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CHARACTERISATION OF SIX ISOLATED BACTERIAL STRAINS FROM GENUS *BACILLUS* APPLICABLE IN ORGANIC FARMING

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Abstract

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The aim of this study was to determine the activity of six isolated strains from genus *Bacillus* applicable in organic farming. These strains were cultivated in two different liquid nutrient media in order to examine their growth. Monitoring of the growth was carried out in order to achieve high concentrations of active cells. The results showed that the better medium for achieving high concentrations of active cells for all tested strains was PDG. The enzymatic potential of six isolated strains was assessed by the API ZYM system (BioMérieux, France) by semi-quantitative scale from 0 to 5. The strains showed phosphatase (acid and alkaline) and phospho-hydrolase activities in the range 4–5. For confirmation of the results qualitative analysis for determination of phosphatase activity by the method of Pikovska was performed. The effect of the strains on the growth of test plant *Pisumsativum* was studied (*in vivo*). The results showed that when *Pisumsativum* was inoculated with strains T 3, T 10 and T 18, the fresh root biomass and fresh shoot biomass increased significantly.

Key words: Bacillus, phosphatase activity, *Pisumsativum*

Introduction

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria which have the ability to colonize the roots and promote plant growth either through direct action or via biological control of plant diseases (Kloepper and Schroth, 1978).They are associated with many plant species and are commonly present in varied environments. Strains with PGPR activity, belonging to genera *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and *Serratia*, have been reported (Hurek and Reinhold-Hurek, 2003). Among these, species of *Pseudomonas* and *Bacillus* are the most extensively studied. These bacteria competitively colonize the roots of plant and can act as biofertilizers and/or antagonists (biopesticides) or simultaneously both.Many species of *Bacillus* and *Paenibacillus* are known to promote plant growth. The principal mechanisms of growth promotion include production of growth stimulating phytohormones, solubilization and mobilization of phosphate, siderophore production, antibiosis, i.e., production of antibiotics, inhibition of plant ethylene synthesis, and induction of plant systemic resistance to pathogens (Richardson et al., 2009; Idris et al., 2007; Gutierrez-Manero et al., 2001; Kumar et al., 2009). It is very likely that plant growth promotion by rhizosphere microorganisms from genus *Bacillus* may be a result of the combined action of two or more mechanisms.

Strains of the genus *Bacillus* exhibit the ability to inhibit the growth of phytopathogenic bacteria and fungi, which favors the growth of plant species (Cuero, 1991) and that has an important economic and agricultural development. Most strains of *Bacillus* species have a high capacity to produce a variety of extracellular enzymes such as amylase, arabinase, cellulase, lipase, protease, and xylanase, and these enzymes play important roles in several biotechnological processes (Sinchaikul et al., 2002; Cherry and Fidantsef, 2003). However, the properties of their enzymes vary from strain to strain.

In this study, an investigation ofsix *Bacillu*s strains that are biotechnologically attractive for industrial application like biofertilizers in biocontrol of pathogenic bacteriawas performed.

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Materials and Methods

Bacterial Strains

Six bacterial strains from genus *Bacillus*(T2, T3, T4, T10, T17 and T18) were selectedfor this study. They were isolated from different types of soils from the northregion of Bulgaria (unpublished data).

Culture Media and Growth Conditions

For all of the experiments, the microbial strains were routinely cultivated at 37°C in nutrient broth (NB) medium (Difco) and composed PDG medium (glucose – 5 g/l, yeast extract – 5 g/l, peptone – 5 g/l) prepared according to the manufacturer's instructions. To observe the growth profiles, a single colony of each isolate was preinoculated in NB and incubated at 37°C under shaking conditions until the stationary phase. The 24 h cultures were inoculated (1%) into NBand PDG medium and cultivated in an incubator shaker (New Brunswick, USA). The bacterial growth was monitored by measuring the optical density at 600 nm (OD600) using a spectrophotometer (Jenway-6305, Essex, England).

Detection of Enzyme Activities

The enzyme production of the isolates was observed via API ZYM system (BioMericux, France) according to the manufacturer's instructions.

