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Rhizosphere yeast *Torulaspora globosa* with plant growth promotion traits and improvement of the development of tomato seedlings under greenhouse conditions

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Yeasts are an interesting group of microorganisms, which occur naturally in soil and on plant surfaces. Few studies have analysed their potential as plant growth promoters. Thus, the aim of this study was to evaluate the indole acetic acid (IAA) production and phosphate solubilization by the yeasts *Torulaspora globosa* (CCA5S51 and CCA5S55), *Meyerozyma guilliermondii* (CCA3C98), and *Rhodotorula mucilaginosa* (CCA2F32), and the influence of *T. globosa* (CCA5S55) in the development of tomato seedlings. The results showed that *T. globosa* strains present both plant growth promotion traits (IAA production and phosphate solubilization). The strains of *T. globosa* (5S51 and 5S55) showed high IAA production (641 and 669 µg.ml-1, respectively) after 48 h of incubation, while *Rh. mucilaginosa* produced 406 µg.ml^{-1} of IAA after 120 h. The strains CCA5S55 and CCA5S51 could also solubilize 47 and 35% of tricalcium phosphate in the medium, respectively, after 12 days of incubation; whereas *M. guilliermondii* (CCA3C98) solubilized only 10% of the tricalcium phosphate after 12 days. The inoculation of tomato seedlings with *T. globosa* stimulates the plant growth; root height was statistically superior when the higher cell concentration was inoculated. The root dry weight was enhanced with addition of glucose and tryptophan. The conclusion is the yeast species *T. globosa* is able to produce IAA in the presence of tryptophan and also solubilize phosphate *in vitro*. The inoculation of tomato seedlings promoted its development. The cell concentration and the addition of glucose and tryptophan must be evaluated in details to attain optimized yields.

Key words: indole acetic acid (IAA) production, phosphate solubilisation, yeast as plant growth promoter.

INTRODUCTION

The soil and plant ecosystem is a complex environment with a very high microbial diversity. It is characterized by

different functional types; the interplay of which is fundamental to different processes, including nutrient cycling, protection, and stimulus to the development of plants. However, the current agricultural practices employ chemical compounds such as mineral fertilizers and chemical defensives, which disrupt the balance of the microbial ecosystem by decreasing biodiversity and consequently diminishing the resilience of the agricultural environment. However, the use of these products is essential, since the plant production process is a vicious cycle: highly dependent on inputs that support the yield while damaging the environment (Zhang et al., 2018).

The role of microorganisms in the soil and plants remains unclear. Several studies on rhizosphere micro-organisms, especially rhizobacteria, have reported encouraging results in the promotion of plant growth and as biological agents in control of phytopathogens and plagues (Vejan et al*.,* 2016; Liu et al*.,* 2017). These studies highlight the potential of rhizosphere micro-organisms to support various economically important crops. This group of microorganisms, known as Plant Growth Promoting Microorganisms (PGPM), can produce compounds capable of stimulating plant development through various mechanisms, such as the production of plant hormones, antagonism against pathogens, induction of plant resistance, and mineral solubilization (Gray and Smith, 2005).

The phytohormone auxin, through its main representative indole acetic acid (IAA), is a class of plant hormones produced by PGPM. IAA is known to stimulate a rapid and sustained response by plants, mainly by promoting the elongation of cell roots (Cleland, 1990). Several microorganisms, including bacteria (Khalid et al., 2004; Ahemad and Kibret, 2014), filamentous fungi (Floch et al., 2003; Gravel et al., 2007; Hoyos-Carvajal et al., 2009), and yeast are able to produce IAA and significantly influence plant growth and development (El-Tarabily, 2004; Nutaratat et al., 2014).

There is also a great interest in the application of PGPM as biofertilizers to eventually phase out chemical fertilizers. These microorganisms can improve plant nutrition by increasing the availability of nutrients in the soil, by the solubilization of inorganic compounds via organic acids production (Wei et al., 2010) and enzymatic mineralization (Alori et al*.,* 2017).

