

Bacterial Community Associated with Autotrophic and Heterotrophic Cultures of Medicinal Plant *Smallanthus sonchifolius* (Yacon)

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Received August 11th, 2012; revised September 8th, 2012; accepted September 18th, 2012

ABSTRACT

Several unfruitful attempts to grow axenic shoot cultures of *Smallanthus sonchifolius*, also known as yacon, were made before healthy shoots grew in association with bacteria on half strength Murashigie and Skoog media supplemented with 2.2 μ M benzylaminopurine. Twenty-one bacterial isolates were obtained from *in vitro* *S. sonchifolius* plantlets, eight of these isolates were identified as *Flavimonas oryzihabitans*, *Curtobacterium pusillum*, *Sphingomonas paucimobilis*, and *Microbacterium imperiale*. These microorganisms produced indole acetic acid (IAA) at amounts varying between 8.89 to 47.45 μ g/mL, reason for being classified as plant growth promoting bacteria (PGPB). The results show that buds associated with bacteria cultured on sucrose free media produced 3.77 new roots measuring 18.33 cm in length after a 30-day growing period. In contrast, buds growing on sucrose supplemented media, the number of roots induced was higher (6.67 to 14 roots/explant) but shorter in length, 4.67 to 5.83 cm. During plant acclimatization to soil, photosynthesis and water efficiency were measured showing that the plants were healthy and vigorous. A slightly higher rate of photosynthesis and water use efficiency was recorded in the plants produced on heterotrophic conditions as compared to plants grown in sucrose free media. Plants adapted well in the soil demonstrating that the PGPB community associated to *S. sonchifolius* in shoot cultures was not harmful to plant production. The purpose of this study was to demonstrate that the bacteria associated with *in vitro* *S. sonchifolius* shoot cultures was not the result of microbial contamination, but rather from symbiotic associations that extended from cultivation in the greenhouse, to culture and back to soil. This is the first report to show that autotrophic cultures may represent a viable alternative to grow healthy plants without eliminating beneficial bacteria associated with the host.

Keywords: Micropropagation; Endophytes; Yacon; IAA; Bacteria

1. Introduction

Micropropagation in the horticulture industry is a routine technique used to propagate healthy and elite germplasm. One of the critical steps in micropropagation is the disinfection procedure. Published reports dedicate special attention to protocols used to disinfect different plant tissues and to avoid microbial contamination during the process of plant multiplication [1-3]. Leifert *et al.* [4] suggest that the elimination of plant parts close to the soil could avoid microbial growth in tissue culture because disinfection of those parts is difficult. According to Haroim *et al.* [5], plant rhizospheres are the hot areas of microbial colonization.

Recently, Miller *et al.* [6] demonstrate that leaf and stem tissues of Traditional Chinese Medicine (TCM) herbs are host to diverse bacteria and fungi with the potential to

synthesize secondary metabolites which contribute to the plant's chemical composition and therapeutic properties. Our studies on *Echinacea* immune enhancing properties revealed that lipoproteins and lipopolysaccharides of bacterial endophytes are the actives responsible for *in vitro* macrophage activation [7].

In reviewing the biology and chemistry of endophytes, Zhang *et al.* [8] report that some bacterial endophytes could originate from the rhizosphere or phylloplane microbiota, then penetrate and colonize root tissue to gain access to the xylem. Several reports describe endophytic microbiota as beneficial, because they improve the host's adaptability, promote growth, and prevent disease through endophyte-mediated *de novo* synthesis of compounds with anti-fungal properties [9-11].

Thomas *et al.* [12] show the ubiquitous presence of

fastidious endophytic bacteria in apparently clean shoot-tip cultures of papaya. Cells of axenic peach palm (*Bactris gasipaes*) microplants were observed by light microscopy and De Almeida *et al.* [13] analyze the movements of small particles within the cells as intracellular bacterial community. The presence of bacteria in tissue culture is not only the result of poor technique but it may be the result of mutualistic association between the host and its associates. Potential applications of beneficial microorganisms associated with plants still restricted to those amenable to cultivation [6,14]. Thus, plant tissue culture may offer a useful system to recover beneficial microorganisms in association with specific organs. Herein, the study describes healthy shoot cultures of *Smallanthus sonchifolius* Poepp. H. Rob, also known as yacon, growing hetero and autotrophically, while maintaining the consortium between host and associated bacteria.

