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Multifaceted Plant Growth Promoting Yeasts Isolated from Rice and its Functional Characterization

Geethanjali Muthuramalingam[1], Shobana Narayanasamy[1], Akihiko Kamoshita[2], Sivakumar Uthandi[1]

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ABSTRACT

Background: Rice is a staple food crop worldwide and enhancing its resilience to abiotic stress such as drought is crucial for sustainable agriculture.

Methods: In this study, yeast strains associated with rice plants were isolated and characterized for their plant growth-promoting (PGP) traits. Yeasts isolated from rice rhizosphere VIR1, DIN2 and VIR3 were evaluated for key PGP traits such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, production of indole-3-acetic acid (IAA), siderophore and mineral solubilization.

Result: Among them, isolate VIR1 demonstrated the highest activity, producing ACC deaminase (112.5±0.8 nmol α-ketobutyrate mg^{-1} protein h^{-1}), IAA (12.5±1.0 $\mu g\ mL^{-1}$), siderophores (36.8±2.3% SU), phosphate solubilization (86.7±0.6 $\mu g\ mL^{-1}$) and zinc solubilization (11.9±0.9 $\mu g\ mL^{-1}$). Molecular identification using 18S rRNA gene sequencing revealed VIR1 and VIR3 as *Candida tropicalis* and *Saccharomyces cerevisiae*, respectively.

Key words: Nutrition, Plant growth promotion, Rice, Soil yeast.

INTRODUCTION

One of the most important foods on Earth, rice (*Oryza sativa* L.) is consumed by around three billion people every day. To keep up with economic demands, rice output must increase in response to the growing population. This prompted a shift in emphasis towards more efficient farming methods, including the introduction of high-yielding rice varieties, better irrigation strategies and cutting-edge agricultural technology (Yang *et al.,* 2019). In spite of these advancements, rice output is still low in several parts of the world due to issues connected to biotic and abiotic stress. According to Narayanasamy and Uthandi (2024), drought is one of the abiotic factors that impacts almost 42 million hectares of rice production in Asia. Damage to rice crops and the production of harmful oxidative stress from reactive oxygen species (ROS) are consequences of drought stress, which interferes with the plant's morpho-physiological, biochemical and molecular responses (Ghosh *et al.,* 2020). A potential solution to these problems is to harness the power of plant growth-promoting (PGP) microbes. These bacteria can improve plant development, increase yield and aid in disease defence while reducing the impact of biotic and abiotic pressures. Environmental factors likely influence the survival, development and interaction of PGPR with host plants, which in turn affects their performance, making the application of PGPR questionable (Gouda *et al.,* 2018). On the other hand, new research has shown that yeasts which are unicellular fungi that occur naturally in soil and on plant surfaces are crucial for the expression of PGP characteristics. These yeast varieties are gaining attention as fascinating microbiological alternatives for use in sustainable agriculture; they are also safe to cultivate (Singh and Gaur, 2021). A wide range of yeast genera isolated from the soil, rhizosphere, phyllosphere and other parts of the plants (Abdel-Kareem *et al.,* 2021; Bright *et al.,* 2022; Muthukrishnan *et al.,* 2024) exhibits various PGP traits, which includes the production of phytohormones such as Indole acetic acid, Gibberellic acid, cytokinin, exopolysaccharides (EPS), nutrient solubilisation, iron chelation, ACC deaminase (ACCD), production of hydrolytic enzymes, antagonistic action against potential phytopathogens (Nimsi *et al.,* 2023; Ruspi *et al.,* 2025; Ruspi *et al.,* 2024) and stimulation of mycorrhizal-root colonisation (Sarabia *et al.,* 2018). Moreover, according to Ruspi *et al.* (2025), the main yeast genera that display PGP features and have the ability to

[1]Biocatalysts Laboratary Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

[2]Asian Research Centre for Bioresources and Environmental Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo.

