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Biosurfactant- Producing Yeast *pichia* sp. Ynchg: Characterization and Plant Growth - Promoting PotentialNahida Fatima¹, Yahya Khan M², Anuradha B S^{1*}¹Department of Microbiology, Chaitanya (Deemed to be University), Himayatnagar, Telangana 500075.²Kalam's Institute of Science, Nallakunta, Hyderabad, Telangana 500044.

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ABSTRACT

Biosurfactants are emerging as a promising biomolecule in sustainable agricultural applications. The primary objective of the present study is to isolate the most potential biosurfactant producing yeast from various exotic fruits and assess for its plant growth promoting trait. Among the yeast isolates YNChG showed the maximum emulsification index (E24%) of 49.93 ± 0.11 and oil displacement activity of 5.03 ± 0.05 cm and hence used in subsequent studies. Based on molecular analysis of 18S rRNA the isolate YNChG is identified as *Pichia kudriavzevii*. The pearl millet and green gram were used to assess the plant growth promoting activity of the biosurfactant derived from YNChG using paper towel method. The seeds were coated with the supernatant, biosurfactant, and the pellet obtained from YNChG isolate. The seeds coated with the biosurfactant showed highest seed vigor index, and enhanced germination compared to other seed coatings. Hence, these findings emphasize on the multi-functional property of isolate YNChG as a potential biosurfactant producer and a plant growth promoter indicating its relevance to sustainable agricultural practices.

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1.0 INTRODUCTION:

To meet the growing demands of human population, sustainable agriculture is the current need. Biosurfactants has shown a significant role in the agricultural industry due to their plant growth promoting property (Markande et al., 2021). Biosurfactants are low molecular weight, surface-active microbial metabolites that have been characterized by the properties such as biodegradability, less toxicity and effective under extreme environment. Because of these advantages of biosurfactants over synthetic surfactants, biosurfactants has been potentially applied in agriculture. Yeast biosurfactants are considered as GRAS (Generally recognised as safe) compared to those produced by bacteria. Common yeast genera

that produce biosurfactants includes *Candida*, *Pichia*, *Saccharomyces*, *Torulopsis*, *Geotrichum*, etc. (Konishi.M et al.,2014). Yeast biosurfactants can be used as an adjuvant and as an active compound in pesticides (Kosaric & Vandan-Sukan,2014). The antimicrobial effect of yeast biosurfactants is due to their ability to disrupt the cytoplasmic membrane of the pathogen (Lahkar et al., 2015; Valotteau et al., 2017). In addition, yeast biosurfactants can be used for the removal of heavy metals (Ahueke et al., 2016; Minucelli et al., 2017). Yeast belonging to Basidiomycota and Ascomycota phyla can form symbiotic association with plants and enhance plant growth by producing siderophores and phytohormones, solubilize inorganic phosphate, oxidize nitrogen and sulphur, stimulate mycorrhizal root colonization and enhances crop resilience (Alonso, L.M., et al 2008; Bhoomika M Karamchandani et al., 2022). Moreover, yeast can induce the systemic defence mechanism of plants to reduce the biotic stress via the production of peroxidase and catalase (De Tenorio, D.A.; et al 2019).

Seed coating technique is used for the application of any active ingredient like biosurfactant or microbial inoculants to the surface of the seed with the help of a binder such as carboxy methyl cellulose (CMC).

This technique is considered as a potential, cost effective, and time saving approach that can be used in sustainable agriculture (Rocha et al 2019). Therefore, the present work aimed to isolate and characterize the most efficient biosurfactant producing yeast from exotic fruits and to assess its plant growth promoting traits using seed coating technique.

2.0 MATERIALS AND METHODS:

2.1 Isolation of Yeast:

Exotic fruits such as Dragon fruit, Blueberries and Cherries were used as a sample source for isolation of yeast. Sample is mixed with sterilized distilled water and incubated overnight at room temperature. Subsequently, 10 mL of the overnight fermented sample is inoculated in 100 mL YEPD broth (1% yeast extract w/v, 2% peptone w/v, 2% dextrose w/v) and incubated on rotary shaker at 180 rpm for 24 hr. Serially diluted samples were inoculated on YEPD agar medium and incubated at 30 °C for 48 hr. Colonies obtained were observed under the microscope.

