacter* as a biofertilizer has been shown to reduce the amount of chemical fertilizer required per hectare by 21-31 kg of nitrogen per hectare [8].

Understanding the indigenous bacterial population, its characteristics, ability to identify native bacteria in a given location is crucial for understanding how they are distributed and their diversity in the rhizosphere of specific crops [9, 10]. Microbial strains that are specific to a particular region can be employed as a growth-promoting inoculum in order to achieve the desired yield. Triticale is a multipurpose crop used for forage,

grain, and ethanol production. The grain can be used for human consumption or animal feed [11, 12].

Materials and methods

In September 2022, the isolation of rhizobacteria from agricultural cereal crop rhizoplane communities was conducted. The study focused on the isolation of rhizospheric bacteria from the rhizosphere of cultivated cereal plants, specifically rye and wheat amphidiploid (X Triticosecale Wittmack). The triticale samples were obtained from the Kazakh Scientific Research Institute of Agriculture and Plant Production, located in the Karasay district of Almaty region. The plants were uprooted and a clod of soil was removed, after which they were placed in clean plastic bags and transported to the laboratory.

Agrochemical soil analysis. The preparation of the analyzed samples involves weighing them on a balance with an accuracy of ±0.0001 g. Prepared samples are then placed in the chamber for irradiation by the X-ray fluorescence spectrometer, ensuring that they are not contaminated [13]. The instrument used in this analysis was the Focus M at the Faculty of Chemistry and Chemical Technology of al-Farabi Kazakh National University. The resulting spectrum of intensities of the characteristic fluorescence of the elements being determined is processed using software according to pre-installed calibration, content of macroelement oxides, expressed in mass percent, and the content of trace elements, expressed in mg/kg of the sample, are estimated [13]. Each sample is subjected to analysis once, with the sample placed within the X-ray fluorescence spectrometer irradiation chamber. The results are then averaged.

Isolation of rhizosphere bacteria. The isolation of microorganisms was conducted as follows. A soil suspension was prepared to isolate PSBs from a dilution cascade. For this purpose, 10 g of rhizosphere soil were suspended in 100 mL of sterile phosphate-buffered saline (PBS) solution (pH 7.2) and shaken at 190 rpm for 45 minutes [14]. Subsequently, serial dilutions were prepared to 10^{-9}. Approximately 100 µL of each dilution was placed on sterile Luria Bertani (LB) agar medium (Himedia, India). The medium was prepared by dissolving 10 g tryptone, 5 g yeast extract, 5 g NaCl, and 20 g agarose in 1 000 ml of distilled water. Subsequently, the pH of the medium was adjusted to 7.0. LB agar plates were incubated for 3 days at 28 °C. Single colonies were picked up and streaked on sterile LB agar plates to obtain pure cultures. Well-isolated colonies were observed for morphological characterization.

Isolation of endophytic bacteria. To isolate endophytic bacteria, the roots of the sample were thoroughly washed with running tap water for 10 min to remove adherent soil particles. The roots were disinfected with 70% ethanol for 1 min and then washed three times with sterile distilled water. Then the roots were surface sterilized with 3% sodium hypochlorite solution for 10 min and washed six times with sterile distilled water [16]. Then 1 g of surface sterilized root tissue was macerated with a sterilized mortar and pestle in 10 ml phosphate buffered saline (PBS), serial dilutions were prepared and 1 ml of tissue extract and diluents were applied to LB medium [17]. The plates were incubated at 28°C and monitored for the development of bacterial colonies within 2-3 days.

Fungistatic activity of isolated bacteria. The fungistatic activity of isolated bacteria was determined on Sabouraud's medium. The phytopathogenic fungus *Fusarium graminearum*, the causal agent of Fusarium ear blight, was selected as the test organism. The antifungal effect was quantified by measuring the width of the inhibition zones around the wells over a period of 4-5 days [18].

