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Isolation and Characterization of Indigenous Yeast Species from Yoghurt and Sugarcane Juice for Production of Bio-ethanol

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Abstract
The yeast strains possess appreciable characteristics for ethanol production. Moreover, Bio-ethanol production from renewable sources to be used is now an increasing demand worldwide due to continuous depletion of fossil fuels, economic and political crises, and growing concern for environmental safety. The main objective of this study was to isolate indigenous yeast strains from yoghurt and sugarcane juice samples which may further be utilized in bio-ethanol production. A total of 20 samples were collected randomly from local markets of different localities of Karachi. These samples were screened using selective medium Yeast Extract Peptone Dextrose Agar (YEPD), from where fifteen yeast strains were isolated. Differential tests were applied, including morphological, cultural and biochemical characteristics, which facilitate the opportunity for identification of the yeast strains. More yeast strains were isolated from sugar cane samples as compared to yoghurt. These strains have shown the potential to efficiently utilization of the Glucose, Fructose, Sucrose, Maltose and Urea as the carbon source. A yeast strain able to produce a good yield of fermentation of sugars into ethanol was identified. The isolation of these fermenting yeasts could attract widespread interest worldwide, as they can be used in the cost-effective production of bio-ethanol. These isolates could be used at the industrial level for fermentation of various raw materials in order to obtain an increased production of bio-ethanol.
Keywords: Yeasts, yeast extract peptone dextrose agar, bio-ethanol, starch, technology.

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INTRODUCTION

A worldwide interest in the increasing demand of bio-ethanol as an energy source from renewable resources has encouraged studies on successful fermentation technology for ethanol production. The economics of ethanol production by fermentation increases by the cost of raw materials which rate for more than half of the manufacturing costs (Roble *et al.,* 2003). For this reason, bioethanol production from agricultural resources has concerned attention in current years. For a larger yield of ethanol production by fermentation, we have to require fermentation substrates, an ideal high yielding strain, and suitable process technology is also essential. In the process of fermentation, Carbohydrate-rich raw materials good for bio-ethanol production can be divided into three groups of agricultural products: the first raw material group is from sugar like sugarcane and molasses, about 60% of the global ethanol is produced from sugar crops (Ghassem *et al.,* 2012); the second one is starch from crops includes cassava, cereals, and potatoes, and the last group is lignocelluloses which cover waste materials such as rice straw, corn cob waste such as bioethanol from lignocellulosic biomass has recently been studied extensively (Mogg, 2004). Moreover, 40% ethanol is produced from starchy grains (Salassi, 2007). Starch constitutes the most abundant source of energy for living organisms. This heterogeneous polysaccharide made up of two high-molecular-weight components: linear amylase and branched amylopectin are ruined predominantly by hydrolytic enzymes known as amylolytic enzymes. A large variety of microorganisms, among them yeasts, are the producers of these enzymes. Various hydrolytic enzymes are also produced by bacterial isolates which have industrial applications (El-Gendi *et al.,* 2016; Imran *et al.,* 2016). Yeasts are eukaryotic microorganisms in the kingdom Fungi, with 1,500 species presently described

(Pastorius, 1997). Yeasts are unicellular, ubiquitous and commonly spoilage fruits, vegetables, and other plant materials (Khattab *et al.,* 2016).

Although a number of yeast species show multicellular forms through the creation of strings in connected budding cells known as pseudo-hyphae, or false hyphae (Barnett, 1975). Yeast size can vary completely depending on the species, normally measuring 3-4 µm in diameter, while some yeast species can range over 40 µm (Walker *et al.,* 2002). Most yeasts reproduce asexually by mitosis, and many do so by an asymmetric separation process called budding. *Saccharomyces cerevisiae* and other yeast species convert carbohydrates to carbon dioxide and alcohol through fermentation, used in baking and alcoholic beverages respectively (Legras *et al.,* 2007). The importance of genus *S. cerevisiae* has been well-known from the early times for fermentation (Qureshi *et al.,* 2007). Other species of yeasts, such as *Candida albicans*, are opportunistic pathogens and can initiate infections in humans.

Microorganisms such as yeasts play an important role in bioethanol production by fermenting a wide range of sugars to ethanol. Yeasts have been used to produce electrical energy through microbial fuel cells (Bergman, 2001) and yield ethanol for the biofuel industry. Bio-ethanol fermentation also referred to as Alcoholic fermentation is a biological process in which elements such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products (Ruhul *et al.,* 2013). At present, the bread industry of Pakistan is exclusively reliant on the import of yeast. So, there is a terrible prerequisite to discover the potential of indigenous Yeast strains in order to meet the national requirements. As an alternative, there is a pronounced potential for commercial biogas sector for mitigating energy crisis in Pakistan (Shaukat *et al.,* 2016).

