Scientific Research Publishing

American Journal of Plant Sciences, 2025, 16(1), 28-34
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Influence of *Bacillus* Strains on Biophysiological Processes in Plants

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How to cite this paper: Kizi, K.U.S.M. (2025) Influence of *Bacillus* Strains on Biophysiological Processes in Plants. *American Journal of Plant Sciences*, **16**, 28-34.
https://doi.org/10.4236/ajps.2025.161004

Received: June 18, 2024
Accepted: January 12, 2025
Published: January 15, 2025

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Abstract

The article presents data on the screening of rhizobacteria and active isolates isolated from the humus layer of natural humus soils against phytopathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* and their resistance to various concentrations of NaCl (0%, 1%, 5%, 7%, 10%). The results of studies on the synthesis of phytohormones with the properties of microorganisms that enhance plant growth and development are presented. Antagonistically active bacterial strains that grew even at high salt concentrations were identified using MALDI-TOF analysis. They were identified as XD 4.3 *Bacillus subtilis* and XDN6 *Bacillus cereus*, respectively.

Keywords

Soil, Rhizobacteria, Phytopathogen, Antifungal Drug, Indole-3-Acetic Acid (IAA)

1. Introduction

A global challenge is that the spread of droughts due to climate change may lead to a reduction in arable land. More than half of the cultivated land on Earth is expected to experience drought by 2050 [1]. As a result of high temperatures and reduced rainfall, the rate of water evaporation increases, and soil salinization occurs in drylands [2]. Pathogens that affect plant growth will also lead to food shortages in the near future. Agriculture is increasingly dependent on agrochemicals to combat these threats. However, the increased use of chemicals has a number of negative consequences, such as the development of resistance to the pathogens used and their impact on the environment - unintended environmental impacts may occur [3]. To prevent this, many measures are taken. Among them, bacterial strains that perform a biocontrol function have been introduced. Microorganisms are found in almost all regions of the Earth. For example, extremophiles

inhabit deserts, rocks, springs, fresh water, marine and arctic environments. with terrestrial and aquatic animals, and a variety of microorganisms live in the internal tissues of the plant and around the roots [4]. Plant growth promoters (PGPRs) play an important role in sustainable agriculture. The use of PGPRs has been shown to be an environmentally friendly way to increase crop yields by facilitating plant growth through direct or indirect mechanisms. The mechanisms of PGPRs include regulation of hormonal and nutrient balance, resistance to plant pathogens, and solubilization of nutrients to facilitate their absorption by plants. In addition, PGPRs synergistically and antagonistically interact with soil microorganisms located in the rhizosphere, which indirectly increases the rate of plant growth [5]. Plant growth-promoting rhizobacteria (PGPR) are rhizosphere bacteria that promote plant growth through various mechanisms including phosphate solubilization, siderophore production, biological nitrogen fixation, and 1-aminocyclic-1-azapropaneC (AC) production, which can enhance plant growth, produce phytohormones with antifungal activity, produce volatile organic compounds (VOCs), induce systemic resistance, stimulate plant-microbe symbiosis, and produce pathogenic toxins [6].

2. Methods

To isolate rhizobacteria from soil, 1 g of soil sample was serially diluted to 10-6 in 9 ml of 0.85% NaCl using conventional microbiological methods. Then 20 ml of the diluted soil suspension was inoculated and plated on solid media. The mixture was incubated in LB agar medium at 28 °C for 5 days. To determine the antifungal activity [7], several isolated colonies were examined in the center of the PDA medium, and the bacterial straining method was used to study the antagonistic activity of rhizobacterial isolates [8]. *Fungal mycelium* grown 3 days ago, *Rhizoctoni solani, Fusarium oxysporum* strains isolated from diseased plants. Symptoms were used as a test object in the form of a disk (diameter 0.6 cm), solid composition PDA (potato dextrose) (g/l): potato - 200 g, dextrose - 0 g, pH 5.6-6.0). The studied isolates were grown opposite each other at a distance of about 3 cm from the phytopathogens. After 72-96 hours of the experiment. The inhibition distance (mm) between the bacterial colony and the pathogenic fungi was measured. Each experiment was repeated three times. Cultivated in LB (Luria Bertani) medium with 0%, 1%, 5%, 7%, 10% NaCl at pH 7.0-7.2 and a temperature of 28 0C. Colonies that were highly sensitive to salt were selected and their cell mass was determined. The number of colonies was determined, and the colony mass after 3 incubations was compared with the standard LB medium (1% Na Cl). To determine the mass of dry cells, a centrifuge tube or filter with sediment of microorganism cells is placed in a drying cabinet, dried and weighed. The drying and weighing modes are the same as those used to determine the mass of tubes or filters. Dry biomass is determined by the formula,

$$M = \frac{(A-B)*1000}{V}$$

Where *M* is the dry mass, g/l; *A* is the mass of the centrifuge tube (filter) with sediment - g; The mass of the sediment of the *B*-centrifuge tube (filter) is g; *V* is the volume of the culture fluid obtained for centrifugation, ml. The accuracy of the method is determined by the completeness of washing the cells from the salts of the medium and the correctness of drying [9].