2. Material and Methods

2.1. Plant Material

Yacon's rhizophores were donated to the National Center for Natural Products Research by the University of Ribeirão Preto (UNAERP), Department of Biotechnology, Brazil. Stem cuttings were planted in pots and kept under mist system for 15 days in the glasshouse located at University Field Station and later the pots were irrigated by a dripping system, 200 ml per pot on a daily basis. The plant material donated by UNAERP was of a single clone that has been propagated by rhizophores and stem cuttings.

2.2. Shoot Cultures of *Smallanthus sonchifolius*

Terminal buds and nodal segments (~0.5 cm long) were harvested from potted plants and washed with running water for 15 minutes. Buds were immersed in 0.5% (w/v) sodium hypochlorite solution for 30 minutes, and then washed three times in sterile water. Surface disinfected buds were inoculated on half strength Murashige and Skoog [15] (MS), media supplemented with 3% (w/v) sucrose and 2.2 μ M benzyladenine (BA) solidified with 0.8% agar, pH was adjusted to 5.8. After the third unsuccessful attempt to obtain axenic *in vitro* cultures of *S. sonchifolius*. Five ml of the third wash used to remove sodium hypochlorite solution of explants, was smeared on bacteriological media showing no signs of bacterial growth.

Half of the shoot cultures associated with bacteria growing epiphytically at the base of explants with no signs of virulence to the host were separated and later half were transferred to sucrose free media using sun cap closures (MKKB7267) to allow gas exchange. These cultures were denominated WOS. The other half of the cul-

tures were sub-cultured to the sucrose media called WS media. Twenty days after the inoculation of the explants, cultures were evaluated (**Table 1, Figure 1E**). After the first evaluation, terminal shoots were transferred to MS/2 supplemented with BA at concentrations ranging from 2.2 μ M, 4.4 μ M and 8.8 μ M to induce multiple shoots. WOS and WS cultures were evaluated after a 30-day growing period (**Table 2**). Each treatment had 6 replicates. The experiment was repeated three times. Presence of bacteria was noticed growing epiphytically at the base of the shoot explants in every subculture. Shoot cultures were sent to the microbiology laboratory at EMBRAPA/Environment, Jaguariuna Brazil for microorganism identification.

S. sonchifolius cultures were maintained in alternating growing conditions with sucrose (WS) and without sucrose (WOS) for more than 12 months in a 30 day interval between subcultures. Thirty day plantlets grown on WOS and WS media (**Figures 1E and F**) were submitted to microscopic analysis. The subcultures were done by removing and discarding the roots and the re-inoculation of the buds on fresh media. Cultures were always incubated at 25°C \pm 2°C, 16-h photoperiod under fluorescent light with a photon flux of 52 μ mol·m⁻²·s⁻¹. After 12 months of *in vitro* alternating cultures (WOS and WS) the *S. sonchifolius* plantlets were acclimatized in the soil and later cultivated in the pots. Photosynthesis rate was measured to evaluate the performance of WOS and WS plants during acclimatization.

2.3. Microscopic Evaluation of *Smallanthus sonchifolius* Shoots

S. sonchifolius/bacteria cultures grown on sucrose free and sucrose containing media were observed both morphologically and micro-morphologically. Hand sections of the shoots were taken for samples growing under autotrophic and heterotrophic conditions; sections were stained with Safranin O and mounted on glass slide with glycerine and observed using Nikon Eclipse E-600. Photomicrographs were taken using Olympus DP-71; images were processed using DP manager (**Figures 1C and D**).

Table 1. Effect of explants and growing conditions on the growth of *S. sonchifolius* shoot cultures.

