

## Evaluation of The Growth-Stimulating Potential of Salt-Tolerant Bacteria Isolated from Saline Soils

Zakiryayeva S.I.

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan

Karimov H.Kh.

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan

Isokulov M.Z.

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan

Abdukarimova I.A.

National University of Uzbekistan

Ergasheva Z.M.

National University of Uzbekistan

Khamidova H.M.

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan

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### Abstract

*The growth-stimulating potential of four salt-tolerant Bacillus strains (B. subtilis 7A, 9A, 10A and B. proteolyticus 33S) on cotton (Gossypium hirsutum L.) was studied in vitro. Seed inoculation with the strains significantly increased germination, shoot and root length, as well as fresh and dry weight compared to the control. The most pronounced growth-promoting effect was observed with the B. proteolyticus 33S strain, which increased shoot length by 4.9 cm, root length by 2.72 cm, fresh weight by 0.20 g, and dry weight by 0.03 g relative to the control. The obtained results allow us to recommend the studied strains for further research with the aim of their future use in microbial biopreparations aimed at stimulating plant growth and improving the properties of saline soils.*

**Keywords:** Salinity, Bacillus, salt-tolerance, cotton, plant growth promotion, indole-3-acetic acid, microbial biopreparation.

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## 1. Introduction

Crop losses due to soil salinity are a growing threat to agriculture worldwide [1]. Soil salinity is becoming an increasingly serious global problem because salt inhibits plant growth and development and reduces crop yields [2]. The detrimental effects of NaCl on plants are due to both the reduced water availability as sodium accumulates in the soil [3] and the toxic effects of sodium and chloride ions on plants. Soil salinity typically inhibits plant growth and reproduction through an initial phase of osmotic stress, followed by ionic toxicity due to the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in the cell cytosol, ultimately leading to oxidative stress and nutrient deficiency [4].

Soil is a complex biologically active system rich in organic carbon and supporting diverse microbial communities. The rhizomicrobiome, formed under the influence of root exudates and ensuring the colonization of the root zone by microorganisms, plays a special role in agroecosystems. Rhizosphere microorganisms participate in the mobilization of nutrients and the synthesis of phytohormones and other biologically active compounds, contributing to improved plant growth and development. Plant inoculation with plant growth-promoting rhizobacteria (PGPR) is considered an effective approach to increasing crop productivity and their resistance to abiotic stresses, including salinity, drought, and high temperatures. This facilitates the development of environmentally friendly biopreparations aimed at reducing the use of chemical fertilizers [5]. Soil microorganisms commonly found in the plant rhizosphere exert a wide range of beneficial effects on plant growth. The best-studied bacteria with this effect belong to the genus *Bacillus* [6].

Phytohormones play a key role in regulating plant growth and development [7]. Many plant growth-promoting rhizobacteria (PGPR) are capable of producing auxins, exerting a significant impact on the growth and architecture of the root system [8-9]. Auxin-producing bacteria induce transcriptional changes in genes associated with hormonal regulation and plant defense responses, promote root elongation and an increase in their biomass, and activate auxin-dependent growth mechanisms [10]. In addition, representatives of the genus *Bacillus* are capable of synthesizing a wide range of enzymes that contribute to increased plant resistance to biotic stress, which determines their important role in sustainable agricultural systems. Due to their ability to adapt to various soil conditions, form

resistant endospores, and produce biologically active compounds, bacteria of the genus *Bacillus* are considered promising agents for stimulating plant growth under unfavorable environmental factors [11-12].

In connection with the above, the aim of this study was to evaluate the effectiveness of new salt-tolerant bacterial strains of the genus *Bacillus*, isolated from saline soils, in the germination of cotton (*Gossypium hirsutum* L.) in vitro, as well as to determine the biosynthesis of indole-3-acetic acid (IAA).

## 2. Methods

The study subjects were four strains of salt-tolerant soil bacteria of the genus *Bacillus* (*B. subtilis* 7A, 9A, 10A, and *B. proteolyticus* 33S) isolated from saline soils [13], as well as clean pubescent seeds of the Zafar cotton variety.

