

Plant Growth Promoting Trait of Yeasts Isolated from Tomato in Relation to IAA Production

ABSTRACT

Yeasts are the useful microorganism widely present in nature and are the common inhabitants of soil, vegetation, and other environments. It also coexists with other microbes. This study outlines the variety of phylloplane, fructoplane and endophytic yeasts that can grow on leaves and fruits of tomato which favours the formation of the plant-friendly compound indole-3-acetic acid (IAA) production. Thirty yeast isolates were isolated from phylloplane, fructoplane and as endophyte of tomato plant. The isolated yeasts were screened for the IAA production which enhances the plant growth parameters effectively ranging from 0.53 to 11.35 $\mu\text{g/mL}$. The most effective one in promoting plant growth was recorded in the isolate PY 15 ($11.35\pm 0.495 \mu\text{g/mL}$) and it was followed by PY14 ($10.38\pm 0.452 \mu\text{g/mL}$). The least IAA production was recorded in the isolate FY1 ($0.53\pm 0.03 \mu\text{g/mL}$). Among the various isolates tested *in vitro* for the promotion of growth, the highest root and shoot length of $7.56\pm 0.007 \text{ cm}$ and $31.79\pm 0.802 \text{ cm}$ was recorded in the effective isolate PY15. The effective isolate (PY15) was identified as *Meyerozyma guilliermondii* (QR485249) based on morphological and molecular characterization. This study result concluded with statement that yeast strains found to promote plant growth by IAA production and could be considered for the development of biological products to enhance plant growth and to replace / reduce the use of synthetic fertilizers in the market.

Key words: Yeast, IAA, Plant growth promotion, *Meyerozyma guilliermondii*

1. INTRODUCTION

Plant hormones play a crucial role in controlling various cellular and physiological functions including cell division, cell expansion, bud dormancy, flowering, fruit maturation, seed dormancy, seed sprouting, and leaf shedding. Among these hormones, the natural auxin known as indole-3-acetic acid (IAA) is responsible for promoting the differentiation of phloem and xylem, initiating root growth in stem cuttings, and facilitating the development of lateral roots. IAA exhibits a wide range of actions and significantly influences the physiological processes of plants (Yallappa *et al.*, 2021) [1]. IAA triggers both rapid and enduring reactions within plants and has been detected in various organisms associated with plants including bacteria, molds and yeasts.

The significance of microbial IAA in the context of plant-microbe interactions has recently gained importance. Consequently, microbes capable of producing IAA have been proposed as potential suppliers of biofertilizers (Pei *et al.*, 2014) [2]. IAA could potentially serve as a mutual signalling molecule in the interactions between microbes and plants. Biologically synthesised IAA could be a more stable alternative to synthetic IAA, as it is expensive and less stable compared to synthetic auxin analogs like IBA and NAA (Pumin Nutaratat *et al.*, 2015) [3].

Yeast has been extensively studied for its ability to produce IAA. Specific yeast species such as *Pichia spartinae* (Nakamura *et al.*, 1991) [4], *Candida valida*, *Rhodotorula glutinis* and *Trichosporon asahii* (El-Tarabily, 2004) [5], *Cyberlindnera saturnus* (Nassar *et al.*, 2005) [6], *Rhodotorula graminis* and *Rhodotorula mucilaginosa* (Xin *et al.*, 2009) [7], *Candida tropicalis* (Amprayn *et al.*, 2012) [8], *Candida maltose* (Limtong and Koowadjanakul, 2012) [9], *Cryptococcus* sp. (Deng *et al.*, 2012) [10] and *Rhodospiridium fluviale* (Limtong *et al.*, 2014) [11] able to produce IAA. Johnson and Echavarri-Erasun (2011) [12] indicated that yeasts can be developed as fermentation products through a well-developed large-scale production process.

Tomato is a short duration crop gives remunerative income to farmers. The yield is drastically reduced when the crop is grown in poor unfertilized soil. To envisage the improvement in tomato production, various IAA producing yeasts have been isolated from the tomato plant, IAA was quantitatively assayed and examined under *in vivo* conditions for the observations of their biometric variations.

2. MATERIALS AND METHODS

2.1 Isolation of Yeasts

2.1.1 Isolation of Phylloplane and Fructoplane Yeasts

The yeasts were isolated from leaves (phylloplane) and fruits (fructoplane). The samples were collected from nearby villages of Madurai district, including Kesampatti, Arittapatti, Sekkipatti, Vallapatti, Kalvelipatti villages at different stages of growth. Yeast isolation was made from leaf discs (30 nos.) prepared by punching the leaves and also from stems and fruits. All the samples were mixed with 100 ml of sterile distilled water. After shaking for one hour, the standard plate count technique was performed separately for the leaf, stem, and fruit samples. Diluted samples of 0.1 ml were used for yeast isolation using the Yeast Peptone Dextrose (YPD) Agar medium (Yeast extract-10g/L, Peptone-20g/L, Dextrose-20g/L, Agar-20g/L) (Cheng *et al.*, 2019) [13]. Dilutions ranging from 10^{-1} to 10^{-3} were used in the experiment. The plates were then incubated at 27 °C for 48 hours and the population was recorded. The dominant yeast isolates were then isolated (Ashiwini and Mallesha, 2022) [14].