2.2 Screening for biosurfactant producing yeast:

2.2.1 Oil displacement assay:

The oil displacement test measures the diameter of the clear zone formed after mixing surfactant with oil-water interface. 30 mL distilled water was taken in a 15 cm diameter petri dish. Add about 10 µL of crude oil on surface of water, to this add 5 µL of culture supernatant. The diameter of the clear halo is measured (Pornsunthorntawee *et al.*, 2008).

2.2.2 Emulsification assay (E24%):

This assay determines the emulsifying capacity of the biosurfactant produced. Take 4 mL of cell free supernatant in a tube to this add 4 mL of oil. Vortex the mixture for 2 minutes and then keep it standstill for 24 hr (Rajesh *et al.*, 2013). Emulsification index is calculated as below:

$$E_{24} = \frac{\text{Height of emulsion layer}}{\text{Total height of liquid}} \times 100$$

2.3 Molecular characterisation and identification of isolate YNChG

Molecular identification of isolate YNChG was done by evaluating the quality of the isolated DNA on 1% agarose gel. The 18S rRNA gene fragments was amplified by NS1 and NS4 primers. The consensus sequence of 18S rRNA gene were generated by aligning the forward and reverse sequences using aligner software. These sequences were further used to carry out BLAST with the GenBank 'nr' database of NCBI. A phylogenetic tree was constructed using the first ten identical sequence using Clustal-W a multiple alignment software program and MEGA 11 software to construct the phylogenetic tree (Archana *et al.*,

2019).

2.4 Production and extraction of biosurfactant produced by YNChG

Inoculate 2% YNChG culture into the Bushnell Haas (BH) broth of composition (w/v) 0.2% MgSO₄, 0.02% CaCl₂, 1% KH₂PO₄, 1% K₂HPO₄, 1% NH₄NO₃, 0.05% FeCl₃. This media is supplemented with 2% glucose, 2% soyabean oil (v/v) and 0.02% and streptomycin. Incubate the inoculated flask at 30 °C on rotary shaker at 150 rpm for 96 hr. Centrifuge the yeast culture grown on BH broth at 10,000 rpm at 4 °C for 12 min. To the supernatant equal volumes of ethyl acetate is added and the biosurfactant is separated by liquid-liquid extraction method. The separated organic phase is vacuum dried using rotary evaporator (Daniel Loeto *et al.*, 2021).

2.5 Paper towel method for evaluating plant growth promotion using isolate YNChG and its produced biosurfactant

Paper towel method for assessment of plant growth was carried out using seeds of the pearl millet and green gram. Surface sterilisation of the seeds is done by using 70% ethanol treatment for 2 minutes and then washed with sterile distilled water for five to seven times. Three types of treatments were done to the seeds, first treatment is done using YNChG pellet, supernatant is used for the second treatment and the third treatment is done using biosurfactant. The surface sterilized seeds were taken in different petri dishes and incubated for 30 minutes with three different types of treatments combined with 1%(w/v) carboxymethyl cellulose (CMC) and then air dried. Untreated seeds were maintained as control. Moisten the paper towel and spread the seeds evenly on one side of the damp paper towel leaving space between them, then fold the paper towel over the seeds and incubate at room temperature for 14 days. Record the growth parameters such as shoot length, root length, and seed vigor index (Bright *et al.*, 2022).

Seed vigor Index (SVI)

$$= (\text{shoot length} + \text{root length}) \times \text{germination percentage}$$

2.6 Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Statistical differences among treatments were analyzed using one-way analysis of variance (ANOVA) using OriginPro 2024 software. Differences were considered statistically significant at $p < 0.05$.

3.0 RESULTS AND DISCUSSION:

3.1 Isolation and screening of biosurfactant producing yeast

About ten morphologically distinct yeasts were isolated from the exotic fruits sample using YEPD medium. Colony characteristics of the isolated yeast were recorded (Table 1). Figure 1 shows the microscopic observation of the oval shaped yeast cells.

Table 1. Colony characteristics of yeast isolates

Isolate s	Colon y colour	Margi n	Elevatio n	Texture/Surfac e
YNDr O	Off-white	Entire	Convex	Glistening, Smooth
YNCh G	Cream	Entire	Raised	Smooth
YNBb G	White	Entire	Flat	Rough



Fig. 1: Pure culture of Yeast isolate YNChG and microscopic image under 100X

In this study, three yeast isolates namely YNDrO, YNChG, YNBbG, obtained from dragon fruit, cherries and blueberries respectively were used. Among these, the yeast isolate YNChG showed the highest oil displacement activity with a zone measuring about 5.03 ± 0.05 cm, and emulsification activity (E24%) of $49.93 \pm 0.11\%$ respectively (Table 2). The oil displacement activity of YNDrO and YNBbG was 4.0 ± 0.07 cm and 4.43 ± 0.11 cm respectively (Fig.2), whereas their emulsification activity was $41.80 \pm 0.13\%$ and $44.40 \pm 0.06\%$ respectively (Fig.3).