Estimation of IAA phytohormone content. The quantity of indole-3-acetic acid (IAA) produced by microorganisms was quantified using a colorimetric method with Salkowski's reagent. 1 ml of the supernatant (filtrate) was combined with 2 ml of Salkovsky reagent, which is a solution composed of 1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% HCl [19]. The color development time was approximately 30 to 40 minutes. The optical density of the colored samples was quantified on a spectrophotometer at a wavelength of 540 nm. The control was an uninoculated medium to which a reagent was added. The concentration of IAA was determined according to a calibration curve constructed in the concentration range of the substance of 10^{-8} to 10^{-2} g/l. IAA content was expressed in µg/ml.

Determination of growth-promoting activity was developed via an inoculation of triticale seeds. The impact of bacterial inoculation on the growth of triticale was evaluated through an experiment conducted in accordance with the conditions of the laboratory setting. The triticale seeds were subjected to surface sterilization with a 2% sodium hypochlorite solution for a period of 15 minutes, after which they were washed five times with sterile water. Subsequently, the inoculated seeds were transferred to test tubes containing the relevant strains. A series of cultures were established by inoculating 50-ml Falcon tubes with 25 ml of LB broth and maintaining

them at 200 rpm for 16 hours on a shaker. Subsequent to this period of growth, the cultures were inoculated onto water agar plates in order to permit germination of the seeds. The control consisted of seeds that had not been inoculated. After 14 days, the length of the shoots and roots was recorded [21].

Results and their discussion

Agrochemical soil analysis. The results of quantitative analysis of the content of trace elements in the soil adjacent to the triticale variety X Triticosecale Wittmack are presented in Table 1.

Table 1 – Trace elements in the soil of X Triticosecale Wittmacktriticale variety

No.	Elements	Concentration, %	Intensity, %
1	Ca	8.928	18.17
2	Ti	1.738	10.23
3	Mn	0.653	718.43
4	Fe	44.781	17.81
5	K	6.837	1.79
6	Si	26.969	0.18
7	Al	9.247	0.47
8	Cr	0.030	3.63
9	Zn	0.342	1.54
10	Sr	0.164	2.95
11	Rb	0.295	3.00
12	V	0.045	18.17

Table 1 indicates that iron (Fe), silicon (Si), aluminum (Al), potassium (K) and calcium (Ca) are prevailing in soil adjacent to X Triticosecale Wittmack.

Isolation of rhizosphere bacteria. Pure cultures of the bacteria were isolated from the rhizosphere of triticale. A total of 22 bacteria were isolated from rhizosphere soil of triticale. The bacteria showed colonies of various sizes and margins, ranging from white to milky white, on LB agar plates. The cells were highly motile and rod-shaped. Among the 22 bacterial isolates, 18 were identified as gram-negative, 4 as gram-positive bacteria, and 18 as non-spore-forming and 4 as spore-forming bacteria based on their spore-forming activity.

Fungistatic activity of strains of rhizophilic bacteria. Of the total number of 22 bacterial strains tested, only three exhibited notable antagonistic activity against the fungus *Fusarium graminearum*, with the highest inhibition of fungal growth observed at 10-12 mm in strains AS4 and AR6, indicating a high degree of antifungal activity. The AS7 strains exhibited a weak, fungistatic effect, with suppression zones found to be within 1-6 mm, while the remaining 19 strains exhibited no effect on the growth of *Fusarium graminearum*. However, they demonstrated tolerance to the fungus, with the wells remaining unovergrown with mycelium.

Amount of phytohormone IAA determination. The rhizobacteria under study exhibited a high level of IAA production, with a maximum observed concentration of 194.99 μg/ml (Table 2).

Eight of the 22 strains were found to synthesize more IAA, including those presented on Figure 1.

Determination of growth-promoting activity. The results of studies investigating the impact of distinct microbial isolates with varying characteristics on the growth and root development of triticale plants can be found in Table 3.

In addition, the data illustrates the disparate effects of the microbial culture fluid of various strains and its dilutions on the growth and maturation of diverse agricultural products, as depicted on Figures 2 and 3.

