

## Investigation of Indigenous Plant Root Associated Bacteria and Yeast Isolates for Plant Growth Promoting Ability

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### ABSTRACT

Several bacteria (BSP41, BSP42, BSP51, BPL41 and BPL51) and yeast (YSP11, YSP31, YSP41 and YPL21) were isolated from the rhizosphere (SP) and rhizoplane (PL) of marigold (*Tagetes erecta* L.) obtained from local nurseries. Each isolate was identified and classified to genus and species levels. BSP41 was *Stenotrophomonas maltophilia*, and the other isolates belonged to *Aeromonas veronii*. The yeasts YSP11 and YPL21 were identified as *Cryptococcus humicolus*, YSP31 as *Candida famata* and YSP41 as *Cryptococcus laurentii*. Marigold seedlings, at the third leaf stage, were treated by dipping their root system in cell suspensions of the isolated bacteria or yeast or bread yeast (*Saccharomyces cerevisiae*) and observed for growth and nutrient composition. The effect of nutrient levels at the ambient solution was also investigated. Results indicated that there was a high degree of inconsistency in plant growth response and tissue nutrient contents of treated plants. However, the yeast isolate YSP31 (*Candida famata*) showed consistency in enhancement of plant growth over the untreated control plants. All tested isolates were found to be able to produce IAA in the culture medium.

**Keywords:** Plant Growth Promoting Organisms, Rhizosphere, IAA, Marigold.

### INTRODUCTION

Plant growth-promoting bacteria (Bashan and Holguin, 1998) and yeast (El-Tarabily, 2004; Nassar et al., 2005) may enhance plant growth indirectly by preventing deleterious effects of phytopathogens or directly by increasing number and length of roots resulting in a more branched root system. An increase in the root surface area leads to enhanced nutrient uptake, resulting in shoot growth promotion. Certain bacteria promote plant growth directly through the release of phytohormones (Koh and Song, 2007), enhancement of mineral uptake (Bashan et al.,

2004), solubilization of P (Dey et al., 2004) and/or decrease in heavy metal toxicity (Belimov et al., 2001). Some bacteria possess 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase (Dey et al., 2004), which hydrolyses ethylene precursor, resulting in root growth reduction (Glick et al., 1998). Yeast may enhance plant growth through releasing auxin (Nassar et al., 2005) and increasing P and Mn uptake by the plant (Mekki and Ahmed, 2005). There is no documented literature regarding such interactions in Jordan. Therefore, this study was conducted to test the effect of indigenous soil bacteria and yeast on growth of marigold (*Tagetes erecta* L.) and determine the possible mechanism(s) of growth promotion.

### MATERIALS AND METHODS

#### Isolation and Identification of Rhizosphere and Rhizoplane Bacteria and Yeast

Marigold plants at the vegetative stage were obtained

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from bedding plants nurseries in Baqa' and Hisban, Jordan. The root system was carefully dug out, shaken free of the extra soil, immersed inside 500 ml flasks containing sterile distilled water and shaken by hand for 30 min in order to obtain a rhizosphere (SP) soil suspension. The roots were then rinsed once with sterile distilled water, transferred into another 500 ml of sterile distilled water and shaken as above to obtain a rhizoplane (PL) soil suspension. Then, 10 fold serial dilutions of  $10^{-1}$ - $10^{-5}$  were prepared from each suspension and 1 ml from each dilution was spread on Potato Dextrose Agar (PDA) plates in 5 replicates under aseptic conditions inside a laminar flow chamber (EQU/03-EVC-UF, Singapore). Inoculated plates were incubated at 28 °C for 48-72 h. The plates were checked periodically for bacteria and yeast growth using a light microscope. Based on microscopic examination, the bacterial colonies are smoother and more shiny and slimy, while yeasts are rough, not so shiny and may be threads or free cells.

The gram reaction of the bacterial isolates was determined by inoculating a colony from a 24-48 h old culture on blood agar and MacConckey agar at 28 °C for 24 h (Murray et al., 1999). MacConckey plates were prepared using 50 g medium/l. MacConckey medium consisted of 20 g/l peptone, 10 g/l lactose, 31.5 g/l bile salts, 5 g/l NaCl, 0.001 g/l neutral red 0.03 crystal violet and 15 g/l agar. Blood plates were prepared using 40 g medium and 100 ml blood sheep/l. The blood medium consisted of 10 g/l peptone, 5 g/l NaCl, 10 g/l lab-lemco powder and 15 g/l agar. An isolate showing growth on MacConckey plates was considered as gram negative, whereas an isolate showing growth on blood plates was considered as gram positive.

