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RESEARCH ARTICLE

Isolation and screening of soil yeasts for plant growth promoting traits

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ABSTRACT

Soil microorganisms are the key player of biochemical and biological processes of the soil and govern the soil health and sustain agricultural production. Yeast is one of the potential plant growth promoting organisms when compared to bacteria and filamentous fungi. In this study, potential plant growth promoting yeast isolates were isolated from garden land soil. All the yeast isolates were screened based on morphological and antibiotic resistance. Several plant growth promoting abilities of the yeast isolates were investigated. The yeast isolates were tested for mineral solubilization of Zinc, phosphate, and potassium (K). Among the isolates, SY7 showed maximum Zn solubilization potential. For P solubilization and K releasing, SY10 yeast isolate showed maximum potential when compared to others. The results revealed that SY2 yeast isolate, produced the maximum amount of indole acetic acid (IAA), Gibberellic acid (GA3). The isolate, SY6 exhibited better siderophore activity and positive for HCN production. Hence, it is concluded that soil yeast may be considered as potential plant growth promoting agents.

Keywords: *Soil yeast, plant growth promotion, solubilization and antibiotic resistance*.

INTRODUCTION

Microorganisms can promote plant growth through direct and indirect mechanisms. Microorganisms form the basis of the ecological balance of the biosphere. The composition of the microbial communities influences the nutrient transformation (Beare *et al*., 1993; Kennedy and Gewin, 1997). Soil microbes, bacteria, archaea, and fungi play diverse and often critical roles in these ecosystem services. Fungi may contribute substantially to soil microbial biomass (Ekelund *et al*., 2001) as well as to the genetic diversity among soil microorganisms (Fierer *et al*., 2007). It is evident from the literature that soil yeasts can exert many beneficial activities *viz.*, plant growth promotion, phosphate solubilization, nitrogen and sulphur oxidation, siderophore production, stimulation of mycorrhizal root colonization, cell wall degrading enzyme production and biocontrol of pathogenic fungi/bacteria (El-Tarabily, 2004; Nassar *et al*., 2005; Falih*et al*., 1995 and Cloete *et al*., 2009). Soil yeasts also play an important role in soil aggregate formation by producing extracellular polymeric substances which bind the soil particles together (Botha, 2006). A wide diversity of soil yeasts have been researched for their potential as bio-fertilizers (Gomaa *et al*., 2007; Eman *et al*., 2008). Culture filtrates of the yeasts detected some beneficial secondary metabolites that could enhance the plant vigour and the physical and chemical properties of the soil (Ramadan *et al*., 2012). The diversity of soil yeasts may be ascribed to the diverse habitat structure of microsites occurring in soils. The studies so far conducted with soil yeast mainly comprise of taxonomical diversity and assessing the functional diversity might be helpful in exploiting this microbiome for sustainable crop production. Hence, the present study was aimed to isolate the yeast from soils and to explored their potential for plant growth promotion.

MATERIAL AND METHODS

Isolation of soil yeasts

Soil samples were collected from garden land soils of TNAU, Orchard (11˚00'34.5" N 76˚55'54.4"E). Isolation of soil yeasts was performed by serial dilution and plating technique. For isolation of yeast, two different media was used *viz.,* YEME (Yeast extract Malt extract) and YPD (Yeast extract, Peptone, Dextrose) (Yarrow, 1998). The plates were incubated at 30˚C for 2-3 days.

Identification and conformation of yeast isolates

Identification was done by morphological

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observation and the yeast isolates were further screened by intrinsic antibiotic resistance test (Van Dijken and Harder, 1974). The selected isolates were streaked on the medium containing streptomycin at the concentration of 100ppm.

Screening of soil yeasts for plant growth promoting traits

Mineral solubilization

Phosphate solubilization

Sperber's hydroxy apatite medium was used for screening the yeast isolates for phosphate solubilization. Plates were inoculated with 10^5 CFU/ml and incubated for 2-3 days at 30°C. *Bacillus megaterium* var. *phosphaticum* Pb1 was used as a reference strain. After the period of incubation, the clear zone was observed around the colony and clearing zone diameter was measured (Sperber, 1958). The ability of the yeast isolates to solubilize insoluble phosphate was described by the solubilization index (SI): the ratio of the total diameter (colony+halozone) and the colony diameter (Premono *et al*., 1996).