Treatments		Plant Height \pm SE	# of Roots \pm SE	Root Length \pm SE
Media/Explant Type [†]		(cm)		(cm)
WOS*	TB	2.93 \pm 0.85	6.90 \pm 4.00	2.95 \pm 1.98
WOS	NS	1.30 \pm 0.76	1.90 \pm 2.57	1.10 \pm 1.34
WS	TB	3.20 \pm 0.72	6.60 \pm 2.76	2.99 \pm 1.66
WS	NS	1.42 \pm 1.13	1.81 \pm 1.72	1.10 \pm 1.24

[†]TB is terminal bud, NS is nodal segment. WOS- ½ MS without sucrose, WS-½ MS media with sucrose; Data was recorded after a 20 days of the inoculation.

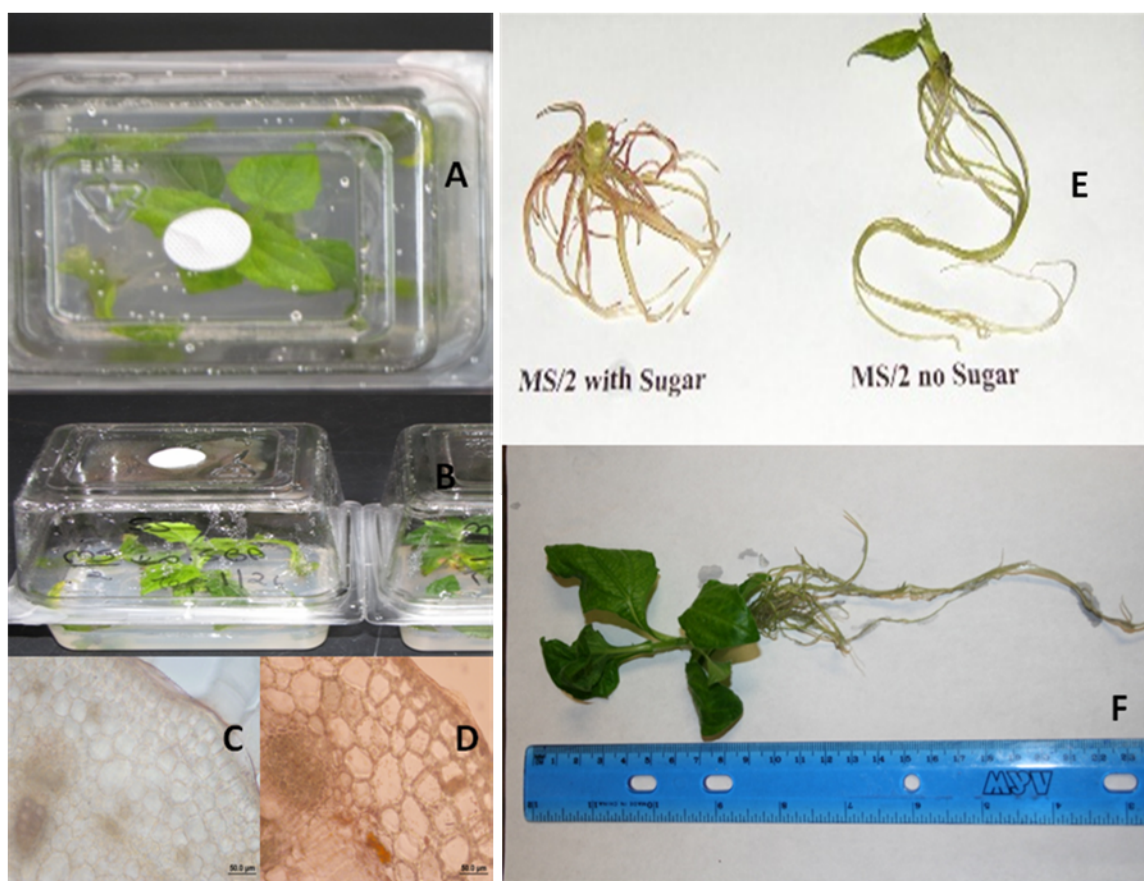


Figure 1. Shows shoots of *Smallanthus sonchifolius*, also known as yacon, growing on autotrophic cultures in association with bacteria (A) and (B), hand sections of shoots taken for samples growing under autotrophic and heterotrophic conditions (C) and (D), root branching of yacon plantlets produced on sugar free and sugar containing $\frac{1}{2}$ MS salts (E); *in vitro* produced yacon plantlet before acclimatization (F).

