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# THE PHYTOHORMONE SYNTHESIZING PROPERTY OF THE BACTERIUM PSEUDOMONAS CHLORORAPHIS

M. A. Qalandarova 1
T. S. Xusanov 2
B. A. Nigmatullayev 3
N. O. Ro‘ziyeva 4
Teacher of The Nizomi National Pedagogical University of Uzbekistan 1,
Senior Researcher, Institute of Microbiology, Uzbekistan Academy of Sciences, PhD 2,
PhD of The Nizomi National Pedagogical University of Uzbekistan 3,
Doctor of Pedagogical Sciences (PhD), Teacher of the Department of "Agrobiology and Cultivation of Medicinal Plants" of the Tashkent Branch of the Samarkand State Veterinary, Animal Husbandry and Biotechnology University 4

**Abstract**
In this study, the mechanisms of phytohormone production of plant hormones, including secretion fluid, chemicals, and plant hormones by the soil microorganism Pseudomonas chlororaphis were observed. The ability of the Pseudomonas chlororaphis strain to synthesize phytohormones was studied based on cultures grown in MPB (Meat Peptone Broth) liquid nutrient medium.

**Keywords**: Pseudomonas chlororaphis, gibberellin, rhizobacterium, auxin, phytohormone, laminar boxing, PGPR, MPB, Folin.

## Introduction

Among the phytohormones, auxin, ethylene, abscisic acid, and gibberellin are well-studied. Gibberellin was first isolated in 1935 from the fungus Gibberella fujikuroi and several soil microorganisms.[1].The role of gibberellin in plant growth and development, its biosynthetic pathways, inheritance, and regulation have been studied [1,2]. Phytohormones such as gibberellin and auxin, produced by plant growth-promoting rhizobacteria (PGPR), promote plant growth and increase the yield of many crops. Pseudomonas chlororaphis is a type of beneficial bacteria that is an integral part of the soil ecosystem, in particular the rhizosphere of the plant root system[3]. This microorganism is included in the PGPR (plant growth-promoting rhizobacteria) group, since it establishes a mutually beneficial relationship with plants and accelerates their development. The widespread distribution of the bacterium in nature is explained by its easy adaptation to various external environmental factors (heat and cold, acidity, drought) and the ability to combat harmful phytopathogens [4]

## Methods

The bacterial strain was cultured in MPB liquid broth medium and Salkovsky reagent (16g/l of FeCl3 crystal was mixed in distilled H2O, after the salt was dissolved, it was mixed with

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concentrated H2SO4 until it reached 20% before use) was used for auxin group phytohormones, and for gibberellin group phytohormones, a solution of folin in distilled H2O (1:1 ratio) and concentrated HCl was prepared[5,6].

The culture grown in MPB (24 hours after inoculation) was passed through filter paper and the filtrate was exposed to reagents. 0.5 ml of filtrate was used for qualitative analysis of auxin, and 1 ml of filtrate was used for qualitative analysis of gibberellin. For control in the spectrophotometer, MPB without pure strain was used[7,8,9].

Qualitative analysis for auxin.

1. 0.5 ml of filtrate from a sample of the strain grown on MPB for 1 day under sterile conditions in a laminar flow hood was separated into a test tube using filter paper.
2. 4 ml of Salkovsky reagent was poured onto the filtrate
3. It was left in a dark place for 20 minutes
4. After 20 minutes, the samples were measured at a wavelength of 400 nm on a spectrophotometer
5. The results of the determined wavelength were recorded in a notebook

This reaction was carried out for 5 days.

Qualitative analysis for gibberellin[9,10,11,12].

6. 1 ml of filtrate from a sample of a strain grown on MPB for 1 day under sterile conditions in a laminar flow hood was separated into a test tube using filter paper.
7. 0.5 ml of folin solution and 2.5 ml of conc. HCl were poured onto the filtrate.
8. It was left in a dark place for 40 minutes.
9. After 40 minutes, the samples were measured at a wavelength of 750 nm on a spectrophotometer.
10. The results of the obtained wavelength were recorded in a notebook.

This reaction was carried out for 5 days [13,14,15].

**Result**

The study investigated the ability of Pseudomonas chlororaphis strain to synthesize phytohormones using cultures grown in MPB (Meat Peptone Broth) liquid nutrient medium. The strain was incubated for 24 hours, and the resulting culture was filtered through filter paper. The resulting filtrate was used to determine phytohormones. Salkowski reagent was used to determine the auxin group of phytohormones. To determine the reaction results, 4 ml of Salkowski reagent was added to 0.5 ml of filtrate and the mixture was left in the dark for 20 minutes. Then the samples were measured in a spectrophotometer at a wavelength of 400 nm. To determine the phytohormones of the gibberellin group, Folin's reagent (diluted 1:1 with distilled water) and concentrated HCl were added to the filtrate. 0.5 ml of Folin's solution and 2.5 ml of HCl were added to 1 ml of filtrate and left in the dark for 40 minutes. Measurements were made using a spectrophotometer at a wavelength of 750 nm. MPB medium without phytohormones (uncultured) was used as a control. The experiment was carried out for 5 days and changes in the amount of phytohormones were recorded.