To determine the activity of the microorganisms degrading inorganic compounds of phosphorus, was used the methodology of Pikovska (Zhdan- Pushkina et al., 1974). After preparing the nutrient medium and spilled it onto the agar plates, was inaculated the bacterial suspension and after 24 hours of cultivation at 28°C were reported lightening zones.

Testing of studied strains on test plant *Pisumsativum in vivo*

Each of the studied bacterial strains was applied at a concentration of 1–1.2x10^8 CFU/mlin order to examine the influence on the generative development of the test plants. The vegetation experiments were carried out in a heated greenhouse for a period of 3 months. Welldeveloped rooted plants (20 days after rooting) were used. The plants were transplanted in a standard peat mixture "Terra Mix" in pots number 11 and treated immediately after transplanting with the studied strains. Each variant had 3 repetitions. Three treatments were done at intervals of one week. Water was used as a control. The bacterial suspension was introduced as 2 ml/l working solution. The biometric indicators of the test plantswere measured. After the end of the experiment the plants were removed from the substrate, the roots were washed with water and their length was measured.The weight of the root and shoot biomass (fresh and dry) was recorded.

Results and Discussion

Dynamics of accumulation of biomass

The results for the growth of the strains in the different variants of the nutrient media as relative optical units corresponding to the accumulation of biomass are presented in Figure 1 (A, B, C, D, E, and F).

A typical S-shaped curve of bacterial growth with a pronounced exponential phase can be observed. The logarithmic phase for the six tested strains lasts 4 hours. The exponential phase is 12 hours for all strains tested, and passes into a stationary phase at the 16th hour for strains T2 and T3 and at the 18th hour for strains T4, T10, T17 and T18. The six strains grow well in both culture media, but a significant increase in the biomass is observed when PDG medium is used.

It can be concluded that PDG is more suitable for biomass accumulation of strains T2, T3, T4, T10, T17 and T18.

Enzyme activity of the tested strains

The biochemical characteristics, which were examinedby API Zym system (BioMericux, France) varied within the strains tested. The results are presented in Figure 2.

Strain T2 was highly positive for alkaline and acid phosphatase, leucine aminopeptidase and β-galactosidase. Strain T3, like strain T2 produced high levels of alkaline phosphatase and leucine aminopeptidase. It also produced, although in smaller quantities,esterase, esterase lipase, trypsin, phosphohydrolase, acid phosphatase and α- and β-glucosidase. Strain T4 exhibited high production of alkaline and acid phosphatase, leucine aminopeptidase, α-glucosidase and β-galactosidase. Strain T10 produced high levels of alkaline and acidic phosphatase, leucine aminopeptidase, β-galactosidase, β-glucosidase and phosphohydrolase. Strains T17 and T18 produced high levels of alkaline and acid phosphatase. All tested stains were negative for lipase (C-14), α-galactosidase, β-glucuronidase α-mannosidase and α-fucosidase.

In order to confirm the phosphatase activity of the strains, they were further examined by qualitative analysis according to the method of Pikovska (Zhdan- Pushkina et al., 1974).

The data from this experiment are presented in Figure 3. The results show a high phosphatase activity exhibited by all strains tested. The greatest area of enlightenment is reported for strains T4 (26 mm), T3 (25 mm) and T2 (23 mm). This makes them interesting for application in the production of bioproducts that encourages the growth and development of plants, improving their phosphorus nutrition (Chen et al., 2006).

Study of the effect of the strains on the growth of *Pisum sativum*

The data for the biometric measurements from the experiment with test plants *Pisum sativum* (pea) are presented in Figures 4 and 5.

