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Original article

Nutritional content analysis of plant growth-promoting rhizobacteria species

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ABSTRACT

Agricultural production requires the continuous application of mineral fertilizers, which not only disrupt the natural balance but also reduce economic efficiency. The objective of this study was to understand the effects of plant growth-promoting rhizobacteria (PGPR) species (*Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Bacillus pumilus* C26, *Paenibacillus polymyxa* RC05, *Azospirillum brasilense* sp245, *Burkholderia cepacia* OSU7, *B. cepacia* OSU7 AMP Res and *Raoultella terrigena* TFi08) on their host plants. The maximum levels of arginine, histidine, tartaric acid, citric acid, and gibberellic acid were observed in *B. megaterium* M3 with maximum levels of glycine and threonine in *B. subtilis* OSU142, maximum lysine and lactic acid levels in *R. terrigena* TFi08 and maximum asparagine, serine, and abscisic acid levels in *A. brasilense* sp245. The highest Ca and P concentrations were observed in *B. megaterium* M3, while high concentrations of K, S, Na, Mn, Cd, and Ni were obtained from *A. brasilense* sp245. These data suggest that the *B. megaterium* M3, *A. brasilense* sp245, and *R. terrigena* TFi08 strains have the potential to be used as organic fertilizers to facilitate plant growth in sustainable and organic farming.

1. Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) is free-living microorganisms that exert beneficial effects on plants by colonizing their rhizospheres or phyllospheres [1]. PGPRs can improve plant growth and yield directly and indirectly. Direct mechanisms include the following: 1) releasing plant growth regulators such as auxins, cytokinins, and gibberellins, 2) lowering ethylene in plants, 3) solubilizing inorganic phosphate, 4) mineralizing organic phosphate, 5) asymbiotic fixation of atmospheric nitrogen, 6) producing organic matter, including amino acids, 7) releasing enzymes and 8) stimulating disease-resistance mechanisms (systemic acquired or induced resistance) [1,2]. As an indirect mechanism, PGPR may have an antagonistic effect against phytopathogenic microorganisms and act as biocontrol agents, controlling plant disease-causing organisms, stimulating beneficial symbioses, and/or protecting the plant by degrading xenobiotics in contaminated soils [3]. Additionally, PGPR alleviate drought, cold, high salinity, and metal toxicity stresses [1–5].

Many PGPR produce phytohormones that enhance root growth and surface area. Phytohormones consist of auxins (IAA), cytokinins and gibberellins produced by natural soil microbial communities [6]. However, hormone and organic acid contributions from PGPR have not been determined, and their involvement in promoting plant growth is speculative. Therefore, PGPR have been formulated for use in agriculture, on the scale of fermentation microorganisms, with management of the quality and effectiveness of the product [7]. To ensure survival and activity of PGPR in the field, seeds have been inoculated with PGPR, or PGPR have been applied as a powder or liquid.

Intensive farming practices, which warrant high yield and quality, require the extensive use of chemical fertilizers, which are costly and create environmental problems. Therefore, there has been a recent resurgence of interest in environmentally friendly, sustainable and organic agricultural practices. This system avoids or largely excludes the use of synthetic fertilizers, pesticides, growth regulators and livestock feed additives. Environmentally friendly organic agricultural systems depend heavily on biofertilization, green manure, farm manure, crop rotation, legumes, mineral-bearing rocks, and aspects of biological pest control to sustain soil productivity. However, yield reduction is a serious problem in the organic production system [8]. The use of organic fertilizers containing sewage sludge, seaweed, and lichen is known

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to improve plant growth and help sustain environmental health and soil productivity [9,10]. Crops, vegetables and fruits are reliably produced using organic fertilizer sources if enough nutrients are available. To our knowledge, no study has previously investigated the chemical composition of PGPR exudates. Therefore, in the present study, our objectives were to understand how PGPR act on their host plant and to evaluate various chemical properties of PGPR strains as a nutrient source for sustainable and organic agriculture.

2. Materials and methods

2.1. Bacterial strains

PGPR strains (*Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Bacillus pumilus* C26, *Paenibacillus polymyxa* RC05, *Azospirillum brasilense* sp245, *Burkholderia cepacia* OSU7, *B. cepacia* OSU7 AMP Res., and *Raoultella terrigena* TFi08) were obtained from the culture collection in the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture at Yeditepe University, Istanbul, Turkey. These bacteria were previously reported to have plant growth-promoting characteristics and to be potential biocontrol agents against a wide range of bacterial and fungal pathogens that cause economically important crop losses in agriculture [11–15]. The bacterial strains used in this study were identified using the Sherlock Microbial Identification System version 6.0 (MIDI, Newark, DE).