Yeasts are unicellular fungi, which are often used in biotechnological processes. This microbial group can also be found in the rhizosphere and on the surface of plants, but in a low number compared to bacteria and filamentous fungi (Yurkov, 2018). Hence, little is known about their role in this ecosystem, with few studies available in the literature. A wide variety of yeast species has plant growth promotion traits (Cloete et al., 2009; Limtong et al., 2014; Nutaratat et al., 2014), including the control of phytopathogens (El-Tarabily, 2004; Sansone et al., 2005; El-Tarabily and Sivasithamparam, 2006; Rosa et al., 2010; Korres et al., 2011; Platania et al., 2012; Yu et al., 2012), production of phytohormones (Nassar et al., 2005), phosphate solubilization (Falih and Wainwright 1995; Alonso et al., 2008; Hesham and Mohamed, 2011; Mundra et al., 2011), nitrogen and sulfur oxidation (Falih and Wainwright, 1995), production of siderophores (Sansone et al., 2005), and the stimulation of root colonization by mycorrhizal fungi (Vassileva et al., 2000; Alonso et al., 2008).

Therefore, it acknowledges that the present challenge is to expand our knowledge about the yeast niches in soil and their association with plants. This study aims at investigating the mechanism of action of this microbial group in assisting plant production, contributing to the balance of the ecosystem, and minimizing the use of toxic chemicals in the agriculture.

MATERIALS AND METHODS

Yeasts strains

The yeasts to be evaluated were isolated from the rhizosphere, stem, and leaf of sugarcane and maize. The isolation of strains was realized, during the prospection of yeasts as biological control agents against phytopathogens. The strains were identified by molecular taxonomy based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene. The strains were screened for their ability to produce IAA and solubilize phosphate *in vitro*, and four yeast strains (*Torulaspora globosa*–strains CCA5S51 and CCA5S55; *Meyerozyma guilliermondii*–strain CCA3C98 and *Rhodotorula mucilaginosa*–strain CCA2F32) were selected (data not published) (Table 1).

Quantitative indole acetic acid (IAA) production

The strains CCA5S51, CCA5S55, and CCA2F32 were evaluated to produce IAA. The assay was carried out in Erlenmeyer flasks (500 mL) containing 200 mL of liquid culture medium Potato Dextrose (BD) (200 mL of potato broth and 20 g of dextrose per litre, pH unadjusted) with or without 0,1% tryptophan solution. The flasks were inoculated with 1 ml of yeast cell suspension (5×10^4 cells.ml^{-1}) and incubated on a shaker at 25°C, 160 rpm for seven days. Samples were collected every 24 h. For quantification of IAA, each sample was centrifuged at 1000 × g for 3 min and 1.5 ml of the culture supernatant was pipetted into microtubes followed by addition of 1.5 ml of the Salkowski reagent (0.5 M ferric chloride and 35% perchloric acid) (Gordon and Weber, 1951). The tubes were kept for 30 min at room temperature, after which the development of pink colour revealed the presence of IAA. The

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Table 1. Characteristics of yeast strains selected and plant growth promoting traits.

Strain code	Source	Identification	GenBank accession no. (closest species)	AIA production	Phosphate solubilization
CCA5S51	Sugar cane rhizosphere	*Torulaspora globosa*	KY109864.1	+	+
CCA5S55	Sugar cane rhizosphere	*Torulaspora globosa*	KY109864.1	+	+
CCA2F32	Sugar cane leaf	*Rhodotorula mucilaginosa*	FJ515212.1	+	-
CCA3C98	Maize stem	*Meyerozyma guilliermondii*	MG323878.1	-	+

intensity of the colour was analysed using a spectrophotometer at a wavelength of 530 nm. To quantify the amount of IAA, a standard curve was prepared based on the optical density (OD) of IAA solutions at known concentrations. For evaluation of yeast growth, yeast cells in every sample were counted by microscopy in a Neubauer chamber, and the results were expressed as the number of cells per millilitre (Camacho-Fernandez et al., 2018).