Table 2. Effect of benzyladenine on the growth and development of micropropagated cultures of yacon initiated only using only terminal shoot as explants.

Treatment		Plant Height \pm SE	# of Roots \pm SE	Root Length \pm SE
Culture condition	BA (μ M)	(cm)		(cm)
WOS	2.2	6.33 \pm 0.58	5.33 \pm 0.58	17.33 \pm 4.93
	4.4	4.67 \pm 1.53	3.33 \pm 1.15	21.00 \pm 6.56
	8.8	4.67 \pm 2.52	2.67 \pm 0.58	16.67 \pm 2.89
WS	2.2	4.00 \pm 0.00	6.67 \pm 3.06	5.83 \pm 2.25
	4.4	3.00 \pm 1.00	14.00 \pm 5.29	4.67 \pm 1.53
	8.8	3.33 \pm 0.58	10.67 \pm 1.15	4.67 \pm 1.53

BA= Benzyladenine, SE = Standard Error; Data was recorded after a 30 day growing cycle.

2.4. Isolation, Identification of the *in Vitro* Community of Bacteria Associated with *in Vitro* Cultures of *Smallanthus sonchifolius*

In vitro *S. sonchifolius* plantlets were cut in small pieces (0.5 - 0.7 cm) and inoculated on Tryptone Soya Agar (TSA) media [16] and on starch (10 g per liter) casein (0.3 g per liter) medium supplemented with KNO₃ 2 g; NaCl 2 g; K₂HPO₄, 2 g; MgSO₄·7H₂O, 0.05 g; FeSO₄·7H₂O, 0.01 g;

agar, 16 g per liter). Cultures were maintained at room temperature varying between 25°C and 27°C. Bacteria growth was evaluated 48 hours after inoculation and for a 10 day-period. Cultures were storage in -70°C on glycerol (20% v/v) stocks.

All bacteria were identified by Gram-staining and whole cell fatty acid analysis (FAME) [17,18]. For the FAME analysis the isolates were cultures on TSBA medium at 28°C for 48 h. Biomass was harvested and the

fatty acids saponificated, methylated and extracted as described by Millar and Berger [17]. Fatty acid methyl esters were analyzed using an Agilent GC (7683) and the identification was done with the Sherlock Microbial Identification System (MIDI Inc. Newark, DE, USA) and GC analysis. The FAME profile of *Xanthomonas maltophilia* ATCC 13637 was used as a reference for the MIDI determinations. FAME identification is highly reliable for similarities higher than 0.70 at species level, while lower levels can affiliate isolates to higher taxonomic groups, like genus or families [18].

2.5. Indole Acetic Acid Production of the Isolated Bacteria from *in Vitro* Shoot Cultures of *Smallanthus sonchifolius*

The production of Indole Acetic Acid (IAA) was determined by the colorimetric methodology described previously by Gordon and Weber [19]. Briefly, isolates were grown in nutrient broth (NB) medium amended with tryptophan (100 µg/mL) in the dark. Cells were harvested by centrifugation (12.000 g for 5 min) and the supernatant was treated with Salkovski reagent for 15 min. The production of IAA was direct related to the absorbance measured at 530 nm. Pure IAA was used in cell experiments as a standard.

2.6. Plantlet Acclimatization

In vitro propagated *S. sonchifolius* plantlets grown under autotrophic and heterotrophic conditions were transferred to a soil substrate composed of a mixture (1:2 v/v) potting soil (Potting Mix MiracleGro 0.14 0.14 0.14) and sand (Garden Basic Play Sand, Sims Bark Co, Tusculumbia, AL). Potted plantlets were maintained under mist-irrigation watering cycle of 1 min every hour for a period of 6 hours similar to the procedure.

