Int.J.Curr.Microbiol.App.Sci (2019) *8*(8): 465-472

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.808.053

Screening of Phyllosphere Yeast of Rice for the Production Enzymes and Solubilisation of Minerals

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Keywords

Phyllosphere, Yeast, Rice, Growth promoting substances

Article Info

Accepted:
07 July 2019
Available Online:
10 August 2019

A B S T R A C T

Rice leaf samples were collected at different critical stages from different places of Tuticorin district for isolation of yeast. Leaf samples were collected and imprinted in various medium viz., Sabourauds dextrose agar, Yeast extract Malt extract, Czapek Dox agar, Yeast extract peptone dextrose agar and Potato dextrose agar. Plates were incubated for 24 hours and observed for yeast population. The yeast colonies were dull white to white in colour, a few colonies were pigmented. Cells were oval to round in shape. Biochemical characterization tests were carried out and the yeast isolates were tested for enzyme production and mineral solubilisation.

Introduction

All aerial plant surfaces are inhabited by diverse assemblages of microorganisms that have profound effects on plant health and impact on ecosystem and agricultural functions. This environment is usually named as phylloplane or phyllosphere (Slavikova *et al.*, 2009). A variety of bacteria, yeasts and filamentous fungi have been isolated from the phyllosphere of several plant species. Microbes residing in the phyllosphere can have various life styles and modes of interaction with the host, being neutral residents, latent pathogens, or plant-health and growth promoters (Kai Wang *et al.*, 2016). In the phylloplane, the growth of microorganisms is dependent on nutrients from plant metabolites that are secreted to the phylloplane or on compounds in materials from external sources that drop on the plant surface. The phytohormones Indole Acetic Acid (IAA) and Gibberellic Acid (GA) are synthesized not only by plants, but also by many microorganisms. Microbial phytohormones are secondary metabolites, which are non-essential to life, but are involved in competition, defence and dispersal (Betina, 1995; Fox and Howlett, 2008).

Exudations are nothing but secretions of living plants, which contain simple sugars, organic acids and other easily utilized compounds which are the main nutrient source for such

epiphytic yeasts. The yeasts from plant leaves belong to both ascomycete and basidiomycete species.

Anamorphic basidiomycete yeasts are known to dominate in plant phyllosphere filobasidiales and tremellales yeasts of the genus *Cryptococcus*, as well as red-pigmented yeasts *Rhodotorula* and *Sporobolomyces*, are the most numerous. Some ascomycetous species have also been found such as *Debaryomyces hansenii, Hanseniaspora uvarum, Kazachstania barnetii, Metschnikowia pulcherima, Metschnikowia reukaufii, Pichia membranifaciens, Saccharomyces cerevisiae* and various species of *Candida* (Kachalkin *et al.,* 2008). The present research work aim to isolate, characterize and screen the yeast from phyllosphere of rice in Tuticorin district.

Materials and Methods

Collection of samples

Rice leaf samples were collected at different stages from different locations in Thoothukudi district of Tamil Nadu.

Isolation and purification of yeasts

Isolation of yeast was carried out using the Sabourauds dextrose agar, Yeast Extract Malt Extract, Czapek Dox agar, Yeast Extract peptone dextrose agar medium with pH 5.5-6.0. The samples were surface sterilized using 70% Ethanol and washed with sterile water and used for isolation. Leaf samples were imprinted and the plates were incubated at room temperature for 24- 48 h at 25° C. Yeast isolates were selected based on morphology, and purified using Potato dextrose agar medium. The pure cultures of yeast were maintained in Potato dextrose agar slants at 4°C and stored at -20°C in 30 % glycerol for further studies.

Morphological and biochemical characterization

Colony and cell morphology

The yeast isolates were observed microscopically for the morphological characters.

By culturing on Potato dextrose agar plates, colony morphology was studied. Gram's staining was performed for yeast isolates by following the standard procedure.

IMVIC test (Indole production, Methyl red, Voges Proskauer, Citrate utilization)

Indole production

Yeast isolates were inoculated in peptone water and incubated for three days. After incubation Kovack's reagent was added and the results were noted.

Methyl red

Yeast isolates were inoculated in the broth and incubated for three days. After incubation Methyl red indicator was added and observed the changes in color of methyl red.