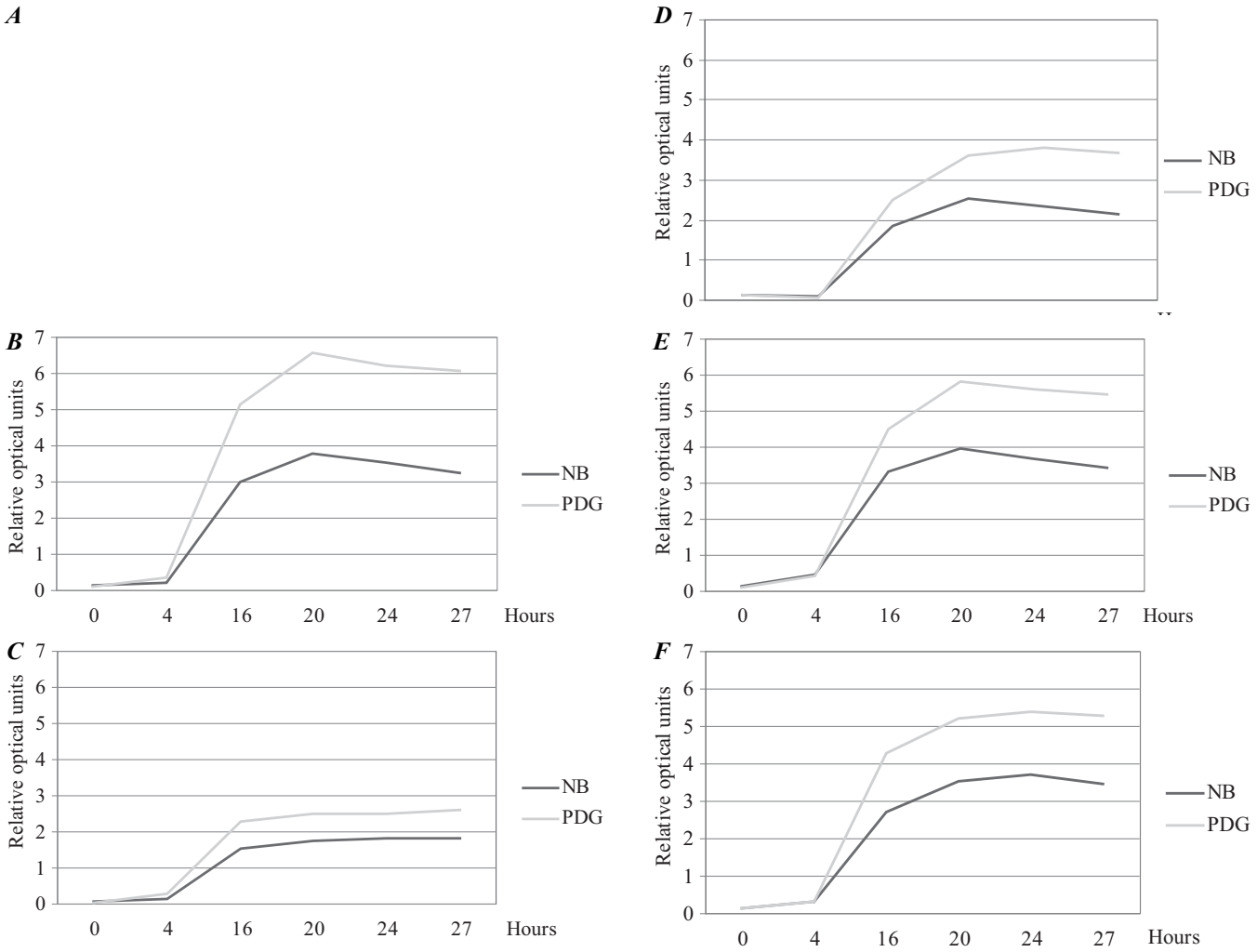

Fig. 1. Biomass accumulation (OU) (*A* – strain T 2, *B* – strain T 3, *C* – strain T 4, *D* – strain T 10, E – strain T 17 and *F* – strain T 18)