The leaf imprint method was also used to isolate yeasts from a leaf sample. Leaf samples were first surface sterilised with 0.1% sodium hypochlorite and repeatedly washed in sterile water. They were then cut into two pieces, each about 2x2 cm in size, and placed on Petri plates containing solidified YPD medium so that the upper surface of one piece and the lower surface of the other piece remained in contact with the medium. Sample pieces were taken using sterilised forceps after the plate was left undisturbed in laminar airflow for 4 hours. The plate was then incubated at 28°C for two days. Every sample is processed in the same way (Surussawadee *et al.*, 2014) [15].

2.1.2 Isolation of Endophytic Yeasts

The isolation of endophytic yeast was made in the laboratory within 24 h of collection of samples. Stem, roots and leaves were separately cut into 2–3 cm long sections. The adhered debris

and epiphytic microorganisms were removed by washing the samples with sterile water. They were then subjected to consecutive 1 min washes with 1% sodium hypochlorite, 70% ethanol and sterile distilled water. Surface disinfected tissue was aseptically deliquesced with pastel and mortar. Serial dilution was made up to 10^{-6} and 0.5 mL from the last two dilutions was spread on YPD medium on plates, and then incubated for 5–7 days at $25\pm 1^{\circ}\text{C}$. The yeast isolates grown were initially observed for their morphological characteristics based on colony colour and texture (Kurtzman *et al.*, 2011) [16].

2.2 Microscopic and Morphological Characterization of the Yeasts

The yeast isolates maintained on YPD agar slants were used for the observations on morphological characteristics which includes colony appearance, shape, nature of growth, type of growth, and colony colour at 10X and 40X magnifications using compound microscope (Labomed Co.) (Ashiwini and Mallesha, 2022) [14].

2.3 Molecular Characterization of Yeast

2.3.1 Isolation of Genomic DNA from Yeasts

For the molecular analysis, the yeast isolates were cultivated in YPD broth under continuous shaking conditions for 24 hours. Subsequently, DNA was extracted using the cetyl trimethyl-ammonium bromide (CTAB) method by following the established protocol described by Doyle and Doyle (1990) [17]. After 24 hours of growth, the yeast cells that had reached the logarithmic growth phase was collected and transferred into 1.5 ml Eppendorf tubes. These cells were then subjected to centrifugation at 6000 rpm for 15 minutes. The resulting cell pellets were ground in CTAB buffer and incubated at 65°C for 30 minutes. Next, an equal volume of chloroform-isoamyl alcohol (24:1) to the mixture was added and centrifuged at 10000 rpm for 10 minutes. The upper aqueous layer containing the DNA was carefully transferred into separate 1.5 ml centrifuge tubes. To this, an equal volume of ice-cold isopropanol was added. The resultant mixture was stored at -20°C overnight and then subjected to centrifugation at 12000 rpm for 10 minutes. The precipitated DNA was washed with an equal volume of ethanol, followed by centrifugation. Afterward, the DNA was allowed to dry and was subsequently dissolved in 50 μl of TE buffer. The concentration of DNA was determined by measuring its optical density at 260 nm (OD 260) using a nanodrop spectrophotometer. Additionally, the DNA concentration was verified by comparing it to standard molecular markers on a 0.8% agarose gel.

2.3.2 PCR Amplification and Identification of Yeasts

The genomic DNA was amplified by polymerase chain reaction (PCR) using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (3'-TCCTCCGCTTATTGATATGC-5') in a thermal cycler (Eppendorf Master Cycler, Germany). The PCR conditions followed are: initial denaturation at 95°C for 1min, followed by 40 cycles which includes a denaturation at 95°C for 1 min, annealing at 56°C for

30 seconds, 1 min extension at 72°C and final extension at 72°C for 10 min. The PCR products were separated in a 1.2% agarose gel by gel electrophoresis, stained with ethidium bromide (0.5 µg/mL) and the gel was photographed and analysed using a Gel Documentation system. The sample was forwarded for the sequencing and the result was analysed by using MEGA7 software as a FASTA format (Kumar *et al.*, 2016) [18]. Sequence homology of the yeast isolates with closest similar isolates was determined by using web based BLAST (Basic Local Alignment Search Tool) program of the NCBI. The sequence was deposited in the GenBank (NCBI).