For further identification, the bacterial isolates were subjected to the oxidase test using the filter paper method (Sinclair and Dhingra, 1995). This was done by

taking a loop full of a 24 h-old colony and spreading it on a filter paper then adding 2 drops of oxidase enzyme. The isolate changing in color to blue was considered as oxidase positive indicating aerial bacteria, whereas an isolate showing no change in color was considered as oxidase negative indicating that the bacteria was not aerial. Pure culture of each bacterial and yeast isolate was prepared by isolating a single colony and plating it on PDA plates at 4 °C, after which a single colony was isolated and plated as above. This was done for 10 times. The isolates were identified using automated microbial identification system (BIOMÉRIEUX VITEK<sup>®</sup>, VITEK 60, France) (Sinclair and Dhingra, 1995).

All isolates were characterized for their ability to produce IAA in the culture medium. Quantitative analysis of IAA was carried out using SHIMADZU<sup>®</sup> (Kyoto, Japan) UV-VIS detector and high performance liquid chromatography columns. A loop full of the tested isolate was placed in a centrifuge tube containing 40 ml autoclaved nutrient broth medium. The tubes were incubated at 27 °C for 48 h, then centrifuged at 6000 rpm for 30 min. The supernatant was collected, adjusted to pH 3 and filtered using 0.2-millipore Whatman. The filtered supernatant (1-2 ml) was injected into a 250 mm x 4.6 mm reverse phase column (C<sub>18</sub>, 5 µm) (Merck Hitachi, Japan) equipped with a differential UV-detector absorbing at 254 nm. The mobile phase was 400 methanol: 8 acetic acid: 592 distilled water (Kamal and Bano, 2008). A standard IAA (Acros Co., Belgium) was used to construct the calibration curve.

#### **Effect of Bacterial and Yeast Isolates on Growth of Marigold**

A standard calibration curve of optical density against colony forming unit (CFU/ml) for each identified isolate was prepared to quantify cell concentration and confirm the correlation between optical density and CFU. Aliquot (0.5 ml) of  $10^{-1}$ - $10^{-7}$  dilution series was

plated on PDA plates and incubated at 27 °C for 24 h. Colonies were counted using the spread plate technique and the dilutions of 30-300 CFU/ml were further diluted with sterile distilled water to 0.5, 0.25, 0.125 and 0.0625. The optical density of each dilution was measured at 650 nm for bacteria and 490 nm for yeast using a spectrophotometer (Shimadzu ICPS-7510, Japan).

Seeds of marigold Royal Juane F1 hybrid (Royal Fleur, France) were germinated in trays containing sterilized peat moss under laboratory conditions (26±2 °C, 60% RH and natural photoperiod). The roots of four seedlings at 3-4 true leaf stage were dipped for 20 min in 20 ml of 10<sup>8</sup> CFU/ml suspension of each of the rhizosphere and rhizoplane isolated bacteria and yeast. In addition, roots of four seedlings were dipped in bread yeast (*Saccharomyces cerevisiae*) suspension of the same concentration. In the control treatment, the roots were dipped in sterile distilled water only. The seedlings were planted into 1/2 L plastic pots filled with equal weight of sterilized peat moss and placed in the laboratory at 23±3 °C, 60% RH, 90 μmol·m<sup>-2</sup>·s<sup>-1</sup> photosynthetic photon flux density (PPFD) and natural photoperiod. The seedlings were irrigated with sterile distilled water to maintain the peat moss moisture content at container capacity (White and Mastalerz, 1966) and allow for a 0.2-leaching fraction. This was done by regularly weighing each pot until its weight reached the weight at container capacity.

A second experiment was conducted to evaluate the response of inoculated plants to fertilization with a nutrient solution. The seedlings were treated as above except that they were irrigated with 50 ml/plant of a full strength Hoagland nutrient solution 3 wk after transplanting and were placed in a growth chamber at 26 °C, 65-70% RH, 90 μmol·m<sup>-2</sup>·s<sup>-1</sup> PPFD and natural photoperiod.

Eight weeks after inoculation, the following data

were collected: plant length (from plant tip to crown), leaf number, leaf area using a leaf area meter (Li-COR 3100, USA), length of the longest root (from tip to crown) and shoot and root fresh and dry (70 °C, 48 h) weight. Dried shoots were ground to pass through a 0.5-mm sieve and analyzed for total N using Kjeldahl digestion, moisture, ash and organic matter. Concentration of P was determined by colorimetry using ammonium vanadate-molybdate method and of K, Mg, Fe, Cu, Zn and Mn by plasma emission spectrometry (Shimadzu Sequential ICPS-7510, Japan) (Chapman and Pratt, 1961).

The layout for each experiment was a completely randomized design with four replicates per treatment (bacterial or yeast isolate). In each experiment, collected data for the bacteria and yeast were separately subjected to analysis of variance by the General Linear Models procedure using SAS (Statistical Analysis System, version 8.2, 2001). Mean separation was performed using the Least Significant Difference (LSD) method at P≤0.05.