Corresponding Author: Sivakumar Uthandi, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
Email: usivakumartnau@gmail.com
ORCID: 0000-0002-7116-1317

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improve plant development and stress resilience include *Rhodotorula spp., Candida spp., Saccharomyces* and *Cryptococcus*. Nevertheless, there is a lack of study on the use of PGPY as bioinoculants and the exact method by which PGPY may affect plant growth remains unclear. Research has shown that certain bacterial and fungal biofertilizers can be used to support sustainable rice production. These include *Azospirillum, Pseudomonas, Bacillus, Phosphobacteria, Methylobacterium, Burkholderia, Azotobacter, Trichoderma* spp., *Gliocladium virens* and Arbuscular mycorrhizal fungi (Adedayo and Babalola, 2023; Nimsi *et al.,* 2023) to name a few. Despite yeasts' reputation for safety and their many useful PGP features, research and development into bioinoculants derived from yeasts for rice is limited. With this background, it is postulated that the multifunctional PGP features exhibited by native yeast strains associated with rice plants could improve plant health and fitness when exposed to harsh climatic conditions. So far, this study has cleared the way for the creation of new yeast-based bio-stimulants for environmentally friendly rice cultivation by identifying and characterising native yeast species in the rice plant's phyllosphere and rhizosphere and by testing their PGP traits.

MATERIALS AND METHODS

Survey details and sample collection

We chose three sites in Tamil Nadu to collect soil and crop samples from because their soil types, cropping patterns, fertilizer management and crop durations were different. The fields of Dindigul (S1), Virudhunagar-Narikudi (S2) and Kundukulam (S3) were sampled for rice rhizosphere soil, roots and leaves at 10°25′18.48″ N, 78°05′32.64″ E, 9°35′06.32″ N and 77°57′28.33″ E, respectively. Samples were taken following the 25th day of crop establishment in all districts that used organic nutrient management. While S1 and S3 grew RNR rice, S2 included white ponni. In S1 and S2, the crop sequence is rice followed by vegetables, whereas in S3, the crop sequence is rice monocropping. The soil and roots of healthy rice plants were extracted from a depth of 10-20 cm, which is known as the rhizosphere. At 4°C, soil samples were collected from five separate plots, combined and sent in a polypropylene sample box for further examination.

Isolation and characterization of yeast from rice

Using the conventional serial dilution technique, yeast was isolated from the rice plant samples (rhizosphere soil, stem, root and leaves). The root and leaf segments were immersed in 70% ethanol for 1 minute, then in 5% sodium hypochlorite for 3 minutes. After that, they were treated with 70% ethanol for 30 seconds and finally, they were rinsed three times with sterile distilled water, in order to isolate endophytic yeast. Following the sterilisation of the root and leaf samples, they were ground in a phosphate buffer (Mahaffee and Kloepper, 1997). The resulting homogenate was further diluted in a series of steps up to a concentration of 10-8. Soil samples were placed in yeast peptone dextrose (YPD) plates after being serially diluted and then incubated at 28°C. The YPD agar plates allowed for the observation and purification of yeast colonies with different morphologies. For long-term maintenance, the cultures were kept in 30% glycerol stocks and stored at -80°C (Robinson and Erasmus, 2016).

Functional characterization of yeast isolates for their PGP attributes

IAA production

Yeast isolates at log phase growth were inoculated into 100 mL of YPD broth containing 1.0 mM L-tryptophan and incubated at room temperature for 7 days. A parallel culture without tryptophan supplementation was maintained as a control. Following incubation, 1.5 mL of yeast culture was centrifuged at 8000 rpm for 10 min and the supernatant was collected separately. Accurately 4 mL of Salkowski reagent comprising 0.5 M ferric chloride ($FeCl_3$) and 35% perchloric acid ($HClO_4$) was added to the supernatant as described (Mohite, 2013). The reaction mixture was incubated for 30 min at room temperature in a darkened environment and the absorbance was measured at 520 nm and expressed as µg mL^{-1}.