2.2. Bacterial growth and laboratory experiment

Pure bacterial cultures were grown on National Botanical Research Institute Phosphate (NBRIP) growth medium [16]. The bacterial culture suspensions were kept in a nutrient broth with 15% glycerol at −80 °C for long-term storage. For these experiments, a single colony was transferred to a 500 mL flask containing NBRIP and grown aerobically on a rotating shaker (Merck KGaA, Darmstadt, Germany) for 48 h at 27 °C and 150 rpm. Next, the culture was diluted to a final concentration of 108 colony forming units (CFU) mL^{-1} using sterile distilled water containing 0.025% Tween 20. Twenty-five samples of PGPR bacterial suspensions in replicate were used to determine the organic acid, amino acid, hormone, and nutrient contents, along with enzyme activity.

2.3. Amino acid analysis

For the amino acid analysis, 5 mL of 0.1 N HCl was added to 5 mL bacterial culture suspensions. The cultures were homogenized and dispersed using an IKA Ultra Turrax D125 Basic homogenizer and incubated at 40 °C for 12 h. Then, the homogenized bacterial culture suspensions were vortexed. After these bacterial suspensions were centrifuged at 1200 rpm for 50 min, the supernatants were filtered using a 0.22 μm Millex Millipore filter. Next, the supernatants were transferred to vials for amino acid analysis using HPLC as described [17]. The quantities of amino acids found in the bacteria samples, including aspartate, glutamate, and asparagine, were determined after 26 min of HPLC derivation and are reported as pmol μl^{-1}.

2.4. Organic acid analysis

For the analysis of organic acids, 10 mL of deionized water was added to 5 mL bacterial culture suspensions, which were homogenized using an IKA Ultra Turrax D125 Basic homogenizer. After centrifugation at 1200 rpm for 50 min, the supernatants were filtered through a 0.22 μm pore Millex Millipore filter and collected in vials. The supernatants were subjected to HPLC analysis using a Zorbax Eclipse-AAA 4.6 × 250 mm, 5 μm column (Agilent 1200 HPLC), and the absorbance at 220 nm was read using a UV detector. The flow speed was 1 mL min^{-1}, and the column temperature was 250 °C. The organic acid contents of the bacterial suspensions, including oxalic and propionic acids, were determined using 25 mM potassium phosphate pH 2.5 as the mobile phase.

2.5. Hormone analysis

The extraction and purification processes were executed as described [18]. For hormone analysis, 5 mL of cold (−400 °C) 80% methanol was added to 5 mL bacterial culture suspensions. The bacterial suspensions were homogenized for 10 min using an IKA Ultra Turrax D125 Basic homogenizer, and then the bacterial suspensions were incubated for 24 h in the dark. The bacterial suspensions were filtered using a Whatman No: 1 filter, and the supernatants were filtered again using a 0.45 μm pore filter. The hormones were analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC), and the absorbance was read at 265 nm using a UV detector. Gibberellic acid, salicylic acid, indole acetic acid (IAA), and abscisic acid (ABA) were determined using 13% acetonitrile (pH 4.98) as the mobile phase.

2.6. Enzyme activities of PGPR

Phosphatase activity was determined using *para*-nitrophenyl phosphate (pNPP) as an ortho-phosphate monoester analog substrate [19]. We calculated the *p*-nitrophenol content using a calibration curve obtained with standards containing 0, 10, 20, 30, 40 and 50 ppm of *p*-nitrophenol.

2.7. Antioxidant enzymes analysis of PGPR

For antioxidant enzyme assays, frozen cell samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer, pH 7.8, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzyme activities in the apoplastic fractions were measured using a spectrophotometer [20].

2.8. Element analysis

The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine the total N content [21] of PGPR strains. The Ca, Mg, Na, K, P, S, Fe, Cu, Mn, Zn, Pb, Ni and Cd contents were determined using an Inductively Coupled Plasma spectrometer (Perkin–Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) [22].

2.9. Statistical analysis

Data were sorted by PGPR species, and differences among species were identified using the Duncan test option in the analysis of variance [23]. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Amino acids and organic acids produced from PGPR

B. megaterium M3 had the highest glutamate (26,814 pmol μl^{-1}), isoleucine (25,562 pmol μl^{-1}), arginine (35,727 pmol μl^{-1}),