Salt-tolerant bacteria were submerged cultured on a shaker at 160 rpm in peptone water with glucose and NaCl at 28°C. The working volume of the nutrient medium was 25 ml. The number of viable bacteria (CFU/ml) in the culture fluid was determined by serial dilution followed by plating on meat-peptone agar (MPA) [14].

The ability of bacterial cultures to induce cotton seed germination was tested in vitro in Petri dishes. Sterile filter paper was placed in Petri dishes, moistened with 1 ml of sterile distilled water, and six cotton seeds were added to each dish. The cotton seeds were pre-washed, sterilized with 70% ethanol for 5 minutes, and then rinsed several times with sterile distilled water to remove residual disinfectant. Seeds soaked in sterile water served as a control. Experimental conditions were prepared by soaking the seeds in a culture suspension of four bacterial monocultures with a titer of 10<sup>6</sup> CFU/ml for 2 hours. The experiment was repeated three times. The Petri dishes were incubated in a thermostat at 26°C. The results were evaluated dynamically over 7 days using the following parameters: shoot and root length, fresh and dry weight, and seed germination (%). The seed germination percentage was calculated according to the method [15] as follows:

$$\text{Germination (\%)} = n/N \times 100$$

where n - is the number of germinated seeds after 7 days, and N - is the total number of seeds.

Indole 3 acetic acid (IAA) production in bacterial strains

was determined using a modified Salkowski method based on the formation of a colored complex between IAA and Salkowski's reagent. Strains were grown in a liquid nutrient medium containing peptone water, glucose, and NaCl, supplemented with 2 mM L-tryptophan, a precursor of IAA. Cultures were incubated at 28°C on a shaker at 160 rpm for 24 h. After incubation, the culture was centrifuged (13,000 rpm, 5 min), and 1 ml of the resulting supernatant was used for analysis. To qualitatively assess IAA production, 1 ml of supernatant was mixed with 2 ml of Salkowski's reagent (2 ml of 0.5 M FeCl<sub>3</sub> solution prepared in 100 ml of 37% HClO<sub>4</sub> solution) and kept at room temperature in the dark for 20 min. The formation of a pink-red colored complex indicated the presence of IAA in the sample. The color intensity was assessed visually [16].

Statistical processing of experimental data was carried out using standard methods for calculating errors, means,

confidence intervals, and standard deviations [17]. All calculations and mathematical analyses were performed using STATISTICA 6.0 and Microsoft Excel 2007.

### 3. Results and Discussion

The effects of four salt-tolerant strains (*Bacillus subtilis* 7A, 9A, 10A, and *B. proteolyticus* 33S) on cotton seed germination, shoot and root length, and seedling fresh and dry weight are presented in Table 1 and Figures 1 and 2.

The experimental results showed that inoculation of cotton seeds with salt-tolerant strains promoted more intensive plant growth and development compared to the control (no inoculation). On the third day of the experiment, seed germination in all treatments inoculated with salt-tolerant strains was higher than in the control treatment, with *B. proteolyticus* 33S exhibiting the greatest activity (Fig. 1).