Table 2 – Detection of phytohormone IAA produced by microbial strains

No.	Isolate	The amount of IAA, mcg/ml	No.	Isolate	The amount of IAA, mcg/ml
1	AS1	63.58±3.14	12	AR6	150.51±4.25
2	AS2	19.09±1.80	13	AS7	3.20±0.71
3	AS3	15.20±1.42	14	AR8	40.31±1.25
4	AS4	103.34±5.10	15	AR9	10.40±1.12
5	AS5	0	16	TS2	9.55±1.01
6	AS6	137.78±3.17	17	TS3	4.81±1.21
7	AR1	194.00±5.70	18	TS4	12.90±1.32
8	AR2	-	19	TS5	28.00±1.90
9	AR3	4.25±1.02	20	TS6	114.46±5.39
10	AR4	17.72±1.61	21	TS7	16.45±1.07
11	AR5	94.33±5.10	22	TR2	12.73±2.20

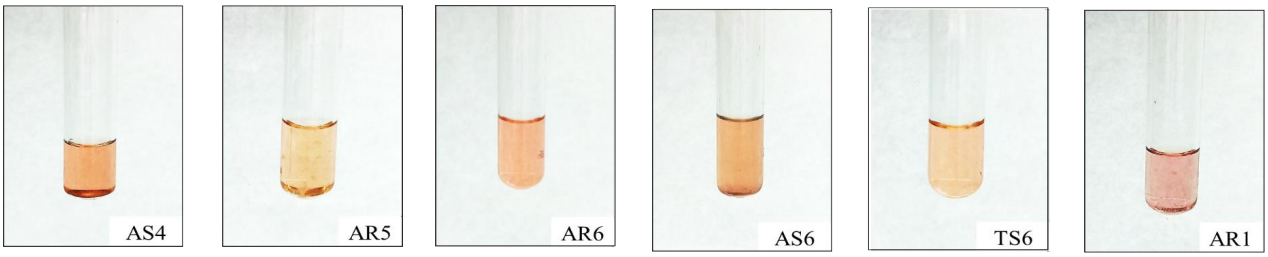

Figure 1 – Strains with high IAA production

Table 3 – Stimulation of the growth of roots and seedlings of winter triticale by rhizobacteria, % of control

No.	Isolate	Dilution	Root length	Seedling length
1	AR1	CF	-0.9	+8.4
		1:10	+10.5	+12.2
		1:100	-13.1	-2.2
		1:1000	-	-
2	AR6	CF	-14.3	-18.2
		1:10	0	+11.6
		1:100	-15.2	+18.6
		1:1000	-	-
3	AR8	CF	+13.4	+21.4
		1:10	+6.2	+147.8
		1:100	+0.1	-9.5
		1:1000	-	-
4	TS2	CF	-8.3	-6.4
		1:10	-6.5	+3.4
		1:100	-2.0	-48.5
		1:1000	-	-
5	TS6	CF	+31.4	0
		1:10	+35.4	+21.8
		1:100	+11.8	+17.9
		1:1000	-	-
6	TS7	CF	+33.0	+7.4
		1:10	-3.4	-9.3
		1:100	+17.5	-8.3
		1:1000	-	-

*CF – cultural fluid

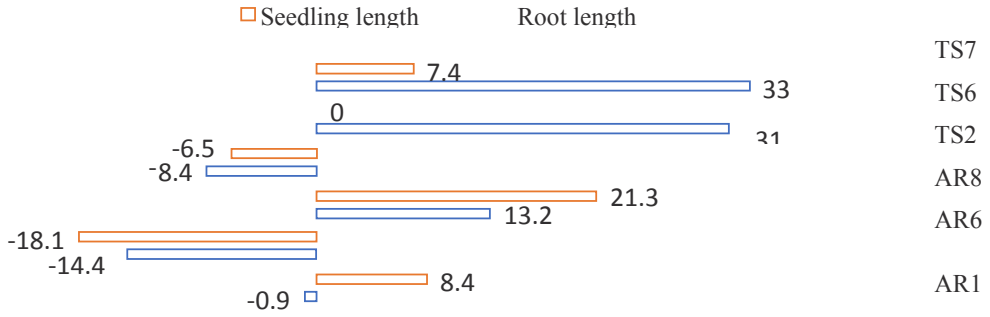

Figure 2 – Stimulation of triticale roots and seedlings growth by rhizobacteria with dilution of cultural fluid