Zinc solubilization

Bunt and Rovira's medium was used. The plates were inoculated with the yeast isolates and incubated for 2-3 days at 30°C. After the incubation period, the plates were observed for the clear zone formation. *Enterobacter cloacae* ZSB 14 was used as a reference strain. Zinc solubilization index was calculated as described previously.

Potassium Releasing potential

Aleksandrov medium (Aleksandrov *et al*., 1967) was used to study the potassium releasing potential. The plates were inoculated with the yeast isolates and incubated for 2-3 days at 30°C. After the incubation period the plates were observed for the clear zone formation. *Bacillus mucilaginosus* KRB 9 was used as the reference strain for this experiment. Potassium releasing index was calculated as described above.

IAA production

Yeast isolates were grown overnight in YEME broth and transferred to fresh YEME broth amended with 0.1% L-tryptophan as a precursor for IAA production. The cultures were incubated for 7 days at 28°C, without any interference of light and then centrifuged at 15,000rpm for 10 mins. One milliliter of the supernatant was mixed with 2 mL of Salkowsi reagent (2 mL of 0.5 M $FeCl_3$·$6H_2O$ in 98 mL of 35% perchloric acid) and incubated in the dark for 45 mins. The concentration of IAA was calculated from a standard curve of IAA obtained in the range of 0.5–10.0 g mL^{-1} by measuring the absorbance of samples and standards at 530nm. Three replication for each sample was maintained (Gordon & Weber, 1951).

Gibberellic acid production

The yeast isolates were grown in YEME broth at 30°C for 7 days. After the period of incubation, the culture broth was centrifuged at 10,000rpm for 10mins and then the supernatant was collected. Then the cell pellet was re-extracted with phosphate buffer (pH 8.0) and then again centrifuged at 10,000rpm for 10mins.The supernatant was collected and pooled and the pH of the supernatant was adjusted to 2.0 using 1N HCl. An equal volume of ethyl acetate was added and the organic phase was extracted using separating funnel. Then 2 ml of Zinc acetate solution was added to 5ml of collected residue. After 2 minutes 2ml of potassium ferrocyanide solution was added and the mixture was centrifuged at 10,000rpm for 10 minutes. Then 5 ml of supernatant was mixed with 5ml of 30% HCl and incubated for 75 minutes. The blank was prepared with 5% HCl. The absorbance was measured at 254nm in a spectrophotometer (Mahadevan and Sridhar, 1982).

Siderophore production

Quantitative assay

Yeast isolates were inoculated in YEME broth and incubated for 48h in a shaker with 125rpm. The supernatant was obtained from 0.5ml of inoculated broth which contains 10^8cfu mL^{-1}. Supernatant and CAS reagents were added at 1:1 ratio and incubated. After incubation period OD was measured at 630nm for each sample (Calvente *et al*., 2001). Four replicates were maintained for each sample.

HCN production

Yeast isolates were streaked on YEME medium containing glycine at the rate of 4.4g L^{-1}. Filter papers are cut into pieces with the size of 1x1cm^2 and dipped in picric acid solution (2.5g of picric acid and 12.5g of Na_2CO_3 in 1litre of water). After soaking the filter paper was placed on the top of Petri plate containing inoculum and allowed for incubation for 48h at 30°C. The positive results were noted by the colour change of the disc from yellow to brown or reddish brown (Millar and Higgins, 1970).

Statistical analysis

All the experimental data were subjected to one-way analysis of variance (ANOVA) and the results are expressed as mean with standard error (mean ± SE). Duncan's multiple range test (DMRT) at $P < 0.05$ was used to compare the mean values. The software package used was SPSS version (16.0).

RESULTS AND DISCUSSION

Isolation and identification of soil yeasts

Totally 18 isolates were selected based on morphological characters and named according to the location of the soil sample collected. The isolates were creamy and formed non-shiny colonies. Among the 18 isolates, 10 isolates were exhibited antibiotic resistance when grown on medium with streptomycin (100ppm) and this antibiotic may suppress the bacteria on growth medium but not in the growth of eukaryotic organisms. The isolates were named according to the culture number from SY1 to SY10. The yeast isolates exhibiting antibiotic resistance were selected for further studies.