There are various sources for the isolation of yeast species. However, their incidence was described mostly from the acidic foods. Among them, citrus juice (Arias *et al.,* 2002), yoghurt (Savova and Nikolova, 2002) and sugarcane juice (Ceccato-Antonini *et al.,* 2004) are deliberated to be the best sources of yeast. Citrus juices are acidic beverages (pH 3 - 4) with high sugar content because of their low pH, selective environment for the growth of microbes (Iqbal *et al.,* 2015; Iqbal *et al.,* 2016a; Iqbal *et al.,* 2016b) and distinctive yeast species found in citrus juices are *Candida intermedia, Candida parapsilosis, Hanseniaspora occidentalis, Hanseniaspora uvarum, Pichia kluyveri* and *Saccharomyces cerevisiae* (Arias *et al.,* 2002). Yoghurt is the most significant fermented milk product. Yeasts are un-desirable microflora in yoghurt. It is an especially favorable environment for the growth of yeasts due to the acidic reaction of the medium. Yoghurt supports the growth of a wider variety of yeast species including *S. cerevisiae*, *S. dairnensis*, *S. unisporus* and *S. octosporus* for their ability to cultivate at low temperature and also lactose dissimilation (Robert *et al.,* 2015). Sugarcane juice is a promising environment for the growth of yeasts owing to high sugar content. Distinctive yeast species related to sugarcane juice are *Saccharomyces cerevisiae*, *S. exigus, S. kluyveri* and *S. ludwigii* (Oliveira *et al.,* 2008). Thus, in industrial ethanol production, there are many important factors to be considered, such as sugar or ethanol tolerance of yeast strains, ability to do fermentation at higher temperatures (thermotolerance) and enzymatic activities for certain transformations (Mobini-Dehkordi *et al.,* 2007; Patrascu *et al.,* 2009).

As there is a worldwide emphasis on ethanol production by the fermentation process and keeping in outlook the importance of yeast, the present study followed isolation of indigenous yeast strains from yoghurt and sugarcane juice samples which may further be used for alcohol production.

MATERIALS AND METHODS

All the research work was carried out in the Microbiology Laboratory of the Department of Microbiology, Jinnah University for women, (JUW) Karachi, Pakistan.

Collection of samples

A total of 20 yoghurt and sugarcane juice samples (10 samples each) were randomly collected in sterile bottles from local markets of different areas of Karachi. These samples were screened for identifying the yeast strains.

Isolation of Yeast strains

The isolation was achieved according to methods used by Kurtzman et al. (2011) and Satish Babu et al. (2010). The sampled were placed on a selective medium, Yeast Extract Peptone Dextrose Agar (YEPD). The inoculated plates were incubated at 28°C for 48 hours. Plates were observed for growth and colonial morphology of the isolates.

Biochemical characterization

Isolates were further biochemically tested for their confirmation, including Catalase, glucose, fructose, sucrose, maltose and urea.

Determination of enzyme synthesizing ability (Starch)

To determine the capability of strains to assimilate polysaccharide, the strains were grown in basal medium at 28°C for 18 hours, supplemented with starch.

Determination of Ethanol producing ability

To confirm the ability of strains to produce alcohol, qualitative estimation of ethanol by sodium hydroxide and potassium iodide (Chatterjee *et al.,* 2011).

RESULTS

Isolation of yeast strains

Fifteen yeast colonies were isolated from twenty samples (two sources) on YPD agar medium. A total of 15 isolates were obtained: yogurt (N=10) and sugar-cane (N=5). The isolates were subjected to further streaking until completely purified; subsequently, fifteen isolates were selected for further studies of characterizations and identification (Table 1).

Morphological Characterization

The morphological characters of selected yeast isolates were summarized in Table 2.

Biochemical Characterization

The biochemical analysis of the strains isolated from yoghurt samples showed that all the fifteen strains could grow in the presence of sugars and urea and ferment them (Table 3).

Assimilation tests

All selected yeast isolates were tested for assimilation of carbon and their catalase activity. The utilization of polysaccharides (starches) was tested. A reaction was detected by observation of turbidity that indicated the presence of starch. All the fifteen strains strongly assimilated starch enzyme. Moreover, the isolates also exhibited catalase ability of various degrees (Table 2 and 3).

Fermentation tests

Five sugars (glucose, fructose, maltose, sucrose and urea) were used to study fermentation abilities of yeast isolates. The change in color indicates production of alcohol. Strains Y.1, Y.2, Y.3, Y.4, Y.5, Y.6, A.1, A.2, A.3, A.4, and A.5 were efficient to ferment all sugars, while Y. 7 and 10 fermented all sugars except maltose and sucrose. On the other hand, A.5 to 10 was unable to ferment any sugar (Table 2 and 3).