Quantitative phosphate solubilization

The strains CCA5S51, CCA5S55, and CCA2F32 were evaluated as tricalcium phosphate solubilizers. The assay was performed in an Erlenmeyer flask (125 mL) containing 50 mL of liquid medium Pikovskaya (0.5 $g.l^{-1}$ yeast extract, 10 $g.l^{-1}$ glucose, 0.5 $g.l^{-1}$ ammonium sulphate, 0.2 $g.l^{-1}$ potassium chloride, 0.1 $g.l^{-1}$ magnesium sulphate, 0.0001 $g.l^{-1}$ manganese sulphate, 0.0001 $g.l^{-1}$ iron sulphate), with or without tricalcium phosphate (5 $g.l^{-1}$). The flasks were either inoculated with the yeast strains or left uninoculated, depending on the treatments. The experiment was carried out in three sets each for the three treatments (T1: yeast + medium + tricalcium phosphate; T2: medium + tricalcium phosphate; T3: medium + yeast). Treatments T1 and T3 received the inoculation of 1 ml suspension with 5×10^4 yeast cells.mL^{-1}. The flasks were incubated for 12 days at 25 °C and 160 rpm, with sampling every 72 h. The evaluation involved the quantification of soluble phosphate in the culture medium, following the method described by Strickland and Parsons (1960) using the molybdenum blue colorimetric method. The intensity of the blue colour of the medium was assessed using a spectrophotometer at a wavelength of 880 nm. A standard curve was prepared using known concentrations of soluble phosphate. The pH of the medium was also determined using a digital pH meter (MS Tecnopon® mPA210).

Pot experiment under greenhouse conditions

The strain 5S55 was selected for inoculation of tomato seedlings. The inoculum was produced in YEPD medium (10 $g.l^{-1}$ yeast extract, 10 $g.l^{-1}$ peptone, and 20 $g.l^{-1}$ dextrose) in a shaker at 160 rpm, 30°C for three days. Twelve different types of treatments, consisting of combinations of three concentrations of cells (1×10^8, 3×10^8, and 9×10^8 cells.$plant^{-1}$), presence or absence of glucose (20 $g.l^{-1}$), and the presence or absence of tryptophan (0,1%) were evaluated. The control group consisted of plants treated with sterile water. The inoculation of tomato seedlings (30-day-old plants) was carried out after transplanting to 5 L plastic pots containing non-sterile commercial organic substrate. The inoculum was sprayed near the root. The plants were maintained in a greenhouse for 45 days, while they were being evaluated for their height and root length (in centimetres) and dry weight (in grams) of shoot and root. The experimental design was completely randomized, with ten repetitions for each treatment. All the results were analysed using Analysis of Variance (ANOVA) and comparison of means by Tukey's test at 5% significance level using the statistical program STATISTICA 6.0 (Statsoft, 2001).

RESULTS AND DISCUSSION

The strains of *T. globosa* and *Rh. mucilaginosa* produced a large quantity of IAA (Figure 1). *T. globosa* (strain CCA5S55) showed maximum production of IAA (669 $\mu g.ml^{-1}$) after 48 h of incubation, which was statistically similar to the strain CCA5S51 (641 $\mu g.ml^{-1}$) after the same incubation period. The IAA production by *T. globosa* was statistically superior to that of *Rh. mucilaginosa* (CCA2F32), which produced 407 $\mu g.ml^{-1}$ after 144 h of incubation.