Voges Proskauer

Yeast isolates were inoculated in the broth and incubated for three days. After incubation 12 drops of VP reagent -1 (Napthol solution) and 2-3 drops of VP reagent- II (40% KOH) was added and observed for change in colour for the VP test

Citrate utilisation test

Yeast isolates were streaked on Simmon's citrate agar and incubated for three days. After incubation the colour change was observed and recorded.

Ammonia production

Yeast isolates were inoculated in peptone water for the determination of Ammonia production. Then test tubes were incubated for 5 days and the change of colour from yellow to brown by adding Nesseler's reagent is positive for ammonia production. (Nutaratat *et al.,* 2014)

H_2S Production

Yeast isolates were streaked on Triple sugar-iron agar and incubated for 7days and observed the change of colour from yellow to black.

Enzyme activity

Amylase test (Buzzini and Martini, 2002)

Yeast isolates were streaked on starch agar and incubated for 48 h at 30±1°C.

Then Petriplates were flooded with Lugol's iodine solution for 30 sec and drained. Formation of yellow zone around the colonies against dark blue background, indicates the positive starch hydrolysis.

Catalase test (Nutaratat *et al.*, 2014)

The Yeast isolates were streaked on the Yeast Extract peptone dextrose agar plates and incubated for 48 h at 30±1°C. After incubation few drops of 3% hydrogen peroxide (H_2O_2) was added to the grown culture plates and observed for appearance of effervescence which indicates positive for catalase test.

Urease (Ebabhi *et al.*, 2013)

The Yeast isolates were spot inoculated on the urease media and incubated for three days. After incubation the plates were observed for the change of colour from red to pink.

Pectinase (Aisha, 2016)

The Yeast isolates were spot inoculated on the Vincent's agar medium and incubated for five days.

After incubation the plates were flooded with Iodine solution and observed for the zone formation around the colony

Cellulase (Buzzini and Martini, 2002)

The Yeast isolates were streaked on the CMC agar medium and incubated for 5 days. After incubation plates were flooded with 1% congo red and discard the congo red. Observed the zone formation around the colony.

Mineral solubilisation

Phosphate solubilisation

The phosphate solublization of the yeast isolates were determined by streaking the isolates on the Pikovskaya's medium.

Then plates were incubated at 30±1°C for 3 days. Clear halo zone was observed around the colony.

Potassium solubilisation

The Potassium solublization of the yeast isolates were determined by Spot inoculation of isolates on the Alexandro medium.

Then plates were incubated at 30±1°C for 3 days. Clear halo zone was observed around the colony.

Results and Discussion

The study was mainly focused to identify, characterize and screen the yeast isolates for the production of enzymes and other plant growth promoting characteristics.

Table.1 Colony Morphology and gram staining of the phyllosphere yeast isolates from rice

Yeast isolates	Gram's stain	Size	Pigment	Form	Margin	Elevation
1	$+^{ve}$	Moderate	Dark pink	Circular	Sharply defined	Dome shaped elevation
2	$+^{ve}$	Large	White	Circular	Sharply defined	Slight elevated
4	$+^{ve}$	Small	Light red	Circular	Sharply defined	Flat
6	$+^{ve}$	Small	Light red	Circular	Sharply defined	Flat
9	$+^{ve}$	Small	Dull white	Circular	Sharply defined	Flat
23	$+^{ve}$	Small	White	Circular	Sharply defined	Flat
27	$+^{ve}$	Small	Light red	Circular	Sharply defined	Slight elevated
50	$+^{ve}$	Pin head	Light red	Circular	Sharply defined	Flat
55	$+^{ve}$	Pin head	White	Circular	Sharply defined	Flat
57	$+^{ve}$	Pin head	White	Circular	Sharply defined	Flat
68	$+^{ve}$	Pin head	White	Circular	Sharply defined	Flat
70	$+^{ve}$	Moderate	Red	Circular	Sharply defined	Slight elevated
71	$+^{ve}$	Small	White	Circular	Sharply defined	Slight elevated
72	$+^{ve}$	Small	Dull	Circular	Sharply defined	Slight elevated
75	$+^{ve}$	Small	Light red	Circular	Sharply defined	Flat
76	$+^{ve}$	Pin head	White	Circular	Sharply defined	Flat
78	$+^{ve}$	Small	Red	Circular	Sharply defined	Slight elevated
79	$+^{ve}$	Moderate	Light red	Circular	Sharply defined	Flat
80	$+^{ve}$	Moderate	Light red	Circular	Sharply defined	Flat