2.4 Functional Characterization of Yeasts

2.4.1 Quantification of IAA Synthesis

Yeast isolates were cultured in YPD broth with one flask containing 1 per cent tryptophan and another without tryptophan. The cultures were then incubated on a rotary shaker at $30 \pm 1^\circ\text{C}$ for 48 hours and subsequently centrifuged at 10,000 rpm for 20 minutes. After that, 50 µL of ortho-phosphoric acid and 2 mL of Salkowski's reagent were added to 2 mL of the supernatant from the centrifuged samples. This mixture was incubated in the dark at $30 \pm 1^\circ\text{C}$ for 25 minutes. The presence of IAA production was indicated by the development of a reddish-pink colour, and its absorbance was measured at 530 nm using a spectrophotometer against a control. The quantification of IAA was determined by comparing the absorbance readings to a standard graph (Gordon and Weber, 1951) [19].

2.4.2 Mass Culturing of Yeast

Yeast inoculum was prepared on YPD broth at pH 6.8 and incubated in the dark in an orbital shaker at 150 rpm and 28°C for two days. Cultures were collected by centrifugation for 5 min at 3,000 g, washed twice and pellets were re-suspended in the same volume of sterile water. The concentration of the inoculum was measured and adjusted to 10^8 CFU mL⁻¹ (Fernandez-San Millan *et al.*, 2020) [20]. The prepared concentration of the cell culture was utilized for the experiment on *in vitro* assessment of IAA on growth promotion by spraying on to the plants.

2.4.3 In vitro Assessment of IAA Producing Yeasts for Tomato Growth Promotion

Tomato seedlings were used for the *in vitro* assessment for the identification of potential IAA producing yeasts. The seedlings were grown under the screen house conditions in the pro-tray. Five different types of isolates *viz.*, FY1, PY15, PY18, FY6 and PY14 were used for assessment. The yeast inoculum was prepared by adding 3 mL of mass cultured yeasts into 100 mL of sterile water which consisted the concentrations of cells 3×10^8 cells mL⁻¹. The control group consisted of plants treated with sterile water alone. The inoculum was sprayed on the foliar portion of the seedlings. The plants were maintained in a greenhouse for 35 days and watered periodically. At the end of the 35th day, the length of the shoots or roots after washing off the substrate were used to measure the effects of yeasts on plant growth (Oliveira *et al.*, 2019) [21].

2.5 Statistical Analysis

Data were subjected to two-way ANOVA using SPSS software for Windows. Statistical differences between means were compared using Tukey's HSD (Honestly Significant Difference) test at $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Isolation of Yeasts

A total of 30 yeasts were isolated from leaves, fruits and as endophytes from samples collected from different locations in the Madurai district by leaf imprinting, washed or unwashed leaf pieces or by fruit washing on YPD medium and further maintained in slants for further experiment. Among the 30 isolates, 10 were phylloplane yeasts which were isolated from different locations such as PY11, PY12 and PY13 from Arittapatti, PY14, PY15 and PY16 from Kesampatti and PY17, PY18, PY19 and PY20 from Kalvelpatti. The majority of fructoplane yeasts were isolated from Arittapatti *i.e.*, FY1, FY2, FY3 and FY4 and from Sekkipatti (FY7, FY8 and FY9). The rest of the isolates *viz.*, FY5, FY6, and FY10 were isolated from the samples collected from Kesampatti and Kalvelipatti villages. Endophytic yeasts were isolated from Kesampatti (EPY21, EPY22 and EPY23), Sekkipatti and Vallapatti (EPY24, EPY25, EPY26, EPY27, EPY28, EPY29 and EPY30) areas (Table 1).

3.2 Microscopic and Morphological Characterization of the Yeasts

All the 30 yeast isolates were observed for their morphological characters like colony colour, colony appearance and nature of growth. They were also observed under microscope for cell shape (Table 2). Most of the colonies of isolates were cremish white in colour, while some were completely whitish and yellowish. Certain isolates were found to be pinkish (EPY23, EPY30, FY7, FY10 and PY18). Yeast isolates were found to be smooth to crusty, glassy and granular. Most of the isolates were found to be fast-growing while few (FY3, FY9, PY14, EPY21, EPY25 and EPY30) were slow growing. Cell shape of most the isolates varied from oval to spherical but cylindrical shape was also observed in certain isolates like FY3, FY8, FY10, PY11 and EPY28. Similar experiment was conducted by Shikha *et al.* (2020) [22], isolated yeasts from fruit and phylloplanes in the summer, winter and rainy seasons to determine their population density. Thirteen different yeast isolates were cultured from various phylloplanes and then characterised for their morphological, physiological and other traits. The findings revealed that colony colour of yeast isolates vary from cremish (Y1 to Y7, Y10 and *Saccharomyces cerevisiae*) to whitish (Y8, Y11 and Y12) and one isolate Y9 gave yellowish colour. Smooth, crusty to granular colony appeared on YPD medium and having different growth nature *viz.*, isolates Y2, Y9 and Y14 showed faster growth as compared to isolates Y1, Y11, Y12 and Y13 which showed slow colony growth. The cell shape and size ranges from spherical, oval to cylindrical (1.9 x 1.06 μm to 4.51 x 3.14 μm).