Table 2. Screening of biosurfactant producing yeast

Isolates	Oil Displacement Test (cm)	E24%
YNDrO	4.0 ± 0.07	41.80 ± 0.13
YNChG	5.03 ± 0.05	49.93 ± 0.11
YNBbG	4.43 ± 0.11	44.40 ± 0.06

Data is given as mean $\pm$ STDV (n=3)

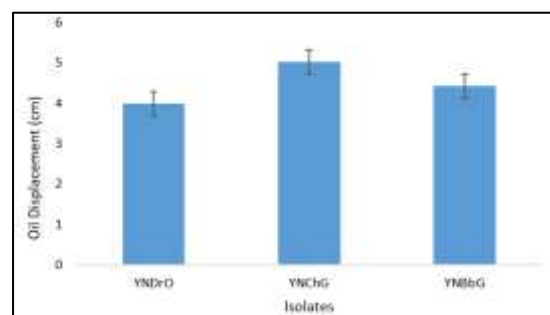


Fig.2: Oil displacement activity of yeast isolates

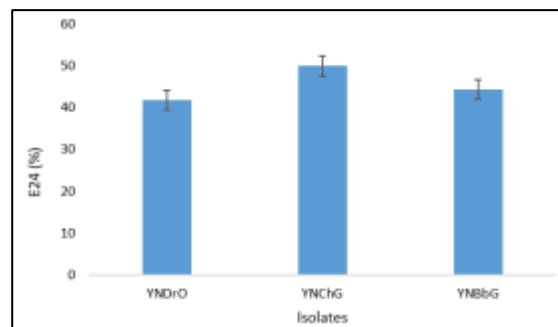


Fig.3: Emulsification activity (E24%) of yeast isolates



Fig. 4: Oil Displacement test of yeast isolates. (1) YNDrO (2) YNChG (3) YNBbG

3.2 Molecular characterisation and identification of isolate YNChG

Based on 18srRNA, molecular characterisation of the potential biosurfactant producing yeast strain YNChG was done. The isolate was identified as *Pichia kudriavzevii* YNChG based on the BLAST analysis of ITS region using NCBI database. The sequence was submitted to NCBI and the accession number were assigned as PV592260. First ten sequences were selected based on maximum identity score and were aligned using multiple alignment software program Clustal W. Distance matrix and phylogenetic tree was constructed using MEGA 11 by applying Neighbor- join method (Fig. 5).

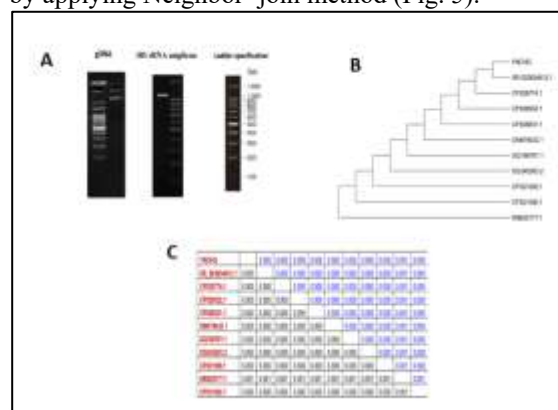


Fig.5: Molecular characterisation based on 18S rRNA gene sequencing and phylogenetic analysis of the *Pichia kudriavzevii* YNChG isolate. A) DNA extraction by Agarose gel electrophoresis B) Neighbor-Joining method Phylogenetic dendrogram constructed based on ITS sequencing showing relationship between isolate YNChG and their related taxa C) Pairwise genetic distance matrix showing the nucleotide divergence between YNChG and reference strains, with lower values indicating higher sequence similarity.