## RESULTS AND DISCUSSION

### Characterization and Identification of Bacterial and Yeast isolates

All selected and tested bacterial isolates were gram negative and oxidase positive. All bacterial isolates were identified as *Aeromonas veronii* except the isolate BSP41 which was identified as *Stenotrophomonas maltophilia* (Table 1). The yeast isolates YSP11 and YPL21 were identified as *Cryptococcus humicolus*, YSP31 as *Candida famata* and YSP41 as *Cryptococcus laurentii* (Table 2).

### Effect of Bacterial Isolates on Growth of Marigold

In Exp. 1, the longest marigold plants (23.5, 23.0 and 24.8 cm) were those inoculated with the bacterial

isolates BSP41, BSP42 and BSP51, respectively (Table 1). The plants that were treated with BSP42 showed the greatest leaf area (443 cm<sup>2</sup>) and shoot fresh weight (13.5 g) and those treated with BSP51 had the greatest leaf number (average of 27) and shoot dry weight (1.23 g).

Furthermore, growth parameters of plants inoculated with BPL41 or BPL51 were generally comparable to those of the control plants. Leaf width was not significantly affected under all treatments.

**Table 1. Identification of the bacterial isolates, amount of IAA produced by the isolates in the culture medium and growth characteristics of non-fertilized marigold (*Tagetes erecta* L. cv. Royal Juane) plants (Experiment 1) and plants fertilized with a Hoagland nutrient solution (Experiment 2) two months after dipping the root system in suspensions of the tested bacterial isolates.**

Isolate	Latin name	IAA (mg/l)	Plant length (cm) <sup>x</sup>	Leaf number	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	Shoot Fwt (g)	Shoot Dwt (g)	Root length (cm) <sup>y</sup>	Root Fwt (g)	Root Dwt (g)
<b>Experiment 1</b>											
Control			18.4 b <sup>z</sup>	22.0 b	19.6	223 c	6.2 c	0.52 c	14.9	7.3	1.20
BSP41	<i>Stenotrophomonas maltophilia</i>	1.798	23.5 a	22.3 b	19.9	245 c	9.2 abc	0.87 abc	14.3	4.1	0.96
BSP42	<i>Aeromonas veronii</i>	3.279	23.0 a	24.5 ab	28.4	443 a	13.5 a	1.19 ab	21.6	10.2	1.97
BSP51	<i>Aeromonas veronii</i>	1.383	24.8 a	27.0 a	26.1	434 ab	12.4 ab	1.23 a	23.3	14.5	3.06
BPL41	<i>Aeromonas veronii</i>	2.607	20.6 ab	20.3 b	21.8	227 c	7.6 c	0.63 bc	19.5	4.0	0.86
BPL51	<i>Aeromonas veronii</i>	3.033	21.3 ab	20.5 b	22.9	267 bc	8.0 bc	0.66 bc	15.3	12.0	1.74
LSD			4.46	4.39	NS	175	4.69	0.566	NS	NS	NS
<b>Experiment 2</b>											
Control			28.9 b	22.3 c	22.6	176	11.7 ab	1.60 ab	24.4	16.9	4.08
BSP41	<i>Stenotrophomonas maltophilia</i>	1.798	35.9 a	31.3 a	19.4	222	12.5 a	1.78 a	21.9	11.1	3.27
BSP42	<i>Aeromonas veronii</i>	3.279	34.6 a	27.8 abc	21.2	280	12.7 a	1.81 a	19.6	16.0	4.68
BSP51	<i>Aeromonas veronii</i>	1.383	29.2 b	24.5 bc	19.3	222	9.6 bc	1.27 c	25.1	13.5	3.01
BPL41	<i>Aeromonas veronii</i>	2.607	28.6 b	29.0 ab	20.7	248	10.4 abc	1.35 bc	31.1	15.5	4.00
BPL51	<i>Aeromonas veronii</i>	3.033	28.6 b	24.8 abc	17.4	190	8.1 c	1.06 c	29.8	13.6	3.34
LSD			4.71	6.55	NS	NS	2.54	0.302	NS	NS	NS

B = bacteria, SP = rhizosphere, PL = rhizoplane.

<sup>x</sup>Measured from plant tip to crown.

<sup>y</sup>Length of the longest root.

<sup>z</sup>Means within columns in each experiment having different letters are significantly different according to LSD,  $P \leq 0.05$ .

In Exp. 2, the greatest plant length (35.9 and 34.6 g) and shoot fresh (12.5 and 12.7 g) and dry (1.78 and 1.81

g) weight were achieved when the roots of marigold seedlings were dipped in suspensions of the bacterial

isolates BSP41 and BSP42, respectively (Table 1). Inoculation with the isolate BSP41 also resulted in the greatest leaf number (average of 31.3). Plants inoculated with BPL41 were comparable to the control plants but showed higher leaf number (average of 29.0 vs. 22.3), and those inoculated with BPL51 were generally inferior to the control. Furthermore, no significant effect of the isolate was detected on leaf width or area.