ACC deaminase activity

Quantitative estimation of ACC deaminase (ACCd) activity was conducted following the protocol outlined (Honma and Shimomura, 1978). The yeast isolates were cultured in 5 mL of tryptic soy broth (TSB) at room temperature for 24 h. Following cultivation, the cells were harvested by centrifugation at 3000 rpm for 5 min, then resuspended and washed twice with 0.1 M Tris-HCl (pH 7.5). The resulting cell pellet was resuspended in 2 mL of modified dworkin and foster (DF) minimal medium supplemented with 3 mM ACC as the sole nitrogen source. Cultures were incubated at 37°C for 2-3 days under agitation on an orbital shaker. ACC deaminase activity was quantified by measuring the concentration of α-ketobutyrate produced and the enzymatic activity was expressed as nanomoles of α-ketobutyrate released per milligram of protein per hour (nmol α-ketobutyrate mg^{-1} protein h^{-1}).

Siderophore production

Quantitative estimation of siderophore was carried out by growing yeast cultures [~10^8 colony-forming units (cfu) mL^{-1}] in Fiss Glucose medium (Vellore, 2001) at 37±2°C in a rotary shaker set at 180 rpm. After 72 h of incubation, the cultures were centrifuged at 14000 rpm for 20 min at 4°C. Subsequently, 2 mL of the culture supernatant was mixed with 4 mL of CAS solution and incubated in the dark for 10 min. The uninoculated broth with CAS reagent served as a reference. The colour change from blue to yellow was observed by measuring the absorbance at 600 nm, using uninoculated broth without reagent as the blank and siderophore desferrioxamine (up to 50 µM) as the standard (Henderson and Payne, 1994).

Nutrient solubilisation

Phosphorus solubilization

The selected isolates were screened for the quantitative estimation of phosphorus solubilization. Exactly 24 h old cultures of yeast isolates (~10^8 cfu mL^{-1}) were inoculated into 50 mL of Pikovskaya's broth containing 0.5% of TCP and incubated in a rotary shaker at 180 rpm for 10 days at 37±2°C (Nautiyal *et al.,* 2000). A blank without inoculation was run simultaneously. Following incubation, the cultures were centrifuged at 12,000 g for 15 min and the supernatant was collected to quantify P solubilisation by the molybdenum blue method (Watanabe and Olsen, 1965). Then, 1 mL of the supernatant was taken for quantification and the pellet was stored at -20°C for protein estimation. To the supernatant (1 mL), 10 mL of ammonium molybdate solution was added and the volume was made up to 45 mL. Furthermore, 5 drops of Chlorostannous acid were added and the final volume was maintained at 50 mL. The mixture was then mixed well and the optical density at 600 nm was determined using a UV-Vis Spectrophotometer. The amount of phosphate solubilized was calculated from the standard curve and expressed as µg mg $protein^{-1}$.

Zinc solubilization

Zinc solubilization was assessed in culture medium fortified with 0.1% insoluble zinc compound (ZnO) as described (Bunt and Rovira, 1955). Flasks were inoculated with 1% of metabolically active yeast culture containing a population of 8 × 10^5 cells. Cultures were incubated under aerobic conditions at 30°C with continuous shaking at 100 rpm for 5 days. Following incubation, the samples were centrifuged at 5000 × g for 15 min and the supernatant was analyzed for pH and soluble zinc content.

Production of ammonia and hydrogen cyanide (HCN)

Ammonia production by yeast isolates was assessed (Hakim *et al.,* 2021). The isolates were cultured in 10 mL of peptone broth overnight and incubated at 30°C for 48 h in a shaker cum incubator. Following incubation, Nessler's reagent (0.5 mL) was added to each culture. A colour transition from yellow to dark brown was indicative of a positive reaction for ammonia production. The absorbance was recorded at 450 nm using a spectrophotometer.

Hydrogen cyanide (HCN) production by yeast cultures was assessed following the method (Ahmad *et al.,* 2008). The isolates were streaked onto nutrient agar medium fortified with 4.4 g/L glycine. Filter paper discs soaked in a 0.5% picric acid solution were placed on the lids of the Petri dishes, which were then sealed to prevent gas exchange. The sealed plates were incubated at 28°C for 4 days. A colour change from yellow to orange in the filter paper is a positive indicator for HCN production by the yeast isolates.