3.3 Paper towel method for assessment of plant growth using isolate YNChG

The seed coating of the pearl millet and green gram

was assessed by paper towel method after 14 days of incubation (Fig. 6). It was observed that the pearl millet seeds achieved 100% germination rate when treated with biosurfactant produced by YNChG isolate and resulted in maximum shoot length of 22.6 ± 0.08 cm, root length of 16 ± 0.08 cm and highest seed vigor index of 3,860. Similarly, the green gram seeds achieved 100% germination rate when treated with biosurfactant produced by YNChG isolate and resulted in maximum shoot length of 23.5 ± 0.04 cm, root length of 14 ± 0.04 cm and seed vigor index of 3,750 significantly high as compared to other seed coating methods used

(Table 3, Fig.7).

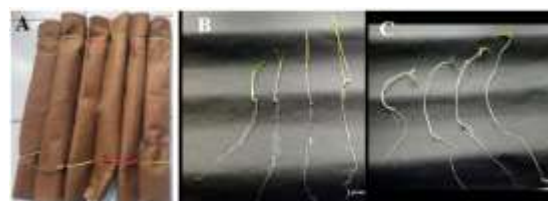


Fig. 6: Assessment of plant growth promoting effects of yeast strain through paper towel method A- Green gram & pearl millet seeds in paper towel B- Pearl millet seeds after incubation C- Green millet seeds after incubation

Table 3. Effect of YNChG derived product treatments on seed germination

Seeds	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seed vigor index
Green gram	Control	85 ± 0.2	12 ± 0.02	10 ± 0.07	1,870
	Pellet	100 ± 0.0	18.5 ± 0.06	12 ± 0.03	3,050
	Supernatant	100 ± 0.0	22.5 ± 0.05	13 ± 0.01	3,550
	Biosurfactant	100 ± 0.0	23.5 ± 0.04	14 ± 0.04	3,750
Pearl millet	Control	90 ± 0.1	12.5 ± 0.03	9 ± 0.02	1,935
	Pellet	100 ± 0.0	15.5 ± 0.07	13 ± 0.09	2,850
	Supernatant	100 ± 0.0	17.4 ± 0.04	14.2 ± 0.05	3,160
	Biosurfactant	100 ± 0.0	22.6 ± 0.08	16 ± 0.08	3,860

Data is given as mean $\pm$ STDV (n=3). Statistical analysis was performed using ANOVA

3.4 Statistical Analysis

One-way ANOVA revealed a significant effect of YNChG-derived product treatments on seed germination, shoot length, root length, and seed vigor index in both green gram and the pearl millet ($p < 0.05$). Among the treatments, biosurfactant treatment produced the highest seed vigor index in green gram (3,750) and pearl millet (3,860).

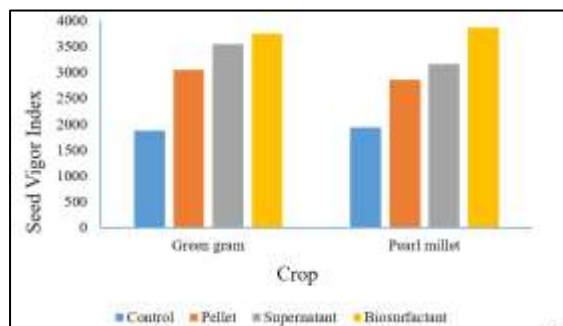


Fig.7: Effect of different treatments on seed vigor index of green gram and pearl millet

4.0 DISCUSSION:

Due to their environmental advantages and multiple functional properties yeast biosurfactant is gaining attention for the application in sustainable agriculture. In this study, the seed coating of cereals like green gram and pearl millet with the biosurfactant from yeast isolate YNChG (*Pichia kudriavzevii*) showed a significant early seedling with highest seed vigor index. In addition, the seed protection from soil pathogens is improved due to the antimicrobial properties of yeast biosurfactant. Moreover, the emulsification property of yeast

biosurfactant enhances the microbial colonization of rhizosphere and nutrient uptake after sowing. Hence, this study finds isolate YNChG as an efficient biosurfactant producing yeast and plant growth promoter. Further studies should be done to upscale the production of yeast biosurfactant to ensure its integration into sustainable agriculture.

5.0 CONCLUSION:

Potential biosurfactant producing yeast were isolated from exotic fruits and screened for emulsifying activity and oil displacement test. Based on the molecular characterisation the most potential isolate YNChG was identified as *Pichia kudriavzevii*. The biosurfactant produced by the isolate YNChG has shown the highest seed vigor index and maximum root length and shoot length indicating the key plant growth promoting traits. Hence, these results indicate the application of isolate YNChG in sustainable agriculture for the early growth of cereals such as green gram and pearl millet.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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