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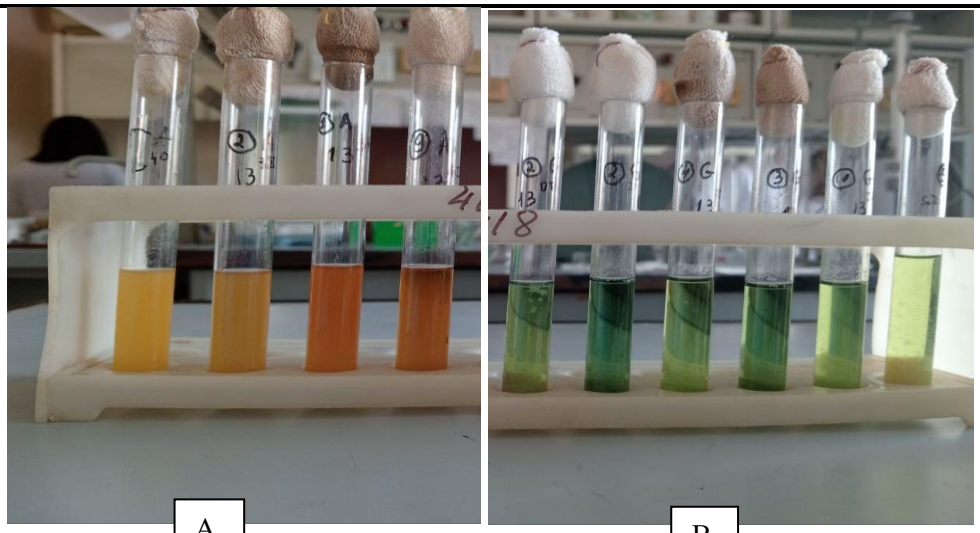


**Figure 1.** Samples pre for viewing in a spectrophotomet for auxin, B-for gibberellin.

According to the results obtained, it was found that the Pseudomonas chlororaphis strain has the ability to synthesize the phytohormones auxin and gibberellin (Table 1).

| T/r | Pseudomonas chlororaphis | Auxin | Gibberellin | Control(MPB) Auxin | Control(MPB) Gibberellin |
|---|---|---|---|---|---|
| 1 | Day 1 | 0,331 | 0,270 | 0,110 | 0,061 |
| 2 | Day 2 | 0,541 | 0,424 | 0,110 | 0,061 |
| 3 | Day 3 | 0,708 | 1,500 | 0,110 | 0,061 |
| 4 | Day 4 | 2,387 | 0,670 | 0,110 | 0,061 |
| 5 | Day 5 | 1,102 | 0,675 | 0,110 | 0,061 |

The auxin content reached its highest level (2.387) on day 4, indicating that active metabolic processes were occurring during the growth phase of the bacteria.

The amount of gibberellin showed the highest value (1,500) on the 3rd day, and a decreasing trend was observed in the following days. This indicates that the synthesis of phytohormone has a dynamic nature depending on time, that is, the growth phase (Figure 2).

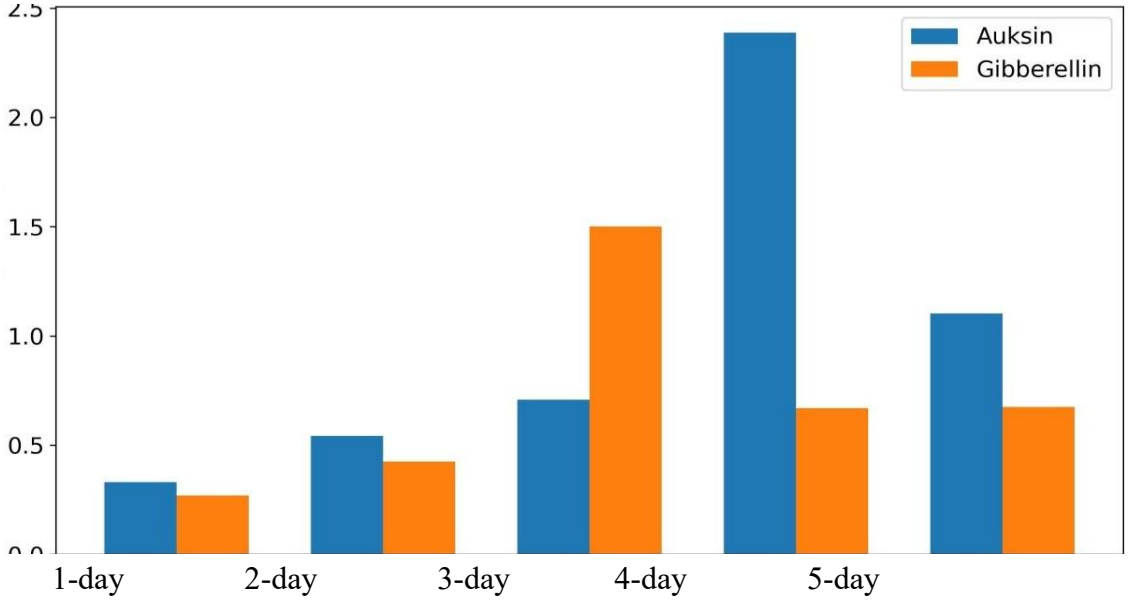


**Figure 2.** Analysis of 5-day results of auxin and gibberellin synthesis by the bacterium Pseudomonas chlororaphis.

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In general, the Pseudomonas chlororaphis strain has the ability to produce phytohormones that stimulate plant growth, confirming its potential as a biostimulant.

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