Molecular characterization and identification of selected yeast isolates

The genomic DNA of yeast isolates was obtained using the cetyl-trimethyl ammonium bromide (CTAB) method for two isolates (VIR1 and VIR3), which were segregated based on morphological and biochemical characteristics. Yeast species were amplified using primers of NL1 (5′ GCATATCAATAAGCGGAGGAAAAG 3′) and NL4 (5′ GGTCCGTGTTTCAAGACGG 3′). Using NCBI Blast, similarity between the sequences was identified. The phylogenetic tree was constructed using MEGA 11.0 to determine the close and distant relationships between sequences.

RESULTS AND DISCUSSION

Isolation of yeasts

A total of three morphologically distinct yeast isolates (VIR1, DIN2 and VIR3) were isolated only from rice rhizosphere soil collected from Virudhunagar (S1 and S3) and Dindigul (S2). Notably, no isolates were recovered from other niches investigated, *viz.*, root, stem and leaves. The colour of the yeast isolates VIR1 and VIR3 was observed to be creamy white and DIN 2 was light yellowish. The margin and elevation of the respective colonies were smooth and raised.

Quantitative assay for IAA production

IAA concentrations of the 3 yeast isolates ranged from 6.21 to 12.55 µg mL^{-1}. VIR1 exhibited the highest IAA production (12.55 µg mL^{-1}), followed by DIN2 (8.68 µg mL^{-1}). The lowest IAA production was recorded in VIR3 (6.21 µg mL^{-1}). However, all values were lower than those of the positive control, *Bacillus altitudinus* FD48, which exhibited a highest IAA concentration of 16.42 µg mL^{-1} (Fig 1).

Quantitative assay for siderophore production

Quantitative estimation of siderophore production was performed using the CAS liquid assay. The results revealed that siderophore production ranged from 14.85% to 36.86% siderophore units (% SU). Among the isolates, VIR1 demonstrated the highest siderophore production at 36.86% SU, followed by VIR3 at 23.42% SU and DIN2 at 14.85% SU (Fig 1).

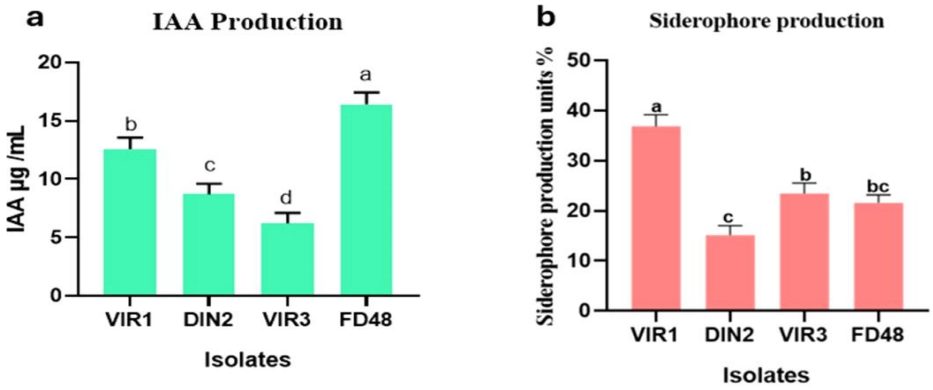
Screening for PGP traits

Quantitative estimation of nutrient solubilization

Quantitative estimation of P and Zn solubilization was carried out in liquid culture under standardized incubation conditions. Phosphorus solubilization was highest in isolate VIR1, with a concentration of 86.78 µg mL^{-1} of soluble phosphate released into the medium. This was followed by VIR3 and DIN2, which recorded 45.78 µg mL^{-1} and 23.42 µg mL^{-1}, respectively. Zinc solubilization followed a similar trend. VIR1 exhibited the highest solubilized Zn content (11.92 µg mL^{-1}), followed by VIR3 (7.21 µg mL^{-1}) and DIN2 (4.92 µg mL^{-1}) (Fig 2).

