

## Estimation of Indole Acetic Acid and Phosphate Solubilisation by the Yeasts Isolated from the Leguminous Crops

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### Authors' contributions

This work was carried out in collaboration between both authors. Author AK designed the study, performed the statistical analysis using OP STAT online software tool, wrote the protocol and wrote the first draft of the manuscript. Authors AK and MBC managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The present study was aimed to estimate the quantity of IAA production and Phosphate solubilisation by the isolated yeasts from the leguminous crops.

**Place and Duration of Work:** The experiments were carried out in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru during 2019-2020.

**Methodology:** Yeasts were isolated from the leguminous crops such as Red gram, Cowpea, Green gram, Black gram and Bengal gram. Thirty-five yeast isolates were obtained using four media and were subjected to IAA production test and Phosphorus solubilisation by Spectrophotometry method. All the yeast isolates were subjected to the temperature tolerance test at 25, 30, 35 and 40°C.

**Results:** Yeast isolate, CP15SI21 has found to produce the highest IAA under in vitro conditions 30.50 µg/ml and the lowest was found to be 17.16µg/ml by yeast isolate BG20SI29. 24 yeast isolates were found to produce above 20 µg/ml. In the case of Phosphate solubilisation highest was observed in GG7SI9 (25.70 mg/l) and lowest in BG6SI8 (1.20 mg/l). Seven isolates showed Phosphate solubilisation above 10 mg/l. At 35°C all the yeast isolates have shown very good

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growth compared to other lower temperatures. All the yeast isolates were found to grow in the media supplemented with micronutrients such as zinc and potassium.

**Conclusion:** Our study highlights the potential of yeasts isolated from the leguminous crops that can help in plant growth promotion as the yeast isolates are capable of producing higher amounts of IAA. Some of the yeast isolates can solubilise phosphate under *in vitro* conditions which in turn helps in the utilization of unavailable P from soils thereby improves plant growth and tolerance to a higher temperature can alleviate abiotic stress.

**Keywords:** Yeast isolates; IAA production; phosphorus solubilisation.

## ABBREVIATIONS

YEPD : Yeast Extract Peptone Dextrose Agar Medium

IAA : Indole Acetic Acid

## 1. INTRODUCTION

In the current agriculture system imbalance of the beneficial microorganism has led to a reduction in the yield of the crops. One of the reasons is increased chemical fertilizers utilization for crop improvement drastically reduced native beneficial microorganisms [1]. Microorganisms play a vital role in plant growth promotion either by direct or indirect mechanisms. Yeasts are found to colonize in diverse habitats, some are beneficial and some are infectious. Some of the yeasts are widely exploited for commercial purposes as they can be easily manipulated genetically [2,3]. Yeasts have wider applications in fields such as food, medicine and biotechnology research but their potential has been exploited lesser in crop improvement practices [4].

Plant growth-promoting microorganisms can be enhanced directly by IAA (Indole acetic acid) and gibberellic acid production and indirectly as bio-control agents, polyamine producers thereby preventing biotic and abiotic stress in plants [5]. IAA regulates various aspects of plant growth and development. Bacteria, fungi and yeasts, are also known to possess IAA-producing ability similar to those of plants [6,7].

Yeasts are known for their ability to produce organic acids, there have been very few reports on their ability to solubilize inorganic phosphate. Phosphorus (P) is an essential macroelement for plants and the total concentration of P in soils ranges from 0.02% to 0.5%. Several mechanisms, such as lowering the pH by acid production and ion chelation have been reported to play a role in Phosphate-solubilisation by microorganisms.

Our knowledge of soil yeasts in improving plant growth was found to be limited. There are reports

suggesting yeasts play an important role in improving plant growth by increased nutrient uptake, phyto-hormones production and some can act as effective bio-control agents [4,8,9]. Therefore, the present study was conducted in isolating yeasts from rhizosphere of different leguminous crops and screening them for plant growth-promoting traits under *in vitro* conditions.

## 2. MATERIALS AND METHODS

### 2.1 Characterization of Yeast Isolates for Plant Growth Promotion

The experiments were conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru. Soil samples and leaf samples were collected for the isolation of yeasts from Bengaluru and Dharwad districts. A total of thirty-five yeasts were isolated from the phyllosphere, endophytes and rhizosphere soil of leguminous crops and were tested for their ability to promote plant growth. Pure culture of the yeast isolates was maintained on Yeast Extract Peptone Dextrose (YEPD) agar medium for further studies.

### 2.2 IAA Production by the Yeast Isolates

Yeast isolates were grown in Yeast Extract Peptone Dextrose (YEPD) and incubated at 26°C. Then the yeast isolates were centrifuged at 8000 g for 10 min and the supernatants were collected. One ml of supernatant was mixed with 2 ml of the Salkowski reagent (1 ml of 0.5 M FeCl<sub>3</sub> in 50 ml of 35% HClO<sub>4</sub>). The mixture was allowed to stand for 30 min for colour development. The intensity of the colour developed was measured at a wavelength of 530 nm using a spectrophotometer [10,11].

