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Growth performance of rhizobacteria on water hyacinth (*Eichhornia crassipes*) juices and dehydrated powder

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ABSTRACT

In the present study, juice of water hyacinth (*Eichhorina crassipes*), either crude or from its successive dilutions (1:1, 1:10, 1:30, 1:50 v/v) supported the *in vitro* development of *Bacillus megaterium*, *Bacillus subtilis*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* biovar *Phaseoli* with doubling time (23.1–63.0 min) which was comparable if not shorter, to that calculated using the standard laboratory -synthetic media (nutrient, N-deficient mannitol and yeast extract agar media; 48.0–64.8 min). Rhizospheric microorganisms of legume and non-legume plants successfully grew on surface-inoculated agar plates of crude and diluted juices of the macrophyte. Tea bags filled with the dehydrated powders (5 and 10 g l^{-1}) of water hyacinth supported the *in situ* recoverability of total rhizobacteria in population densities (3×10^7 - $>10^8$ cfu.g^{-1}), which were found to be comparable, if not excessive, to those enumerated on the recommended culture media. Morpho-physiological identification of some isolates that had developed on the plant juice and tea bag culture media, revealed that they are not akin to those cultured on the chemically-synthetic culture media; they possibly represent a portion of recommended media - unculturables.

Introduction

A number of plant juices and extracts was found suitable as growth media for bacterial growth as they contain the necessary nutrients and growth factors e.g., amino acids, vitamins and minerals. Nour et al. (2012) and Youssef et al. (2016) indicated the possible use of plant materials including juices, saps and powders to stimulate the culturability of a great number of microorganisms. In comparison with the chemically- synthetic culture media, plant materials did promote growth of bacteria and efficiently recovered their *in situ* population. Apart from that, plant dehydrated powders that are packed in tea bags and satisfy the growth requirements of various microbial communities, are highly recommended (Sarhan et al., 2016).

This is when the idea to provide further support to the original approach of the sole use of the water hyacinth (*Eichhornia crassipes*) juice and dehydrated powder tea bags to replace the recommended standard culture media arose. Such media were investigated for culturing some pure bacterial isolates and recovering the rhizobacteria population associated with roots of representative legume and cereal plants.

Materials and methods

Plant material

Samples representing the whole fully-grown water hyacinth (*Eichhornia crassipes*) plants were taken from heavily-covered sites along the River Nile at Kome Hamada, Behera governorate. Plants were washed in water then transferred in paper bags. One set of plants was kept fresh while another one was dried overnight at 70 °C. The nutritional composition of dried weed was determined according to Gunnarsson and Petersen (2007) and Akinwande et al. (2013).

Preparation of plant juice

Adopting the procedure of Nour et al. (2012), the plant juice was prepared as follows: the whole succulent mature plant was sliced and blended with distilled water (4:1 w/v fresh weight plant: water) for *ca.* 5 min in a Warring blender. The obtained juice was coarse –filtered through cotton tissue and stored at −20 °C. The juice in this state or diluted with distilled water (1:1, 1:10,

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1:30 and 1:50 v/v) was exclusively used to prepare liquid culture media. Juices were adjusted to pH 7.0 from pH 6.1 and autoclaved at 121 °C for 20 min.

Preparation of tea bags

The water hyacinth dehydrated powder tea bags were prepared as mentioned by Sarhan et al. (2016). Oven-dried plant substances were ground to 2.0 mm screen to get the required powder. Five or ten grams of the fine material were distributed into each bag and sealed by stapling. The tea bags were immersed to secure the liquid plant infusion in one liter of distilled water. Two percent (w/v) of agar was added to obtain agar culture media and adjusted to pH 7.0 then autoclaved for 20 min at 121 °C. The tea bags were kept in the culture media during autoclaving to guarantee more plant extraction.

Tested pure isolates

Four representative pure isolates: *Bacillus subtilis*, *Bacillus megaterium*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* biovar. *phaseoli* provided by the Department of Microbiology, Faculty of Agriculture, Cairo University and Department of Microbiology; Soils, Water and Environment Institute, Agricultural Research Center, Giza, Egypt were used. Strains were maintained on the recommended liquid media of both.

Standard chemically-synthetic culture media

The recommended nutrient (Jensen, 1962), modified N-deficient mannitol (Abd-el-Malek and Ishac, 1962) and yeast extract mannitol, YEM (Vincent, 1970) agar media were used to count the CFUs of the pure isolates of *Bacillus* spp., *Azotobacter chroococcum* and *Rhizobium leguminosarum* biovar. *phaseoli,* respectively.