M. guilliermondii (CCA3C98) was unable to produce IAA *in vitro* under the established conditions of this study. In contrast, Limtong and Koowadjanakul (2012) reported that an isolate of *M. guilliermondii* (strain LM120) grown in YEPD culture medium supplemented with tryptophan could produce 68.1 $\mu g.ml^{-1}$ of IAA after seven days of incubation. Nakayan et al. (2013) and Nutaratat et al. (2014) also observed that IAA was produced by the isolates of *M. guilliermondii* (strains CC1 and DMKU-RP168) under the same conditions. These studies reported an average production of 10.6 $\mu g.ml^{-1}$ after five days and 45 $\mu g.ml^{-1}$ after seven days of cultivation, respectively. Thus, our results highlight two important issues related to the production of IAA by yeasts: first, varying results are obtained from diverse strains of the same species, especially if they are isolated from different environments (soil, rhizosphere, or phyllosphere); secondly, the specific conditions necessary for the IAA production, such as sources of nutrients and pH of the medium may influence the production significantly, even preventing it (Apine and Jadhav, 2011; Scarcella et al., 2017).

The production of IAA by all strains was dependent on tryptophan. This is common among yeasts, as reported by Nassar et al. (2005), who evaluated 24 yeast strains for IAA production in the presence and absence of tryptophan and observed production only in the presence of the amino acid. Contrasting results were reported by

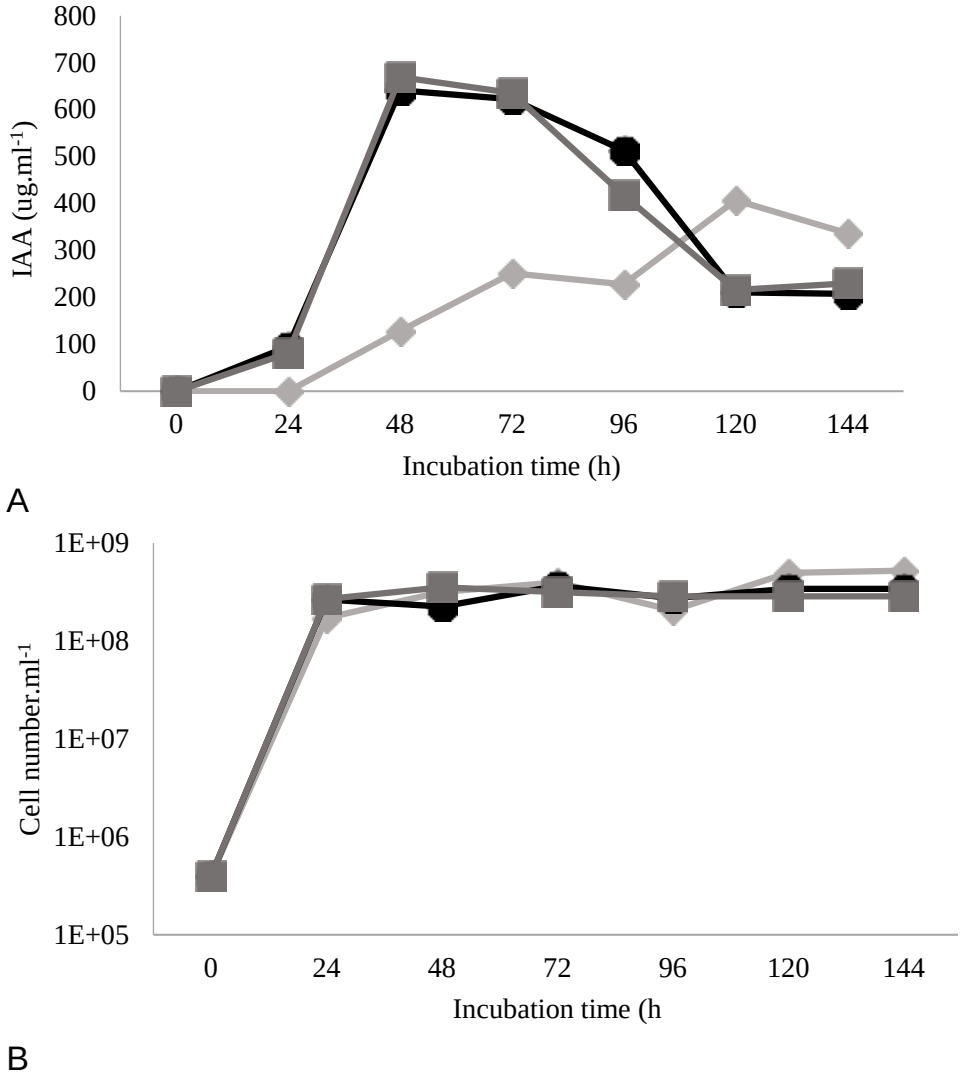

Figure 1. IAA production (A) and cell growth evaluation (B) of yeasts strains 2F32 (R. mucilaginosa) (◆), 5S51 (■) and 5S55 (●) (T. globosa).