3.3 Molecular Characterization of Yeast isolate

Accordance with the spectrometric reading, the higher IAA producing yeast strain PY15 with the value of $11.35 \pm 0.495 \mu\text{g/mL}$ was molecularly confirmed as the beneficial yeast. The gene sequence data of PY15 exhibited 91 % homology with *Meyerozyma guilliermondii*. The sequence of

this isolated strain was deposited to the NCBI GenBank and assigned with the accession number (QR485249).

3.4 IAA Quantification

The IAA production in the yeast was determined by two methods *i.e.*, YPD broth with L-tryptophan and without L-tryptophan, where all the yeasts produced IAA. The production ranged from 0.53 to 11.35 $\mu\text{g/mL}$. The maximum IAA production ($\mu\text{g/mL}$) was recorded in the isolate PY 15 (11.35 ± 0.495) and it was followed by PY14 (10.38 ± 0.452). The least was recorded in the isolates FY1 (0.53 ± 0.03) and FY8 (0.87 ± 0.068). Most of the isolates lies within the range of 3.12 to 3.95 which includes FY2, FY10, PY19, EPY23 and EPY27 (Table 3). The notable set of yeasts found to produce appreciable IAA were represented graphically in the Fig. 1 (without tryptophan) and Fig. 2 (with tryptophan).

Pumin Nutaratat *et al.* (2014) [23] isolated and screened a total of 1035 yeast isolates from rice and sugar cane leaves primarily for IAA production. Thirteen isolates were selected due to their IAA production ranged from 1.2 to 29.3 mg g^{-1} . Among the thirteen yeast isolates, four yeast species were molecularly confirmed as *Cryptococcus flavus*, *Rhodospodium paludigenum*, *Torulaspora globosa* and *Hannaella sinensis*, capable of high IAA production. The yeast *R. paludigenum* was found to be best in IAA production yielding 29.3 mg g^{-1} . Different levels of IAA production were also observed in four strains of *C. flavus* DMKU-RE12, DMKU-RE19, DMKU-RE67 and DMKU-RP128, which were isolated from rice leaf as epiphyte. In addition, four *C. flavus* were also found to show optimal IAA production.

Rou-Yun Chen *et al.* (2020) [24] used wild cherry tomatoes and reported that yeast strains isolated from rhizosphere having the potential for enhancing plant growth. A total of 118 yeast strains were isolated from the rhizosphere and evaluated based on the production of IAA which ranged from 17.65 to 789.41 μM using tryptophan as a precursor. Jeberlin *et al.* (2022) [25] characterized twenty seed-borne yeasts and tested for growth promotion of black gram. Among them, *Pichia kudriavzevii* POY5 and *Issatchenkia terricola* GRY4 produced IAA. Inoculation of these two yeast isolates and *Rhizobium* BMBS1 improved the seed germination, physiological parameters and yield of black gram.

3.4 In vitro Assessment of IAA Producing Yeasts for Growth Promotion

The effective strains of the isolated yeast were selected on the basis of the spectrometric reading and taken forward for the *in vitro* study for ensuring the remarkable changes in the growth parameters of the tomato. The yeast strains such as PY15, PY 18, PY14 and FY6 produced maximum amount of IAA. The FY1 isolate produced the least amount of IAA comparatively with others. The spraying of these yeast strains on tomato seedlings increased the shoot length and root length (Table 4). The isolate PY15 which recorded the higher value for IAA in the spectrometric reading also increased the plant growth traits such as shoot (cm) and root length (cm) as 31.79 ± 0.802 and 7.56 ± 0.007 , respectively. The relativeness of quantitative assay against the growth parameter is again justified by the check (control) maintained, which measures 4.38 ± 0.058 as root length (cm) and 23.91 ± 0.51 as shoot length (cm). Fernandez-San Millan *et al.* (2020) [20] isolated 69

yeast strains from Spanish vineyards. Among which, three isolates (*Debaryomyces hansenii* Dh-67, *Lachancea thermotolerans* Lt-69, and *Saccharomyces cerevisiae* Sc-6) found to promote maize seedling development in addition to 10% increase in dry weight and chlorophyll content.

Yallappa *et al.* (2021) [1] identified superior yeast isolates capable of producing IAA (MZL-8 and TCL-1) and assessed their impact on tomato plant growth. The application by spraying the yeast isolate TCL-1 exhibited the most favorable outcomes, resulting in the highest values for plant height, the number of leaves per plant, the number of branches per plant, root length, fresh shoot biomass, dry shoot biomass, fresh root biomass and dry root biomass as well as IAA content. This finding justified the plant growth promotion ability of the yeast isolates producing IAA. This study also confirms that several yeast strains isolated from tomato have the potential to enhance the plant growth and suggesting their consideration for the development of biological fertilizer treatments.