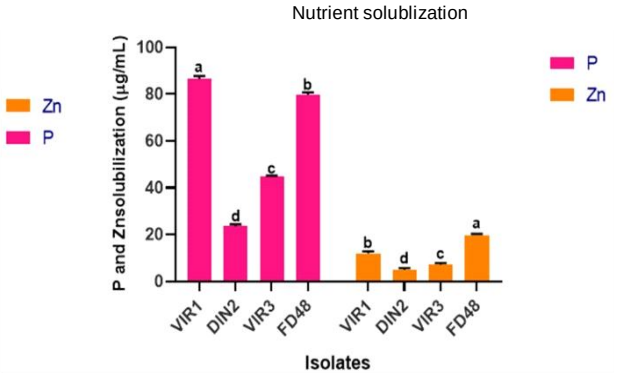
Ammonia and HCN production

Among the selected yeast isolates, ammonia production was detected in VIR1 and VIR3. The presence of ammonia was confirmed by the development of a brown to yellow coloration



a: IAA production (µg/mL); b: Siderophore production (units). Values are mean (±standard error) (n = 5) and values followed by the same letter in each column are significantly different from each other.

Fig 1: Quantitative assay for IAA and siderophore production by yeast isolates VIR1, DIN2 and VIR3 from rice rhizosphere.



DIN2, VIR3 and FD48 (control). Each bar represents the mean (±) S.E. of 3 independent replicates of each treatment.

Fig 2: Quantitative assessment of P and Zn solubilization by yeast strains-VIR1.

upon the addition of Nessler's reagent to the culture supernatant, indicating positive ammonia synthesis. In contrast, none of the three isolates demonstrated hydrogen cyanide (HCN) production, as evidenced by the absence of colour change in the picrate assay.

ACC deaminase activity

Among the three yeast isolates tested, VIR1 exhibited the highest ACC deaminase activity, recording 112.58 nmol α-ketobutyrate mg^{-1} protein h^{-1}, followed by VIR3 with 91.94 nmol α-ketobutyrate mg^{-1} protein h^{-1}. The lowest activity was observed in DIN2, which recorded 71.38 nmol α-ketobutyrate mg^{-1} protein h^{-1}. In comparison, the positive control, *B. altitudinus* FD48, showed a significantly higher activity of 173.45 nmol α-ketobutyrate mg^{-1} protein h^{-1}, indicating a strong ACC deaminase potential (Fig 3).

Molecular identification

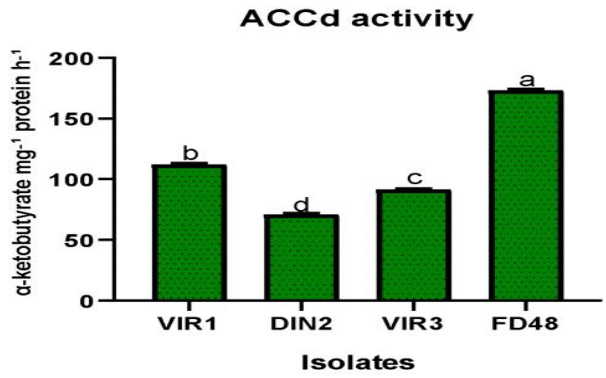
Using sequencing of the internal transcribed spacer (ITS) region, the chosen yeast isolates VIR1 and VIR3 were molecularly characterised. Virtually every amino acid in VIR1 was identical to *C. tropicalis*, according to phylogenetic analysis, multiple sequence alignment and BLASTn. Fig 4 shows that VIR3 was quite similar to *S. cerevisiae*, with a similarity score of 99%. Under the accession numbers PQ455181 for *C. tropicalis* and PQ455185 for *S. cerevisiae*, the validated sequences were uploaded to the NCBI GenBank database.

Plant growth and health can be enhanced by microbial communities culled from a variety of soil types and biological niches. Because of their capacity to generate enzymes, antioxidants, phytohormones and amino acids, all of which contribute to plant health and biomass accumulation, PGPYs have lately gained a lot of interest as potential bio-stimulants (Koza *et al.,* 2022). Despite the diversity and abundance of yeasts in natural ecosystems, very little is known regarding their presence in agricultural soil (Sarabia *et al.,* 2018). This study set out to identify yeasts in three different places in Tamil Nadu-Sengulathupatti (Dindigul), Kundukulam and Narikudi by isolating them from soil in the rhizosphere as well as stem, root and leaf samples taken from rice plants. Surprisingly, no yeast isolates were obtained from any other plant niche; the three morphologically different isolates, VIR1, DIN2 and VIR3, were all obtained from the soil of the rice rhizosphere. This finding lends credence to the idea that diverse microbial populations in the rhizosphere promote plant health by way of different PGP properties (Sarabia *et al.,* 2018).