Root characteristics (length, fresh weight and dry weight) were not significantly affected by inoculation with the tested bacterial isolates in both experiments (Table 1), contrary to findings on other species of bacteria, which revealed increased oilseed rape (*Brassica napus* L.) seedling root dry weight (Bertrand et al., 2000), black pepper (*Piper nigrum* L.) root biomass and length (Paul and Sarma, 2006) and peanut (*Arachis hypogaea* L.) root length (Dey et al., 2004). Although the tested bacterial isolates BSP42, BSP51, BPL41 and BPL51 in the current study were of the same species (*Aeromonas veronii*), their impacts on marigold growth were different in both experiments (Table 1), most likely because of the complex relationships between the strain and the host plant (Belimov et al., 2001). Performance of BSP42 and BSP51 in enhancing growth of marigold plants compared with the control was generally inconsistent; they enhanced growth in Exp. 1 but not in Exp. 2 (Table 1). *A. veronii* was previously isolated from rice (*Oryza sativa* L.) roots and shoots (Mehnaz et al., 2001). A related species (*A. hydrophila*) was found to increase photosynthetic rate, plant dry weight and pod number in soybean (*Glycine max* L.) (Zhang et al., 1997).

Performance of *Stenotrophomonas maltophilia* (the isolate BSP41) in enhancing growth of marigold plants compared with the control was unsatisfactory; it only increased plant length in both experiments and leaf number in Exp. 2 (Table 1). Our results are in agreement with those reported by Taghavi et al. (2009) which demonstrated no difference in growth index in poplar cuttings (*Populus deltoides* x *Populus nigra* DN-34) between non inoculated plants and those inoculated with *S. maltophilia* R551-3. Furthermore, Suckstorff and Berg (2003) found that all strains of *Stenotrophomonas* applied to strawberry (*Fragaria x ananassa* Duchesne) cv. Rügen Selecta seedlings negatively influenced stem length and leaf development, but promoted root growth and hair development. On the contrary, Sturz et al. (2001) reported significant increase in weight of potato (*Solanum tuberosum* L.) shoots and roots upon bacterization with *Stenotrophomonas*.

The reason for inefficiency of *S. maltophilia* in enhancing plant growth may be related to the concentration of the inoculum. Suckstorff and Berg (2003) observed a strong dose-dependent effect of all *Stenotrophomonas* strains on all growth parameters and reported for the first time that at higher concentrations, *Stenotrophomonas* strains were able to damage plants. The influence of the inoculum concentration on plant growth has been reported for other bacteria such as *Pseudomonas brassicacearum*, which increased tomato (*Lycopersicon esculentum* x *Solanum lycopersicum*) root length and biomass at low concentrations, but had negative effects at higher concentrations (Belimov et al., 2007).

**Table 2. Identification of the yeast isolates, amount of IAA produced by the isolates in the culture medium and growth characteristics of non-fertilized marigold (*Tagetes erecta* L. cv. Royal Juane) plants (Experiment 1) and plants fertilized with a Hoagland nutrient solution (Experiment 2) two months after dipping the root system in suspensions of the tested yeast isolates.**

Isolate	Latin name	IAA mg/l	Plant length (cm) <sup>x</sup>	Leaf number	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	Shoot Fwt (g)	Shoot Dwt (g)	Root length (cm) <sup>y</sup>	Root Fwt (g)	Root Dwt (g)
<b>Experiment 1</b>											
Control			18.4 b <sup>z</sup>	22.0 b	19.6 b	223 c	6.2 c	0.52 c	14.9	7.3	1.20
YSP11	<i>Cryptococcus humicolus</i>	1.082	25.8 a	29.0 a	26.9 a	503 a	13.7 a	1.35 a	28.8	16.9	2.81
YSP31	<i>Candida famata</i>	2.828	23.4 a	27.3 a	26.4 a	459 ab	13.7 a	1.23 ab	21.5	7.5	1.54
YSP41	<i>Cryptococcus laurentii</i>	2.839	24.0 a	25.0 ab	25.4 a	407 ab	11.8 ab	1.14 ab	25.3	14.0	2.68
YPL21	<i>Cryptococcus humicolus</i>	1.288	24.0 a	25.8 ab	28.1 a	469 ab	13.5 a	1.21 ab	26.8	13.7	2.87
YF	<i>Saccharomyces cerevisiae</i>	0.423	21.9 ab	22.3 b	23.7 ab	322 bc	8.8 bc	0.81 bc	26.5	12.5	1.95
LSD			4.04	4.15	4.96	170	4.15	0.464	NS	NS	NS
<b>Experiment 2</b>											
Control			28.9 bc	22.3	22.6 a	176 c	11.7 b	1.60 b	24.4 c	16.9	4.08
YSP11	<i>Cryptococcus humicolus</i>	1.082	32.0 ab	25.0	20.7 ab	259 ab	11.5 b	1.62 b	20.8 c	19.3	4.61
YSP31	<i>Candida famata</i>	2.828	34.6 a	25.5	21.8 a	280 a	13.8 a	2.09 a	38.1a	17.0	5.17
YSP41	<i>Cryptococcus laurentii</i>	2.839	25.5 c	23.3	18.6 bc	186 c	7.8 c	0.98 c	31.5 ab	19.5	3.96
YPL21	<i>Cryptococcus humicolus</i>	1.288	25.4 c	23.0	15.6 d	157 c	7.8 c	0.98 c	23.4 c	16.2	3.59
YF	<i>Saccharomyces cerevisiae</i>	0.423	30.3 b	26.8	17.8 cd	195 bc	10.0 b	1.38 b	25.9 bc	15.4	4.47
LSD			4.08	NS	2.55	67	1.77	0.322	6.85	NS	NS