Table 1. Isolation of yeasts

S. No.	Location name	Plant used part	Soil type	Variety name	Isolate code
1.	Arittapatti	Leaves	Red soil	Sivam	PY11, PY12, PY13
		Fruit			FY1, FY2
		Fruit			FY3, FY4
2.	Kesampatti	Fruit	Red sandy loam	Sivang	FY5, FY6
		Leaves			EPY21, EPY22, EPY23
		Leaves			PY14, PY15, PY16
3.	Sekkipatti	Fruit	Red soil	Paiyur-1	FY7, FY8
		Fruit			FY9
		Leaves			EPY24, EPY25, EPY26
4.	Vallalapatti	Leaves	Red loam	CO 3	EPY27, EPY28, EPY29
		Leaves			EPY30
5.	Kalvelipaati	Fruit	Black clay	CO 2	FY10
		Leaves			PY17, PY18
		Leaves			PY19, PY20

*PY-Phylloplane yeast; EPY-Epiphytic yeast; FY-Fructoplane yeast

Table 2. Microscopic and morphological characterization of yeasts

S. No.	Isolate code	Colony colour	Colony appearance	Nature of growth	Cell shape
1	FY1	Whitish	Smooth, raised	Fast	Spherical

2	FY 2	Yellowish	Smooth and soft	Fast	Oval to Spherical
3	FY3	Yellowish	Granular	Slow	Cylindrical
4	FY4	Whitish	Smooth and soft, Chalky	Fast	Spherical
5	FY5	Cremish white	Smooth, Butyrous and small lobes	Fast	Oval
6	FY6	Cremish white	Smooth, raised, entire margin	Fast	Oval
7	FY7	Pinkish	Smooth and soft, Chalky	Fast	Oval
8	FY8	Cremish white	Granular	Fast	Cylindrical
9	FY9	Yellowish	Folded, rough	Slow	Oval
10	FY10	Pinkish	Smooth and soft	Fast	Cylindrical
11	PY11	Whitish	Smooth, raised	Fast	Cylindrical
12	PY12	Cremish White	flat, with volcano-shaped	Fast	Oval
13	PY13	Cremish white	Granular	Fast	Spherical
14	PY14	Cremish white	Smooth, raised	Slow	Oval
15	PY15	Whitish	Smooth and soft	Fast	Oval to Spherical
16	PY16	Cremish white	Granular	Fast	Spherical
17	PY17	Yellowish	Smooth, raised	Fast	Spherical
18	PY18	Pinkish	Smooth, raised, entire margin	Fast	Oval to Spherical
19	PY19	Cremish white	Smooth, raised	Fast	Oval
20	PY20	Whitish	Granular	Fast	Spherical
21	EPY21	Cremish white	Smooth and soft ,Chalky	Slow	Oval to Spherical
22	EPY22	Whitish	Granular	Fast	Spherical
23	EPY23	Pinkish	Folded, rough	Fast	Oval
24	EPY24	Cremish white	Glassy, smooth	Fast	Oval to Spherical
25	EPY25	Cremish white	Smooth and soft, Chalky fringed with filaments	Slow	Spherical
26	EPY26	Cremish white	Smooth surface, raised	Fast	Spherical
27	EPY27	Cremish white	Glassy, smooth surface, fringed with filaments	Fast	Oval
28	EPY28	Cremish white	Smooth, Butyrous and small lobes	Fast	Cylindrical
29	EPY29	Cremish white	Smooth and soft, Chalky fringed with filaments	Fast	Spherical
30	EPY30	Pinkish	Granular	Slow	Oval to Spherical

*PY-Phylloplane yeast; EPY-Epiphytic yeast; FY-Fructoplane yeast

Table 3. IAA production of yeasts ($\mu\text{g/mL}$)