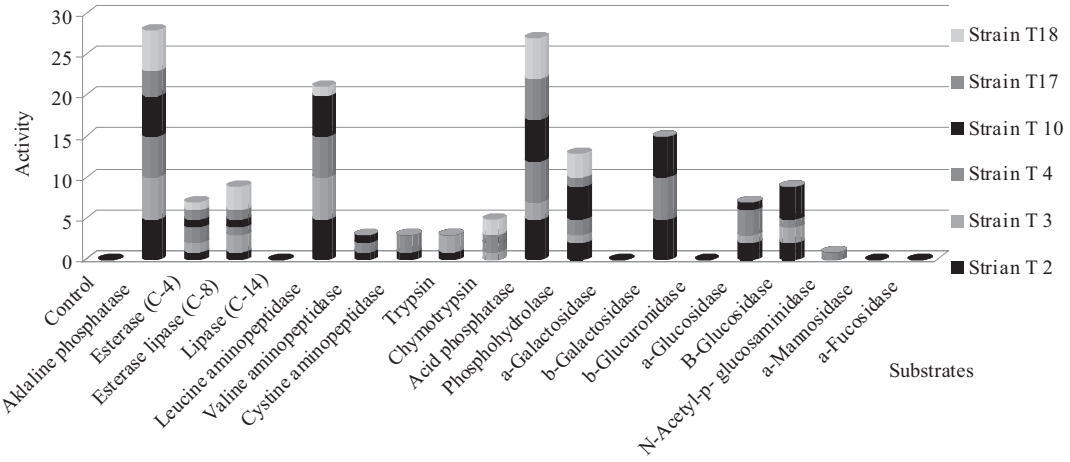

Fig. 2. Biochemical characteristics of the tested strains

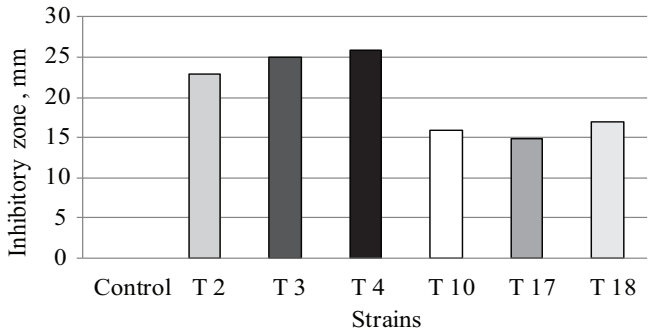

Fig. 3. Phosphatase activity of tested strain

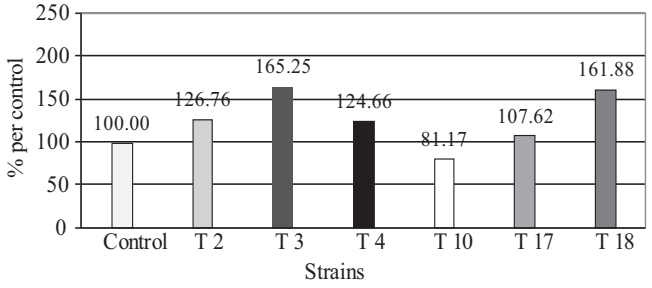

Fig. 4. Effect of the tested bacterial strains (T 2, T 3, T 4, T 10, T 17 and T 18) on the fresh shoot biomass of test plant *Pisumsativum*

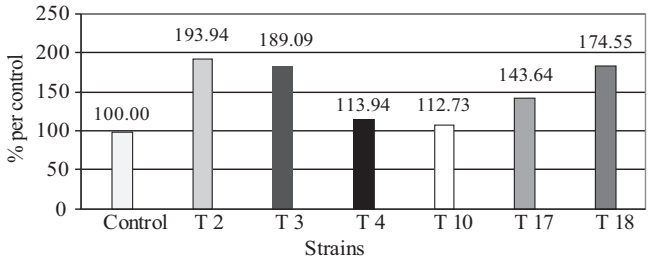

Fig. 5. Effect of the tested bacterial strains (T 2, T 3, T 4, T 10, T 17 and T 18) on the fresh root biomass of test plant *Pisumsativum*

The results demonstrate that strains T2, T3, T4 and T18 increase the fresh above-ground biomass of *Pisum sativum* in comparison with the control. The highest values are observed when strains T3 and T18 are applied – the fresh above-ground biomass is increased by up to 65% compared to the control. For the other inoculated strains demonstrating positive results, values range up to 26% compared to the control variant.

All inoculated strains show positive effect on the root system of test plant *Pisum sativum*. The highest positive effect is observed when strains T2, T3 and T18 are applied–the fresh root biomass is 93.94%, 89.09% and 74.55% heavier than that of the control variant. For the other inoculated strains the values for the root weight vary from 13 to 44% more than the control variant.

Conclusions

All six studied strains from the genus *Bacillus* (T2, T3, T4, T10, T17 and T18) produce acid and alkaline phosphatases and phosphohydrolases andincrease the root system of test plant *Pisum sativum*. On the basis of the obtained results it can be concluded that the strains are suitable to be included in the composition of biopreparations for soil improvement, leading to increased absorption of phosphorus by plants and promotingplant growth.

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