The ability of the yeast isolates to produce ACC deaminase, which helps plants extend their roots and remain vigorous in the face of abiotic stress, further demonstrated their drought tolerance (Yim *et al.,* 2010). According to Sessitsch *et al.* (2005), yeast isolates with higher ACC deaminase activity lower ethylene levels and aid plants in surviving abiotic stress. VIR1 had the highest ACCD activity in this investigation, measuring 112.58 n moles α-ketobutyrate mg $protein^{-1}$ h^{-1}. The results were consistent with those of Hussein's previous research, which found that

the ACCD gene was expressed by *Cyberlindnera subhashii* YEAST-17, which in turn boosted salinity tolerance in *Triticum aestivum* (Hussein *et al.,* 2022). Likewise, under stress, pea seedlings (*Pisum sativum*) were able to grow better when exposed to the ACCd-producing yeast strain DH16 (Kaur and Manhas, 2022).

For plants to develop and grow better, they must be able to solubilise nutrients, produce growth-promoting chemicals such IAA, hydrogen cyanide and siderophore and synthesise these substances (Ma *et al.,* 2009). Yeast isolates VIR1 and VIR3 exhibited the highest P and Zn solubilisation in mineral medium supplemented with insoluble sources, according to the present study's PGP trait screening. By lowering the pH and producing organic acids, phosphorus is more easily soluble and made available to plants (Fankem *et al.,* 2006). Zinc, in contrast to phosphorus, is essential for plants in very little amounts; however, it is an essential element for many plant metabolic and enzymatic processes. This agrees with earlier results, for example, that *Cryptococcus laurentii* JYC370 successfully dissolved zinc oxide (ZO) and dicalcium phosphate (DCP) with SE units of 1.25 and 1.86, respectively (Fu *et al.,* 2016).



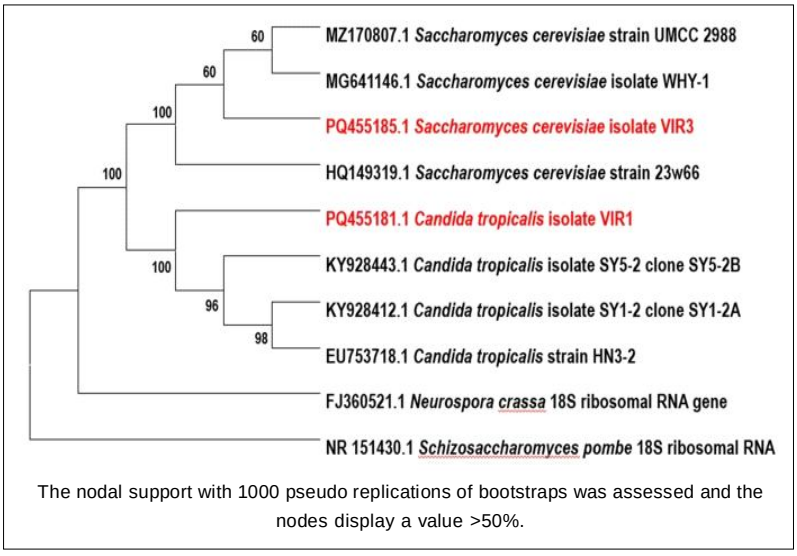
Values are mean (±standard error) (n = 5) and values followed by the same letter in each column are significantly different from each other.

Fig 3: Quantitative assay for ACCd production (n moles α-ketobutyrate mg^{-1} protein h^{-1}) by rice yeast isolates, where VIR1, DIN2 and VIR3 are yeast morphotypes.

It is proposed that auxin production by the isolates is an important strategy for promoting growth. Initiation of root development, regulation of fruit ripening and leaf fall and overall plant growth and development are all impacted by it (Trotsenko *et al.,* 2001). There has been a lot of buzz about the possibility of using microbes that produce IAA as biofertilizers recently (Saharan and Nehra, 2011). The VIR1 strain produced the most IAA (12.55 µg mL^{1}) out of all the isolates. A range of IAA concentrations, from 38.6±1.7 to 103.9±21.2 µg/mL, has been found in Cryptococcus flavus strains, according to Sun *et al.* (2014).