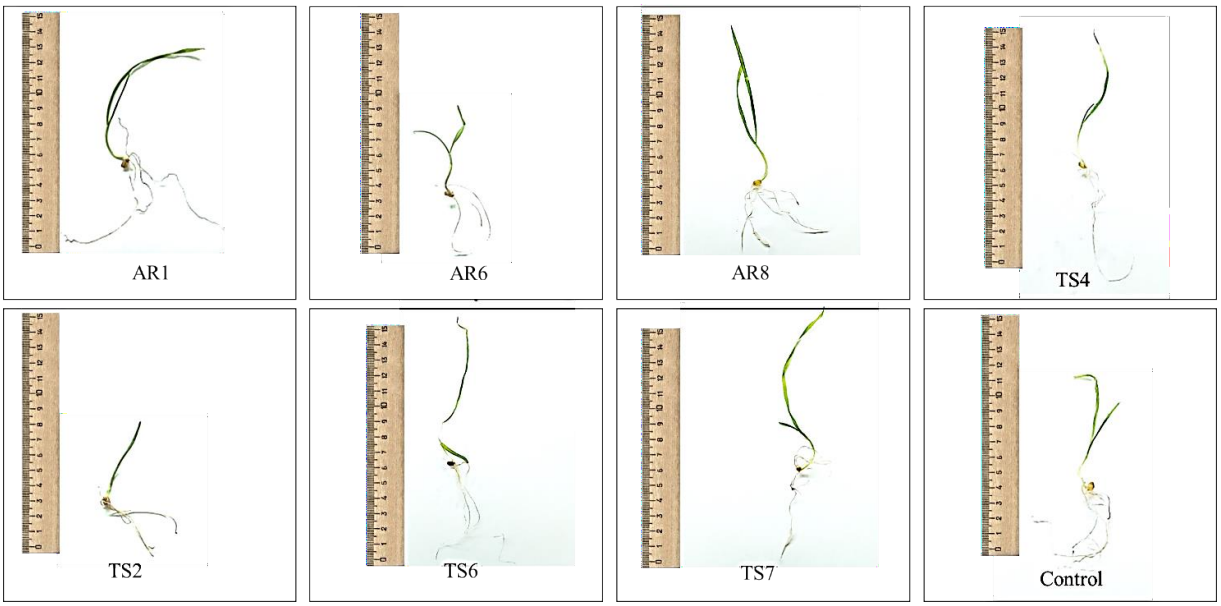

Figure 3 – Measurement of triticale roots and seedlings growth on 14th day with dilution of cultural fluid

Development of above-the-ground and under-the-ground organs of winter triticale was most favorably affected by the cultural liquid and its 1:10 dilution of strains AR8, TS6, and AR1 and TS7. In other variants, the development of the culture was inhibited, with the exception of variant AR6.

Conclusion

PGPR isolated from the triticale rhizosphere was reflected in their antifungal effect against *Fusarium graminearium*, which could potentially induce resistance of triticale plants. In examining the capacity of microorganisms to control the phytopathogen *Fusarium graminearum*, it was demonstrated that three strains were capable to actively inhibit the growth of the phytopathogen, exhibiting fungistatic properties. These strains are AR6, AR2, and AS7. However, the antifungal activity of the AR2 strain was found to be particularly noteworthy, with a phytopathogen inhibition zone of 12 ± 0 mm. In addition isolated rhizobacteria demonstrated the ability to synthesize the phytohormone auxin (IAA), which stimulates triticale growth, i.e. strains AS4 (103.33 ± 5.10 µg/ml), AS6 (137.77 ± 3.16 µg/ml), AR6 (150.49 ± 4.23 µg/ml), TS6 (114.45 ± 5.37 µg/ml) have the ability to synthesize phytohormones. The most prolific producer of IAA was strain AR1 (194.99 ± 5.69 µg/ml). An investigation into the impact of strains on the initial stages of plant growth and development revealed that the strains AR8, 38-22, TS6, AR1, and

TS7 had promoting effect on triticale growth and development. The findings of the studies indicate that five strains were identified as having growth-promoting properties (AS4, AS6, AR1, AR6, and TS6), three as having antifungal activity (AR2, AR6, and AS7), and one as exhibiting combined effect (AR6). The outcomes of the studies serve as a foundation for the further utilisation of rhizophilic bacteria in a range of agricultural technology applications.

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