**Fig. 1. Effect of salt-tolerant soil bacteria on cotton seed germination (on the 3rd day of the experiment)**

**1- control; 2- *B. subtilis* 7A; 3-*B. subtilis* 9A; 4-*B. subtilis* 10A; 5- *B. proteolyticus* 33S:**

**Table 1.**

**Effect of salt-tolerant strains of the genus *Bacillus* on germination and biometric parameters of cotton seedlings**

Variant	Germination, %	Shootlength, cm	Rootlength, cm	Freshweight, g	Dryweight, g
Контроль	83.3± 2.9	3.8 ± 0.16	1.48 ± 0.09	0.19 ± 0.03	0.04 ± 0.003
<i>B. subtilis</i> 7A	100 ± 0.0	5.1 ± 0.21	2.18 ± 0.12	0.23 ± 0.05	0.06 ± 0.002
<i>B. subtilis</i> 9A	83.3± 2.9	4.5 ± 0.18	1.76 ± 0.11	0.21 ± 0.03	0.05 ± 0.002
<i>B. subtilis</i> 10A	100 ± 0.0	5.6 ± 0.22	2.68 ± 0.12	0.25 ± 0.06	0.06 ± 0.004
<i>B. proteolyticus</i> 33S	100 ± 0.0	8.7 ± 0.25	4.20 ± 0.16	0.39 ± 0.08	0.07 ± 0.006

LSD <sub>0.05</sub>	8.5	0.75	0.52	0.06	0.01
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Notes: Values represent the mean  $\pm$  SE (n = 3);  $p \leq 0.05$ .

All tested strains stimulated cotton seed germination to varying degrees. Maximum germination (100%) after 7 days (Table 1) was observed when seeds were treated with *B. subtilis* 7A, *B. subtilis* 10A, and *B. cereus* 33S, which was 16.7% higher than the control (83.3%). Meanwhile, *B. subtilis* 9A demonstrated the lowest germination rate - 83.3%, which was the same as the control.

The most pronounced growth-promoting effect was observed with *B. proteolyticus* 33S. The average shoot length was  $8.7 \pm 0.25$  cm, 4.9 cm longer than the control, and the root length was  $4.20 \pm 0.16$  cm, 2.72 cm longer than the control. The increase in biometric parameters was accompanied by an accumulation of biomass: the average fresh weight of one seedling was  $0.39 \pm 0.08$  g,

which is 0.20 g more than the control, and the dry weight was  $0.07 \pm 0.006$  g, which is 0.03 g more than the control. The *B. subtilis* 10A strain also demonstrated a significant stimulating effect, but it was less pronounced. The average shoot length was  $5.6 \pm 0.22$  cm, which is 1.8 cm longer than the control, and the root length was  $2.68 \pm 0.12$  cm, which exceeded the control value by 1.2 cm ( $p \leq 0.05$ ). Moreover, the fresh weight increased by 0.06 g, and the dry weight by 0.02 g relative to the control variant.

Strains *B. subtilis* 7A and *B. subtilis* 9A showed a similar, but less pronounced tendency to increase biometric indicators, which indicates strain-specificity of the growth-stimulating effect (Table 1 and Fig. 2).



**Fig. 2. The effect of salt-tolerant soil bacteria on the growth and development of cotton seedlings (on the 7th day of the experiment)**

**1-control; 2- *B. subtilis* 7A; 3-*B. subtilis* 9A; 4-*B. subtilis* 10A; 5- *B. proteolyticus* 33S:**

Similar results were obtained in studies by M. Luqman et al., who demonstrated that bacteria of the genus *Bacillus* enhance plant growth, increase biomass, and activate photosynthetic processes [18].

These results can be explained by a number of physiological and biochemical mechanisms characteristic of rhizobacteria of the genus *Bacillus*. In particular, the stimulating effect may be associated with the production of phytohormones, primarily indole-3-acetic acid (IAA), which enhances cell division and cell elongation [19].

In light of the above, we studied IAA production by the studied strains. It was found that all studied strains are capable of producing IAA. This is confirmed by a change in the color of the medium after the addition of Salkowski's reagent, indicating IAA synthesis.

The high efficiency of the *B. proteolyticus* 33S strain is

apparently due to more intense IAA production, as well as the combined action of other growth-promoting mechanisms, including phosphate mobilization.

#### 4. Conclusion

Thus, strains of *Bacillus* bacteria isolated from saline soils have the ability to stimulate seed germination, growth and development of the aboveground part and root system of plants, and also contribute to an increase in cotton biomass. These results allow us to recommend the studied strains for further research with a view to their future use in microbial biopreparations aimed at stimulating plant growth and improving the properties of saline soils.

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