Synthesis of IAA is a rhizobacterial method based on Gordon and Weber [10]. Rhizobacteria were grown on Kinga B medium containing 5 mg/L L-tryptophan. The cultures were grown in a shaker-incubator rotating at 120 rpm at 28˚C for 48 h. At the end of the rotation period, the cell in the medium was centrifuged at 3000 rpm for 30 min at 4˚C, 35% $HClO_4$ was added and 1 ml of 0.5 M $FeCl_3$*$6H_2O$ was added and the mixture was incubated in the dark. It was recovered after 30 minutes. The appearance of cherry-red color in the test tubes indicates the presence of bacteria synthesizing indole-3-acetic acid (IAA). Sterile nutrient medium (King B) without bacterial isolates was used as a control [10].

3. Results and Discussion

In our studies, 16 bacterial cultures were isolated from vermicompost of Californian worm farms. To check the antifungal activity of the isolated isolates, their activity was tested against phytopathogenic fungi: *Fusarium oxysporum* and *Rhizoctonia solani*. The results showed that 8 isolates (XD1, XD3, XD1.1, XD1.2, XD2.1, XD3.1, XD4.2, XD4.3, XDN6) were resistant to *Fusarium oxysporum* (**Figure 1**). However, the remaining isolates were inactive. It was experimentally established that isolates XD4.1, XD4.2, XD4.3, XD4.4, XDN6 are resistant to *R. solani* (**Figure 2**).

As can be seen from the data in **Table 1**, the bacterial isolate XD4.3 is characterized by greater inhibition of the growth of the phytopathogenic fungus *F. oxysporum* at a distance of 0.2 mm compared to other isolates. Inhibition was observed up to a distance of 18.4 ± 0.3 mm. According to the results, the rhizobacterial isolate XDN-6 inhibited *R. solani* up to 20.4 ± 0.1 mm and *F. oxysporum* up to 12.5 ± 0.2 mm.

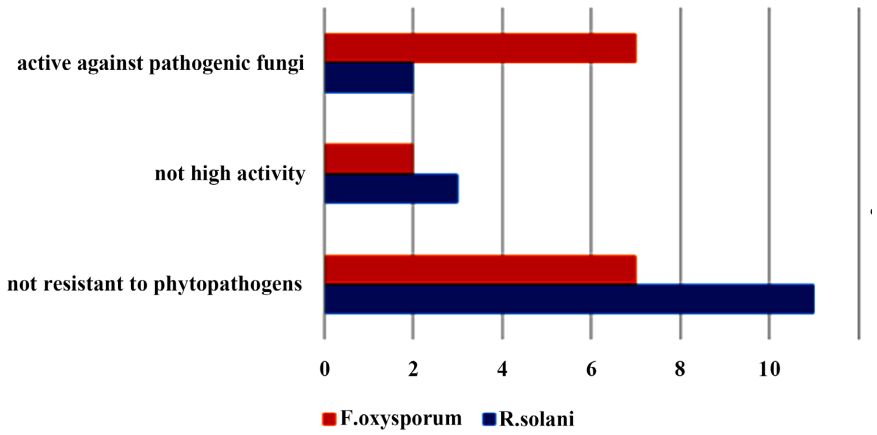

Figure 1. Antifungal properties of rhizobacterial isolates.

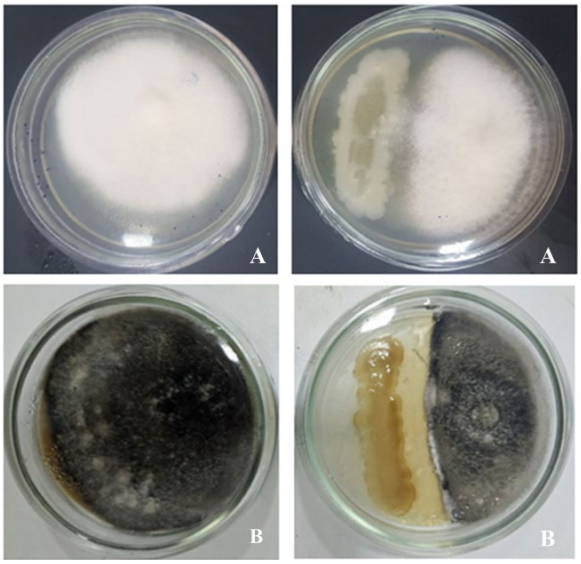

Figure 2. Antigonesis (antifungal activity of isolate A-XD4.3 against *F. oxysporum* and isolate B-ХДN6 against *R. solani*) resistance test of the isolated bacterial isolates.

