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PLANT PATHOLOGY

Plant Growth Promoting Activity and Biocontrol Potential of Soil Yeast

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ABSTRACT

Among soil microorganisms, yeasts have received little attention as bioonoculant and biocontrol agents in comparison to bacterial, actinomycetes, and filamentous fungal antagonists. The ability of certain taxa of yeasts to multiply rapidly, to produce antibiotics and cell wall-degrading enzymes, to induce resistance of host tissues, and to produce plant growth regulators indicates the potential to exploit them as biocontrol agents and plant growth promoters. We investigated the ability of the soil yeast *Rhodotorula* sp. and *Candida tropicalis* to stimulate plant growth promoting characters and biocontrol potential. *In vitro* culture experiments found that *Rhodotorula* sp. SY3 (623.14 ug/ml with tryptophan: 150.12 ug/ml without tryptophan) and *Candida tropicalis* SY5 (580.25 ug/ml - with tryptophan: 120.24 ug/ml - without tryptophan) produces large quantities of indole acetic acid (IAA), but grows rapidly on aminocyclopropane-1-carboxylate (ACC) as a sole source of nitrogen, indicative of high ACC deaminase activity. The strain also tested positive for hydrogen cyanide production, solubilisation of phosphorus and zinc. The yeast isolates significantly inhibited the *Alternaria* sp. and *Colletotrichum* sp. pathogen with Per cent inhibition of mycelial growth over control ranging between 32% and 55%. The application of the yeast crude extract could suppress population of *M. javanica* under laboratory conditions. In conclusion, our data confirm that soil yeast strains can promote plant growth and control pathogens, it could be considered for the development of biological fertiliser treatments.

HIGHLIGHTS

- *Rhodotorula* sp. and *Candida tropicalis* produces plant growth regulators, siderophore and mobilizes insoluble phosphate, zinc.
- Biocontrol potential against plant pathogens and root knot nematode.

Keywords: Soil yeast, *Candida tropicalis*, *Rhodotorula* sp, Plant growth promotion, Biocontrol

Rhizobacteria are the most studied PGP microbes (Gouda *et al*. 2018) and research has resulted in multifunctional formulations for commercial agriculture (Backer *et al*. 2018). However, usage of PGP rhizobacteria remains controversial due to the variability in their performance, which is probably subject to environmental factors that affect their growth and proliferation in the plant (Backer *et al*. 2018; Gouda *et al*. 2018).

Yeasts are unicellular fungi that proliferate primarily through asexual means and grow rapidly on simple carbohydrates, often through fermentative as well as respiratory pathways (Botha 2011). As a consequence of their nutritional preference, yeast populations are generally an order of magnitude higher in the rhizosphere as opposed to the bulk soil

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(Botha 2011). Plant growth promoting characteristics are exhibited by a diverse range of yeasts. It includes pathogen inhibition (El-Tarabily and Sivasithamparam, 2006); phytohormone production (Nassar *et al.* 2005); phosphate solubilisation; N and S oxidation; siderophore production and stimulation of mycorrhizal-root colonization (de Oliveira *et al.* 2019).

Genera of soil yeasts most frequently isolated include *Candida*, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Lipomyces*, *Pichia*, *Aureobasidium*, *Rhodotorula*, *Saccharomyces*, *Schizoblastosporion*, *Sporobolomyces*, *Torulaspora*, *Torulopsis*, *Trichosporon*, *Kluyveromyces*, and *Zygosaccharomyces*. Some of these genera, such as *Aureobasidium* and *Trichosporon*, can also have mycelial phases (de Souza *et al.* 2019).

Biocontrol of plant pathogenic organisms by soil yeast is carried out, on the one hand, by improving the uptake of water and mineral elements nitrogen, phosphorus, potassium by the plant, on the other hand – through the production of antifungal agents and the displacement of pathogenic bacteria and fungi in the rhizosphere by inhibiting their growth (Fernández *et al.* 2021). Potential use of yeast fungi as biocontrol agents of soil-borne plant pathogens and plant growth promoters was recently investigated (Azzam *et al.* 2012). El-Tarabily (2004) reported that *Rhizoctonia solani* infection of sugar beet plants was greatly suppressed by using different yeasts. Many types of yeast were tested for biological control of post-harvest diseases of fruits and vegetables (Zhang *et al.* 2005), moulds of stored grains and powdery mildews of different crops .The yeast fungus, *S. cerevisiae*, reduced infection of *M. incognita* on Egyptian henbane, *Hyoscyamus muticus*, and increased its growth.

Here, our aim of investigation was to identify potential multifunctional plant growth promoting soil yeast for plant growth and biocontrol that would help in sustaining for crop production.