S. No.	Isolates code	IAA with Tryptophan ($\mu\text{g/mL}$)	IAA Production without Tryptophan ($\mu\text{g/mL}$)
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1	FY1	0.53±0.033 ^p	0.49±0.019 ^o
2	FY 2	3.23±0.161 ^{lm}	1.23±0.021 ^{lm}
3	FY3	5.56±0.338 ^{gh}	1.18±0.052 ^m
4	FY4	6.89±0.206 ^f	1.79±0.006 ^l
5	FY5	8.12±0.354 ^e	3.03±0.026 ^f
6	FY6	9.38±0.409 ^{bcd}	3.02±0.109 ^{tg}
7	FY7	4.32±0.188 ^{jl}	3.02±0.095 ^{tg}
8	FY8	0.87±0.068 ^{nop}	4.00±0.173 ^c
9	FY9	5.67±0.354 ^{gh}	2.60±0.100 ^h
10	FY10	3.12±0.156 ^{lm}	0.81±0.006 ⁿ
11	PY11	4.87±0.296 ^{hi}	0.35±0.007 ^o
12	PY12	6.93±0.208 ^f	3.27±0.038 ^{def}
13	PY13	9.26±0.4039 ^{cd}	1.42±0.025 ^{klm}
14	PY14	10.38±0.452 ^{ab}	3.35±0.130 ^{de}
15	PY15	11.35±0.495 ^a	6.43±0.174 ^a
16	PY16	8.42±0.657 ^{de}	3.10±0.075 ^{ef}
17	PY17	9.12±0.569 ^{de}	1.29±0.013 ^{kim}
18	PY18	10.23±0.511 ^{bc}	1.53±0.040 ^{jk}
19	PY19	3.58±0.218 ^{kim}	5.35±0.174 ^b
20	PY20	0.97±0.029 ^{nop}	3.40±0.100 ^d
21	EPY21	0.67±0.029 ^{op}	0.90±0.075 ⁿ
22	EPY22	2.84±0.124 ^m	2.58±0.030 ^h
23	EPY23	3.95±0.172 ^{kl}	3.15±0.046 ^{ef}
24	EPY24	6.43±0.502 ^{tg}	2.75±0.074 ^h
25	EPY25	1.63±0.102 ^{no}	1.47±0.037 ^{kl}
26	EPY26	2.78±0.139 ^m	1.29±0.042 ^{klm}
27	EPY27	3.87±0.235 ^{kl}	3.03±0.052 ^f
28	EPY28	4.12±0.123 ^{kl}	2.78±0.058 ^{gh}
29	EPY29	5.98±0.260 ^{tg}	2.64±0.026 ^h
30	EPY30	1.73±0.075 ⁿ	1.54±0.038 ^l
		CD (0.05) = 0.512	CD (0.05) = 0.127

Table 4. Effect of yeast treatment on growth parameters (shoot and root length) of tomato

S. No.	Isolate code	Shoot length (cm)	Root length (cm)
1.	PY14	27.32±0.345 ^{bc}	6.01±0.130 ^c
2	PY15	31.79±0.802 ^a	7.56±0.007 ^a
3	PY18	28.14±0.624 ^b	6.65±0.180 ^b
4	FY6	26.45±0.477 ^c	5.95±0.209 ^c
5	FY1	23.91±0.517 ^d	4.38±0.058 ^d
6	Control	22.84±0.247 ^e	3.99±0.162 ^e

		CD (0.05) = 1.04	CD (0.05) = 0.27
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Fig 1. IAA production (without tryptophan) by effective isolates of yeast

