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INDOLE-3-ACETIC ACID PRODUCING BACTERIA AND THEIR EFFECT ON THE GROWTH OF *PISUM SATIVUM*

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ABSTRACT. Indole-3-acetic acid (auxin) is a well known phytohormone which is involved in the regulation of plant growth and development. A high number of plant growth promoting rhizobacteria (PGPR) produce auxin, thus play major role in plant development. In this study we characterized 25 bacterial strains by their auxin producing ability. The growth promoting effect of two selected bacterial strains on pea (*Pisum sativum*) was studied.

Keywords: *indole-3-acetic acid, phytohormones, plant-growth promoting rhizobacteria*

INTRODUCTION

Plant growth promoting bacteria enhance and regulate plant growth by different mechanisms such as solubilisation of phosphorous, production of phytohormones, stimulation of certain metabolic pathways (nitrogen fixation or control the pathogenic microorganisms) [1, 2, 3].

Plants regulate and control their development by using chemical signals, such as hormones. Among these hormones auxin (represented by indole-3-acetic acid - IAA) is the most active. It is responsible for the division, expansion and differentiation of plant cells and tissues [4].

Attachment of bacteria to the roots is beneficial for plant growth and productivity. In their interaction with plants, these microorganisms interfere with plant development by disturbing the auxin balance in plants. Two major pathways for auxin biosynthesis have been proposed: the indole-3-acetamide and the indole-3-pyruvate pathway [3, 5, 8]. The ability of auxin production has been found among various microorganisms, representatives of the

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genera *Pseudomonas*, *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Rhizobium*, *Bradyrhizobium* etc. [3, 7, 8, 9].

The increasing interest in PGPR microorganisms led to an extensive use of different biofertilizers. These products represent an alternative for chemical fertilizers [10]

The aim of the current study is to determine the auxin production ability of selected bacterial strains using a colorimetric method. It is well known that one of the main mechanisms of plant growth regulation is production of different phytohormones; thus we studied the effect on the growth of pea (*Pisum sativum)* of two selected bacterial strains, which proved to be the best auxin producers.

RESULTS AND DISCUSSION

In this study the beneficial properties of 25 bacterial strains was assayed; bacteria were isolated from the rhizosphere of *Sphagnum* plants. The ability of auxin production was analyzed. Auxin is a phytohormone which plays crucial role in plant growth and development. During our work we examined the effect of two selected bacterial strain on the growth and development of pea (*Pisum sativum)*.

In Table 1 are presented the auxin concentrations, produced by the studied bacterial strains. The amount of auxin produced is between 2.29 – 17.79 μg ml^{-1}; the highest amount of auxin was produced by *Pseudomonas fluorescens E8*. *Serratia nematodiphila P17* found to produce auxin in the less concentration.

Table 1. The values of auxin concentration produced by the examined bacterial strains

Bacterial strain	Absorbance (530 nm)	Auxin concentration (μg ml^{-1})
Bacillus cereus P2	0.172	6.79
Bacillus mycoides B6	0.073	2.66
Bacillus thuringiensis B11	0.159	6.25
Lysinibacillus fusiformis P24	0.317	12.83
Viridibacillus arenosi P13	0.214	8.54
Pseudomonas fluorescens E8	0.436	17.79
Pseudomonas fluorescens B14	0.285	11.5

Pseudomonas jessenii E20	0.264	10.62
Pseudomonas koreensis P8	0.313	12.66
Pseudomonas koreensis P15	0.316	12.79
Pseudomonas lurida P20	0.166	6.54
Pseudomonas stutzeri P23	0.294	11.87
Serratia fonticola P5	0.106	4.04
Serratia fonticola B17	0.327	13.25
Serratia marcescens E11	0.066	2.375
Serratia nematodiphila P17	0.064	2.29
Serratia plymuthica B19	0.209	8.33
Serratia plymuthica E5	0.15	5.87
Stenotrophomonas rhizophila B21	0.317	12.83
Stenotrophomonas rhizophila E3	0.265	10.66
Paracoccus yeei P3	0.072	2.62
Cedecea neteri P4	0.072	2.62
Delftia acidovorans P12	0.326	13.20
Microbacterium hydrocarbonoxydans P21	0.133	5.16
Enterobacter sp.E6	0.295	11.91

Inoculation of *P. sativum* plants with *S. fonticola B17* and *P. fluorescens E8* stimulated plant growth. A significant increase ($p<0.001$) in shoot length was observed between treated plants and control sample (Figure 1.).

Figure 1. The mean values of shoot length

In case of pea plants a significant difference were obtained for the shoot and root fresh weight between plants treated with *P. fluorescens E8* and *S. fonticola B17* and control plants (Figure 2.); no differences were observed between the shoot and root dry weights of the control and treated plants.