2.7. Photosynthesis Measurements

S. sonchifolius plantlets produced on WOS and WS cultures were hardened and used for carbon assimilation and water vapor exchange studies. Measurements were carried out on five upper undamaged, fully expanded and healthy leaves of each plant with the help of a closed portable photosynthesis system (Model LI-6400; LI-COR, Lincoln, Nebraska, USA). Following preliminary experiments on these plants, ~30°C was found optimum temperature for growth, and therefore all subsequent gas and water vapor exchange measurements were carried out at 30°C ± 0.5°C. To determine the effect of light on gas and water vapor exchange, leaves were exposed to different photosynthetic photon flux densities (PPFD) viz., 0, 500, 1000, 1500 and 2000 µmol·m⁻²·s⁻¹ with the help of an artificial light source (Model LI-6400-02; light emitting

silicon diode; LI-COR), fixed on the top of the leaf chamber. Photosynthetically active radiation (PAR) was recorded with the help of a quantum sensor kept in the range of 660 - 675 nm wave radiation, fixed at the leaf level. The rate of dark respiration was measured by maintaining the leaf in the cuvette at zero irradiance. The leaf chamber was covered with a black cloth throughout the course of respiration measurements to avoid any external radiation. Air flow rate (500 mmol·s⁻¹), CO₂ concentration inside the leaf chamber (350 ± 5 ppm), temperature (30°C ± 0.5°C) and relative humidity (55% ± 5%) were kept nearly constant throughout the experiment. Since steady state photosynthesis is reached within 30 - 45 min, the leaves were kept for about 45 - 60 min under each set of light conditions before the observations were recorded. Water use efficiency (WUE) was calculated as a ratio of the rate of photosynthesis and transpiration. The data were analyzed using SYSTAT software package [20].

3. Results

3.1. Morphological Evaluation of Plantlets Growing on WOS and WS Cultures

Preference was given to terminal and nodal segments removed from plants cultivated in the greenhouse to initiate *in vitro* cultures over rhizophores (the underground tissue containing fructans in Asteraceae). According to Laifert *et al.* [4] aerial tissues are easier to surface sterilize than underground parts (**Table 1**). In less than two weeks after *in vitro* inoculation of the explants, all cultures showed bacteria growing around the shoots. All cultures were discharged and new attempts were made to obtain axenic shoot cultures of *S. sonchifolius* sugar containing media.

In the third attempt the third wash was smeared on bacteriological media, showing no signs of bacterial growth. Results of **Table 1** revealed that buds/bacteria transferred to WOS media grew taller produced fewer and longer roots than buds growing on WS cultures during three weeks (**Table 1**). Plant height, root length, number of roots, leaf size and shape were morphological parameters evaluated in this study. Compiled data (**Tables 1, 2 and 3**) show shoots cultured on WOS media produced taller plantlets, parenchyma cells were more elongated. **Figure 1** shows *in vitro* cultures of yacon (A & B) a transversal section of the shoots of plants growing in media with and without sucrose (C & D), morphological differences on root branching of plantlets grown on media with and without sucrose (E & F). Leaf size, shape and petiole, softness and hyper-hydricity of the tissues were morphological differences noticed on plantlets grown under autotrophic conditions. Epidermis and vascular bundles did

Table 3. Morphological characters of *in vitro* yacon shoot cultures grown on half strength MS media supplemented with 2.2 μM benzyladenine with or without sucrose (WS and WOS).

Shoot Cultures	WS media	WOS media
Leaf size	3.5 \times 2.5 cm	4.5 \times 3.5 cm
Shape	Ovate oblong	Oblong
Petiole	Cuneat, ca 2 cm, winged	Cuneat ca 2.5 cm long,
Leaf margins	Slightly undulate rounded teeth with irregular, wedge shaped	Margins undulate with irregular shaped margins
Leaf apex	Acute	Acute
Hair type abaxial	Densely hairy on adaxial surface, sparse on abaxial	Densely hairy on adaxial surface, sparse on abaxial

not show any significant differences in the two treatments. No sign of toxicity was noticed on the parenchyma cells, epidermis or vascular bundles of shoots grown in association with bacteria.