Table.2 Biochemical and enzyme activity of phyllosphere yeast isolates from rice

Yeast isolates	IMViC Test	Ammonia production	H_2S production	Amylase	Catalase	Cellulase	Urease	Pectinase
1	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
2	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve
4	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve
6	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
9	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve
23	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
27	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
38	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
50	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
55	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve
57	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve
68	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
70	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
71	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve
72	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
74	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
75	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
76	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
78	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
79	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
80	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve

Table.3 Mineral solublisation ability of yeast isolates

Yeast isolates	Potassium solubilisation	Phosphate solubilisation
1	-ve	+ve
2	-ve	-ve
4	-ve	+ve
6	-ve	+ve
9	-ve	+ve
23	-ve	+ve
27	-ve	+ve
38	+ve	+ve
50	-ve	+ve
55	+ve	+ve
57	+ve	+ve
68	-ve	+ve
70	-ve	+ve
71	+ve	+ve
72	-ve	-ve
74	-ve	-ve
75	+ve	+ve
76	-ve	+ve
78	-ve	+ve
79	-ve	-ve
80	-ve	+ve

Plate.1 Yeast isolates and stained cells

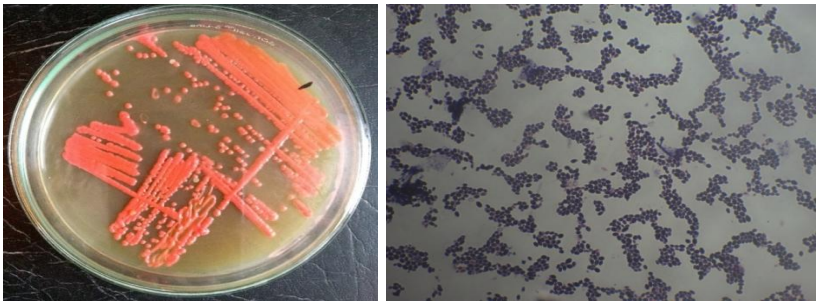


Plate.2 H_2S production and Ammonia production of yeast isolates from phyllosphere of rice

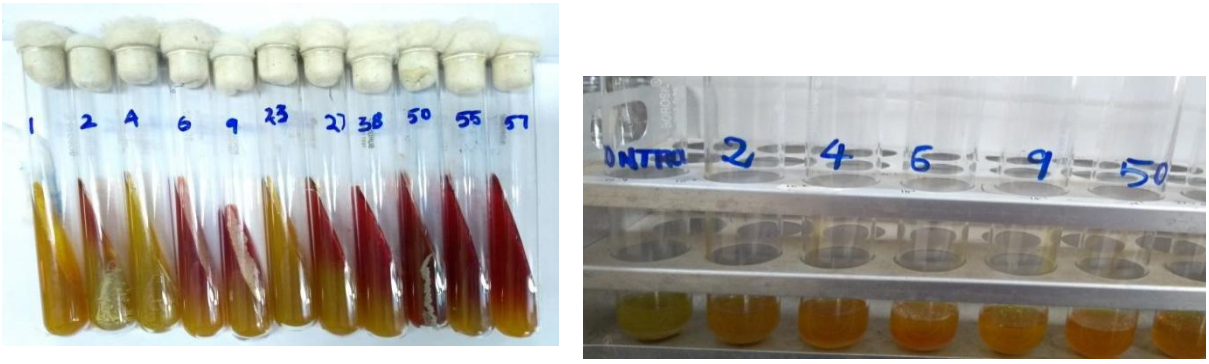
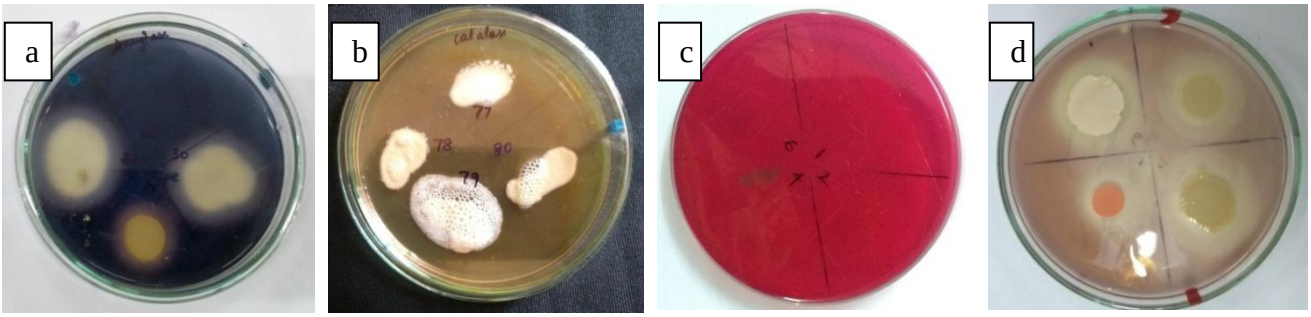
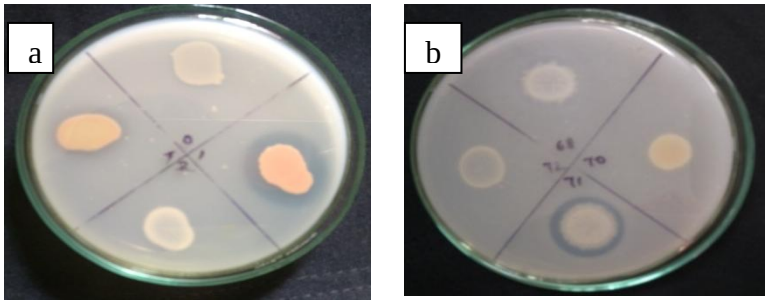