In addition, soil extracts (Parkinson et al., 1971) and nutrient agar media were used for enumeration of total bacterial communities in rhizospheres and root-free soils of representative cereal and legume plants. Nutrient agar contains (g l^{-1}): peptone, 5.0; beef extract, 3.0; glucose, 1.0; yeast extract, 0.5; agar, 15; pH, 7.0 ± 0.2. N-deficient mannitol medium comprises (g l^{-1}): mannitol, 10.0; sucrose, 10.0; K_2HPO_4, 0.2; $MgSO_4$, 0.2; NaCl, 0.2; KSO_4, 0.1; $CaCO_3$, 5.0; agar, 15.0; pH 7.4 ± 0.2. Yeast extract mannitol contains (g l^{-1}): K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; mannitol; 10.0; yeast extract, 0.4; agar, 15; pH 6.8 ± 0.2. Soil extract contains (g l^{-1}): glucose, 1.0; peptone, 1.0; yeast extract, 1.0; K_2HPO_4, 1.0; soil extract, 400 ml; tap water up to 1 L; agar, 15; pH, 7.0 ± 0.2.

Water hyacinth juices as culture media for bacterial isolates

The growth patterns of selected bacterial candidates were monitored in liquid culture media depending on either crude water hyacinth juice or its successive dilutions. For comparison, the reference nutrient, N-deficient mannitol, and YEM liquid culture media were included. The liquid culture media were prepared (100 ml in 250 ml capacity Erlenmeyer flasks), inoculated with tested bacterial liquid cultures (2%, v/v) and incubated at 30 °C in a rotary shaker (100 rpm) for 120 h. At 12- hour intervals, suitable serial dilutions prepared from the resulting liquid cultures were surface-plated, in triplicate, on agar plates of water hyacinth juices and on agar plates of reference media. After incubation at 30 °C for 2–5 days, the CFUs were counted on the inoculated plates. Growth curves were plotted and doubling times were estimated (Wistreich, 2003) as the following: Growth rate (K) = Log Nt-Log N0/Log 2 (Tt-T0); Doubling time (dt) = 1/K; where: N0 = viable cell counts at T0, T0 = time at the begging of determination; Nt = viable cell count at Tt, Tt = time of determination.

Culturability of rhizospheric microorganisms (RMOs) on plant juice–based culture media

The rhizosphere samples, representing the root surface together with the closely-adhering soil, and root-free soils of legume (broad bean and lentil) and non-legume (wheat and maize) plants, were determined for total bacterial communities using the plant juice either as such or in its distilled water-successive dilutions of 1:1, 1:10, 1:30 and 1:50 (v/v) as culture media. For rhizospheres, roots with adhering soils were transferred into glass bottles containing sterile distilled water. Serial dilutions were prepared after shaking for 30 min. For root-free soils, 10 g of crushed air-dried soil sample were transferred to 90 ml sterile distilled water in glass bottles. These were then thoroughly shaken and serial dilutions were prepared. For both spheres, 1 ml aliquots of suitable dilutions was taken to surface-inoculate agar plates along with 3 replicates from each dilution that represented the plant-based culture media which had been prepared from the tested juices as well as the chemically synthetic nutrient and soil extract agar media. After incubation at 30 °C for 5 days, developing colonies were enumerated. Dry weights (105 °C) of suspended soils were determined.

Recovery of rhizobacteria associated with plant roots on water hyacinth tea bag culture media

The legume and non-legume plant rhizosphere and root-free soil samples were determined for total bacterial populations using the water hyacinth dehydrated powder tea bags (5 and 10 g l^{-1}) adopting the procedures previously mentioned with the plant juices. The produced CFUs were counted against those developed on the recommended nutrient and soil extract agar media.

Morphological identification of rhizobacteria developed on agar plates

Some agar plates containing separated colonies were selected to represent the water hyacinth juices and dehydrated powders besides the standard recommended media including nutrient agar and soil extract. Those colonies were examined for cultural characteristics encompassing color and spreading. The successfully subcultured colonies were purified and tested for cell morphology, Gram stain, motility and sporulation as well as catalase and oxidase activities (Benson, 1985; Kovacs, 1956).

Statistical analysis

Statistical analysis was made by STATISTICA 10.0 (Statictica 10.0. StatSoft Inc, Tusla, USA.). Differences among treatments were statistically compared using one-way ANOVA and Fisher's least significant difference (LSD; at level of 0.05).

Results

Nutritional profile of water hyacinth

The chemical analyses of the water hyacinth indicate that the plant is rich in total carbohydrates (42%) and proteins (24%). Macro-nutrients (Na, K, Ca, Mg) were present in quantities of 2.1–14.9%; lower amounts of micro-nutrients (Cd, Cu, Cr, Fe, Ni, Zn) were found with pH of 7.2.

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