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Research Article

MORPHOLOGICAL AND BIOCHEMICAL PROFILES OF YEASTS FROM LEGUMINOUS CROPS

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Abstract- The study was conducted to isolate yeasts from phyllosphere and rhizosphere soil of different leguminous crops from Bengaluru and Dharwad districts. Thirty-five yeast isolates were obtained using four different media. Microscopic observations revealed that most of the yeast isolates were oval and the color of the yeast isolates varied from white, milky white, dull-white, pink and yellow. Nearly 85 percent of the yeast isolates were found to be acid producers and capable of utilizing six different sugars as carbon source. All the yeast isolates showed positive growth for tryptophan and indole production test. Among thirty-five yeast isolates, twenty-five isolates showed positive for starch hydrolysis, seventeen isolates were positive for cellulase production test and six for urease test. About 35-40 percent of the yeast isolates were found to grow fairly in all the supplemented nitrogen sources.

Keywords- *Yeast isolates, Sugar fermentation, Nitrate reduction, leguminous crop*

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Introduction

Pulses are an important dietary protein source for the human diet. It covers nearly 20 per cent of the area under food grains and contributes around 7 to 10 per cent of the total food grain production in India. To meet the requirements of the growing population, it is necessary to improve the yield of pulses in a sustainable way [1]. With this account, beneficial microorganisms have been employed to improve production even in adverse environmental conditions [2].

Soil is the ultimate repository for microbial growth and development. Microorganisms help in plant growth promotion by both direct and indirect means. Yeasts are unicellular, eukaryotic, polyphyletic microfungi that belong to the Ascomycota and Basidiomycota. Yeasts are chemoorganotrophs, reproduce asexually, grows rapidly on simple carbohydrates and often measuring about 3–4 µm in diameter [3, 4]. Yeasts are naturally present in rhizosphere soil and on the leaf tissues [5]. Yeasts are known to improve soil structure, nutrient cycling and even plant growth. When compared to the application of bacteria and mycorrhizal fungi in the areas of plant growth promotion, the investigation of yeasts is quite less [6]. In this article, the isolated yeasts are studied for their morphological and biochemical characters which will be helpful to conduct further research in screening them for their plant growth-promoting traits.

Materials and Methods

The experiments were carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru.

Sample collection

Leaf and rhizosphere soil samples were collected from Bengaluru and Dharwad districts along with geographical positions (GPS). Yeasts were isolated from the rhizosphere and phyllosphere of different leguminous crops such as Red gram, Cowpea, Green gram, Black gram and Bengal gram. The soil samples were air-dried, sieved using 0.2 mm sieve and stored at 4°C.

Isolation of yeasts from the phyllosphere and rhizosphere

From the leaf samples, phyllosphere yeasts were isolated by the imprinting method. Endophytic yeasts were isolated from the leaf samples by following surface sterilizing leaf samples with sodium hypochlorite (4%) for 1 minute, ethanol (70%) for 30 seconds and rinsed with distilled sterile water [7]. Yeasts were isolated from rhizosphere soil by serial dilution and spread plate method on Sabouraud Dextrose Agar (SDA), Potato dextrose agar (PDA), Czapek-Dox agar, Yeast Extract Peptone Dextrose (YEPD) Agar medium as detailed by Swapan [8].

Characterization of yeast isolates

Microscopic observation of yeast isolates

Yeast isolates were purified by streaking on Yeast Extract Peptone Dextrose (YEPD) Agar and maintained on slants for further studies. Yeast isolates were studied for colony morphology such as color, nature and shape. Lactophenol cotton blue staining was done and observed under the compound microscope (40X) as described by Swapan [8].

Biochemical tests

Starch hydrolysis

The yeast isolates were streaked on the starch agar medium and incubated for 72-96 hours at 25°C in the inverted position. The ability of yeast isolates to degrade starch was used as a criterion for the determination of amylase production. The incubated plates were flooded with iodine solution, a yellow zone formation around the colony indicates positive whereas the blue coloration of the medium with no zone formation indicates negative for starch hydrolysis was observed [9].

Indole production test

Yeast isolates were inoculated into tryptone broth and incubated at 35°C for 48 hours. Indole produced during the reaction was detected by adding Kovac's reagent which produces a cherry red reagent layer in the top of the tube. No cherry red reagent layer will be formed if no indole is produced in media[10, 11].