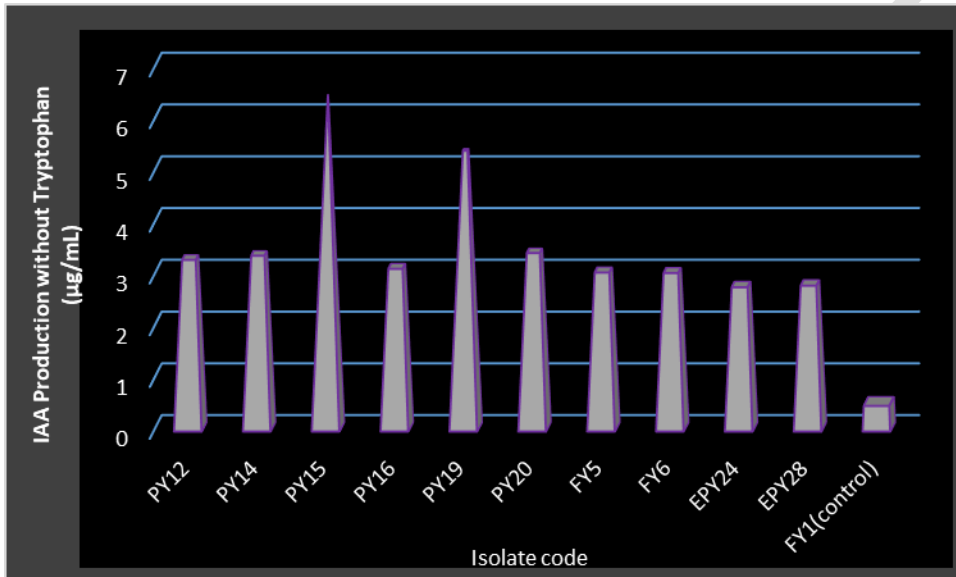


Fig 2. IAA production (with tryptophan) by effective isolates of yeast

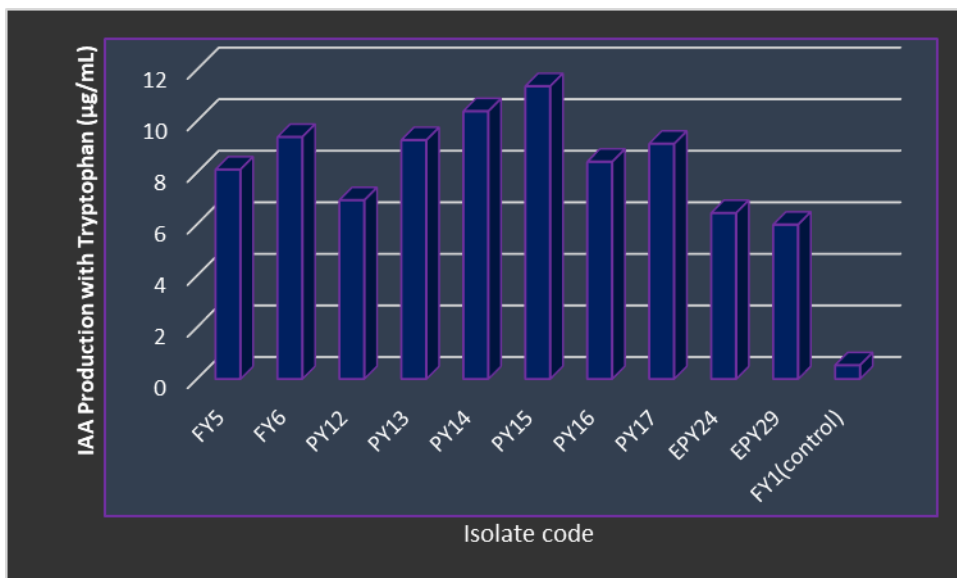


Fig 3. Efficacy of effective yeast isolates on the growth parameters of tomato

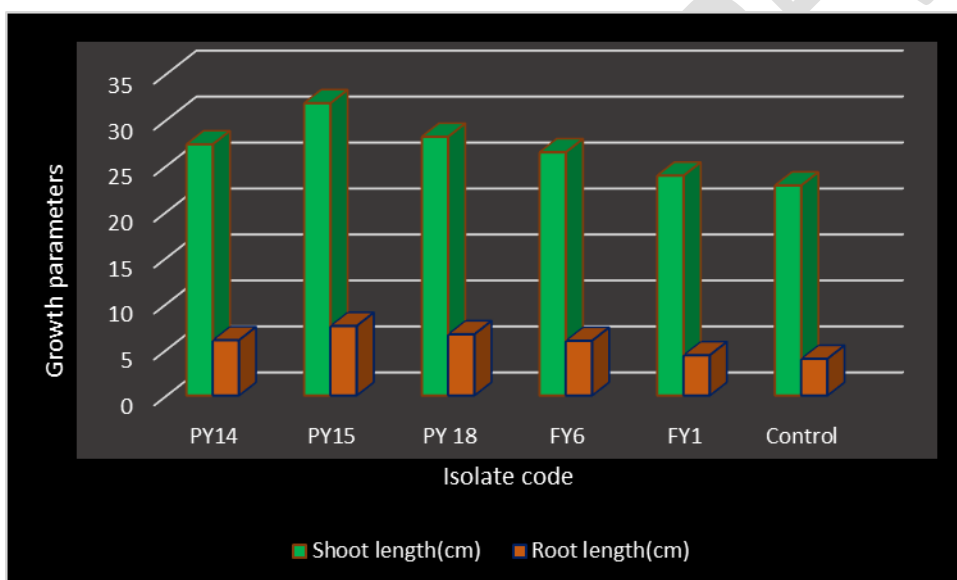


Plate1. *In vitro* efficacy of effective yeast isolates on the growth of tomato



Control-water spray; T1- FY1 spray; T2- PY15 spray; T3- PY 18 spray; T4 FY6 spray; T5-PY14 spray

CONCLUSION

The phylloplane, fructoplane, endophyte yeasts were isolated from tomato samples collected in various locations and assessed for the IAA production. The higher IAA production was observed in the isolate PY15 ($11.35 \pm 0.495 \mu\text{g/mL}$). The least was reported in the isolate FY1 ($0.53 \pm 0.03 \mu\text{g/mL}$). The maximum IAA producing isolates further tested by *in vitro* assessment confirmed the growth promotion by these yeasts. The maximum shoot and root length was recorded as 31.79 ± 0.802 and 7.56 ± 0.007 cm, respectively in the isolate PY15. The isolate with the higher plant growth promoting trait was molecularly confirmed. The gene sequence data of PY15 exhibited 91 % homology with *Meyerozyma guilliermondii*. The sequence of this isolated strain was deposited to the NCBI GenBank and assigned with the accession number (QR485249). This study concluded that the isolated yeast strains contained greater amount of plant growth promoting IAA, which may be used as biofertilizer and also a greater alternative to the synthetic fertilizers in the market.

REFERENCES

1. Yallapa M, Mallesha BC, Rekha KR, Swathi M. Evaluation of IAA and GA3 producing yeast isolates on growth of tomato crop by spraying under green house. International Journal of Agricultural Sciences. 2021; 17(1):273-276.
2. Sun PF, Fang WT, Shin LY, Wei JY, Fu SF, Chou JY. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant *Drosera indica* L. PloS one. 2014; 9(12):e114196.
3. Nutaratat P, Amsri W, Srisuk N, Arunrattiyakorn P, Limtong S. Indole-3-acetic acid production by newly isolated red yeast *Rhodospiridium paludigenum*. The Journal of General and Applied Microbiology. 2015; 61(1):1-9.