Buds/bacteria on sugar containing (WS) media supplemented with 4.4 μM produced more roots than any other treatment while under autotrophic condition the roots were fewer and longer (Table 2, Figures 1E and F). In addition, plantlets grown on WS cultures show browning of the lower leaves with small lateral roots induced at the nodes while WOS plantlets had roots that reached 21 cm long. Most importantly, the shoots showed differences in root branching due to presence of sugar associated with colonies of bacteria growing epiphytically. The elongated roots may be the result of levels of indole acetic acid (IAA) produced by the plant growth promoting bacteria (Table 4).

3.2. Isolation and Identification of Epiphytic and Endophytic Bacteria Associated with *in Vitro* Propagated *Smallanthus sonchifolius*

A total of 21 bacteria were isolated from *in vitro* yacon plantlets. Of these bacteria, only two isolates did not match any known species by the FAME technique. Two isolates matched with low similarity index; *Rhizobium radiobacter* (0.167) and *Pseudomonas aeruginosa* (0.2548). Included in Table 4 are eight isolates that were identified to the species level with similarity index above 0.75. These isolates synthesized IAA, with *in vitro* production varying between 8.89 $\mu\text{g/mL}$ to 47.45 $\mu\text{g/mL}$. Our data suggest that the isolates recovered from *in vitro* yacon cultures were endophytes except for *F. oryzihabitans*, which grew endo and epiphytically. Several reports indicate that plant growth promoting bacteria are beneficial endophytes growing in association with the host [21-23]. Among the *S. sonchifolius* isolates, *Microbacterium imperiali* and *Sphingomonas paucimobilis* were the highest IAA producers (Table 4).

3.3. Photosynthesis Performance of *in Vitro* Propagated *Smallanthus sonchifolius* during Acclimatization

Autotrophically (WOS) and heterotrophically (WS) *in vitro* produced plantlets were evaluated *ex vitro* by measuring the effect of different photosynthetic photon flux densities (PPFD) on photosynthesis (P_n), dark respiration (R_d), transpiration (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), ratio of intercellular to external CO_2 concentration (C_i/C_a) and water use efficiency (WUE). The rate of photosynthesis increased with increasing light intensity up to 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in both types (WOS and WS) plants (Figure 2). However, increment was more pronounced at PPFD below 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Maximum rate of photosynthesis ($P_{n_{\max}}$) was recorded to be $17.08 \pm 2.60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the plants produced on WS media with active growing bacteria whereas, it was found to be slightly lower ($15.37 \pm 2.80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for the plants that were produced on WOS cultures at 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Furthermore, exchange of CO_2 was found to be adversely affected by light intensities beyond 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD in both types of plants. Dark respiration (at 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) was also found slightly higher in the plants produced under heterotrophic conditions with active growing bacteria as compared to the plants without sugar. The rate of transpiration and therefore, stomatal conductance (Figures 2(a) and (b), respectively) increased considerably with an increase in PPFD up to highest level tested (2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in both sets of plants. Values of E and g_s were however found to be higher in the plants grown under sucrose media (Figure 2(b)). Contrary to g_s , C_i was highest, *i.e.* 364.00 ± 22.83 and $360.00 \pm 19.71 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at zero light for *in vitro* and *ex vitro* propagated plants, respectively (Figure 2(b)). The values gradually decreased with increase in PPFD in both cases. Similar to C_i , C_i/C_a ratio also gradually decreased with PPFD up to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and then became nearly

Table 4. Identification of endophytic bacteria isolated from micropropagated yacon cultured on half strength MS media supplemented with sugar and benzylamine.