Table 1. Mineral solubilization potential of yeast isolates

Isolates	Zn solubilization (%)	P solubilization (%)	K releasing (%)
SY1	ND	29.86 (±7.64)^{bcd}	ND
SY2	53.71 (± 0.14)^{bc}	29.68 (±1.90)^{bcd}	ND
SY3	53.87 (± 2.02)^{bc}	36.23 (±2.90)^{bc}	ND
SY4	38.30 (± 7.53)^{d}	29.17 (±4.17)^{bcd}	ND
SY5	ND	28.89(±1.11)^{bcd}	10.99 (±3.30)^{d}
SY6	ND	ND	ND
SY7	61.90 (±4.76)^{ab}	ND	ND
SY8	50.13 (±6.13)^{c}	ND	54.91 (±1.34)^{abc}
SY9	50.00 (±4.55)^{c}	ND	ND
SY10	57.17 (±1.17)^{bc}	60.92 (±3.78)^{a}	59.41 (± 0.59)^{ab}
Reference strain	58.79 (±1.21)^{bc}	56.67 (± 10.00)^{a}	61.11 (± 5.56)^{a}

Data represent by mean ± SE. Values are means of three replicates, and the values with the same lower case letter within a column indicate, there is no significant difference according to Duncan's test ($P < 0.05$). ND – Not Detected.

Screening of soil yeasts for pgpr traits

Mineral solubilization

Among 10 yeast isolates, 6 isolates have the ability to solubilize the inorganic phosphate and the solubilization percent ranged from 28 to 60 based on clear zones around their colonies. Among all the isolates, SY10 showed a higher P solubilization efficiency (60.92%) comparable to the reference strain (Pb1) (57%). Seven isolates possessed the ability to solubilize Zinc. The isolate SY7 excelled in zinc solubilization with maximum solubilization index of 61.90%. When compared with Phosphate and Zinc solubilization, Potassium releasing potential of the yeast isolates were less (Table 1).

It has been concluded that among 10 yeast isolates, 70% of isolates showed a positive result for Zn solubilization, 60% were positive for P solubilization and 30% of isolates showed a positive result for releasing potassium (Table 2). Vassileva *et al*. (2000) reported that *Yarrowia lipolytica* could be successfully applied for rock phosphate solubilization. A number of other yeast strains have also been characterized for their ability to mobilize insoluble inorganic P sources, including calcium, iron and rock phosphates (Vassileva *et al*., 2000; Mirabal Alonso *et al*., 2008). Amprayn *et al*. (2012) also reported that *Candida tropicalis* HY has a better P solubilization efficiency of 119±10g mL^{-1}. Hesham *et al*. (2010) also reported that yeasts isolates exhibited the P solubilization potential. According to Alonso *et al*. (2008) yeasts like *Rhodotorula* and *Cryptococcus* have the ability to solubilize the phosphate.

IAA production

The results indicated that among 10 yeast isolates, four isolates possessed the ability for higher IAA production. IAA production of all yeast isolates ranged from 1 to 32 µg mL^{-1}. Among the isolates, SY2 recorded the higher IAA (32.80µg mL^{-1}) production (Figure 1). The isolates *viz.,* SY3, SY5, SY6 and SY4 also recorded higher IAA accumulation ability. Amprayn *et al*. (2012) reported the ability of soil yeast *C. tropicalis* HY for IAA production and also indicated that IAA production increased with time. According to Xin *et al*. (2009), no detectable IAA was produced by any of the tested yeast strains after 7 days of incubation without the addition of L-tryptophan. When incubated with 0.1 %

L-tryptophan, 3 yeast strains isolated from *Populus* sp. and reference strain *Rhodotorula glutinis* ATCC exhibited IAA production. Among the eight IAA producing yeast isolates screened by Nassar *et al*. (2005), the most promising growth promoting isolate (*Williopsis saturnus*) registered the highest IAA production in the presence or absence of L-TRP (9.67 µg mL^{-1}). From the present study, it is evident that the ability of soil yeast isolates for IAA production is significantly higher than the strains so far reported.

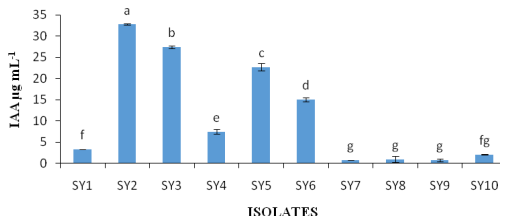

Figure 1. IAA Production by yeast isolates. Same letters in the different bars indicate there are no significant differences between the production of IAA according to Duncan's test (P < 0.05).