In order to promote plant growth and minimise plant diseases, several microbes release tiny molecular molecules called siderophores. These chemicals have a strong affinity for ferric iron. The isolates that were part of this study produced siderophores in different ways. Our results corroborated those of Nutaratat and colleagues, who found that the yeast *R. paludigenum* DMKU-RP301 produced the highest amount of IAA (29.3 mg g^{-1} DCW) together with NH3 and siderophore (Nutaratat *et al.,* 2014).

Additionally, all of the yeast isolates tested here produced ammonia. The generation of ammonia by microbes is a promising PGP activity that could greatly benefit plant growth promotion by increasing nitrogen availability (Mpanga *et al.,* 2019). Two possible PGPYs, VIR1 and VIR3, were isolated from soil in the rice rhizosphere. Using 18S rRNA analysis, they were able to be identified to the species level; VIR1 showed sequence resemblance to



The nodal support with 1000 pseudo replications of bootstraps was assessed and the nodes display a value >50%.

Fig 4: Phylogenetic analysis of 18S rRNA of yeast isolates VIR1 and VIR3 from rice rhizosphere constructed using Neighbour Joining Method in MEGA 11.0.

C. tropicalis, while VIR3 showed sequence closeness to S. cerevisiae. Our research showed that when these isolates are under stress, they exhibit more PGP traits. This jibes with prior research suggesting that prevalent yeast species *such Rhodotorula mucilaginosa, Moesziomyces aphidis, C. tropicalis and Aureobasidium pullulans* can stimulate plant development (Fu *et al.,* 2016). In addition, commercial biofertilizer formulations have acknowledged and utilised yeasts including *Candida, Geotrichum, Rhodotorula, Saccharomyces* and *Williopsis* for their different advantageous qualities (Nimsi *et al.,* 2023). This study's findings suggest that *C. tropicalis* VIR1 and *S. cerevisiae* VIR3, two yeasts found in the rice rhizosphere, may have PGP properties that could be useful in rice farming. This method encourages ecologically sound, climate-resilient rice cultivation when integrated into the agro-ecosystem. Future research into yeast-based biostimulants in various agro-ecosystems should help with the creation of more productive and environmentally friendly rice cultivation methods.

CONCLUSION

This research set out to isolate and describe yeast strains from soil in the rice rhizosphere throughout several parts of Tamil Nadu. We obtained three isolates VIR1, DIN2 and VIR3 and tested them for PGP characteristics. The *C. tropicalis* VIR1 strain had the greatest potential for PGP features, such as IAA (12.5±1.0 μg/ mL^{-1}), ACCd (112.5±0.8 n moles α-ketobutyrate mg $protein^{-1}h^{-1}$) and siderophore synthesis (36.8±2.3% units). Supporting sustainable rice agriculture, these findings lead to the creation of newer yeast-based bio-stimulants employing *C. tropicalis* VIR1. These bio-stimulants can boost plant growth in demanding situations. With the help of system biology methods, future research should investigate the signalling pathways linked to yeast-rice interactions. Furthermore, next-generation biostimulants for climate-resilient, sustainable crop production will open new possibilities with large-scale adoption across varied ecosystems and the development of appropriate yeast-based formulations and delivery modalities.

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Declarations

Ethical approval

Not applicable.

Author's contribution

Geethanjali Muthuramalingam: Conceptualization, Methodology, Investigation, Software, Writing- original draft. Shobana Narayanasamy: Data curation, Formal analysis, Software, Writing-review and editing. Akihiko Kamoshita: Supervised the workflow, Reviewing, Editing and assisted in manuscript preparation. Sivakumar Uthandi: Conceptualized the workflow, Experimental designing, Investigation, Project supervision. All authors read and approved the final manuscript.

Availability of data and materials

All data of this manuscript are included. No separate external data source is required. Any additional information required will be provided by communicating with the corresponding author *via* the official mail: usiva@tnau.ac.in.

Conflict of interest

The authors declare that they have no competing interests.

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