Cellulase production test

A modified Czapek-mineral salt agar medium was used to test the ability of yeast

Table-1 *Morphological characteristics of the yeast isolates*

SN	Isolates	Colony Morphology			Sl. No.	Isolates	Colony Morphology		
		Colour	Nature	Shape			Colour	Nature	Shape
1	GG1SI1	White	Non-spreading	Oval	19	RG13SI19	White	Spreading	Oval
2	GG2SI2	White	Non-spreading	Oval	20	CP14SI20	White	Spreading	Oval
3	GG3SI3	White	Non-spreading	Oval	21	CP15SI21	Milky White	Non-spreading	Oval
4	GG4SI4	White	Non-spreading	Oval	22	CP16SI22	White	Non-spreading	Oval
5	GG4Lfl5	White	Non-spreading	Oval	23	CP17SI23	Milky White	Non-spreading	Oval
6	GG4SI6	White	Non-spreading	Oval	24	CP17SI24	White	Non-spreading	Oval
7	RG5SI7	White	Non-spreading	Oval	25	GG18SI25	White	Spreading	Oval
8	BG6SI8	White	Non-spreading	Oval	26	GG18SI26	Dull White	Spreading	Oval
9	GG7SI9	White	Non-spreading	Oval	27	RG19SI27	White	Spreading	Oval
10	BG8SI10	Dull White	Non-spreading	Oval	28	RG19SI28	White	Non-spreading	Oval
11	RG9SI11	White	Spreading	Oval	29	BG20SI29	White	Non-spreading	Oval
12	RG9SI12	White	Non spreading	Oval	30	RG3Lf1IE30	White	Spreading	Oval
13	RG9Lfl13	White	Non-spreading	Oval	31	GG5Lf2IE31	White	Non spreading	Oval
14	RG10SI14	White	Non spreading	Oval	32	GG9Lf3IE32	Pink	Spreading	Oval
15	RG11SI15	White	Non spreading	Oval	33	GG12Lf4IE33	White	Spreading	Oval
16	RG11SI16	Pink	Non spreading	Oval	34	CP14Lf5IE34	Yellow	Spreading	Oval
17	RG12SI17	White	Spreading	Oval	35	GG15Lf6IE35	White	Spreading	Oval
18	RG13SI18	White	Spreading	Oval					

** S - Rhizosphere soil; Lf - leaf samples; RG-Red gram, GG-Green gram, CP Cowpea, BG-Black gram, BgG-Bengal gram; E-endophytes*

Table-2 *Pattern of sugar utilization by yeast isolates*

Isolates	Fermentation of sugars					
	Glucose	Sucrose	Maltose	Starch	Fructose	Lactose
	Growth and gas production					
GG1SI1	A+	A-	A-	A+	A-	A+
GG2SI2	A+	A+	A-	A-	A+	A+
GG3SI3	A+	A-	A+	A-	A-	A+
GG4SI4	A+	A-	A-	A+	A-	A+
GG4Lfl5	A+	A-	A-	A+	A+	A+
GG4SI6	B-	A+	A-	A-	A-	B-
RG5SI7	A+	A+	A+	A+	A+	A+
BG6SI8	A-	A+	A+	A-	A-	A-
GG7SI9	A+	A-	A+	A+	A-	A+
BG8SI10	A-	A-	A-	A-	A+	A-
RG9SI11	A-	A-	A-	A-	A-	A-
RG9SI12	A-	A+	A-	A-	A+	A-
RG9Lfl13	A-	A-	A-	A-	B-	A-
RG10SI14	A+	A+	A+	A-	A+	A+
RG11SI15	A+	A+	A-	A+	A-	A+
RG11SI16	A-	A-	B-	B-	A+	A-
RG12SI17	A+	A+	A-	A-	A-	A+
RG13SI18	A-	A+	A+	A-	B-	A-
RG13SI19	A+	A+	A-	A-	A-	A-
CP14SI20	A-	A-	A-	A-	A+	A-
CP15SI21	A+	B-	B-	B-	A-	A+
CP16SI22	A+	A+	A+	A-	A-	A-
CP17SI23	A+	A-	A-	A+	A+	A+
CP17SI24	A+	A-	A+	A-	A+	A+
GG18SI25	A-	A-	A+	A-	A-	A-
GG18SI26	A-	A-	A-	A+	A+	A+
RG19SI27	A+	A-	A+	A-	A-	A+
RG19SI28	A+	A+	A-	A+	A+	A+
BG20SI29	A-	A-	A+	A-	A-	A-
RG3Lf1IE30	A+	A-	A-	A-	A+	B-
GG5Lf2IE31	A+	A+	A+	A-	A+	A+
GG9Lf3IE32	A-	A-	B-	B-	B-	B-
GG12Lf4IE33	A-	B-	A-	A-	A-	A-
CP14Lf5IE34	B-	B-	B-	B-	B-	B-
GG15Lf6IE35	A+	B-	B-	B-	B-	B-

**Growth was observed as acid (yellow) and alkali (red) producers, *(A+) acid production positive and gas production positive; (A-) acid production positive and gas production negative*
GG-Green gram; BG-Black gram; RG-Red gram; CP-Cowpea

isolates to decompose cellulose. Microbial utilization of cellulose was detected by flooding with hexadecyltrimethylammonium bromide, as this forms a clear zone around the colony. The absence of a clear zone around the colony indicates that the yeast isolates lack cellulase enzyme production [12].

