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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* STRAINS, OBTAINED FROM SUGAR-CANE JUICE AND H_2S SCREENING FROM DIFFERENT AREAS OF INDIA

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ABSTRACT
The aim of the present study was to isolate Saccharomyces cerevisiae from Saccharum officinarum commonly known as Sugar cane. Saccharomyces cerevisiae was collected from sugarcane juice from different areas that have different atmosphere, soil composition and temperature. Sample of sugarcane juice preserved at 0°C ice bugs. Sample were shifted to the laboratory and stored at 4°C for 15 hours and proceed further for study. Saccharomyces cerevisiae species was identified by applied for different tests including morphological, cultural and biochemical characteristics, which facilitate the opportunity for identification of the yeasts.These strains were observed to produce various extra cellular enzymes. Qualitative analysis of H_2S production and which Mutants were screened using colony color on (BSA) agar.

Keywords: Saccharomyces cerevisiae, Sugarcane, Biochemical test, Qualitative analysis

I INTRODUCTION

Saccharomycescerevisiae is universal organism. *Saccharomyces* strain is harmful and useful of human being. It has long history used in the fermentation food, non-food and including cosmetic and pharmaceutical industries (Y. Sobrun). This taxon consists of four yeast species, namely *Saccharomycesbayanus*, *Saccharomyces cerevisiae*, *Saccharomycesparadoxus* and *Saccharomyces pastorians.* [1]The fermentation process involves conversion of sugars to alcohol and carbon dioxide by the yeast *Saccharomycescerevisiae. Saccharomycescerevisiae* has braked of sugar cane juice molecule during to fermentation increase the carbohydrate and decrease of oxygen as

$$(C_6H_{12}O_6 + H_2O \; C\cancel{O_2 \rightarrow} CH_3CH_2OH \text{ (or } C_2H_5OH))$$

The yeast has classified and isolated from sugar cane juice. The sugar cane was grown in over 120 countries in the all world. Brazil is the world's Biggest Sugarcane producing country in all over the nations with a total production of 672,157,000 tonnes, While India is the 2nd biggest producer after Brazil (http://www.whichcountry.co). [2, 3] Sugar cane, as a raw material, is used for 60% of global ethanol production.[2] The sugar cane is used as the source of table sugar juice, rum, fuel ethanol, sugar molasses, vinegar (Siraka) and directly as fresh sugar cane. The high sugar content of sugar cane makes it an ideal source for the production of alcoholic beverages.[3] Thus the sugarcane juice is an excellent medium for fermentation in order to elaborate alcoholic beverages as it is a rich source of sucrose, glucose and fructose. Fresh sugarcane juice has been used as a thirst quenching drink in some places such as South East Asia and also in Mexico and some parts of South America *et al.* (Mayuri K. 2011). [3] The Brazilian spirit and Caribbean countries was a sugarcane spirit obtained by the distillation of cooked fermented sugar cane juice and molasses *et al*. (M. de Araujo Vicente *et al*. 2006). [5]

Saccharomycescerevisiae species is yeast belonged to Kingdome of fungi and isolated from sugar cane juice that was identified by studying specific morphological, biochemical and physiological characteristics as given by (Kurtzman and Fell 2013). [6]*Saccharomyces cerevisiae* (brewer's yeast) has been used in classical food fermentation applications such as production of beer, bread, yeast extract/vitamins, wine, sake, and distilled spirits supplements in human and animal diets as well as in the production of single cell proteins.[2] The *Saccharomyces cerevisiae* yeast is largely used fermentation of ethanol production using such renewable biomass as sugarcane or sugar beet molasses as the main

carbon source (Echegaray *et al.* 2000, Sanchez and Cardona 2008 and Periyasamy S. 2009, Noroul Asyikeen 2013).[7,8,19] The Hydrogen sulphide was produced by various mechanisms of *Saccharomyces cerevisiae*. Itcan degrade sulphur-containing amino acids to utilize the nitrogen, and the release of H_2S or other volatile sulphur compounds as by-products (Linderholm *et al*. 2008). [10] The protoplast fusion was produced recombinant yeast negative for H_2S (hydrogen sulphide) production and maintaining the flocculation trait (Ribeiro & Horii 2004, Noroul Asyikeen2013). [11,12,19] These characteristics were discussed in the fermentation studies of sugarcane juice, in the quality of sugarcane spirit and alcoholic fermentation process.

The objective of this study was to isolate *Saccharomyces cerevisiae* from sugar cane juice and the isolated yeast strains were identified by studying specific morphological, biochemical and physiological to screen these isolates for desirable traits such as homofermentative ability and the production of various enzymes.