Table 1. The antifungal activity (mm) of the selected rhizobacteria strains against phytopathogens was assessed.

Strains	Inhibition distance. mm	
	F. oxysporum	*R. solani*
ХД4.1	0.3 ± 0.1	14.3 ± 0.3
ХД4.2	10.2 ± 0.2	12.6 ± 0.1
ХД4.3	18.4 ± 0.3	17.4 ± 0.2
ХД4.4	0.3 ± 0.2	13.6 ± 0.3
ХДN 6	12.5 ± 0.2	20.4 ± 0.1

Note: Statistically significant at $P \leq 0.05$.

In the following experiment, the salt tolerance of 16 isolates was tested at different NaCl concentrations (0%, 1%, 5%, 7%, 10%) (Figure 3).

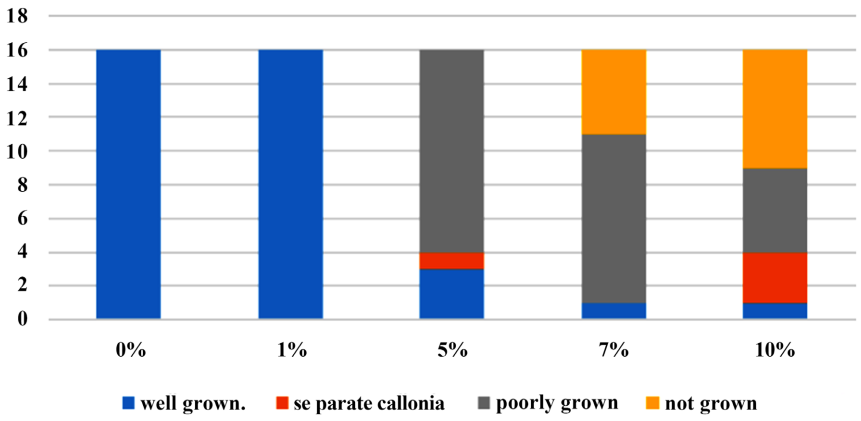

Figure 3. Growth of 16 bacterial isolates in different concentrations of NaCl.

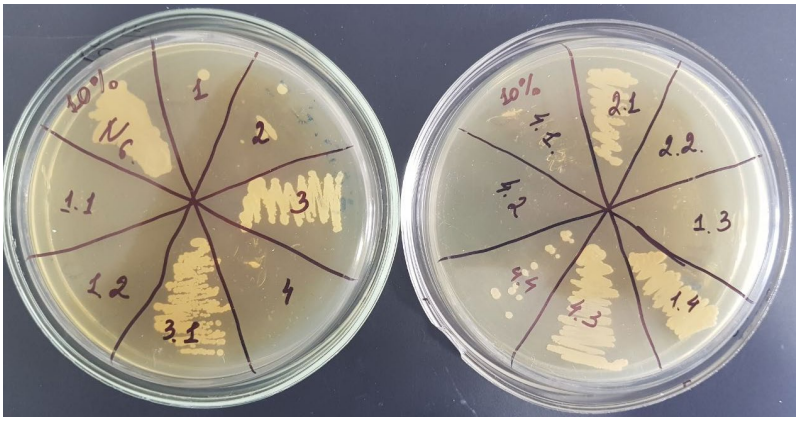

Figure 4. Growth of 16 isolates in 10% NaCl concentration.

The results showed that all isolates grew well at 0% and 1% concentrations. Later, as the concentration increased, their growth slowed down (**Figure 4**). Isolates XD4.3 and XDN6 also showed good colony growth at high NaCl concentrations. Some of the remaining isolates even stopped growing. In 3 of 16 isolates (XD1, XD2, XD4.4), single colony growth was observed on LB agar medium with high NaCl content. The purpose of this experiment was to visually observe the colony growth and select their cells. The mass of two strains: XD4.3, XDN 6 was determined. The mass at different concentrations is presented in **Table 2**.

Table 2. Mass of XD4.3, XDN 6 at different NaCl concentrations (g).