Nakayan et al. (2013), who observed that *M. guilliermondii, Rh. mucilaginosa*, and *M. caribbica* were able to produce low rates of IAA, reaching a maximum of 3 μg.ml^{-1} in the absence of tryptophan. Tryptophan is the main precursor for IAA synthesis and in most of the cases, indispensable for the IAA production (Ahmad et al., 2005). It is worth considering that the production of IAA by microorganisms while associated with plants is directly related to the availability of exudates from the roots or leaves (Melo, 1998). The production and concentration of tryptophan may vary in root exudates across various plant species (Patten and Glick, 1996).

The maximum IAA production obtained from both strains (CCA5S51 and CCA5S55) occurred after 48 h of incubation, during the stationary phase of cell growth (Figure 1). After this period, there was a drop in the level of IAA in the medium. This could be explained by the consumption of IAA as a nitrogen source by the yeast. Previous studies have reported that several microorganisms can produce and degrade IAA in the medium (Faure et al., 2009; Scott et al., 2013; Zúñiga et al., 2013). The degradation of IAA, besides being a source of nutrition, must also be a self-protective action of the yeasts, given that excessive IAA in the medium could be toxic to the cells due to acidification of the cytoplasm (Tromas and Perrot-Rechenmann, 2010). A high concentration of IAA could also be toxic to plants (Biswas et al., 2000). As observed in all phytohormones, IAA stimulates plant growth only at low concentrations (Biswas et al., 2000), and is ineffective and toxic at higher