II MATERIALS AND METHODS

Sample Collection

Samples of sugarcane were collected randomly from different sub-regions like as Agra, Faridabad, Noida, Greater Noida, Mainpuri, different Geographical temperature, soil composition, in sterile bottles at 4°C in ice bugs and have used different variety of sugar cane to produce *Saccharomyces* species. The collected of Sugar cane *(Saccharum officinarum)* juice byMr. Munshi Lal Yadav from mainpuri district 300km to capital of India.

Geographical location of the five distilleries in Utter Pradesh, Haryana state (India). Distances of area are as around 185km, 30km, 35km, 60km, and 300km from capital of India.

Yeast Isolation

Samples were collected from Sugarcane in previously sterilized 250-ml flasks. They were kept and transported in ice-cold water and processed as fast as possible. Triplicates of decimal dilutions sample (.5:4.5, .5:45, .5:450 and .5:4500) in sterilized water were inoculated on YPDA medium containing yeast extract (1% [wt/vol]), potato infusion form (20% [wt/vol]), dextrose (2% [wt/vol]) and agar (1.5% [wt/vol]) supplemented with chloramphenicol (0.01% [wt/vol]) and with ethanol (8% [wt/vol]), Plates were incubated at 30°C for 3-5 days. These special conditions (high sugar concentration and higher temperatures) were chosen because *Saccharomyces* yeast strains are normally subjected to similar conditions during the fermentative process (2% dextrose). The isolates were first classified on the basis of morphological characteristics and then tested for their ability to grow at higher temperatures (35°C) in the presence of 8% (wt/vol) ethanol, conditions similar to the stressing environmental conditions that occur during sugar cane fermentation. Isolated colonies were replicated on YPDA medium containing dextrose (2% [wt/vol]), ethanol (8% [wt/vol]), and chloramphenicol (0.01% [wt/vol]). Plates were then incubated at 35°C for 48 hours.

Identification of *Saccharomyces cerevisiae*

Yeast Viability Staining

The cultures take off with help of loop on slide. The culture was subjected to Yeast Viability staining produced and observed microscopically under high power objectives. Spherical or budding yeast cells were observed.

Lacto Phenol Cotton Blue Staining

Help of loop picked a colony culture on slide. The *S. cerevisiae* culture was stained by Lacto phenol Cotton Blue Staining (Panneerselvam A. *et al.* 2014) [4] producer and observed under high power objectives. The colonies were checked on the basis of color and other features.

Simple Staining of Microbes

The isolated culture picked up with help of loop of culture on slide. The isolated culture was subjected to Sample staining produced and observed under high power objective.[15,16] Spherical or Budding yeast cells were observed.

Gelatine Liquefaction Test

Biochemical characteristics of the *Saccharomyces cerevisiae* strain which is used of Christensen's Urea Broth method.[17] The dispense 10 ml into each autoclaved test tube and inoculated fresh culture (3-5day old), incubated in orbital shaker at 25°C for 200 rpm. The regularly checked for up to 1-2 wks for sign of liquefaction.

Test for Hydrolysis of Urea

The urea media has prepared, that not boil or autoclave the broth which is used of Christensen's Urea Broth method.[17] Urea broth has pour 10 ml into autoclaved each test tubes (12mm) aseptically. Add a loopful of cells from 2-3 day old cultures is suspended in the broth, and incubated shaker at 25°C for 200 rpm. Check every 30 min and 2 hours for up to 24 hours for no color changed. Check again after 36 hrs from inoculation.

Screening of Deletion Strains for H_2S During Fermentation Conditions

Culture Media

The isolation strains of yeast were used and storage on yeast extract Potato dextrose agar (YPDA), while the selection of the H_2S trait was used dehydrated on bismuth sulphite agar (BSA-Difco) incubated at 30°C for 48 hrs. The white colonies had non-sulphide producing strains, while the H_2S producers presented various colony colors that ranged from light brown to black, depending upon the intensity of the production. It is clarified sugarcane juice (Ribeiro & Horii, 2004). [11]

III RESULT AND DISCUSSION

Isolation of *S. cerevisiae*

S. cerevisiae strain has been obtain from sugar cane juice for different areas in India, previously described. Isolated samples were Triplicates of decimal dilutions in sterilized water which is used of streaking method on YPDA medium were inoculated at 35°Cfor 3 days. Yeast was identifying according to standard methods and shape, size, and individually yeast cells appear colorless, grown on YPDA media (Fig.1). In the previous study, they produce may be white, cream color, or tinged with brownish pigments. Most of the isolated colonies have observed smooth surfaces with circular margins and showed a wide variation of creamy white and pinkish. [19] The cells were found to be of various shapes such as round, oval, spherical and ellipsoidal (Table no. 1).[17] Comparative to previous study, The isolated strains grown on YPDA medium, that isolated colonies have founded smooth surfaces with circular margins with creamy, creamy white and yellow white. The Colonies characteristic was useful in the taxonomy of yeasts that is very difficult group to classify. The yeast species are also determined by great extent of physiological characteristics. [12]