Y = yeast, SP = rhizosphere, PL = rhizoplane, YF = food yeast.

<sup>x</sup>Measured from plant tip to crown.

<sup>y</sup>Length of the longest root.

<sup>z</sup>Means within columns in each experiment having different letters are significantly different according to LSD,  $P \leq 0.05$ .

*S. maltophilia* was previously classified as *Pseudomonas maltophilia* and was also grouped in the genus *Xanthomonas* before eventually becoming the type species of the genus *Stenotrophomonas* in 1993. *S. maltophilia* has been shown to have biocontrol traits against phytopathogenic fungi including *Pythium ultimum* (Dunne et al., 1997), *Magnaporthe poae* (Kobayashi et al., 2002) and *Bipolaris sorokiniana* (Zhang and Yuen, 1999). Strains of *S. maltophilia* have

been isolated from sweet potato (*Ipomoea batatas* (L.) Lam.) (Khan and Doty, 2009), coffee (*Coffea arabica* L.) (Vega et al., 2005), tomato, lettuce (*Lactuca sativa*), eggplant (*Solanum melongena*), Chinese cabbage (*Brassica campestris*), Japanese pepper (*Zanthoxylum piperitum*) (Kumatani et al., 2009), weed species (Sturz et al., 2001), rice (Sun et al., 2008) and the rhizosphere of oilseed rape (Berg et al., 1996).

Inoculation with the tested bacterial isolates showed

no significant effect on tissue contents of moisture, ash, organic matter or nutrients in Exp. 1 (Table 3). On the other hand, tissue contents of N, P, Mg and Mn were significantly affected by the isolate in Exp. 2 in which the plants were supplemented with a nutrient solution (Table 3). Concentrations of N (1.065% dry weight) and P (0.3% dry weight) were the highest in plants inoculated with BPL41 and BSP41, respectively. Control

plants and those inoculated with BPL51 had the highest Mg content (0.23% dry weight). Irrespective of the bacterial isolate, inoculated plants had less Mn content (3.0-6.3 ppm) compared with control plants (14 ppm). This is in agreement with findings of Pacovsky (1990) which revealed reduced tissue Mn content in sorghum [*Sorghum bicolor* (L.) Moench] plants upon inoculation with *Azospirillum brasilense*.

**Table 3. Amounts of moisture, ash, organic matter (OM) and nutrients in non-fertilized marigold (*Tagetes erecta* L. cv. Royal Juane) plants (Experiment 1) and plants fertilized with a Hoagland nutrient solution (Experiment 2) two months after dipping the root system in suspensions of the tested bacterial isolates.**

Isolate	Moisture	Ash	OM	N	P	K	Mg		Fe	Cu	Zn	Mn
	(% dry weight)								(ppm)			
<b>Experiment 1</b>												
Control	91.5 <sup>z</sup>	18.7	81.3	1.64	0.37	4.22	0.31		129	38.3	163.8	21.5
BSP41	91.0	19.0	81.0	1.74	0.42	4.46	0.26		129	27.3	142.8	18.0
BSP42	91.3	16.0	84.0	1.68	0.45	3.55	0.27		135	17.0	152.5	27.5
BSP51	90.1	16.0	84.0	1.36	0.42	2.84	0.28		165	17.8	186.5	22.8
BPL41	92.2	18.6	81.4	1.83	0.46	4.22	0.31		129	41.7	171.6	26.0
BPL51	92.3	18.0	82.0	1.41	0.37	3.94	0.26		192	42.8	221.5	19.7
LSD	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS
<b>Experiment 2</b>												
Control	86.4	15.0	85.0	0.664 c	0.27 ab	3.42	0.227 a		84	15.5	68.0	14.0 a
BSP41	85.7	13.0	87.0	0.728 bc	0.30 a	3.20	0.186 b		38	19.5	41.0	5.0 b
BSP42	85.8	12.0	88.0	0.635 c	0.27 ab	2.87	0.209 ab		49	13.3	37.5	6.3 b
BSP51	86.8	13.5	86.5	0.826 bc	0.22 bc	3.20	0.201 ab		138	14.3	59.3	5.5 b
BPL41	86.9	14.5	85.5	1.065 a	0.24 b	3.44	0.202 ab		56	13.0	42.0	5.8 b
BPL51	86.9	11.5	88.5	0.917 ab	0.18 c	3.00	0.226 a		73	13.5	44.5	3.0 b
LSD	NS	NS	NS	0.204	0.056	NS	0.028		NS	NS	NS	2.11

B = bacteria, SP = rhizosphere, PL = rhizoplane.