NaCl %	ХД4.3	ХДN 6
0	0.016	0.019
1	0.020	0.024
5	0.014	0.018
7	0.013	0.016
10	0.010	0.012

Note: Cell biomass was calculated 72 hours after seeding.

According to the results of this table, when calculating the dry mass of cells, the number of cells decreased as the NaCl concentration increased. It has been proven that 1% NaCl concentration is the most useful concentration for cell growth and high biomass. In our studies, to determine the dependence of ISC synthesis by active strains on tryptophan, the ISC strains were grown on a tryptophan-enriched liquid King B medium, without the addition of tryptone, in a minimum salt content. Bacteria grown on liquid. Medium synthesized ISC. Spectrophotometric analysis showed that the amount of ISC is different for each strain. Active strains synthesized relatively high amounts of ISC in a tryptophan medium. It was found that some strains synthesize ISC even on a nutrient medium without tryptophan. According to the results, it was found that the amount of ISC was higher in the variants with tryptophan than in the variants without tryptophan. Each

strain was found to produce ISK in culture fluids containing 0.5, 5, 10 mg/l tryptophan (Table 3).

Table 3. The effect of growing on a nutrient medium enriched with L-tryptophan on the change in the amount of ISC synthesized by bacterial strains (μg/ml).

Stammlar	Tryptophansis	L-tryptophan (mg/l)			
		0.1	1.0	5.0	10
ХД4.1	12.9 ± 0.15	14.4 ± 0.22	23.4 ± 0.31	30.1 ± 0.13	26.3 ± 0.10
ХД4.2	7.5 ± 0.63	10.5 ± 0.35	17.3 ± 0.14	22.3 ± 0.11	18.5 ± 0.33
ХД4.3	8.4 ± 0.61	12.3 ± 0.49	19.2 ± 0.25	24.1 ± 0.12	21.5 ± 0.39
ХД4.4	8.7 ± 0.10	11.5 ± 0.11	18.5 ± 0.60	26.2 ± 0.43	23.3 ± 0.14
ХДN 6	5.5 ± 0.41	10.4 ± 0.32	15.1 ± 0.71	18.1 ± 0.23	13.3 ± 0.55

Note: The amount of ISK in cultures was determined on the 10th day of incubation.

In our studies, ISC strains were cultured in tryptophan-enriched King B liquid medium containing minimal salt without tryptone addition to determine whether the synthesis of the active strain is dependent on tryptophan. A spectrostometric analysis of ISC synthesized in the culture liquid showed that the amount of ISC varies for each strain. Active strains synthesized relatively high amounts of ISC in tryptophan medium. It was found that some strains synthesized ISC even on the nutrient medium without tryptophan. The results showed that the amount of ISK was higher in the variants with tryptophan than in the variants without tryptophan. Each strain was found to produce ISK in culture liquids containing 0.5, 5, 10, and 10 mg/L tryptophan.

1. The antifungal activity of 16 isolates was determined, according to which 8 isolates (XD1, XD3, XD1.1, XD1.2, XD2.1, XD3.1, XD4.2, XD4.3, XD4.6) were resistant to *Fusarium oxysporum*, but the remaining isolates were inactive. It was experimentally established that isolates XD4.1, XD4.2, XD4.3, XD4.4, XDN6 were resistant to *R. solani*.

2. In the next experiment, the salt tolerance of 16 isolates was checked at different concentrations of NaCl (0%, 1%, 5%, 7%, 10%).

3. All isolates grew well at 0% and 1% concentrations. Later, their growth slowed down with increasing concentration. XD4.3 and XDN6 isolates grew well at high NaCl concentrations. Some of the remaining isolates even stopped growing.

4. An experiment was conducted to determine whether the synthesis of ISK by active strains was dependent on tryptophan. The results showed that the amount of ISK was higher in the tryptophan-containing variants than in the tryptophan-free variants. Each strain was found to produce ISK in culture fluids containing 0.5, 5, 10 and 10 mg/L of tryptophan.

5. From these strains, two isolates were selected that were resistant to fungicides, grew at high concentrations of NaCl and had a higher ISC content than other isolates. Based on their data, the isolated microorganism culture was analyzed

using mass spectrometry. XD4.3 *Bacillus subtilis*, XDN 6 *Bacillus cereus* were identified.

6. Based on the data obtained, it was concluded that the treatment of agricultural crops with active strains of seeds and soil and, as a result, the extension of the vegetation period of plants, the prevention of diseases caused by pathogenic microorganisms, and the increase in growth rates help to increase the yield. These bacteria can be used for plant growth on lands with different salinity levels.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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