Urease test

Microorganisms possessing urease enzyme will react with urea in the media and liberates ammonia which raises the pH of the medium which changes the color of the medium to red. The development of red color reveals the production of urease enzyme by the respective microorganism.

Table-3 *Biochemical characteristics of the yeast isolates*

Isolates	Starch Hydrolysis		Indole production test	Cellulase production test	Urease test
	+/-	Culture colour	+/-	+/-	+/-
GG1SI1	+	Yellow	+	+	-
GG2SI2	+	Yellow	+	+	-
GG3SI3	+	White	+	+	-
GG4SI4	-	White	+	-	-
GG4Lfl5	+	Yellow	+	+	-
GG4SI6	+	Yellow	+	-	-
RG5SI7	+	Yellow	+	+	-
BG6SI8	-	White	+	-	-
GG7SI9	+	White	+	+	-
BG8SI10	+	Yellow	+	-	-
RG9SI11	+	White	+	-	-
RG9SI12	+	Yellow	+	+	-
RG9Lfl13	+	Yellow	+	+	-
RG10SI14	+	Yellow	+	+	-
RG11SI15	+	Yellow	+	+	-
RG11SI16	-	White	+	-	+
RG12SI17	+	Yellow	+	+	-
RG13SI18	+	Yellow	+	+	-
RG13SI19	+	Yellow	+	+	-
CP14SI20	+	Yellow	+	+	-
CP15SI21	-	White	+	-	-
CP16SI22	+	Yellow	+	-	-
CP17SI23	-	White	+	-	-
CP17SI24	+	Yellow	+	-	-
GG18SI25	+	Yellow	+	-	-
GG18SI26	+	Yellow	+	-	-
RG19SI27	-	Yellow	+	+	+
RG19SI28	+	Yellow	+	+	-
BG20SI29	+	Yellow	+	+	-
RG3Lf1IE30	+	Yellow	+	-	-
GG5Lf2IE31	+	Yellow	+	+	-
GG9Lf3IE32	-	White	+	-	+
GG12Lf4IE33	-	White	+	-	+
CP14Lf5IE34	-	White	+	-	+
GG15Lf6IE35	-	White	+	-	+

**Starch hydrolysis (+) Zone formation; (-) No-Zone formation*
**Indole production test (+) Development of cherry red color*
**Cellulase production test: (+) Zone formation; (-) No Zone formation*
**Urease test: (+) Red; (-) Yellow; GG-Green gram; BG-Black gram; RG-Red gram; CP-Cowpea*

If there is no color development indicates that the yeast isolates lack production of urease enzyme as described by Hakim [13].

Fermentation of sugars

All thirty-five yeast isolates were tested for fermentation of different sugars such as Glucose, Starch, Maltose, Lactose, Fructose and Sucrose at one per cent to the basal media. The isolates were characterized as fermentative acid or alkali producers as described by Gordon and Keith [14].

Nitrate reduction test

All thirty-five yeast isolates were subjected to nitrate reduction test by supplementing different nitrogen sources such as KNO_3, $NaNO_3$, L-lysine, Glutamine and Tryptophan at one per cent to the basal media. The Colour change of the broth to red color indicates that the microorganism is capable of reducing the available nitrogen source to nitrite by reacting with sulfanilic acid [15].

Results

Isolation of yeast from the phyllosphere and rhizosphere of different leguminous crops Microscopic observation of the yeast isolates

Thirty-five yeast isolates were obtained from leaves and rhizosphere soil samples using four different media. Two isolates were obtained by the imprinting method. Six endophytic yeasts were isolated by following surface sterilization procedures. Total of 27 isolates were obtained from the rhizosphere soil samples. All the isolates were purified on Yeast Extract Peptone Dextrose Agar medium by Streak plate method both as slants and glycerol stock and were maintained at 4°C.

Microscopic observation of the yeast isolates

Microscopic observations were done using compound microscope. Culture slides were stained using lactophenol cotton blue stain and observed under 40X. Most of the isolated colonies are found to be oval in shape. Morphologically, the color of yeast isolates varied from white, milky white, dull-white, pink and yellow with budding whereas the nature of the colony ranging from spreading to non-spreading [Table-1].