Identification and characterization of *Saccharomyces cerevisiae*

S. cerevisiae strain were obtained and preliminarily identified based on morphological characteristics. *Saccharomyces cerevisiae* strain was Identified on the basis of biochemical test. Frist up all, all isolates of yeast colonies were observed under microscope compound. This strain was stained by yeast viability staining (Methylene blue staining) [13] that the cell morphology was morphologically observed under a microscope as shown in Figure 2. *S. cerevisiae* culture was subjected to Lacto phenol cotton blue staining, [14] used of producer and observed under highpower objectives. [3] Budding *S. cerevisiae* cells were seen in figure 3. The morphological characterization of yeast was subjected to simple staining (Crystal violet staining) developed by Teresa Thiel, (Ph.D).[15] The morphology of the *Saccharomyces cerevisiae* isolatewas seen at the time of microscopic observation of theslide (Figure 4). In the presently results, *S. cerevisiae* stain was properly on the slide, the microorganism colonies is observed by microscope

according to different method of staining but previous studies were not describe staining of microorganism morphology.

The yeast was observed positive result for urea according to *et.al. S. Chatterjee*2011. *Saccharomyces* yeast has hydrolysis of urea by a urease enzyme, In Rapid Urease test Broth used the urease reaction given by *H. pylori*.[8] The gelatin hydrolysis test detects the ability of bacteria to produce gelatinises.In the present result,*Saccharomyces cerevisiae*has observed urea negative and also gelatin result, the founded no sign liquefaction (See table 2).

In the previous study, *S. cerevisiae*strains was produce of hydrogen sulphide during to fermentation, determine of lead acetate medium (LA) or BiGGY agar.[20,21,]During to H2S production observed increase the (Darker colony color) and decreased (Lighter colony color) isolated strain color was found white, light tan, light brown, brown, and black. That hydrogen sulphide production property was not useful for wine yeasts. The production of hydrogen sulphide is undesirable for bread making because confer flavor and an undesirable compound that must be absent in fermented beverages (M. de Araujo Vicente *et al.*2006).[22,23] The alcohol was contamination during to fermentation caused by hydrogen sulfide producing microorganisms. The utilization of the differential BSA medium should not have favoured any positive results (Ribeiro & Horii 2004).[23] In the present study, *Saccharomyces cerevisiae* has produced hydrogen sulphite, which isolated strains have given brown and black color colonies on bismuth sulphite agar medium (Table 2 and fig.8).

Table 1: Physiological and Morphological characteristics of the *Saccharomyces* strain isolated from sugarcane juices for different area

S. No.	Strain's	Surface	Margin	Color	Cells
1	Agra	smooth	circular	Yellow white	Spherical
2	Faridabad	smooth	circular	Yellow white	Round
3	Greater Noida	smooth	circular	Creamy white	Spherical
4	Noida	smooth	circular	Creamy white	Spherical
5	Mainpuri	smooth	circular	Creamy white	Spherical

Table 2: Biochemical analysis of the *Saccharomyces* strain isolated from sugarcane juices for different area

S. No.	Strain's	Geletin Liquefaction Test	Urea Test	H_2S Test
1	Agra	-	-	Black
2	Faridabad	-	-	Brownish, black
3	Greater Noida	-	-	Black
4	Noida	-	-	Brownish, black
5	Mainpuri	-	-	Brownish, black