Plate.3 Determination of Enzyme activity using plate assay method



a. Amylase activity, b. Catalase activity, c. Urease activity, d. Pectinase activity

Plate.4 Determination of mineral solubilisation ability of yeast isolates from phyllosphere of rice



a. Phosphate solubilisation, b. Potassium solubilisation

Isolation and purification of yeast

Yeast isolates were isolated from the leaf samples collected from various regions of Tuticorin district using the leaf imprinting technique in various medium. Yeast isolates were obtained and were purified. After purification the isolates were characterized (Table 1; Plate.1). Various studies reported the isolation of yeast from the phyllosphere. About 1035 members of epiphytic and endophytic yeast were isolated earlier from leaves of rice and sugarcane (Nautarat *et al.*, 2014) and also from Vettiver (Limtong *et al.*, 2015), different vegetables (Zhang *et al.*, 2010), different fruit trees (Sláviková *et al.*, 2009).

Characterization of yeast isolates

The isolates were studied for their morphological and biochemical characteristics.

Morphological and Biochemical characterization

All the yeast isolates were observed for the morphological characters such as colony size, colour, form, margin and elevation. The colour of the colonies were pink, white, dull white, red, light red and were mostly circular, flat, raised at the centre (Table 1; Plate 1).

Earlier reports of Ghosh *et al.*, (2013) explorated the morphological and biochemical characteristics of yeast isolated from fruit surface of Jamun. Mukadam *et al.*, (2016) reported the isolated yeast produce the pink coloured, gram positive and showed the negative for the urease activity.

Biochemical characterization

All the isolates showed negative results for the IMViC test and H_2S production. Among the isolates, 7 isolates showed negative results for ammonia production (Table 2; Plate 2).

Enzyme activity

For enzyme activity most of the isolates showed positive results except the cellulase activity. For cellulase activity only 7 isolates showed positive results (Table 2; Plate 3). Carrasco *et al.*, 2016 reported that the most of the psychrotolerant yeast showed the positive for amylase and catalase activity. Fu *et al.*, 2016 isolated the 32 yeasts from the phyllosphere and rhizosphere of *Drosera spatulata Lab.* and reported 34 strains showed positive for the ammonia production, 29 strains showed positive for the solublisation of tricalcium phosphate, 19 strains showed positive for the cellulase activity, 31 strains showed positive for the catalase activity.

Mineral solublisation

Except the 4 isolates, all the isolates showed the zone formation around the colony after the incubation which indicates the yeast isolates solublized the tricalcium phosphate.

Only 5 isolates showed the zone formation around the colony after the incubation which indicates the yeast isolates solublize the potassium alumino silicate (Table 3; plate 4).

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How to cite this article:

Jeyashri, M., M. Gomathy, K.G. Sabarinathan, R. Subhashini and Suresh, S. 2019. Screening of Phyllosphere Yeast of Rice for the Production Enzymes and Solubilisation of Minerals. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 465-472. doi: https://doi.org/10.20546/ijcmas.2019.808.053