### 2.3 Phosphate Solubilisation by the Yeast Isolates

Yeast isolates were grown in Pikovskaya's broth and incubated at 26°C. Then yeast isolates were centrifuged at 7000 g for 10 min and

the supernatants were collected. One ml of supernatant was mixed with 10 ml of ammonium molybdate and diluted to 45 ml with distilled water. 0.25 ml of Chlorostannous acid was added and the volume was made up to 50 ml. The intensity of the colour developed was measured at a wavelength of 600 nm using a spectrophotometer [12,13].

#### 2.4 Zinc Solubilisation and Potassium Solubilisation by the Yeast Isolates

Compared with phosphorous, zinc and potassium are required in extremely small amounts for plants, as they are a critical component of various enzyme activity for driving several plant metabolic reactions [6]. Yeast isolates were plated on the Modified pikovskaya and Aleksandrov medium for Zinc and Potassium solubilisation respectively. The plates were incubated for 2-3 days at 30°C.

#### 2.5 Temperature Tolerance Test

Yeast isolates were streaked on Yeast Extract Peptone Dextrose (YEPD) agar medium and were incubated at temperatures such as 25, 30, 35 and 40°C for 72hrs [14,15]. After incubation growth of the yeast isolates was recorded.

### 3. RESULTS AND DISCUSSION

#### 3.1 IAA Estimation

IAA concentration produced by yeast isolates ranged from 17.16 - 30.50 µg/ml. Among 35 yeast isolates, 24 were attributed to high IAA-producing yeasts (above 20 µg/ml) 538 yeast strains were obtained from dark chestnut soil collected under the plants of the legume family. Among them, fifteen strains were attributed to high IAA-producing yeasts (above 20 µg/ml) [16].

The results were found to be similar in the case of yeast isolates obtained from the phyllosphere and rhizosphere of the *Drosera spatulata* Lab. The IAA concentration ranged from 610.63 ± 54.7 to 8.63 ± 1.4 µg mL<sup>-1</sup> when cultured on YPD medium with Trp as the biochemical precursor. The concentration of the IAA produced was strain-dependent, as all eight *Aureobasidium pullulans* strains produced IAA, ranged from 610.63 ± 54.7 to 56.06 ± 2.9 µg mL<sup>-1</sup> [17]. Similarly, the IAA-producing *Candida tropicalis* SSm-39 strain increased the growth and yield of maize [14]. Whereas in the case of soil yeast *Candida tropicalis* HY (CtHY) IAA production was found to be in the lesser quantity 2.57 µg mL<sup>-1</sup> [12,17]. Endophytic yeast *Williopsis saturnus* isolated from maize roots found to produce indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) in vitro in a chemically defined medium [1].

**Table 1. Quantitative estimation of Indole acetic acid (IAA) and phosphorus by the yeast isolates**

Isolates	IAA (µg/ml)	Soluble Phosphorus (mg/l)	Isolates	IAA (µg/ml)	Soluble Phosphorus (mg/l)
GG1SI1	20.02	03.89	RG13SI19	20.33	04.94
GG2SI2	20.17	17.80	CP14SI20	21.12	06.58
GG3SI3	20.65	02.61	CP15SI21	30.50	10.07
GG4SI4	20.65	03.43	CP16SI22	23.66	02.43
GG4Lf5	18.91	12.69	CP17SI23	23.82	09.35
GG4SI6	22.08	00.82	CP17SI24	20.33	09.67
RG5SI7	17.32	01.89	GG18SI25	26.36	08.31
BG6SI8	17.64	01.20	GG18SI26	18.43	07.05
GG7SI9	24.3	25.70	RG19SI27	26.36	01.87
BG8SI10	18.59	04.39	RG19SI28	26.91	16.27
RG9SI11	26.04	10.63	BG20SI29	17.16	04.32
RG9SI12	19.38	00.36	RG3Lf1IE30	22.17	09.45
RG9Lf13	23.66	05.45	GG5Lf2IE31	17.48	00.16
RG10SI14	20.65	11.03	GG9Lf3IE32	25.09	10.56
RG11SI15	20.49	06.00	GG12Lf4IE33	23.50	07.46
RG11SI16	22.39	01.21	CP14Lf5IE34	17.80	02.46
RG12SI17	17.95	01.85	GG15Lf6IE35	23.34	09.27
RG13SI18	19.70	02.20	C.D.	0.066	0.207
			SE(m)±	0.023	0.073