Methodology

A total of twenty different yeast were isolated from Acid lime, Pomegranatr, Sapota, Banana, Manila Tamarind, Mango, Guava,Amaranthus, Bhendi, Chilli, Tomato, Cauli flower, Ocimum, Solanum, Aloe, Henna, Marigold, Hibiscus, Jasmine, Tuberose, Balsom samples using the Yeast Extract Peptone Dextrose (YEPD) agar medium supplemented with 250 µg/ml chloramphenicol with pH 6.5-6.7 (Nassar *et al.* 2005). Among the twenty, Morphological and biochemical characterization was done for the five selected efficient yeast isolates.

Indole acetic acid production by the yeast isolates

IAA production was estimated using the modified method of Gordon and Weber, 1951. The isolates were grown overnight in YEPD broth and transferred to fresh broth supplemented with 1% tryptophan incubated for 48h at 30±1°C in a rotary shaker. Then, the cultures were centrifuged at 15,000 rpm for 10 min. One millilitre of the supernatant was mixed with 2 mL of Fe-H_2SO_4 solution (1 mL of 0.5 M $FeCl_3 \cdot 6H_2O$ in 75 mL of 6.13 M H_2SO_4) and incubated in the dark for 45 min. under dark condition. The reddish pink colour developed was read at an absorbance of 530 nm and the amount of IAA produced was calculated from the standard graph obtained with 0.5 to 10 µg ml^{-1} of IAA.

Phosphorus and zinc solubilization by the yeast isolates

Yeast inoculum was prepared by cultivation in a 5 ml YPD broth (10 g /l yeast extract, 20 g/ l peptone and 20 g/ l dextrose) and the culture was incubated on a reciprocal shaker at 180 strokes/min at 28±2°C for 24 h. The inoculum density was adjusted to an optical density at 600 nm (OD 600) of 0.10 and 3 ml was spotted onto Pikovskaya's agar (Zaidi *et al.* 2009), zinc oxide agar (Saravanan *et al.* 2003), The phosphate and zinc oxide solubilisation efficiency (SE) was calculated as a ratio between the diameter of the halo zone and the diameter of the colonies

Siderophore production by the yeast isolates

Siderophore productions by all the isolates were tested qualitatively by Chrome Azural S (CAS) plate assay (Schwyn and Neilands 1986). Freshly grown bacterial isolates were inoculated on CAS agar plates and incubated at 30±2°C for 24-48 hours. After proper incubation period, siderophore production was confirmed by the presence of orange colour zone around the colony on CAS agar plates and total four positive colonies were isolated.

Hydrogen cyanide (HCN) production by the yeast isolates

The yeast isolates were streaked onto King's B medium amended with glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed inside the lid of each Petriplate. The plates were then sealed air-tight with parafilm and incubated at 30±1°C for 48 h. A change in colour of the filter paper from deep yellow to reddish-brown indicates the production of Hydrogen cyanide (Bakker and Schipperes 1987).

ACC deaminase activity of the yeast isolates

The presence of ACC deaminase activity was determined as described by Dell'Amico *et al.* (2005). About 24 h old yeast isolates grown in nitrogen free DF medium with ACC as carbon source. After inoculation, the cultures were grown at 28°C with continuous shaking and optical density at 600 nm was read for 8 days. Growth indicates the potential for the microorganism to use ACC as nitrogen source through deamination

In vitro Screening of antagonistic yeast against plant pathogens

The antifungal efficacies of PGPY strains were tested by dual culture technique (Dennis and Webster 1971) using PDA medium. A mycelial disc of the pathogen (5 mm dia.) *Collectotrichum* was placed at one end of the Petriplate. The bacterial antagonists were streaked 1 cm away from the periphery of the Petriplate just opposite to the mycelial disc of the pathogen. Visual observation on the inhibition of the growth of fungal pathogen in PDA plates were recorded after 96 hours of incubation in comparison with simultaneously inoculated with fungus alone.

Egg hatching and juvenile mortality

The culture filtrate of soil yeast was prepared. The broths were centrifuged at 2000 rpm for 15 min. The supernatant solution was filtered through bacterial filter and used as crude. The culture filtrate of each concentration and distilled water to serve as control were taken separately in Petri dishes of 5 cm diam. and egg masses of *M. incognita* were transferred @ 5 egg masses / Petri dish. Observations were made on number of juveniles hatched at 12h interval for three days.

Similarly the juvenile mortality was assessed exposing 100 second stage juveniles (J2) as crude. The number of immobile juveniles was counted and expressed in percentage at an interval of 12 h after exposure. The immobile nematodes were also transferred to distilled water to assess the recovery of juveniles.