Isolates [†]	Similarity Index	Plant Tissue	Absorbance A 530	IAA (µg/mL)
<i>Microbacterium imperiale</i>	0.769	Leaf	0.4614	41.70
<i>Sphingomonas paucimobilis</i>	0.788	Leaf	0.4727	42.74
<i>Curtobacterium pusillum</i>	0.771	Leaf	0.1051	8.89
<i>Microbacterium imperiale</i>	0.892	Root	0.524	47.45
<i>Sphingomonas paucimobilis</i>	0.750	Stem	0.3838	34.55
<i>Flavimonas oryzihabitans</i>	0.898	Endo-Root	0.4285	38.67
<i>Flavimonas oryzihabitans</i>	0.915	Epi-Root	0.4084	36.82
<i>Flavimonas oryzihabitans</i>	0.965	Endo-Stem	0.4084	38.82

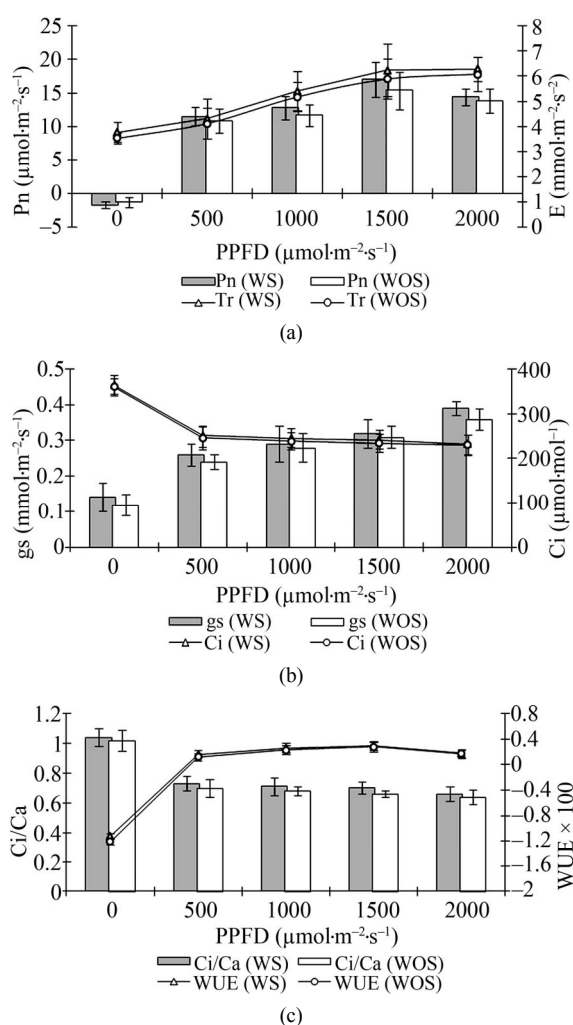


Figure 2. Effect of photosynthetic photon flux density (PPFD) on (A): photosynthesis, Pn and transpiration, E; (B): stomatal conductance, Gs and intercellular CO₂ concentration, Ci; and (C): ratio of intercellular to ambient CO₂ concentration, Ci/Ca and water use efficiency, WUE at optimum (30°C) temperature and relative humidity (55% ± 5%) in micropropagated and soil hardened plants of yacon (*Smallanthus sonchifolius*) produced autotrophically (WOS and heterotrophically (WS).

constant for both sets of plants at higher (1000 to 2000 µmol·m⁻²·s⁻¹) PPFDs (Figure 2(c)). A gradual increase in WUE was recorded with increase in PPFD up to 1500 µmol·m⁻²·s⁻¹ and maximum values (0.30 ± 0.05 and 0.28 ± 0.08) for plants produced on media with or without sucrose, respectively were recorded at this level (Figure 2(c)). However, increment was more pronounced at PPFD below 500 µmol·m⁻²·s⁻¹. A slight decline in WUE was observed at light intensities higher than 1500 µmol·m⁻²·s⁻¹ in both sets of plants. Overall, the values of all these parameters were found slightly higher in plants produced on WS media due to presence of actively growing bacteria as compared to the plants growing on WOS media with lesser bacterial growth. It has been shown that plants with higher Pn and WUE have the potential to grow faster and yield more than the species with low Pn and WUE [24,25].