<sup>z</sup>Means within columns in each experiment having different letters are significantly different according to LSD,  $P \leq 0.05$ .

#### Effect of Yeast Isolates on Growth of Marigold

In Exp. 1, plants inoculated with suspensions of the tested yeast isolate YSP11, YSP31, YSP41 or YPL21 exhibited the greatest plant length and leaf width (Table

2). Plants inoculated with YSP11 and YSP31 had the greatest leaf number (average of 29.0 and 27.3, respectively), and along with those inoculated with YPL21 showed the greatest shoot fresh weight (13.7,

13.7 and 13.5 g, respectively). The greatest leaf area (503 cm<sup>2</sup>) and shoot dry weight (1.35 g) were obtained when marigold roots were dipped in suspension of YSP11. In Exp. 2, inoculation with suspension of the yeast YSP31 resulted in the greatest plant length (34.6 cm), leaf area (280 cm<sup>2</sup>), shoot fresh weight (13.8 g), shoot dry weight (2.09 g) and root length (38.1 cm) (Table 2). Those plants, along with control plants exhibited the greatest leaf width (21.8 and 22.6 cm, respectively).

Inoculation with the tested yeast isolates had no significant effect on root fresh or dry weight in both experiments (Table 2). Results are not in agreement with those of previous studies. For example, Cloete et al. (2009) reported that *Cryptococcus laurentii* was able to increase root growth by 51% when inoculated to seedlings of *Agathosma betulina* (Berg.) Pillans, a medicinal plant adapted to low-nutrient soils. El-Mehalawy (2004) demonstrated that addition of rhizosphere yeast *Candida steatolytica* and *Saccharomyces unispora* to the soil seeded with kidney bean (*Phaseolus vulgaris* L.) increased root fresh and dry weights. Furthermore, El-Mehalawy et al. (2004) reported that *Candida glabrata*, *Candida maltosa* and *Candida slooffii* obtained from the rhizosphere of maize (*Zea mays* L.) significantly increased maize growth measurements including root weight as compared with the control. However, increased root length upon inoculation with YSP31 (*Candida famata*) in Exp. 2 in the current study confirms results obtained by El-Mehalawy (2004) and El-Mehalawy et al. (2004).

Dipping marigold roots in suspensions of YSP11, YSP41 or YPL21 resulted in growth enhancement compared with the control in Exp. 1 (Table 2). On the other hand, growth parameters in Exp.2 were generally comparable to those of the control in the case of the isolate YSP11 (except leaf area which was increased

upon inoculation), and were comparable or inferior compared to the control in the case of the isolates YSP41 (except root length which was increased upon inoculation) and YPL21 (Table 2). *Cryptococcus laurentii*, which was the species of the yeast isolate YSP41, is a common soil yeast, was previously isolated from the rhizosphere and rhizoplane of *Agathosma betulina* (Cloete et al., 2009) and was a prevalent yeast extracted from sugarcane (*Saccharum officinarum* L.) rhizosphere, stem and leaves (De Azeredo et al., 1998). *C. laurentii* was also reported to have a great commercial potential as a biological control product against postharvest diseases caused by pathogenic fungi such as *Alternaria alternata* and *Monilinia fruticola* in Jujube (*Zizyphus jujuba* cv. Dongzao) fruits (Qin and Tian, 2004), *Penicillium expansum* and *Monilinia fruticola* in sweet cherry (*Prunus avivum*) fruits (Qin and Tian, 2005) and *Penicillium expansum* in apple (*Malus domestica* Borkh.) fruits (Yu et al., 2009). In the current study, plants inoculated with YF were comparable to the control in both experiments (Table 2).