RESULTS AND DISCUSSION

Soil yeast and yeast-like fungi produce a variety of biologically active compounds (phytohormones, vitamins, amino acids, enzymes etc.) that have active stimulating effect on the plant growth and development and help to increase their productivity. In addition, yeasts produce antimicrobial substances helping to reduce phytopathogenic infection (Fu *et al.* 2016). A total of 20 yeast isolates were obtained from Acid lime, Pomegranate, Sapota, Banana, Manila Tamarind, Mango, Guava, Amaranthus, Bhendi, Chilli, Tomato, Cauli flower, Ocimum, Solanum, Aloe, Henna, Marigold, Hibiscus, Jasmine, Tuberose, Balsom All the isolates were tested for the plant growth promoting traits such as production of IAA, siderophore, hydrogen cyanide, zinc solubilization and phosphate solubilisation; biocontrol potential against plant pathogen and Root knot nematode.

IAA producing micro organisms is receiving increasing attention as favourable candidates for use as bio fertilizers. IAA producing microorganisms are believed to enhance root growth resulting in increasing root surface area, thus enabling increased access to soil base nutrients. This phytohormone is commonly produce by PGPR. IAA concentration was strain dependent and range from with tryptophan (7.1 to 623.14 ug/ml) and without tryptophan (0.8-166.12 ug/ml). Among 20 high level producers two strains gave higher results *Rhodotorula* sp SY3 (623.14 ug/ml with tryptophan: 150.12 ug/ml without tryptophan) and *Candida tropicalis* SY5 (580.25- with tryptophan: 120.24- without tryptophan) (Table 1). IAA production by strains YA05 andYR07 was comparably higher than that reported for the some other analyzed yeast strains such as *Candida valida*, *Rhodotorula glutinis* and *Trichosporon asahii* (29.5, 24.1 and 31.7 µg/ml respectively) (El-Tarabily 2004); *Williopsis saturnus* (22.5 µg IAA/ml) (Nassar *et al.* 2005); *Hannaella sinensis*, *Cryptococcus flavus*,

Rhodosporidium paludigenum and *Torulaspora glabosa* (up to 29.3 mg/g) (Nutaratat *et al.* 2014).

Phosphorous and zinc are vital for plant growth and productivity. Phosphorous solublizing microorganisms play a role phosphorous nutrition by enhancing its availability to plant through solublization and mineralization in organic and inorganic soil. Among 20 strains *Rhodotorula* sp exhibited strongest solublizing ability with an area of 4.5 cm phosphorus and 3.0 cm of zinc (Fu *et al.* 2016).

Table 1: Screening of plant growth promoting soil yeast by IAA production

Sl. No.	Soil yeast isolates	Presence of Tryptophan (µg/ml)	Absence of Tryptophan (µg/ml)
1	*Candida* sp SY01	7.1	0.8
2	*Candida* sp SY02	54.02	8.12
3	*Rhodotorula* sp SY03	623.14	150.12
4	*Sacharomyces* sp SY04	25.12	1.8
5	*Candida tropicalis* SY05	580.25	120.24
CD		16.32	12.38

Candida tropicalis SY5 and *Rhodotorula* sp SY3 were positive for hydrogen cyanide production that the ability to produce HCN could be considered as a growth promoting trait. All the five isolates utilise the ACC deaminase and the presence of growth (Table 2).

Table 2: Mineral Solubilizing ability of Plant Growth Promoting soil Yeast

Sl. No.	Soil yeast isolates	Phosphorus solubilizing area (cm)	Zinc oxide solubilizing area (cm)
1	*Candida* sp SY01	2.0	2.5
2	*Candida* sp SY02	1.2	3.0
3	*Rhodotorula* sp SY03	4.5	3.0
4	Sacharomyces sp SY04	0.0	2.0
5	*Candida tropicalis* SY05	3.5	4.5
CD		0.12	0.18

Growth indicates the potential for the microorganism to use ACC as nitrogen source through deamination The function of ACC deaminase in plant-microbe systems, including those using the reference *Pseudomonas putida* strain, has been well studied and results in decreased ethylene production and consequent stimulation of plant root elongation (Glick *et al.* 2007). To our knowledge, high ACC deaminase activity has previously been reported in only two other yeast strains, *Hansenula saturnus* and *Issatchenkia occidentalis* (Prabina *et al.* 2019). The siderophore positive isolates were screened, Out of 20 yeast isolates, five bacterial isolates were positive for siderophore production. Siderophore produced by rhizospheric bacteria improve rhizosphere colonization and play an important role in iron mineralization &supplement to plant. Moreover it also play important antagonistic role against phytopathogens (Freimoser *et al.* 2019).