Gibberellic acid production

Gibberellins production by plant growth promoting rhizobacteria (PGPR) promotes the growth and yield of many crop plants (Pandya and Desai, 2014).

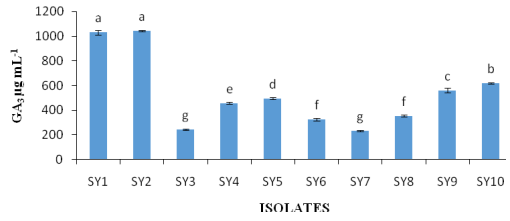

Figure 2. GA3 production by yeast isolates. Same letters on different bars indicate there are no significant differences between the production of GA3 according to Duncan's test (P < 0.05).

The results of the present study indicated that all ten yeast isolates possessed the ability to produce gibberellic acid. The highest gibberellic acid producer was found to be SY1 and SY2 (Figure 2). Tawfiq *et al.* (2018) reported the ability of baker's yeast to produce GA3. Literature indicating gibberellic acid production by yeasts is negligible and this is the first study to report gibberellic acid production by soil yeasts. As gibberellic acid production is an important characteristic of plant growth promoting microbes, this result indicated that the selected soil yeasts may serve as potential plant growth promoting yeasts (PGPY).

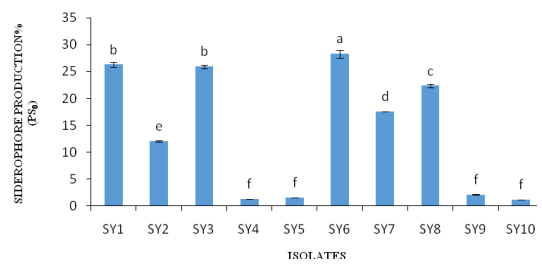

Figure 3. Siderophore production by yeast isolates. Same letters on different bars indicate there are no significant differences between the production of GA3 according to Duncan's test (P < 0.05).

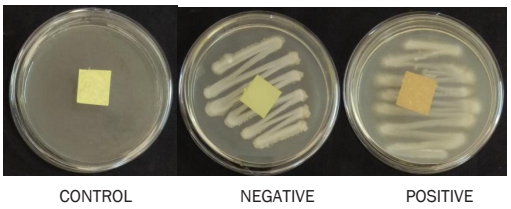

Plate 1. HCN production by yeast isolate SY6

Siderophore production

Calvente *et al*. (2001) reported that yeasts produce hydroxamate-type siderophores (iron-binding compounds) in response to Fe-stress conditions and these siderophores are important to the biocontrol of postharvest diseases of apple and pears. The results indicated that 6 isolates produced siderophore in considerable amounts whereas four isolates produced siderophores in negligible amounts (Figure 3). The isolate SY6 recorded the highest siderophore production than all other isolates followed by SY1 and SY3. In the field of agriculture, different types of siderophores promote the growth of several plant species and increase their yield by enhancing the Fe uptake to plants. From the present study, it is evident that the selected soil yeasts possess the ability to produce siderophore and hence can promote the plant growth to a certain extent.

HCN production

Among the ten yeast isolates, the only isolate SY6 showed positive for HCN production in picric acid solution test (Plate 1). Indeed, the hydrogen cyanide is part of powerful antifungal compounds produced by PGPR and involved in biological control (Haas and Defago, 2005). This supports our finding that the yeast isolate SY6 may be exploited for biological control after detailed analysis.

CONCLUSION

Soil microbes play an essential role in ecosystem functioning and predominantly influence the diversity and structure of above ground communities. The role of soil yeasts in soil ecosystem is not yet fully understood although it is known that they influence soil aggregation, contribute to nutrient cycles, involves in plant growth promotion, protects the crop plants from diseases and involved in the mineralization process. A growing number of

studies indicate that soil and plant system are directly or indirectly influenced to a large extent by the soil yeasts, but the experimental research in this field is still in its infant stage. The results of the present study confirm the presence of plant growth promoting traits in soil yeasts. This indicates that a detailed investigation of these isolates could lead to the identification of a potential yeast candidate that can be successfully exploited as a commercial inoculant for sustainable crop production.

FORMULAE

Siderophore production was calculated by using the formula,

$$\text{Siderophore production}(\%) = \frac{(Ar - As)}{Ar} x100$$

Ar – Absorbance of reference (CAS solution and uninoculated broth)

As – Absorbance of sample (CAS solution and cell free supernatant)

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