It is apparent that the isolate YSP31 (*Candida famata*) enhanced growth compared with the control in both experiments (Table 2). Other *Candida* species were reported to have growth promoting potential. El-Mehalawy (2004) demonstrated that addition of rhizosphere yeast *C. steatolytica* and *Saccharomyces unispora* to the soil seeded with kidney bean increased shoot height, fresh and dry weight. El-Mehalawy et al. (2004) also reported that the yeast isolates *C. glabrata*, *C. maltose* and *C. slooffii* obtained from the rhizosphere of maize significantly increased maize shoot height and weight as compared with the control. Those yeast species were also found to be antagonistic to *Cephalosporium maydis*, a causal agent of late wilt disease of maize (El-Mehalawy et al., 2004). Furthermore, isolates of *C. valida* from the rhizosphere

of sugar beet (*Beta vulgaris* L.) significantly promoted sugar beet growth and protected the seedlings and mature plants from *Rhizoctonia solani*, which causes root diseases (El-Tarabily, 2004). *C. tropicales* was able to increase yield and yield attributes of soybean plants when applied with organic manure (Mekki and Ahmed, 2005) and to increase growth, yield and yield components of wheat (*Triticum aestivum* L.) (Zaki et al., 2007). In a study done on the effect of foliar application of *C. tropicalis* on potato growth and yield, Gomaa et al. (2005) reported that the combined application of organic fertilizers and yeast increased yield significantly (21-73%, depending on the type of organic fertilizer) in comparison with the organic fertilizers applied singly. *C. tropicalis* was found to be antagonistic to white fly (*Bemisia tabaci*) and thrips (*Thrips tabaci*) (Gomaa et al., 2005).

Only tissue K content was affected by inoculation with the tested yeast isolates in Exp. 1; the highest (4.5% dry

weight) being recorded in plants treated with suspensions of YSP31 or YSP41 (Table 4). In Exp. 2, where marigold seedlings were supplemented with a nutrient solution, all evaluated parameters in the plant tissue, except P, Fe and Zn, were affected by the isolate (Table 4). However, there was inconsistency in measurements of tissue parameters among the isolates. The highest moisture, N and K contents were detected in plants inoculated with YSP41. Plants treated with a suspension of YSP11, YSP31 or YF had the highest organic matter content and those treated with YF had the highest Mg. Control plants contained the highest Cu and Mn concentrations. Data do not confirm previous findings which revealed that seed P and Mn contents increased when soybean plants were treated with *Candida tropicales*, singly or in combination with a biofertilizer, whereas Zn and Fe increased when the plants were treated with a combination of the yeast, biofertilizer and organic manure (Mekki and Ahmed, 2005).

**Table 4. Amounts of moisture, ash, organic matter (OM) and nutrients in non-fertilized marigold (*Tagetes erecta* L. cv. Royal Juane) plants (Experiment 1) and plants fertilized with a Hoagland nutrient solution (Experiment 2) two months after dipping the root system in suspensions of the tested yeast isolates.**

	Moisture	Ash	OM	N	P	K	Mg		Fe	Cu	Zn	Mn
Isolate	(% dry weight)								(ppm)			
	Experiment 1											
Control	91.5 <sup>z</sup>	18.7	81.3	1.64	0.37	4.22 ab	0.31		129	38.3	163.8	21.5
YSP11	90.2	15.5	84.5	1.44	0.41	2.56 c	0.26		79	16.3	110.3	18.0
YSP31	91.1	18.0	82.0	1.78	0.44	4.49 a	0.30		90	17.0	241.3	28.5
YSP41	90.2	19.0	81.0	1.54	0.43	4.52 a	0.25		74	16.8	120.0	26.3
YPL21	91.3	18.1	81.9	1.73	0.39	2.99 bc	0.26		91	17.1	115.4	25.7
YF	91.1	18.5	81.5	1.25	0.46	2.92 bc	0.26		153	22.6	127.6	29.3
LSD	NS	NS	NS	NS	NS	1.43	NS		NS	NS	NS	NS
	Experiment 2											
Control	86.4 abc	15.0 a	85.0 b	0.664 cd	0.27	3.42 ab	0.227 ab		84	15.5 a	68.0	14 a
YSP11	85.9 bc	11.5 b	88.5 a	0.671 bcd	0.30	3.03 bc	0.206 bc		116	14.0 abc	127.0	8.3 b

	Moisture	Ash	OM	N	P	K	Mg		Fe	Cu	Zn	Mn
Isolate	(% dry weight)								(ppm)			
YSP31	84.9 c	11.0 b	89.0 a	0.650 d	0.30	2.73 c	0.214 ab		64	13.0 c	54.0	5.5 b
YSP41	87.6 a	15.5 a	84.5 b	0.993 a	0.30	3.66 a	0.215 ab		62	15.0 ab	50.5	7.8 b
YPL21	87.4 ab	14.5 a	85.5 b	0.796 bc	0.29	3.2 abc	0.190 c		61	12.8 c	44.8	6.8 b
YF	86.3 abc	12.0 b	88.0 a	0.802 b	0.22	2.76 c	0.229 a		50	13.5 bc	48.8	5.3 b
LSD	1.54	2.45	2.45	0.133	NS	0.512	0.022		NS	1.67	NS	5.69

Y = yeast, SP = rhizosphere, PL = rhizoplane, YF = food yeast.