Table 1. Total isolates from Yoghurt and Sugarcane Juice Samples

Sources tested	No. of samples	No. of strains isolated
Yoghurt	10	10
Sugarcane	10	5
Total	20	15

Table 2. Morphological, Biochemical and Physiological Characteristics of the Yeast Strains Isolated from Sugar cane Samples

Strains	Surface	Margin	Color	Cells	Glucose		Fructose		Sucrose		Maltose		Urea		Starch	Catalase Activity
					Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc		
Y1	Smooth	Irregular	Creamy White	Round	++	+	+++	+	+++	+	++	+	+	+	+	++
Y2	Rough	Circular	Creamy White	Oval	++	+	+++	+	+++	+	+++	+	+	+	+	+
Y3	Smooth	Circular	Creamy White	Round	++	+	+++	+	+++	+	+++	+	+	+	++	+
Y4	Rough	Irregular	Creamy White	Spherical	++	+	+++	+	+	+	++	+	+	+	+	++
Y5	Rough	Irregular	Creamy White	Oval	++	+	+++	+	++	+	+	+	+	+	+	+
Y6	Rough	Circular	Creamy White	Round	+	+	+++	+	++	+	++	+	+	+	+	+
Y7	Rough	Irregular	Whitish Black	Spherical	+	+	+++	+	+	_	+	-	+	+	+	++
Y8	Rough	Irregular	Creamy White	Spherical	++	+	+++	+	+++	+	+++	+	+	+	+	+
Y9	Rough	Irregular	Creamy White	Oval	++	+	+	+	+++	+	+++	+	+	+	+	+
Y10	Rough	Irregular	Blackish	Oval	++	+	+++	+	+	_	+	-	+	+	+	++

Gr: Growth; Alc: Alcohol; +: moderate; ++: very good; +++: extremely good

Table 3. Morphological, Biochemical and Physiological Characteristics of the Yeast Strains Isolated Sugarcane Juice Samples

Strains	Surface	Margin	Color	Cells	Glucose		Fructose		Sucrose		Maltose		Urea		Starch	Catalase Activity
					Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc		
A1	Rough	Irregular	Creamy White	Oval	+	+	+++	+	+++	+	+	+	+	+	+	+
A2	Rough	Irregular	Creamy White	Oval	++	+	+++	+	+++	+	++	+	+	+	+	+++
A3	Smooth	Circular	Creamy White	Oval	++	+	+++	+	+++	+	++	+	+	+	++	+
A4	Rough	Circular	Creamy White	Round	++	+	+++	+	+++	+	++	+	+	+	+	++
A5	Smooth	Circular	Creamy White	Oval	++	+	+++	+	+++	+	++	+	+	+	+	++

Gr: Growth; Alc: Alcohol; +: moderate; ++: very good; +++: extremely good

DISCUSSION

Yeasts isolated from natural resources have abilities to utilize and ferment various exogenous compounds (Banat *et al.,* 1998; Rodriguez *et al.,* 2011). The isolated strains grown on YPDA medium possess smooth surfaces, circular margins with creamy, creamy white and yellow-white color. The cells were found to be of various shapes such as round, oval, spherical and ellipsoidal. Our findings were found to be consistent with previous findings (Chatterjee *et al.,* 2011).

The yeast species are also determined by the great extent of physiological characteristics (Khattab *et al.,* 2016). An isolated strain has an ability to assimilate all tested sources of carbon, in addition to, ferment glucose, fructose, maltose, sucrose and urea (Yun *et al.,* 2001). The isolates Y.1, Y.2, Y.3, Y.4, Y.5, Y.6, A.1, A.2, A.3, A.4, and A.5 were found to possess the ability to produce alcohol of significant quantity. Yeast strains can be utilized in alcohol production. The best result in alcohol production was given by sugarcane isolates. A previous study reported the production of alcohol, which is quite similar to the present study and provides supportive evidence to our work (Gasmalla *et al.,* 2012).

A number of Yeast species should be used in a different food manufacturing products; also it is a source of ingredients and additives for food processing. By the experiment, we examined yeast strains showed good fermentation attributes, which might enhance ethanol yield that would contribute to the cost-effective role in the production of bio-ethanol and enzymes of industrial importance. At the industrial level, it should be used as a good fermenter (Turner *et al.,* 2007).

However, better yield depends on the selection of microorganisms, fermentation mode and techniques as well as the influence of various factors. In addition, selection of strains and juice producing crops will also improve the commercial ethanol production. However, because of the large volumes of ethanol that is produced and traded each year, any small improvement in the process could represent a savings of billions of dollars. The present study revealed that the yoghurt and sugarcane juice samples produce yeast strains that be utilized in enzyme production and alcohol production.

CONCLUSION

Since thousands of years ago, yeasts such as *S. cerevisiae* have been used in the brewery and wine industries. In this study, indigenous yeast strains were isolated from yoghurt and sugarcane juice samples that produce starch enzyme and bio-ethanol. Therefore, it could provide an economical and easy process industrially for suitable production of ethanol that will solve our energy crisis by producing more ethanol in a stable way.

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CONFLICT OF INTEREST

The authors verify having no interest in competition and have no conflicts of interest.

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