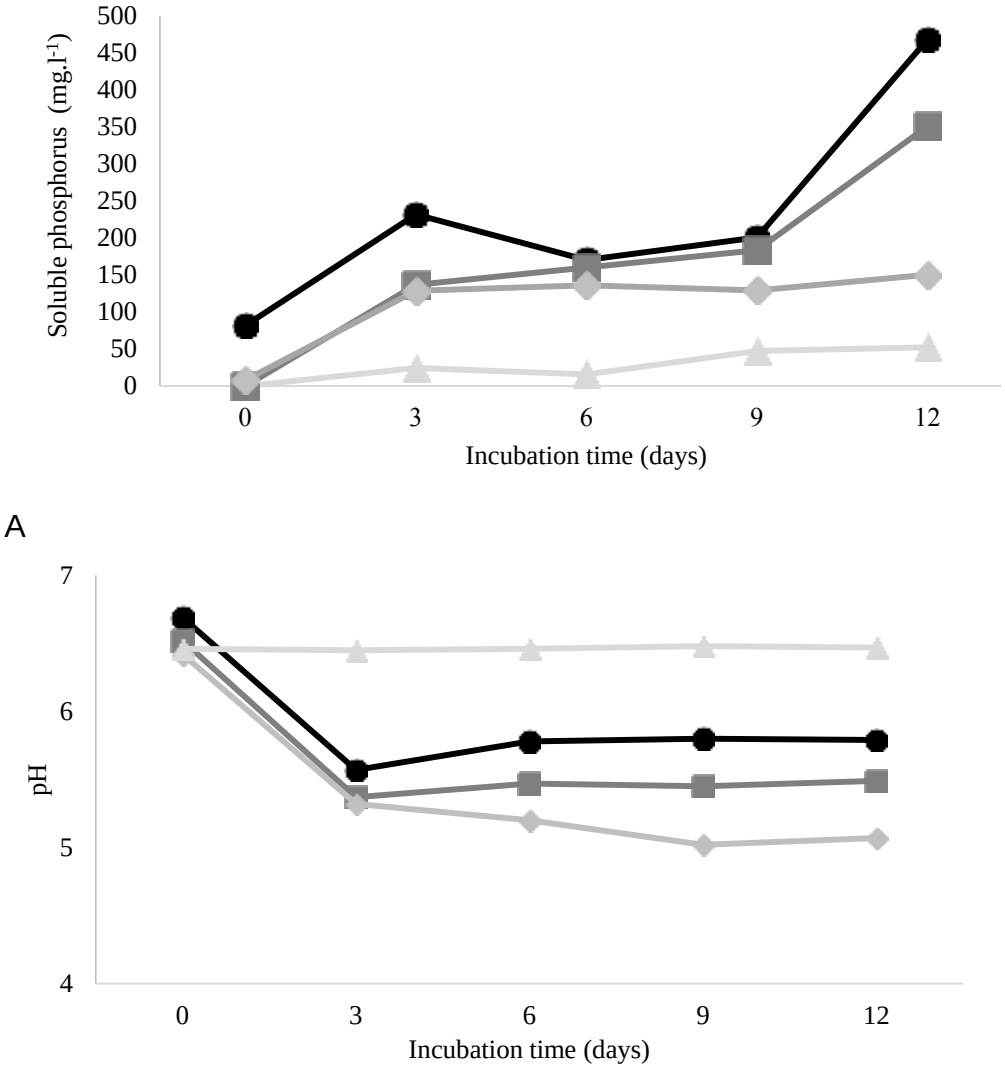

Figure 2. Soluble phosphate (A) and pH (B) observed in the medium of yeast strains cultures 2F32 *(Rh. mucilaginosa) (◆), 5S51 (■) and 5S55 (●) (T. globosa), control (without inoculation) (▲).*

concentrations. Ahmad et al. (2005) inoculated *Sesbania aculeata* and *Vigna radiata* seedlings with *Azotobacter* sp. (IAA producer strain) and found an increase in root elongation at concentrations between 4.4 $\mu g.ml^{-1}$ and 24.8 $\mu g.ml^{-1}$ of IAA for the former and 4.4 $\mu g.ml^{-1}$ to 14.4 $\mu g.ml^{-1}$ for the latter. Similarly, Barazani and Friedman (1999) reported that rhizobacteria-produced IAA at a concentration around 13.5 $\mu g.ml^{-1}$ had a deleterious effect on lettuce seedlings. This harmful effect on the plants occurs when the concentration of IAA exceeds the desirable level of stimulation of plant development; IAA in excess has herbicidal action (Fargasova, 1994).

T. globosa (strain 5S55) solubilized 468.2 $mg.l^{-1}$ of phosphate in 288 h (12 days), which was significantly higher than the amount of phosphate solubilized by the other strains. *T. globosa* (strain 5S51) solubilized 350.8 $mg.l^{-1}$ of phosphate, while *M. guilliermondii* (strain 3C98) solubilized 150.4 $mg.l^{-1}$ of phosphate during the same period.

Previous studies in literature attributed the solubilization of minerals to the production of organic acids by the yeast strains. In our study, the flasks with only yeast culture showed a major decrease in the pH of the medium during the incubation, probably due to the release of organic acids. The treatment of yeast culture supplemented with the insoluble phosphorus (tricalcium phosphate) also showed a decline in the pH, but lower probably due to the release of calcium in the culture medium. The flasks with only insoluble phosphorus did not show considerable pH change. The yeast *M. guilliermondii* promoted the highest reduction in pH (Figure 2), reducing it from 6.0 to 2.17 after 72 h of

Table 2. Effect of yeast inoculation, cell concentration, presence and absence of triptophan and glucose on height and dry-weight of tomato seedlings cultivated under greenhouse experimental conditions.