<sup>2</sup>Means within columns in each experiment having different letters are significantly different according to LSD,  $P \leq 0.05$ .

### Plant Growth Promotion Mechanism

It is apparent that the tested bacterial or yeast isolates did not show potential for increasing P uptake by the plants, which is in disagreement with findings of previous studies (Mekki and Ahmed, 2005; Paul and Sarma, 2006). There was a high degree of inconsistency in nutrient content in the plant tissue upon inoculation implying that mineral uptake by plants is dependent on the strain-plant combination (Bashan et al., 1990) and it is unlikely that this ability was a general mechanism responsible for plant improvement.

Plant growth enhancement may have occurred due to increased level of IAA, which stimulates cell division and elongation (Kende and Zeevaart, 1997), as was reported for several bacteria (Koh and Song, 2007; Suckstorff and Berg, 2003). In fact, all tested bacterial and yeast isolates were found to be able to produce IAA in the culture medium (Tables 1 and 2). It is noteworthy that *Stenotrophomonas maltophilia*, which is the species of bacterial isolate BSP41 in the current study, was reported to have metabolic pathway for producing IAA (Taghavi et al., 2009) although Khan and Doty (2009) reported that *S. maltophilia* isolated from sweet potato shoots did not produce IAA. *Aeromonas veronii*, which was abundant in the present study, was also shown to produce IAA (Mehnaz et al., 2001).

Amount of produced IAA varied among the different tested bacterial and yeast isolates, the highest (3.28 and 3.03 mg/l) being produced by the bacterial isolates BSP42 and BPL51, respectively (Table 1) and the lowest (0.423 mg/l) by the yeast isolate YF (Table 2). The variation in the amount of produced IAA was observed even among the different bacterial isolates belonging to *Aeromonas veronii* (Table 1). However, response in marigold growth to the isolates did not match the amount of IAA produced by the isolates in the culture medium. For example, BSP42 and BPL51 produced comparable amounts of IAA but resulted in different responses in plant growth in both experiments. Furthermore, the isolates BSP51 and BPL41 produced different amounts of IAA but resulted in comparable growth responses in Exp. 2. Likewise, the yeast isolates YSP11 and YPL21 were of the same species (*Cryptococcus humicolus*), produced comparable amounts of IAA (1.08 and 1.29 mg/l) and resulted in comparable growth response in Exp.1 but not in Exp.2 (Table 2). Furthermore, the yeast isolates YSP31 (*Candida famata*) and YSP41 (*Cryptococcus laurentii*) produced similar amounts of IAA (2.83 mg/l) and resulted in comparable plant growth in Exp. 1 but different plant growth responses in Exp. 2.

A positive correlation between strawberry root length

and root hair development and production of IAA by *Stenotrophomonas* was reported by Suckstorff and Berg (2003). However, it is apparent that the interaction between IAA-producing microorganisms and plants leads to diverse outcomes on the plant side. Xie et al. (1996) reported that a wild strain of *Pseudomonas putida* and one mutant, which produced IAA at three times the rate of the wild species, was found to have growth promoting effect, whereas another mutant that produced four times the amount of IAA of the wild species had growth inhibiting effect. This was attributed to the interaction of IAA with ACC synthase, which is activated by the large amounts of IAA produced by bacteria together with endogenously produced plant IAA, leading to production of ACC, a precursor of ethylene which inhibits root growth (Glick et al., 1998).

In conclusion, growth promoting abilities of the isolated and tested bacterial and yeast isolates were not consistent, except the yeast isolate YSP31 (*Candida famata*) which showed consistency in plant growth promotion. Further research is needed to determine the

optimal inoculant dose and assess potential use of that isolate as a biofertilizer. Previous studies indicated potential use of *Candida* as a biofertilizer. El-Tarabily (2004) reported that *C. valida*, *Rhodotorula glutinis* and *Trichosporon asahii* promoted growth of sugar beet when applied individually, but their combination resulted in a significantly better plant growth promotion and concluded that those yeast species had the potential to be used as biofertilizers. Zaki et al. (2007) studied the effect of *C. tropicalis*, in the presence of half the recommended dose of NPK, on yield of some wheat cultivars and concluded that the biofertilizers of efficient strains could save 50% of the recommended dose of NPK without decreasing yield of crops in a newly cultivated land.

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YSP11, YSP31, ) (BSP41, BSP42, BSP51, BPL41, BPL51)  
 (*Tagetes erecta* L.) (PL) (SP) (YSP41, YPL21  
 .(*Stenotrophomonas maltophilia*) BSP41 (*Aeromonas veronii*)  
*Candida* ) YSP41 (*Cryptococcus humicolus*) YPL21 YSP11  
 .(*Cryptococcus laurontii*) YSP41 (*famata*  
 (*Saccharomyces* *cerevisiae*)  
 (*Candida famata*) YSP31  
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