Antagonistic Potential of PGP soil yeast against plant pathogens

The isolated yeast significantly inhibited the pathogen *Colletotrichum gloeosporioides* and *Alternaria sp*.with inhibition values ranging between 2 -22%. The most effective isolates were *Rhodotorula* sp SY3 (*Alternaria* 3.5cm, *Colletotrichum sp* (2.0 cm) and *Candida tropicalis* SY5 (*Alternaria* 2.8cm, *Colletotrichum* sp (2.8 cm) (Table 3,4,5). The yeast isolates significantly inhibited the *Colletotrichum gloeosporioides* pathogen with Per cent inhibition of mycelial growth over control ranging between 32% and 55%. Similar results obtained by Abd Alla *et al*. (2007) indicated that 21.8% of the yeast isolates significantly inhibited the pathogen with inhibition values ranging between 13% and 35%. The yeast able to control the *Alternaria* at 25 % level by the action of volatile antibiotics.

Antagonistic Potential of PGP soil yeast against root knot nematode *M. incognita*

The culture filtrates of all the five isolates had varying effect on *M. incognita* egg hatching and juvenile mortality. The period of exposure, concentration of filtrate showed significant difference among the cultures of Soil Yeast over *M. incognita*. Among the treatments isolate of *Candida tropicalis* SY5 (Egg hatching 2.6% and Juvenile mortality 96.5 %) performed superior over others and it was followed by *Rhodotorula* sp SY3 (Egg hatching 6.2% and Juvenile mortality 90.2%) (Table 6). A commercial product, containing cells of *S. cerevisiae*, at the rate of 5 g/plant significantly affected J_2s of *M. incognita*

Table 3: Siderophore production, Hydrogen cyanide production and ACC deaminase ability of Plant Growth Promoting soil Yeast

Sl. No.	Soil yeast isolates	Siderophore production	Hydrogen cyanide production	ACC deaminase
1	*Candida* sp SY01	Positive	Negative	Positive
2	*Candida* sp SY02	Positive	Negative	Positive
3	*Rhodotorula* sp SY03	Positive	Positive	Positive
4	*Sacharomyces* sp SY04	Positive	Negative	Positive
5	*Candida tropicalis* SY05	Positive	Positive	Positive

Table 4: Antagonistic Potential of PGPYeast Culture againt *Alternaria sp* plant pathogens

Sl. No.	Soil yeast isolates	Diameter of inhibition zone (cm)	Volatile antibiotic production (cm)
1	*Candida* sp SY01	1.0	0
2	*Candida* sp SY02	1.5	0
3	*Rhodotorula* sp SY03	3.5	4.5
4	Sacharomyces sp SY04	1.0	2.0
5	*Candida tropicalis* SY05	1.2	4.0
CD		0.05	0.04

Table 5: Antagonistic Potential of PGP Yeast Culture against *Colletotrichum gloeosporioides*

Sl. No.	Soil yeast isolates	Per cent inhibition of mycelial growth over control	Inhibition zone of *Colletotrichum* sp (cm)
1	*Candida* sp SY01	38.00 (4.3309)	1.7
2	*Candida* sp SY02	32.22 (4.1660)	1.5
3	*Rhodotorula* sp SY03	50.00 (4.6053)	2.0
4	*Sacharomyces* sp SY04	30.00 (4.0946)	1.1
5	*Candida tropicalis* SY05	55.00 (4.7006)	2.8
CD	Figures in the parenthesis are arcsine transformed values	0.021	0.12

Table 6: Egg hatching and juvenile mortality of PGP Yeast Culture against Root knot nematode *Meloidogyne incognita*

Sl. No.	Soil yeast isolates	Egg hatching %	Juvenile mortality %
1	*Candida* sp SY01	56.8 (4.7328)	10.5 (3.0468)
2	*Candida* sp SY02	65.9 (4.8813)	56.8 (4.7328)
3	*Rhodotorula* sp SY03	6.2 (2.5241)	90.2 (5.1952)
4	*Sacharomyces* sp SY04	78.4 (5.0550)	64.3 (4.8568)
5	*Candida tropicalis* SY05	2.6 (1.6837)	96.5 (5.2627)
	CD	0.243	0.176

Figures in the parenthesis are arcsine transformed values.

in soil and root galling in squash and increased yield of the plant under field conditions (Noweer and Hasabo 2005). The application of the yeast (*S. cerevisiae*) could suppress population of *M. javanica* and root gall formation on cucumber through its effects on nematode infection and reproduction and through inducing plant resistance and enhancing fruit production of cucumber under field conditions (Karajeh 2013).

CONCLUSION

As a result of research among the 20 yeast isolates 2 strains *Rhodotorula* sp SY03 and *Candida tropicalis* SY05 with high plant growth-promoting and biocontrol activities were selected. Conducted studies are of interest in the development of multifunctional biological preparations for agriculture, in which microorganisms have different biological activities.

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