GG – Green gram; BG – Black gram; RG – Red gram; CP – Cowpea

The numbers followed by the crop name indicates the location identity from which the soil and leaf samples were collected

**Table 2. Growth of the yeast isolates at different temperatures**

Isolates	Growth of yeast at different temperatures			
	25°C	30°C	35°C	40°C
GG1SI1	+	+	++	+
GG2SI2	+	+	++	+
GG3SI3	+	+	++	++
GG4SI4	+	+	++	++
GG4Lf5	+	+	++	++
GG4SI6	+	+	++	++
RG5SI7	+	+	++	+
BG6SI8	+	+	++	++
GG7SI9	+	+	++	++
BG8SI10	-	+	++	++
RG9SI11	+	+	++	++
RG9SI12	+	+	++	++
RG9Lf13	+	+	++	+
RG10SI14	-	+	++	++
RG11SI15	-	+	++	++
RG11SI16	+	+	++	+
RG12SI17	+	+	++	++
RG13SI18	+	+	++	++
RG13SI19	-	+	++	+
CP14SI20	+	+	++	++
CP15SI21	-	+	++	++
CP16SI22	+	+	+	++
CP17SI23	-	+	++	+
CP17SI24	+	+	++	++
GG18SI25	+	+	++	+
GG18SI26	+	+	++	++
RG19SI27	+	+	++	++
RG19SI28	+	+	++	++
BG20SI29	+	+	++	++
RG3Lf1IE30	-	+	++	+
GG5Lf2IE31	+	+	++	+
GG9Lf3IE32	+	+	++	+
GG12Lf4IE33	+	+	+	++
CP14Lf5IE34	-	+	++	++
GG15Lf6IE35	-	+	++	++

\*(+)- good growth; (-) – no growth; (++)- very good growth; GG – Green gram; BG – Black gram; RG – Red gram; CP – Cowpea

The numbers followed by the crop name indicates the location identity from which the soil and leaf samples were collected

### 3.2 Phosphate Solubilisation by the Yeast Isolates

Seven yeast isolates found to solubilize phosphorus above 10 mg/l. Phosphate solubilisation by the yeast isolates ranged from 1.20 – 25.70 mg/l. Phosphate solubilisation was found to be highest in yeast isolate, GG7SI9 (25.70 mg/l) and lowest in BG6SI8 (1.20 mg/l). In the case of yeast strains obtained from *Drosera spatulata* Lab only two strains exhibited 1.33 and 1.17  $\mu\text{g mL}^{-1}$  under in vitro conditions supplemented with tricalcium phosphate (TCP) [5]. Some of the yeasts like *Rhodotorula* and *Cryptococcus* found to solubilize the phosphate have been reported [18].

Among the 250 yeast isolates obtained from rhizosphere soil of maize, 53 isolates were found to solubilize phosphate and forms a clear zone around colonies supplemented with tricalcium phosphate [19]. Mobilization of phosphate from insoluble tri-calcium phosphate was found to be highest in soil yeast *Candida tropicalis* HY (CtHY) ( $119 \mu\text{g mL}^{-1}$ ). Forty yeast strains isolated from different places of Egypt were tested for their P-solubilizing activities. Only Nine isolates have exhibited P-solubilisation potential with an SI ranging from 1.19 to 2.76 ( $\mu\text{g/ml}$ ) [20].

### 3.3 Zinc Solubilisation and Potassium Solubilisation by the Yeast Isolates

All the thirty-five yeast isolates were found to grow on the Modified pikovskaya and

Aleksandrov medium. Micronutrient uptake and growth of the yeast isolates on the respective media was found to be slower. Among 10 soil yeast isolates, 70 per cent of isolates showed a positive result for Zn solubilization, 60 per cent were positive for P solubilization and 30% of isolates showed a positive result for releasing potassium [7].

### 3.4 Temperature Tolerance Test

Among 35 yeast isolates, nine isolates have shown negative growth at 25°C whereas 18 isolates showed very good growth at 35 and 40°C. At 30°C, all the yeast isolates have shown good growth. Similarly, *Saccharomyces cerevisiae* from palm wine tested for temperature tolerance had shown positive growth at 30 and 35°C and negative growth as the temperature raised to 45°C [15].

## 4. CONCLUSION

Although, the role of yeasts in the soil ecosystem is not understood completely they are known to influence soil aggregation, contributing to nutrient recycling, involve in plant regulation and promotion of plant growth, protect crop plants from soil-borne diseases and involved in the mineralization process. The ability of the yeast isolates obtained from leguminous crops had been shown to improve plant growth and solubilizing nutrients for easy uptake by plants through IAA production, Phosphate, Zinc and Potassium solubilisation. Therefore, detailed studies on these yeast isolates can help in improving leguminous crop growth sustainably thereby reducing chemical fertilizers up to a great share.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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