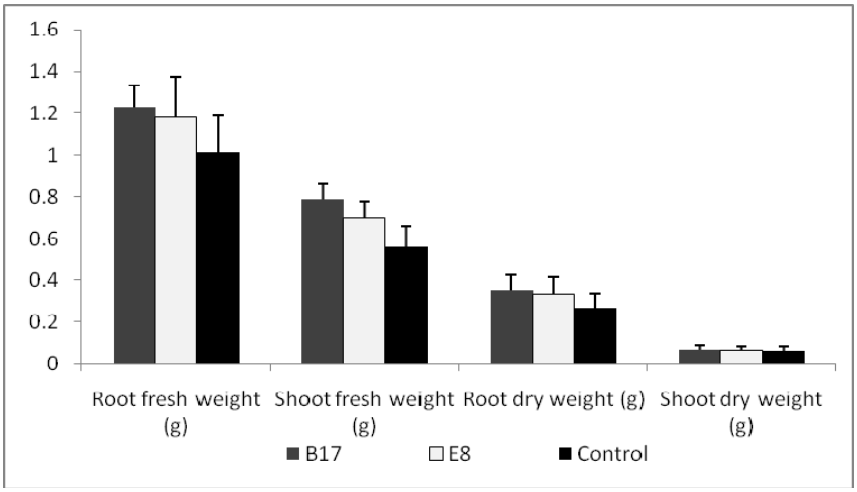

Figure 2. Mean values of root and shoot fresh and dry weight

CONCLUSIONS

During our study the auxin production ability was examined. The best auxin producers proved to be strains belonging to *Pseudomonas* genera: *P. fluorescens*, *P. koreensis*, *P. stutzeri*, *P. jessenii*.

Bacterial strains belonging to *Delftia*, *Serratia*, *Stenotrophomonas* and *Lysinibacillus* were also good auxin producers. Our results are in correlation with results found in the scientific literature. Ali et al. (2009) [11] studied the auxin production property of different bacterial strains belonging to *Bacillus*, *Pseudomonas*, *Escherichia*, *Micrococcus* and *Staphylococcus* genera; these strains were demonstrated having potential to increase the growth of wheat (*Triticum aestivum)*. It is known that auxin producing *Enterobacterium sp.* and *Bacillus sp.* promoted the growth of orchids (*Cattelya walkeriana*) [12]. Husen et al. [8] studied bacteria isolated from rhizosphere and plant roots in Bohol and Tarlac, Philippines, which showed high IAA and siderophore producing ability.

Sphagnum associated bacteria found in raised bogs from Germany and Norway, are known as good antagonists of plant pathogens like Verticillium or Ralstonia, according to Opelt and Berg (2004) and Opelt et al (2007), but none of them showed auxin producing properties [13, 14]. Our data indicates that bacterial strains isolated and identified as *Pseudomonas fluorescens E8* and *Serratia fonticola B17* were able to develop plant growth promoting effects in laboratory conditions.

EXPERIMENTAL SECTION

Bacterial strains

25 bacterial strains, isolated from rhizosphere of Sphagnum plants in Borsáros raised bog natural reserve (Harghita County, Romania) were examined. Bacterial strains were grouped using RFLP (restriction fragment length polymorphism) method, by digesting the 16S ribosomal DNA fragments with *MspI* and *HaeIII* restriction enzymes. Identification of 16S rDNA fragments were realized by sequencing. To identify similar sequences that are available in the NCBI GenBank, sequences were compared with BLAST algorithm. The sequences obtained are deposited in the European Molecular Biology Laboratory – European Bioinformatics Laboratory (EMBL-EBI) European Nucleotide Archive (ENA) database (data not shown).

Auxin production

Production of the well known plant hormone indole acetic acid was determined through colorimetric analysis using Salkowsky reagent [15]. Bacterial strains were grown in Triptone-Soy Broth (triptone 15 g, peptone from soy-meal 5 g, sodium chloride 5 g, distilled water 1000 ml) supplemented with tryptophan (10 mg/ml final concentration), for 3 days at 28 °C, shaken at 150 rpm. After incubation 1.5 ml from each bacterial suspension was centrifuged at 5000 rpm, for 15 minutes. The amount of indolic compounds was estimated adding 1 ml of culture supernatant to 2 ml of Salkowsky reagent (concentrated sulfuric acid 300 ml, 0.5 M $FeCl_3$ 15 ml, distilled water 500 ml). The mixture was incubated in dark, at room temperature for 30 minutes (during this period the mixture color become red, darker red indicated a higher amount of indole compounds). The color intensity was measured at an absorbance of 530 nm. The auxin concentration was estimated using a standard curve prepared with known amounts (5, 10, 15, 20, 25 and 35 µg/ml) of auxin solutions, that generated the equation $y=0.024x + 0.01$, with an $R^2 = 0.993$.

Plant growth experiment

Beneficial properties of two selected bacterial strains, identified as *Pseudomonas fluorescens E8* and *Serratia fonticola B17* was examined on pea (*Pisum sativum*) plants.

Seeds were surface steriliesed and germinated for three days at 28 °C. Soil samples were sterilised at 105 °C, for 60 minutes, for three times, with a 24 h interval. Bacterial strains were grown in liquid Nutirent medium (peptone 5 g, sodium chloride 5 g, yeast extract 2 g, meat extract 1 g), for 1 day and concentration was adjusted to 10^7 colony forming units (CFU) in every ml of suspension.

20-20 germinated seeds were sown in the sterile soil and 1 ml of bacterial suspension was added to every seed. Plants were grown for 10 days in a growth chamber 16 h light at 25 °C and 8 h dark at 20 °C, 70% relative humidity.

As a control germinated seeds were sown in soil samples, without bacterial inoculation.

Shoot length, root and shoot fresh and dry weight was measured after incubation. Statistical analyses were conducted using F and T test ($p<0.001$) of Past statistic software package.

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