Treatment			Heigth (cm)		Dry-weight (g.plant^{-1})	
inoculum	**trp***	**glu***	**Shoot**	**Root**	**Shoot**	**Root**
-	-	-	39.37^{b}**	18.25^{b}	0.45^{c}	0.16^{b}
1×10^{8} cells.ml^{-1}	+	+	43.05^{ab}	21.87^{ab}	0.57^{b}	0.39^{ab}
	-	+	42.37^{ab}	22.12^{ab}	0.49^{b}	0.15^{b}
	+	-	45.02^{ab}	23.63^{a}	0.72^{a}	0.22^{b}
	-	-	46.87^{ab}	22.75^{ab}	0.57^{b}	0.21^{b}
3×10^{8} cells.ml^{-1}	+	+	46,62^{ab}	23.37^{ab}	0.64^{a}	0.59^{a}
	-	+	40.75^{ab}	21.37^{ab}	0.54^{b}	0.18^{b}
	+	-	44.50^{ab}	20.62^{ab}	0.69^{a}	0.35^{ab}
	-	-	41.75^{ab}	20.08^{ab}	0.63^{b}	0.21^{b}
9×10^{8} cells.ml^{-1}	+	+	41.87^{ab}	25.37^{a}	0.58^{b}	0.35^{ab}
	-	+	41.62^{ab}	20.51^{ab}	0.59^{b}	0.39^{ab}
	+	-	42.07^{ab}	24.12^{a}	0.65^{a}	0.28^{b}
	-	-	49.12^{a}	23.87^{a}	0.72^{a}	0.28^{b}

*Triptophan present (trp+), triptophan absence (trp -), glucose present (glu+), glucose absence (glu-). **Mean followed by same letters within the column did not differ significantly at P<0.1 according to Tukey`s test.

incubation. *T. globosa* (strain 5S55) however, had the highest pH value and maximum phosphate solubilization.

The inoculation of seedlings with the strain 5S55 promotes a significant increase in the dry-weight of shoots, which was seen in all the yeast cell concentrations evaluated. The treatment with an inoculum of the highest yeast cell concentration (9×10^{8} cells.ml^{-1}) affected higher root length in the absence of glucose or in the presence of tryptophan. Thus, tryptophan appears to exert a positive effect in improving the dry-weight of shoots. The treatment with tryptophan, glucose, and 3×10^{8} cells.ml^{-1} improved the dry-weight of the root by 72.8% compared to control (Table 2).

The treatments with glucose and/or tryptophan were significantly superior to those without the treatment. The best results of treatments without glucose and tryptophan were obtained only when the higher yeast cell concentrations were inoculated. This emphasizes the importance of the presence of a source of glucose and/or tryptophan as a stimulus for the yeasts for the establishment of the rhizosphere. This reiterates the importance of the presence of tryptophan in the soil, which is necessary for the promotion of plant growth. Nassar et al*.* (2005) also observed that the yeast *Williopsis saturnus* (isolate#4), endophytic to maize roots, promoted growth of maize plants, which in the presence of tryptophan was significantly superior to all other treatments and controls in all the comparative parameters. The same study reported that the inoculation of *Rhodotorula glutinis*, a non-IAA producer, in the presence of tryptophan, presented significantly superior results to those from treatments wherein tryptophan was absent.

From the findings of this study, it can be concluded that the yeast species *T. globosa* (strains 5S51 and 5S55) and *R. mucilaginosa* (strain 2F32) produced IAA in the presence of tryptophan, while the species of *T. globosa* (strains 5S51 and 5S55) and *M. guilliermondii* (strain 3C98) solubilized phosphate under *in vitro* conditions. *T. globosa* strain 5S55 showed the best plant growth promotion traits, with maximum IAA production reaching 669 μg.ml^{-1} after 48 h of incubation, and the solubilization of 47% of phosphorus after 12 days of incubation. The development of the tomato seedlings was also improved by inoculation of the 5S55 strain. However, the cell concentration and the presence of glucose and tryptophan significantly influenced the plant growth and must be evaluated in details to attain optimized yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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