4. Discussion

Other *in vitro* propagation protocols have also described the enrichment of plant growth by endophytic bacteria [26,27] Recently, De Almeida *et al.* [13] reported that microscopy observations of inner tissues of axenic cultures of *Bactris gasipaes* were colonized by bacterial cells. Abreu-Tarazi *et al.* [27] reported the presence of endophytic bacteria in long-term *in vitro* cultivated “axenic” pineapple microplants. Our study corroborates with these reports by demonstrating that some of bacteria associated with yacon were too difficult to eliminate by surface sterilization procedures. According to Kloepper *et al.* [28], *Pseudomonas*, a plant growth promoting bacteria (PGBP) contributed to increase growth and yield of root crops, and seed inoculation with different isolates of PGBP significantly increased plant height, stem diameter, number of branches, number of pods per plant, grain yield and oil content [29]. Most importantly, *Vitis vinefera* disease-free *in vitro* produced plantlets were inoculated with PGBP for host protection against plant diseases. Barka *et al.* [30] reported the beneficial effects of bacterial inoculation aiding plantlets acclimatization to soil condition.

Soil attached to plant root systems are a hot spot of microbial abundance due to the presence of exudates. According to Compant *et al.* [31], rhizosphere derived bacteria colonize internal tissues migrating through the xylem to the terminal buds and lateral buds. Thus, surfaced sterilization of terminal buds and nodal segments of yacon did not eliminate the bacteria associated *in situ* with these tissues. Herein we reported that bacterial growth in association with *in vitro* shoot cultures was epiphytically and endophytically on WS media. We believe that media containing sugar may have induced bacterial growth out of host tissue, gaining access to other sources of carbon. Although plantlets on WOS cultures did not show any visible signs of bacteria, *in vitro* culture condition may have contributed to bacterial growth epiphytically. Microscopy evaluations of epidermis and vascular bundles did not show any significant differences between WS or WOS plantlets excepted by the host cell elongation (**Figures 1C and D**).

Thomas *et al.* [12] reported that papaya shoot cultures displayed variation in growth and rooting potential, such variation was attributed to the associated microorganisms. Shoots growing on WS media produced more roots than shoots cultured on WOS. Bacterial colonies surrounding the explants were noticed only on WS media. This observation suggests that media containing sucrose media has stimulated bacterial growth associated to the *in vitro* rhizospheres. More bacterial growth may have induced greater IAA production leading to great root induction. In fact, browning of the older leaves was noticed on WS plantlets due to accelerated bacterial growth which may have decreased nutrient availability to the host. *S. sonchifolius* shoot cultures derived from a single clone, thus we attributed the differences in root branching to the presence of sucrose and the enhanced bacterial growth, and perhaps to growth promoters compounds released to the media. Results in **Table 4** confirmed that the bacteria associated with yacon were IAA producers, however further studies are needed to determine that bacteria have released auxin in large amounts to shoot cultures causing differences in root branching.

Acclimatization of WS plantlets to soil out performed to WOS plantlets with their long roots (data not shown). The rate of photosynthesis was slightly better for WS plants than WOS. Although, the WOS plantlets had the chloroplast fully functional during *in vitro* cultivation and prior to acclimatization to soil, the WS plants had more roots to support the transference to soil. In addition, the outgrowth of bacteria associated with WS plantlets rhizosphere was highly beneficial during acclimatization providing better resistance to stress.

In conclusion, to our knowledge this is the first report to demonstrate plant growth promoting bacteria (PGPB)

in vitro shoot cultures of *S. sonchifolius*. Autotrophic cultures may represent a viable alternative to grow healthy plants without eliminating beneficial bacteria associated with the host. In fact, *in vitro* protocols may become tool to examine cryptic endophyte consortia associated with embryonic plant tissues.

5. Acknowledgements

This research was partially funded by a grant from the USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012. The authors thank Dr. Luiz Rosa for his critique.

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