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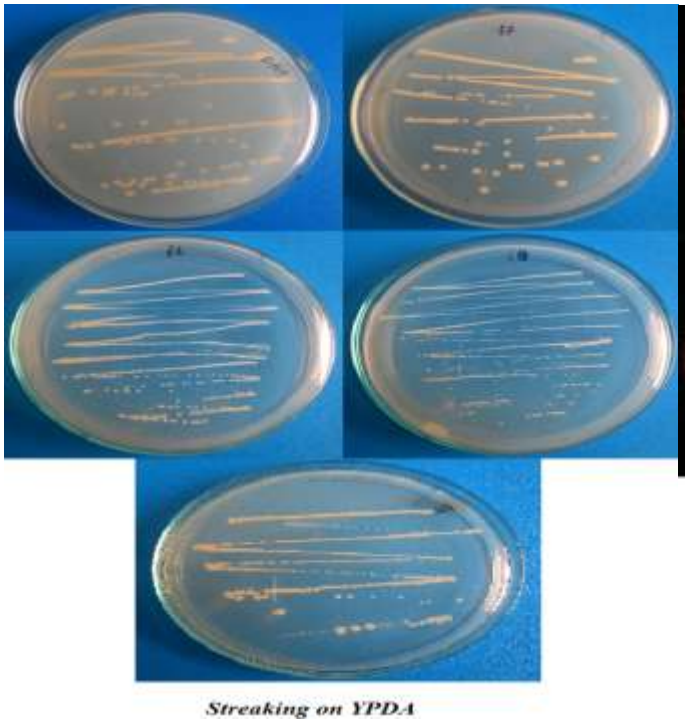

Fig. 1: *Saccharomyces* strain streaking on YPDA media different region such as Agra (1), Faridabad-7, Gr No-9, Noida-11, Mn-15

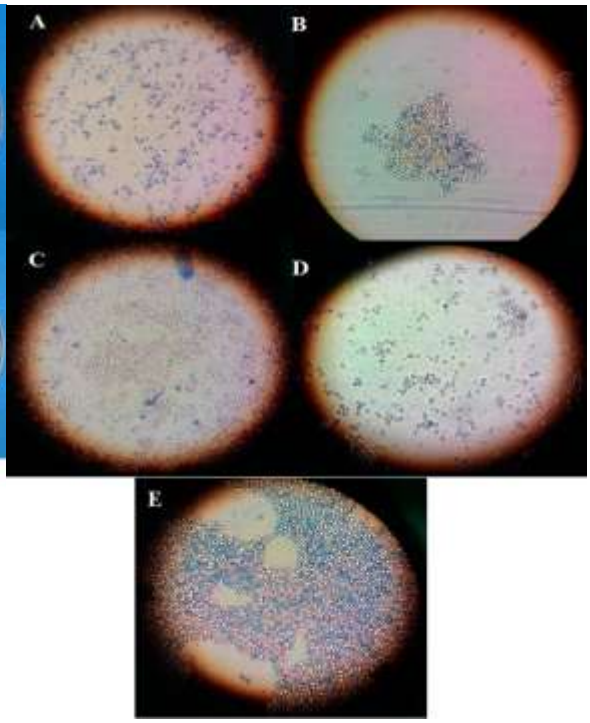

Fig. 2: *Saccharomyces cerevisiae* staining by the yeast viability staining different region such as Ar (A), Fad (B), Gr Noida (C), Noida (D), Mn (E)

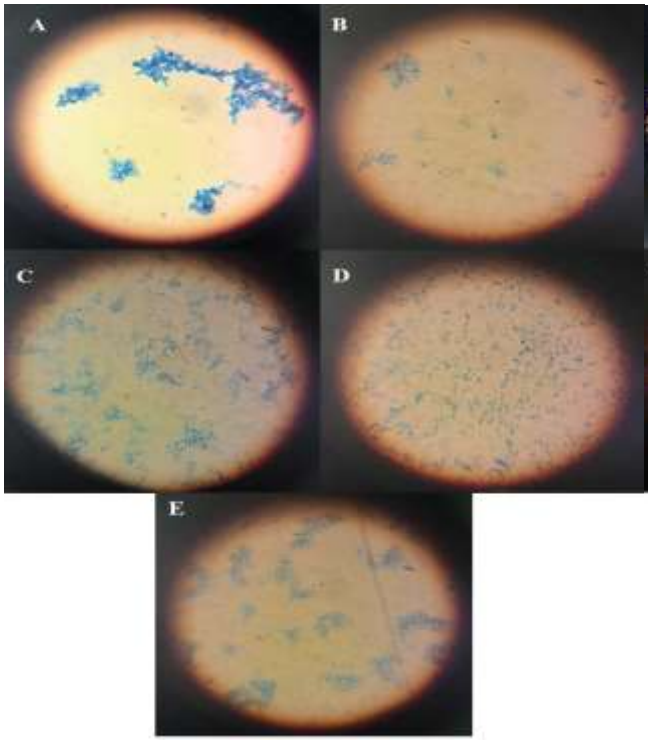

Fig. 3: *Saccharomyces cerevisiae* staining by the Lacto phenol staining different region such as Agra (A), Faridabad (B), Noida (C), Noida (D), Mainpuri (E)