Fermentation of sugars by the yeast isolates

Yeast isolates growing on media supplemented with different sugars at one per cent were observed for color change and gas production and the results are represented in [Table-2]. The pattern of sugar utilization by the yeast isolates revealed that nearly 85 per cent of the isolates were found to be acid producers with or without gas production whereas the remaining isolates were found to be alkali producers with no gas production. The percentage of alkali producers was found to be very low in the media supplemented with glucose. Fermentation of a wide range of carbon sources by different bacterial genera has been extensively studied and reported by Gordon and Keith [14]. Prasad *et al*., [12] also reported that ten cellulolytic actinomycetes strains isolated from the soil were also found to utilize a wider range of carbon sources.

Biochemical characterization of the yeast isolates

All thirty-five yeast isolates were tested for various biochemical tests such as starch hydrolysis, indole production, cellulase production and urease test and the results are presented in [Table-3]. Among thirty-five yeast isolates, twenty-five isolates showed positive and ten isolates showed negative for starch hydrolysis. All thirty-five yeast isolates found to be positive for the indole production test. Liu *et al*., [10], reported in *Saccharomyces* sp. yeasts were found to have a stimulatory growth when Indole acetic acid (IAA) was added exogenously. Seventeen yeast isolates were capable of producing clear zones around the colony which is positive for cellulase production test. Prasad *et al*., [12], screened the actinomycetes isolates for cellulolytic activity based on their clear zone formation around the colonies. In the case of the urease test, six yeast isolates were found to be positive and the color change of the medium was observed.

Nitrate Reduction test by the yeast isolates

All the yeast isolates were subjected to nitrate reduction test by supplementing basal media with Nitrogen sources at one per cent. Nearly, 35-40% of the yeast isolates were found to grow fairly in all the supplemented nitrogen sources [Table-4]. In the basal media supplemented with Tryptophan, all the isolates had shown positive growth. In the case of KNO_3, Na NO_3, L-lysine and Glutamine nitrogen sources, fifteen, fourteen, three, and seven isolates had shown good growth respectively. In contrast, yeasts of the genera *Saccharomyces* and *Schizosaccharomyces* are unable to use nitrate or nitrite as the sole nitrogen source whereas in the recent studies, some other genera of yeasts were found to utilize nitrite and their molecular mechanisms have been delineated [15].

Table-4 *Nitrate reduction test by the yeast isolates*

Isolates	Nitrate Reduction Test				
	KNO_3	Na-NO_3	L-lysine	Glutamine	Tryptophan
GG1SI1	+	+	±	±	+
GG2SI2	±	±	±	±	+
GG3SI3	-	-	±	±	+
GG4SI4	±	±	±	±	+
GG4LfI5	±	±	±	±	+
GG4SI6	±	±	-	+	+
RG5SI7	±	±	-	+	+
BG6SI8	±	±	-	-	+
GG7SI9	+	+	+	+	++
BG8SI10	±	±	+	+	+
RG9SI11	+	+	+	-	+
RG9SI12	+	+	±	-	+
RG9LfI13	+	+	±	-	+
RG10SI14	-	-	±	+	+
RG11SI15	+	+	±	+	+
RG11SI16	+	+	±	-	+
RG12SI17	-	-	-	-	+
RG13SI18	+	+	-	±	+
RG13SI19	+	+	-	±	+
CP14SI20	+	+	±	±	+
CP15SI21	-	-	-	-	+
CP16SI22	+	+	±	±	+
CP17SI23	+	+	-	±	+
CP17SI24	±	±	-	±	+
GG18SI25	±	±	-	-	+
GG18SI26	±	±	-	±	+
RG19SI27	±	±	-	±	+
RG19SI28	+	+	+	+	++
BG20SI29	-	-	-	-	+
RG3Lf1IE30	+	+	±	-	+
GG5Lf2IE31	±	±	±	-	+
GG9Lf3IE32	+	±	-	±	+
GG12Lf4IE33	-	-	-	±	+
CP14Lf5IE34	-	-	±	±	+
GG15Lf6IE35	±	±	-	-	+

**(±) - weak growth; (+) - 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

Conclusion

The present investigation revealed that most of the yeast isolates were capable of utilizing a wide range of carbon and nitrogen sources. These features would help them to survive under nutrient-limited conditions. Biochemical tests revealed that some of the isolated yeasts were capable of performing the cellulolytic and amylolytic activity. Preliminary screening of the yeast isolates for IAA production by indole production test had shown that the isolates can be further employed in future studies for harnessing their ability to promote plant growth and yield in the leguminous crops.

Application of research: Yeasts obtained from leguminous crops can be used to promote plant growth and also as biocontrol agents

Research Category: Bioinoculants, Plant growth promoters

Abbreviations: YEPD: Yeast Extract Peptone Dextrose

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Author Contributions: All authors equally contributed

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Study area / Sample Collection: Bengaluru and Dharwad districts

Cultivar / Variety / Breed name: leguminous crop

Conflict of Interest: None declared

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