4. Nakamura T, Murakami T, Saotome M, Tomita K, Kitsuwa T, Meyers SP. Identification of indole-3-acetic acid in *Pichia spartinae*, an ascosporeogenous yeast from *Spartina alterniflora* marshland environments. *Mycologia*. 1991; 83(5):662-664.
5. El-Tarabily KA. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. *Journal of Applied Microbiology*. 2004; 96(1):69-75.
6. Nassar AH, El-Tarabily KA, Sivasithamparam K. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. *Biology and Fertility of Soils*. 2005; 42:97-108.
7. Xin G, Glawe D, Doty SL. Characterization of three endophytic, indole-3-acetic acid-producing yeasts occurring in *Populus* trees. *mycological research*. 2009; 113(9):973-80.
8. Amprayn KO, Rose MT, Kecskés M, Pereg L, Nguyen HT, Kennedy IR. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. *Applied Soil Ecology*. 2012; 61:295-299.
9. Limtong S, Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. *World Journal of Microbiology and Biotechnology*. 2012; 28:3323-3335.
10. Deng Z, Wang W, Tan H, Cao L. Characterization of heavy metal-resistant endophytic yeast *Cryptococcus* sp. CBSB78 from rapes (*Brassica chinensis*) and its potential in promoting the growth of *Brassica* spp. in metal-contaminated soils. *Water, Air, & Soil Pollution*. 2012; 223:5321-5329.
11. Limtong S, Kaewwichian R, Yongmanitchai W, Kawasaki H. Diversity of culturable yeasts in phylloplane of sugarcane in Thailand and their capability to produce indole-3-acetic acid. *World Journal of Microbiology and Biotechnology*. 2014; 30:1785-1796.
12. Jhonson EA, Echadarri-Erasun C. Yeast Biotechnology. In: Kurtzman C, Fell JW, Boekhout T,(Eds), Elsevier, Amsterdam. The yeasts, a taxonomic study. 5th ed. 2011; 9: 21-44.
13. Cheng BQ, Wei LJ, Lv YB, Chen J, Hua Q. Elevating limonene production in oleaginous yeast *Yarrowia lipolytica* via genetic engineering of limonene biosynthesis pathway and optimization of medium composition. *Biotechnology and Bioprocess Engineering*. 2019; 24:500-506.
14. Ashiwini G, Mallesha BC. Biocontrol Activity of Yeasts against *Alternaria solani* and Plant Growth Promotion of Tomato Plants. *Journal of Experimental Agriculture International*. 2022; 44(10):185-193.
15. Surussawadee J, Khunnamwong P, Srisuk N, Limtong S. *Papiliotrema siamense* fa, sp. nov., a yeast species isolated from plant leaves. *International Journal of Systematic and Evolutionary Microbiology*. 2014; (Pt-9):3058-3062.
16. Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for isolation, phenotypic characterization and maintenance of yeasts. In: *The Yeasts*. Elsevier.2011; pp. 87-110.

17. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus*. 1990;12:13-15.
18. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. 2016; 33(7):1870-1874.
19. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. *Plant physiology*. 1951; 26(1):192.
20. Fernandez-San Millan A, Farran I, Larraya L, Ancin M, Arregui LM, Veramendi J. Plant growth-promoting traits of yeasts isolated from Spanish vineyards: Benefits for seedling development. *Microbiological Research*. 2020; 237:126480.
21. Oliveira BT, Bizarria R, Silva LG, Rosa-Magri MM. Rhizosphere yeast *Torulaspota globosa* with plant growth promotion traits and improvement of the development of tomato seedlings under greenhouse conditions. *Afr. J Agric Res*. 2019; 14(22):935-42.
22. Shikha S, Bhutia DD, Gupta PK. Isolation and characterization of phylloplane yeasts. *Journal of Pharmacognosy and Phytochemistry*. 2020; 9(2):1434-8.
23. Nutaratat P, Srisuk N, Arunrattiyakorn P, Limtong S. Plant growth-promoting traits of epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand. *Fungal Biology*. 2014; 118(8):683-94.
24. Chen RY, Jiang W, Fu SF, Chou JY. Screening, evaluation, and selection of yeasts with high ammonia production ability under nitrogen free condition from the cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*) rhizosphere as a potential bio-fertilizer. *Rhizosphere*. 2022; 23:100580.
25. Jeberlin PB, Karunanadham K, Maheshwari HS, Karuppiyah Eraivan AA, Thankappan S, Nataraj R, Pandian D, Ameen F, Poczai P, Sayyed RZ. Seed-borne probiotic yeasts foster plant growth and elicit health protection in black gram (*Vigna mungo* L.). *Sustainability*. 2022; 14(8):4618.