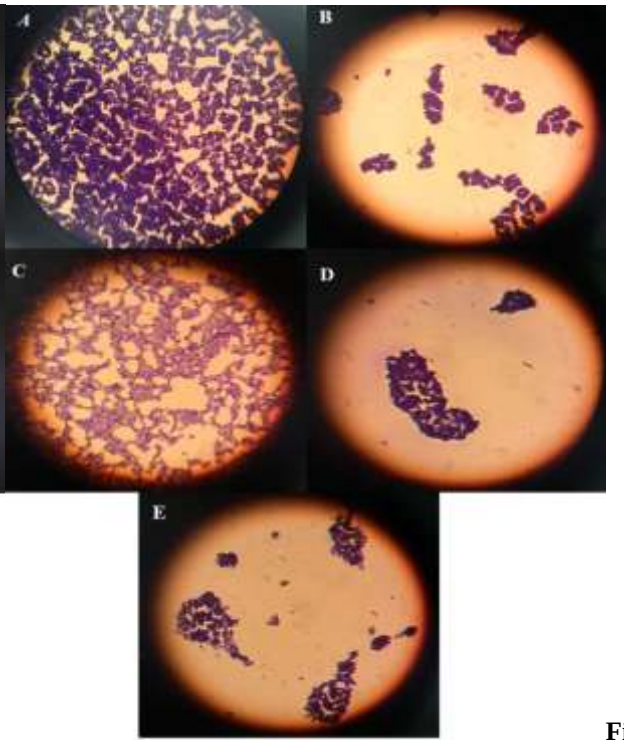

Fig. 4: *Saccharomyces cerevisiae* staining by the Simple staining cotton blue of microbes different region such as Agra (A), Faridabad (B),Greater Greater Noida (C), Noida (D), Mainpuri (E)

Fig. 5: Gelatin liquefaction test again *Saccharomyces cerevisiae* from different region such as Agra, Faridabad, Greater Noida, Noida and Mainpuri

Fig. 7: Urea broth test again *Saccharomyces cerevisiae* from different region such as Agra, Faridabad, Greater Noida, Noida and Mainpuri

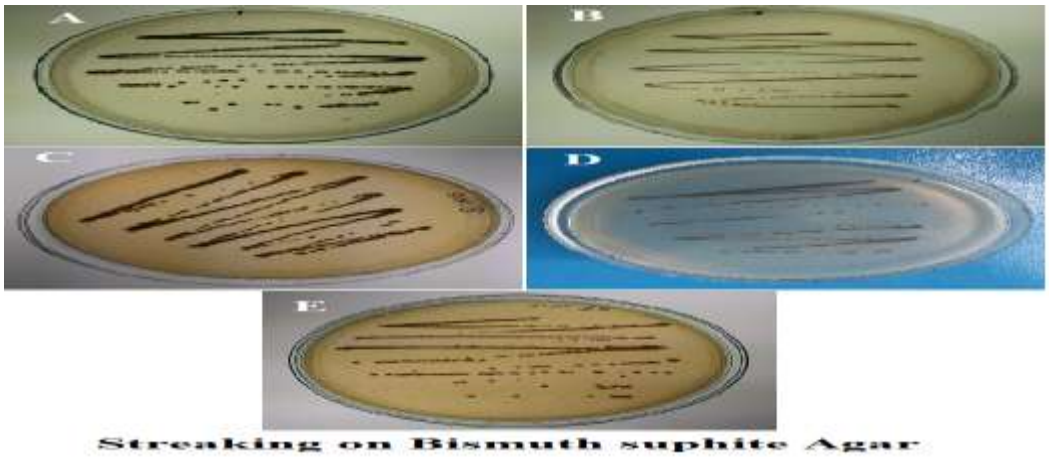

Fig. 8: Bismuth sulphite test again *Saccharomyces cerevisiae* from different region such as Agra (A), Faridabad (B), Greater Noida (C), Noida (D) and Mainpuri (E)

IV CONCLUSION

Saccharomyces cerevisiae strains obtained for sugar cane juice from different sub-region as Agra, Faridabad, Greater Noida, Noida and Mainpuri District. These strains were isolated on YPDA media, incubated at 30°C for 48hrs. These isolated strains have identity by on the basis morphological, physiology and biochemical characterization. Isolated of

yeast colonies were observed under microscope compound. This strain was stained by yeast viability staining (Methylene blue staining), Lacto phenol cotton blue staining and sample staining, that the cell morphology was morphologically observed under a microscope for highpower objectives. Physiology and biochemical diagnosis by deferent methodology, *Saccharomyces cerevisiae* negative result has been found on the gelatin and urea. *Saccharomyces cerevisiae* has produced H_2S on bismuth sulphite agar media, which is screening blackish brown black smooth colonies.

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